NIU Journal of Health Sciences



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Published in October, 2026

ISSN: 3136-6457 (Print)

ISSN: 3136-6449 (Online)

Published by:

Nexus International University,
Kampala, Uganda.

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Nexus International University ISSN: 3136-6449; 12(2) 3

Editorial

This edition of NIU *Journal of Health Sciences* touches on health and medically related issues such as Disease Control, Harmful Traditional Practices, Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) among admitted patients at Baqubah Teaching Hospital in Diyala, among others.

One of the papers, in this issue, reveals that that despite relatively high educational levels among participants, some harmful traditional health practices are still believed and practiced. It therefore, recommends that increasing awareness and providing accurate health education are essential to reduce the spread of these misconceptions.

Another paper also reveals that a high prevalence of multidrug-resistant *S. aureus*, including a substantial proportion of methicillin-resistant (MRSA) strains, at Baqubah Teaching Hospital. The significant variations in susceptibility patterns across different antibiotics and anatomical infection sites emphasize the necessity of tailored empirical therapy. These alarming resistance rates warrant the immediate implementation of strict antimicrobial stewardship programs and regular local surveillance to curb the dissemination of these refractory pathogens.

On the whole, this edition of *NIU Journal of Health Sciences* features many empirical and theoretical based articles which can be of great benefit to every reader.

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October, 2026.

NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 5-14

Apoptosis as the Primary Elimination Mechanism for Cancer Cells: Mechanisms, Evidence, and Therapeutic Implications

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Abstract. Apoptosis constitutes the principal mechanism controlling the elimination of cancer cells. Apoptosis arises as a fundamental capability of multicellular organisms regulating cell number and maintaining homeostasis. It is the best-studied form of programmed cell death and broadly participates in multiple physiological processes embryogenesis, tissue remodeling, and immune cell maturation, among others. According to accumulated evidence, apoptosis acts as a potent tumor-suppressor mechanism curtailing cancer cell expansion. Cancer cells are subjected to multiple biological insults continuously exerted by their micro- and macro-environment and, when left unresolved, such detrimental events drive cells toward a neoplastic transformation. Cancer cells often acquire opposing alterations that foster adaptation to these events, evade replication barriers, and expand into tumors. Recent studies have argued that when cancer clones develop resistance, pro-apoptotic signals become a prominent component of antagonistic programs, reinforcing the notion that a majority of cancer clones are still susceptible to apoptotic removal during tumor establishment.
At least three forms of terminal cancer-cell elimination have been identified: apoptosis, senescence, and necrosis. Apoptosis distinctively leads to the generation of an immunogenic signal that facilitates the elimination of cancer-cell clones even from resistant tumors. Available genetic, molecular, and pharmacological evidence supports the notion that apoptosis operates as a major mode for the elimination of cancer cells during malignant transformation. Importantly, a wide variety of therapeutic modalities induce death predominantly through apoptosis. Because apoptosis appears to still remain a critical backup option during cancer prevention, continued further investigation into the mechanisms of apoptosis and the establishment of new therapeutics for its induction holds considerable promise for improving cancer treatment.
A multitude of stresses imparted by tumor microenvironments destabilizes normal tissues and triggers apoptosis. Treatments that cause further stresses, including DNA lesions, directly promote massive apoptosis, underscoring the still-latent vulnerability of cancer cells. Collectively, compounds that elicit extensive, unmanageable apoptosis in cancer cells, either through direct activation or relay of biochemical signals, can become new agents or be combined with existing ones for more effective therapy.

1. Introduction

Apoptosis is widely recognized as a central elimination mechanism for cancer cells. Most anti-cancer treatments induce cancer cell death, primarily because damage inflicted on malignant cells activates apoptotic pathways, which are often dysregulated in tumors. Thus, fading of these damage signals or the acquisition of pro-survival mutations allows tumor cells to escape the primary elimination mechanism that therapies are designed to engage. This pivotal role of apoptosis in tumor eradication arguably positions it among the most important hallmarks of cancer, especially when discussing therapeutic options. This work compiles the existing evidence that links apoptotic execution to the clearance of tumor cells and considers the implications for the development of therapeutics that target these pathways toward the elimination of cancer (Morana *et al*., 2022). There are some types of cancer that result from chromosomal aberration, and apoptosis plays a major role in their treatment (Al-Samarraie, 2026).

Methods have been developed to induce apoptosis in cancer cells, aiming to restore pathway engagement and tumor-cell elimination. These methods are classified as pro-apoptotic agents and BH3 mimetics. Pro-apoptotic agents activate the apoptotic machinery by targeting specific players at various stages of the core pathway. The rapidly evolving landscape of BH3 mimetics directly targets pro-survival proteins of the BCL-2 family, freeing pro-apoptotic proteins from tight control and unleashing apoptosis upon cells still possessing functional executioners. Both classes have potential for improved cancer therapy, yet ensuring selective action on tumor cells remains critical to safe application (Franzese *et al*., 2024).

Apoptosis is a well-defined form of programmed cell death involving a highly regulated series of biochemical events leading to the characteristic morphological and molecular features of cellular disassembly. Apoptosis is triggered by a variety of stimuli, including developmental signals or pathogen-derived signals, and plays a crucial role in normal biological processes such as embryogenesis, tissue homeostasis, the immune response, and the response to injury (Morana *et al*., 2022). Apoptosis also acts as a key tumor-suppressive mechanism, faithfully executing developmental and homeostatic cell-death programs. Cellular transformation typically involves alterations in apoptosis control—such as the emergence of oncogenic signalling or the disruption of p53 activity—that promote cell cycle entry, progenitor-like cell states, and resistance to stress (Lim et al., 2019).

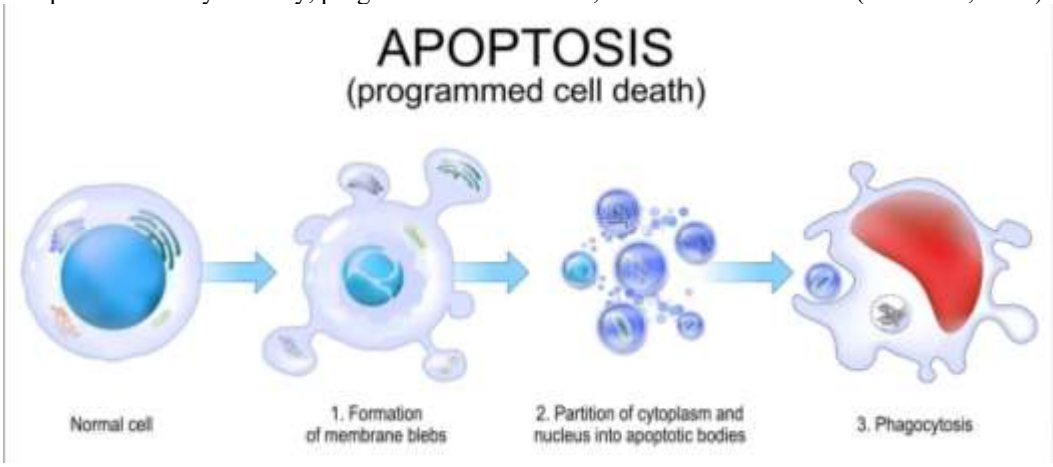


Figure (1) *Stages of Apoptosis*

2. Historical Perspective on Apoptosis in Cancer

Cell death has been an object of biological curiosity since the 1850s, when histologists began documenting the microanatomical pathways leading to the demise of individual cells in cultured tissues (Jacobson *et al*., 1997). Initial concepts of cell death were simplistic on both mechanistic and teleological levels, but advances in light microscopy and biochemistry in the 1960s prompted speculation that physiological apoptosis had evolved to facilitate selective removal of cells during development and tissue homeostasis (Boehm & Bock, 2019). Following these developments, British zoologist Sir Victor Charles Tcharles Huxley undertook pioneering, sophisticated, and painstaking studies on avian red blood cell development in the 1920s—able to distinguish and name "decidual corpuscles" of abortive red blood cells (Huxley, 1922). The discovery that excessive Huxley pathology also accompanied oncogenesis came only later (Huxley, 1949).

Apoptosis (programmed cell death) acts as a primary mechanism responsible for initiating cancer cells; it occurs in physiologically discrete stages, in both intrinsically (mitochondrially) and extrinsically (death receptor-initiated) activated forms. Current evidence substantiates the crucial function of apoptotic clearance in cancer cell initiation, instructing robust therapeutic approaches aimed at reinstating apoptosis to address the pervasive resistance to this death program within tumors. Cancer is a leading global cause of morbidity and mortality, necessitating urgent efforts to prevent and treat rampant tumor proliferation. Although exciting new therapies are emerging to tackle this reflex, observing cancer cell alterations in response to such approaches remains a fundamental challenge. Insights derived from these considerations may inform the ongoing development of more robust and transformative therapies, enabling tumors to be thwarted and precociously eradicated (Hajdu, 2011).

3. Core Mechanisms of Apoptosis

Apoptosis is a tightly regulated, conserved process that removes unwanted or damaged cells without provoking an inflammatory response. The fundamental importance of apoptosis is evident in multicellular organisms: such sensitivity to apoptotic cues renders cells highly vulnerable to therapies. A wealth of studies has illuminated apoptosis' central role in cancers arising in response to DNA damage and oncogenic signaling. In particular, both intrinsic and extrinsic apoptotic pathways can be activated during treatment, yet acquired resistance to these pathways has evolved as a major challenge in the search for effective cancer therapies (Morana *et al*., 2022).

Elimination of unwanted cells occurs via two antagonistic cell-death mechanisms: necrosis and apoptosis. Necrosis leads to cellular swelling or lysis and initiates inflammation, while apoptosis is characterized by cellular shrinking and the preservation of membrane integrity without inflammatory damage. These distinct characteristics secure the existence of the apoptotic mechanism in multicellular organisms, and substantial evidence supports its role in removing unwanted cancer cells. Whereas these three sections outline the general mechanisms for apoptosis, extensive mechanisms concerning necrosis remain to be elucidated (Sazonova *et al*., 2021).

Mammalian apoptosis is divided into three functional stages: initiation, execution, and removal. The initial stage distinguishes between two distinct pathways, termed intrinsic (mitochondrial) and extrinsic (death receptor), which are defined by non-overlapping upstream signals. The second phase generally employs cysteinyl proteases (caspases) as the primary hydrolyzing enzymes. The critical differences in the upstream signalling mechanisms determine how agonistic and antagonistic signals regulate apoptosis. The final stage facilitates the clearance of apoptotic cells by neighbouring or professional phagocytes (Morana *et al*., 2022).

3.1. Intrinsic (mitochondrial) pathway

The mitochondrial or intrinsic pathway of apoptosis is initiated by signals generated within stressed or damaged cells that lead to mitochondrial outer membrane permeabilization (MOMP). First, pro-apoptotic BH3-only proteins such as BIM, BID, and PUMA concentrate at the mitochondria, where they activate the pore-forming BCL-2 proteins BAX and BAK, leading to MOMP. Subsequently, apoptogenic factors from the intermembrane space, including cytochrome c, SMAC, and Omi, are released into the cytosol. Cytochrome c promotes apoptosis by binding to apoptotic protease-activating factor 1 (APAF-1) to form the apoptosome, which recruits pro-caspase-9 and activates the caspase cascade (Lopez & Tait, 2015). Other pro-apoptotic proteins, such as apoptosis-inducing factor (AIF), macroautophagy-related protein 2 (MAP-LC3), and arginase-1 (ARG-1) orchestrate the clearance of the dying cell. At both the cell and organismal level, apoptosis is resolved through coordinated removal of the dying cells (Wani *et al*., 2023).

3.2. Extrinsic (death receptor) pathway

The extrinsic or death receptor pathway of apoptosis occurs through ligation of death receptors on the cell's surface, which are members of the TNF superfamily, such as TNF-α/TNFR1 and FasL/FasR. Upon ligand binding, FADD forms a complex with caspase-8, creating the death-inducing signaling complex (DISC). Caspase-8 acts as the initiator caspase, cleaving effector caspases-3 and -7, which cause apoptosis typically through activation of the endonuclease CAD. Caspase-8 also functions as a molecular switch regulating apoptosis, necroptosis, and pyroptosis. Effector caspases induce chromatin condensation and cell death by cleaving various substrates, with ICAD being integral to chromatin condensation (Devoe, 2020).

The extrinsic pathway of apoptosis involves two models: the TNF-induced model and the Fas-Fas ligand-mediated model. TNF-α, produced by macrophages, is a key extrinsic apoptotic mediator. Human cells have TNFR-1 and TNFR-2; binding of TNF-α to TNFR-1 initiates a pathway leading to caspase activation. The TNFR1-associated death domain protein (TRADD) and FAS-associated death domain protein (FADD) can bind to TRAF-2, inhibiting caspase-8 activation, which may activate inflammatory and survival pathways. The first apoptosis signal, Fas (CD95 or APO-1), binds to Fas ligand, forming the DISC complex with caspase-8, caspase-10, and FADD. In type I cells, caspase-8 directly activates other caspases to execute apoptosis. In type II cells, Fas-DISC triggers mitochondrial release of pro-apoptotic factors, amplifying caspase activation (Wani *et al*., 2023).

3.3 Execution phase and caspases

The central caspases 3, 6, and 7 act as downstream executioners that mediate the final destruction of a wide variety of cellular structures and processes common to virtually all apoptotic stimuli and pathways. Evidence indicates that activation of these effector caspases marks the point of no return for cells communicating an apoptotic signal. Once active, these effectors invariably lead cells to the distinctive morphologic and biochemical endpoints of apoptosis, with a considerable degree of conservation across different species (Boland *et al*., 2013; Eskandari & J. Eaves, 2022).

4. Regulation of Apoptosis in Cancer

Cancer cells acquire several traits during their evolution, facilitating unrestricted proliferation, metabolic changes, and immune evasion. They also engage in mechanisms to evade the intrinsic and extrinsic signals that drive apoptosis, promoting tumor initiation and malignant transformation. Tumor cells often modify oncogene and tumor suppressor signaling; for example, the inactivation of p53, a critical regulator of apoptotic cell death, commonly occurs during the emergence of tumorigenesis (Morana *et al*., 2022). Even if p53 escapes inactivation and remains functional, the upregulation of anti-apoptotic mechanisms can still confer resistance to apoptotic signals. Cancer cells also reprogram the mitochondrial response of the intrinsic pathway, manipulating BCL-2 family proteins that govern mitochondrial priming and the progression of apoptosis (Wani *et al*., 2023).

Apoptosis is the target of multiple alterations favouring tumor progression. The BCL-2 family emerges as one of the main regulators of apoptosis. BCL-2, which restrains apoptosis, is overexpressed in many human cancers. The p53 gene, mutated in the majority of human tumors, regulates pro-apoptotic and anti-apoptotic factors, whereas the FAS-associated death domain protein (FADD), a central mediator of the extrinsic death receptor pathway, is downregulated in several cancer types. Survivin is ubiquitously expressed in human cancers and inhibits apoptosis by interfering with caspase activation and regulating cell division. In cancer, the signalling networks controlling pro-apoptotic and anti-apoptotic factors become rewired, shifting the balance towards pro-survival. This enables tumor cells to resist drugs that induce apoptosis and thus cooperates with other strategies to evade therapy (Jan & Chaudhry, 2019; Morana *et al*., 2022; Franzese *et al*., 2024).

4.1. Oncogenic shifts and p53 status

Only a single oncogenic shift has been shown to induce tumors. The p53 transcription factor plays a crucial role in guarding against cancer by regulating the expression of pro-apoptotic genes, and its loss of function or null mutation is often characteristic of aggressive and difficult-to-treat tumors. Active repression of the p53 transcript or of the signaling pathway by oncogenes has been reported (Mirzayans *et al*., 2012).

4.2. BCL-2 family proteins and mitochondrial priming

In the BCL-2 protein family, anti-apoptotic members inhibit the pro-apoptotic effect of multi-domain and BH3-only proteins. Even in the presence of pro-apoptotic signals, these proteins maintain mitochondrial integrity and inhibit permeabilization of the outer mitochondrial membrane (OMM). Cells reside at varying degrees of mitochondrial priming, defined by the relative expression of pro- and anti-apoptotic BCL-2 family members and the environment determined by upstream signaling to pro-apoptotic effectors. Compounds targeting the anti-apoptotic BCL-2 family have demonstrated selectivity for cancerous tissues, therapeutic enhancement when combined with classical chemotherapeutics, and the ability to overcome acquired resistance—opening a promising avenue for the development of anti-cancer treatments. Potent and selective inhibitors of individual BCL-2 family members have also been developed and shown to target various tumor types. These properties suggest that the compounds could have major implications for a wide array of malignancies, including hematological malignancies and solid tumor types, in both the preclinical and clinical stages (Campbell & Tait, 2018).

Among various therapeutic strategies, agents mobilizing the Intrinsic apoptosis pathway have been extensively studied, including the use of pro-apoptotic N19 and N4 peptides, which disrupt BCL-2:BAX and BCL-2:BAK complexes respectively. BH3 mimetics such as ABT-737, ABT-263 (Navitoclax), and Wehi-539, which selectively inhibit BCL-2 and BCL-XL, have also been explored as single agents in preclinical and clinical studies targeting a variety of hematological malignancies and solid tumors. Although initial results have been promising,

many tumors ultimately develop resistance, and preclinical models have revealed a range of intrinsic and acquired resistance mechanisms (Jan & Chaudhry, 2019).

4.3. Death receptors and extrinsic signal modulation

According to a widely accepted conceptual framework, a cancer cell is considered eliminated when its death, independent of neighboring cells, leads to a significant reduction in tumor burden. Consequently, cancer cells remain capable of proliferating, especially in the initial phases of tumor formation (Papenfuss *et al*., 2008).
Many studies have indicated that the execution stage of the apoptotic process, evidenced by features such as chromatin condensation, cell shrinkage, plasma membrane blebbing, and the exposure of phosphatidylserine residues, leads to the extinction of at least 60% of treated tumor cells. This decisive loss of cellularity contrasts with necroptosis or autophagy, two other mechanisms known to promote tumor suppression. Removal can occur in either lymphoid or nonlymphoid tissues, and substantial tumor regressions have been observed across numerous genetically diverse models, including solid malignancies like lymphomas, leukemias, gliomas, tumors driven by Ras mutations, and Ewing sarcomas characterized by an aberrant EWS–FLI fusion (Jan & Chaudhry, 2019).

Apoptosis may be directly engaged following treatment with cisplatin, etoposide, or various conventional anticancer agents. Such foundational insights into the stringent connection between an apoptotic fate and robust tumor clearance support the notion of apoptosis as the preeminent mechanism for cancer cell elimination (Mirzayans & Murray, 2024).

5. Evidence Linking Apoptosis to Cancer Cell Elimination

The anticancer activity of many therapeutics depends on their capacity to trigger cancer cell apoptosis. Numerous drugs and targeted therapies routinely used to treat various cancers are known to induce apoptotic cell death in tumor cells. For example, γ-irradiation causing DNA double-strand breaks and chemotherapeutic agents such as cisplatin, which form bulky DNA adducts and induce DNA crosslinking, readily activate apoptosis-inducing pathways in tumors (Sazonova *et al*., 2021). Further, when examined in living organisms, treatment regimens that induce the greatest levels of apoptosis often lead to the most bountiful and prolonged tumor regressions. Finally, detailed analyses of the importance of p53, a key tumor suppressor commonly lost during tumor development, have shown that p53-dependent apoptotic signaling plays a prominent role—often the predominant role—in the clearance of tumors in response to a wide range of different therapeutic agents (Morana *et al*., 2022).

Collectively, these observations suggest that, when the capacity to trigger apoptosis is added into the equation, the connection between tumor elimination and cancer cell apoptosis becomes considerably more interconnected than was previously assumed. Thus, a clearer and more compelling case can be made that cancer cell eradication emerges as one of the principal functions fulfilled during p53-dependent tumor suppression, indicating that restoring active p53 signaling in late-stage malignancies still leaves considerable preserved functionality towards cellular clearance (Chen *et al*., 2026).

5.1. In vivo models demonstrating apoptotic clearance of tumors

The association of apoptosis with cancer cell elimination is supported by multiple in vivo model systems demonstrating tumor clearance following apoptotic induction. Various experimental manipulations, including transgenic overexpression of pro-apoptotic regulators, treatment with pro-apoptotic agents, and genetic deletion of anti-apoptotic factors, have been employed to precipitate apoptosis in solid tumors of different origin. Collectively, the data show that apoptosis can mediate substantial tumor reduction across several cancer types and suggest patterns of viral, genotoxic, or metabolic stress that drive apoptotic signaling from a previously ideal state (Schirrmacher, 2019).

Tumor clearance and simultaneous reduction in proliferative marker expression or specific tumor burden measurement are frequently reported endpoints across different animal studies employing distinct transgenic models, xenografts, and drug administration strategies. Tumor volumes are typically determined by caliper measurement of external tumor diameter, with the final volume derived from $(4/3)\pi$ (D1/2) (D2/2) for ellipsoid tumor shape approximation. The time elapsed between induction of committed apoptosis and ablation of the terminally injured tumor cell population varies across settings but generally ranges from days to weeks. In terms of translational relevance, either the MCF7 or 4T1 triple-negative mammary tumor cell types or other models

could be adopted for extensive murine screening of the broad class of BH3 mimetic or other apoptosis-inducing agents (Franzese *et al*., 2024).

5.2. Clinical observations correlating apoptotic markers with outcomes

Studies of various preclinical models show that clearance of the majority of tumors in vivo correlates with apoptotic indicator expression in cancer cells. In p53-deficient models of B-cell lymphoma, tumor eradication is triggered by p53 restoration or c-Myc deletion; clearance occurs through rapid induction of apoptosis in most cancer cells, while a few resist and survive (Morana *et al*., 2022). When MDM2 is inhibited, MYC-activated B-cell tumors regress and most cells undergo apoptosis solely via p53; the majority of tumors are again eliminated from the host. In MYC-driven adult T-cell leukemia, malignant cells undergo massive apoptosis with rapid clearance and tumor-free survival when MDM2 is deleted, confirming that extensive apoptosis coupled with clearance universally marks this model. For several lymphomas and epithelial tumors, apoptosis induction through different signaling modes consistently leads to wide-scale clearance without any other manipulation (O'Donovan *et al*. 2004).

5.3. Resistance mechanisms and therapeutic escape

In most cases, cancer therapies are designed to induce apoptosis in cancer cells. However, many cancer cells, particularly cancer stem cells (CSCs), develop resistance mechanisms that block this process and increase the formation of aggressive metastatic tumors. Cancer cells can evade apoptosis through diverse genetic and epigenetic mechanisms, including increased expression of anti-apoptotic proteins and mutations in pro-apoptotic factors. For instance, upregulation of survivin, a member of the inhibitor of apoptosis (IAP) protein family, occurs in many malignancies and facilitates resistance to apoptosis triggered by diverse chemotherapeutic agents and radiotherapy (Safa, 2022). CSCs arise during tumor development and treatment and are associated with poor prognosis. They frequently acquire resistance to apoptosis and anticancer agents, further promoting tumor relapse and progression. Blocking the expression of the pro-apoptotic gene, Bax, prevents cancer cells from undergoing apoptosis. Mutations that impair the function of the tumor-suppressor genes, p53 and PTEN, are also involved in CSC biology. Downregulation of FAS and FAS-L signaling pathways occurs in multiple malignancies and promotes therapeutic escape from FAS-mediated apoptosis (Franzese *et al*., 2024). Suppression of FADD expression also assists cancer cells in avoiding apoptosis, whereas delivery of the FADD protein restores the apoptotic signal. Inhibition of other principal apoptotic genes hinders the execution of the signaling cascade. Overactivity of the onco-protein, MYC, leads to the downregulation of the miR-15/16 family, which targets BCL-2, IAP-2, and Cyclin D. Diminished levels of these targets further enhance resistance to apoptosis. Anti-apoptotic proteins such as BCL-2, MCL-1, and BCL-xL are often overexpressed in CSCs. Moreover, the phosphatidylinositol-3-kinase (PI3K) pathway, which is frequently hyperactivated in CSCs, induces resistance towards apoptosis through the down-regulation of pro-apoptotic members of the BCL2 family or upregulation of the anti-apoptotic member, BCL-XL. Depending on cell context and context, the extrinsic death receptor pathway can also be executed or inhibited. Activation of this pathway by extracellular signals can contribute to tumor initiation, maintenance, and relapse (Fulda, 2010).

Upregulation of hypoxia-inducible factors (HIF-1/2) and the levels of cytosolic IK-1– and ER–associated IRE-1α critically involved in the hypoxia, oncogenic RAS, or endoplasmic reticulum (ER) stress-responses, are also major orchestrators of both stemness maintenance and survival upon therapy. Pharmacological inhibitors of either HIF or IRE-1α render resistant cells sensitive to therapies and devoid of stemness features. Numerous other genes affect the formation and activity of CSCs (Zheng & Wang, 2025).

6. Therapeutic Strategies Targeting Apoptosis

Promoting apoptosis in cancer cells is a viable approach for boosting anticancer therapeutic strategies. Resistance to apoptosis is a hallmark of cancer and often occurs due to oncogenic activation of key components in the apoptotic suppression machinery. For example, oncogenic mutations in RAS, RAF, MYC, and the cell cycle regulator, RB, as well as loss-of-function mutations in TP53, are frequently observed. These mutations impose a greater reliance on pro-survival BCL-2 family proteins, leading to the emergence of distinct apoptotic signatures in different tumor types (Lim *et al*., 2019). More than 70% of tumors exhibit aberrant oncogenic signaling that independently regulates the BH3-only proteins BIM and PUMA, thereby facilitating the development of therapies that selectively target tumor cells by targeting the expression of these essential mediators of apoptosis. Synthetic

lethality strategies aimed at re-engaging suppressed apoptosis after targeted inhibition of early growth control oncogenes are also of considerable interest (Montero & Haq, 2022). Hope exists that combination therapies targeting tumor-specific apoptotic vulnerabilities discovered at the pre-clinical stage will soon allow further clinical testing in humans (Franzese *et al*., 2024).

Apoptotic cell death is tightly regulated by signaling systems that integrate pro-apoptotic and anti-apoptotic cues during normal physiology. In healthy cells, factors promoting survival are favored over those inducing apoptosis due to stressful conditions. In cancer cells, growth factors or similar cues are preferentially integrated to maintain malignant behaviour and oppose apoptotic cues. Thus, cancer cells often develop mutations that promote the pro-survival programme, leading to treatment resistance and facilitating adaptation to other hostile cues, such as the hypoxia encountered in detached tumors (Lim *et al*., 2019).

6.1. Pro-apoptotic agents and BH3 mimetics

Pharmacological agents that promote the intrinsic apoptotic pathway are currently being pursued as anticancer strategies. Several classes of such agents have been identified, including small-molecule agonists of the pro-apoptotic BCL-2 family of proteins (often referred to as "BH3 mimetics"). These agonists bind selectively to the hydrophobic cleft of BCL-2, BCL-XL, BFL-1, or MCL-1 proteins, thereby preventing them from sequestering their cognate BAx and bAk activator functions. This mechanism of action has been exploited in the preclinical and clinical development of a number of potent and selective BCL-2 family antagonists (Koss *et al*., 2016). Preclinical studies have demonstrated that these compounds can elicit a robust apoptotic response in a variety of cancer models and have greater efficacy in cancer cell lines than in normal cells. However, the excessive activity of these agents may lead to profound peripheral toxicities in the clinic (Lee & Fairlie, 2021) a limitation that can often be circumvented by combining them with other agents that activate "indirect" upstream signals that converge on activation of BAX and BAK. In these combined treatments, the BH3 mimetic is still required for optimal efficacy in broad-spectrum mixtures of encoding proteins. These results also demonstrate that, despite this convergence, signalling pathways normally regulated by BCL-2 family members are nevertheless disrupted (Koss *et al*., 2016).

6.2. Reinstatement of p53 signaling

Targeting p53, a potent tumor suppressor gene, presents a compelling strategy for restoring apoptosis. Loss-of-function mutations in the p53 gene occur in the majority of human cancers, illustrating the oncogenic potential of this tumor suppressor alongside RB and PTEN. Re-establishing the p53 signaling network at any point within the signal-processing hierarchy has been shown to elicit an apoptotic response in cell models devoid of functional p53 (Mirzayans *et al*., 2012). Other studies have highlighted the role of p53 in enabling and executing apoptosis during the formation of genotoxic stress, such as events associated with DNA-damage. Furthermore, a one-two hit regimen has identified apoptosis as a key mechanism in p53-driven clearance of Mas, MDM2, RAS, and E2F. Together, these findings suggest that reinstating p53 signaling in malignant cancer cells invokes a p53-centric switch and promotes cell death through the intrinsic apoptotic pathway (Feldser & Evan, 2008).

6.3. Enhancement of death receptor pathways

Apoptosis offers a means to safely eliminate unwanted cells in multicellular organisms. In mammals, cells subjected to developmental signals or damage, such as DNA breaks, misfolded proteins or oncogene activation, are eliminated through apoptosis. Two pathways have been identified for executing apoptosis: both converge on a caspase cascade, but different upstream signalling networks operate those pathways: the intrinsic pathway mediated mainly by BCL-2 proteins and the extrinsic pathway mediated by death receptors (Papenfuss *et al*., 2008).

Death receptors are members of the tumor necrosis factor (TNF) receptor superfamily. Those that have been extensively studied in the context of cancer therapy are TNF-related apoptosis-inducing ligand (TRAIL) receptor 1 (TRAIL-R1), TRAIL-R2, CD95 (Fas), and TNF receptor 1 (TNF-R1). Various human tumors exhibit resistance to apoptosis, precluding the use of currently available anticancer therapeutic agents. In many instances, the resistance is attributable to mutations of tumor suppressor genes, the most prominent of which is p53. However, the emergence of death receptors has highlighted new approaches to trigger directly apoptotic signaling in cancer cells (Jan & Chaudhry, 2019).

6.4. Combination therapies and biomarker-guided approaches

In view of a considerable body of evidence supporting the central role of apoptosis in the initiation of malignant cells, agents and drug combinations aimed at restoring apoptosis are intensively investigated as cancer treatments. To date, success has accompanied the development of monotherapies, and several major challenges that impede broad application remain unresolved. Preclinical data nonetheless point to a notable capacity for inducing significant and sustained tumor clearance when various pro-apoptotic agents are used in pairwise combinations. The lack of widely applicable tests, however, hampers rational combination design by impeding the identification of the specific pro-apoptotic signaling pathways, cellular targets, and corresponding drug combinations on which diverse tumor types depend (Ventura *et al*., 2007).

The situation has been further complicated by the discovery that even malignant cells under extreme selective pressure may engage apoptosis pathways widely considered incompatible with their survival. Cells treated with either BCL-2 or BCL-XL inhibitors, for instance, often escape death despite the loss of both anti-apoptotic molecules, by switching to the p53 network or to additional BCL-2 family members. Clarification of these aspects would enhance the efficacy of proposed combination therapies and support their translation into the clinic (Junttila *et al*., 2010). Formation of a broad international consortium could expedite progress by making all recovery data freely accessible, thus permitting comprehensive analysis of tumor dependency on different apoptosis pathways across diverse experimental models. This understanding would in turn facilitate identification of rationally selected pro-apoptotic agents and candidate combinations for each tumor type. Finally, growing insight into strategies employed by cancers to counter specific pro-apoptotic signals would inform the design of co-inhibitors aimed at overcoming such resistance mechanisms (Lim *et al*., 2019; Montero & Haq, 2022).

7. Challenges and Future Directions

The first significant challenge facing therapeutic approaches targeting apoptosis in cancer is the marked heterogeneity within tumors that arise from different patient tissues. This heterogeneity extends to the mechanisms that underpin apoptosis resistance in malignant cells, including variability in the expression and activity of pro- and anti-apoptotic proteins belonging to the BCL-2 family (Lim et al., 2019).

Apoptosis serves as the principal mechanism initiating cancer cells; mechanisms, evidence, and therapeutic implications are synthesized from rigorous, evidence-based analysis. Despite progress, critical knowledge gaps remain. Elucidation of the roles of apoptosis in oncogenesis and metastasis is necessary. Further dissection of factors, aside from pro-apoptotic signals, that prime cancer cells to elicit apoptotic responses will enhance understanding of drug responses and aid development of more effective therapeutic strategies. Insights into the positions of pro-apoptotic, anti-apoptotic, and apoptosis-independent signals along the oncogenic and metastatic continuum will also facilitate identification of exploitable targets to sensitize tumors to therapeutic agents (Morana *et al*., 2022).

Drivers of metastasis or other pro-oncogenic alterations can enable tumors to evade death. The biologically relevant contexts in which apoptosis is pro- vs. anti-tumorigenic remain to be fully delineated. Exploration of the interplay between apoptosis and other cell fate decisions—such as proliferation, quiescence, senescence, and differentiation—may illuminate non-canonical routes cancers adopt to evade cell initiation. Enhancing knowledge of these and other facets of apoptosis will further substantiate its status as the most important mechanism through which anti-cancer therapies exert their impact today (Lim *et al*., 2019).

Cascades leading to apoptosis feature prominently in the reactions activated when mutational and other carcinogenic events occur. Apoptotic signalling is initiated early in the evolution of most cancers. Yet, even the most advanced tumors often successfully avoid this fate and, therefore, remain in the population, prolonging their capacity to accumulate additional alterations. Consequently, the elucidation of even earlier events in the life of a cancer and the identification of additional cellular states on the path to full malignancy represent high-priority objectives (D'Arcy, 2015). The aggregated knowledge regarding the mechanisms of cancer initiation and the evidence linking these processes to apoptotic signalling therefore vastly outweighs that available for other routes. Apoptosis is thus regarded as the primary mechanism by which anti-cancer agents exert their effect (Boccia *et al*., 2021).

8. Conclusion

Apoptosis is a major mechanism through which cancer cells are eliminated. This synthesis of the latest evidence, reflecting a substantial body of work, demonstrates that successful cancer therapies act predominantly by inducing the apoptotic death of cancer cells, and discusses the implications this has for drug development, patient stratification, and combination therapies. Extensive datasets and multiple experimental approaches, including systems biology, live cell imaging, and mathematical modeling, indicate that apoptotic clearance of cancer cells is the main mechanism by which tumor cells are eliminated, for many different types of cancer and anticancer treatment. Analysis of the literature on clearance measurements confirms that these measurements are particularly valuable for understanding and predicting the mechanisms of action of anticancer compounds. Researchers who wish to identify compounds that act by apoptosis inducers can concentrate on those that produce a rapid drop in tumor bioluminescence or fluorescence provided that the reporter is adequately validated. A series of patent applications describing innovative strategies to enhance tumor clearance by exploiting the apoptotic mechanism following drug administration is in preparation.

Four small molecules targeting the well-established pro-apoptotic pathway have received considerable attention: the BH3-mimetics ABT-737 and navitoclax, which bind to pro-survival members of the BCL-2 family; the p53-reactivating agent APR-246; and the SMAC-mimetic LCL-161, which promotes the degradation of IAP proteins. Abiding by the principles outlined, combination regimens incorporating such agents along with conventional chemotherapy or targeted therapies are being explored for trans-national implementation. Sufficient preclinical evidence has accumulated to suggest that pro-apoptotic agents function as bona fide sensitizers across various tumor types and therapies, on the condition that cancer cells remain equipped for limited apoptotic execution. However, the shift in apoptotic sensitivity from sufficient to insufficient remains an inevitable fate for evolutionarily successful high-grade tumors that no longer require protection against unwanted apoptotic death.

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Evaluation of the Effectiveness of Citrus aurantifolia Leaf extract as a Botanical Larvicide against mechanical vector of Human Parasitic Diseases

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Abstract
Musca domestica (housefly) is a medically important insect that act as a mechanical vector of many parasitic diseases affecting humans and animals worldwide. Increasing resistance to conventional chemical insecticide and their negative environmental impacts have created the need for eco-friendly alternatives. This study evaluated the larvicidal potential of Citrus aurantifolia leave extract against housefly larvae. Plant leave sample were collected, washed, air-dried under shade, ground into powder, and extracted using maceration by dissolving 250g of powdered bark in 4.5L of distilled water. Photochemical screening revealed the presence of alkaloid, saponins, flavonoids, and tannins, which are known for insecticidal properties. Laboratory reared larvae were exposed to different concentration of the extract (250-1250ppm) with four replicate each and mortality was recorded after 24, 48, and 72 hours. Result showed concentration and time- dependent larvicidal activity. The highest mortality (72%) was observed at 1250ppm after 24hhours, while lower concentration produced reduced mortality rate.LC_{50} and LC_{95} values indicated high larvicidal potency. No mortality was observed in the control group. The findings suggest that citrus aurantifolia leave extract has promising anti-larvicidal properties and could serve as a sustainable, eco-friendly alternative for controlling housefly population.

Keywords: Housefly, Larvicidal, Citrus aurantifolia, Mortality, Maceration.

1. Introduction and Background of the Study

Citrus is a fruit that is widely consumed due to its numerous nutrients' benefits. C. aurantifolia, is a species that widely utilized, as a raw material for cosmetics, food flavouring, beverage flavour enhancement, and traditional medicine. It is a member of the Rutaceae plant family, which has 156 genera and 900species, and cultivated widely throughout the world (Indriyani et al., 2023). The most prevalent fly species worldwide is the housefly, or Musca domestica. Over 100 pathogenic parasites have the potential to infect humans and animals and cause various parasitic diseases in which housefly serves as a mechanical vector of transmitting the parasite to human.

Houseflies (Musca domestica) are known to transmit pathogens that cause cholera, typhoid, and dysentery in Nigeria. These diseases continue to be common, especially in places with open defecation and inadequate sanitation, creating ongoing public health problems (Banjo et al., 2010). Synthetic pesticides, which are the mainstay of common housefly management techniques, have over time led to resistance, environmental contamination, and harm to non-target species (Izah,2016) . (Adebayo, et al 2015). This has led to an urgent demand for alternative, eco-friendly pest control techniques. Therefore, the objective of this is to determine the Larvicidal potential of Citrus aurantifolia leave extract for sustainable control of disease-transmitting mechanical vector, in other to provide a natural and environmental sustainable solution for controlling housefly's population, to reducing the risk of parasitic disease transmission.

2. Methodology

2.1 Study Design

The study adopted a laboratory based experimental design to evaluate the larviciadl activity of Citrus aurantifolia leave extract against Musca domestica larvae. The experiment was involve different concentrations of the extract and varying exposure time (24, 48, and 72hours) with mortality recorded and statistically analyzed

2.2 Collection and Preparation of the Plant Material

Citrus aurantifolia leave was purchased from Oja Oba market in Osogbo Osun state, because the market is a major plant material market authenticated by (Adeyemi, et al 2020) in Nigeria and the plant material also authenticated by a botanist in the department of plant Biology of Osun State university Osogbo with a specimen voucher No 34331.
3000(g) of the citrus aurantifolia leaves was washed with clean water to remove debris, Air –dried under shade for 7-14 days to preserved phytochemicals, Ground into fine powered using a sterile electric blender, stored in airtight container until extraction.

2.3 Preparation of Citrus aurantifolia leave Extract (Maceration Method).

250g of the powdered Citrus aurantifolia leaves was macerated in 3.5litres of distillated water and vigorously stirred at one (1) hour interval for a period of four (4) hours, and it was allowed to stand on bench for an hour without disturbance.
The solution obtained was refrigerated for (24) hours and sieved with a laboratory sieve of 0.5usize,and allowed to stand for 1hour for its heavy particles to settle down then the supernatant was decanted and filtered using whatman filter paper and the residue was transferred into an open tray and dried in the oven at 100 degree for 2 days scraped with spatula and grinded into fine powder using laboratory pestle and mortar (Abiodun et al., 2021)
Percentage yield was calculated using this formula.

$$\%\ \text{yield} = \frac{\text{weight of the extract}}{\text{weight of powdered seed}}\ x\ \frac{100}{1}$$

Phytochemical Screening of Extract
Qualitative phytochemical analysis was conducted using standard procedure to detect major bioactive compound
Test of Major Phytochemicals
Alkalods (Mayer Test)
Extract + Mayer reagent
Cream precipitate indicates presence of alkanoid
Flavonodis (Alkaline Test)
Extract + NaOH solution
Yellow coloration that disappears with acid confirm flavonods
Tannins (Ferric Chloride Test)
Extract + 1% $FeCl_2$
Blue- black coloration indicated tannins
Saponins (Froth Test)
The extract was shaking with distilled water
Persistent froth indicated saponins
Terpenoids (Salkowski Test)
Extract + Chloroform + Concentrated H_2S0_4

Reddish brown interface indicated terpenoids
Phenols (Ferric Chloride Test)
Dark coloration indicated presence of phenolic compound

My scientific note: These tests provide preliminary qualitative confirmation of bioactive compounds responsible for larviciadl activity, although they do not concentration quantified

Rearing of Musca domeditca Larvae

The housefly larvae was collected from poultry decomposed manure sample and it was maintained under laboratory conditions at a temperature of 28+2 c and 60-70% relative humidity feed standard larvae diet (Wheat brain+ yeast+ water) in a sack covered with cheese cloth, The third instars larvae was used for bioassay due t their high sensitivity and uniform development stage. (Ahmed, et al., 2018).

Larvicidal bioassay Procedure

The Larvicidal bioassay ones were carried out by following the method of Biovain (2011) and Palacin et al (2019) respectively with little modification. The aqueous extract was dissolved in 1000ml of distilled water to form different concentrations such as 250ppm, 500ppm, 750ppm, 1000ppm; 1250ppm for plant extract in the plastic cup, 25 larvae was transferred into each test solution prepared. Four replicates were maintained for different. Concentrations and mortality of the larva was recorded after 24, 48 and 72 hours for the plant extract. Dead larvae were detected when appendages did not move when probed with needle. The mortality rate of the tested larva was then calculated after 72 hours by using the below formula.

$$\text{mortality percentage} = \frac{\text{No of dead larvae}}{\text{No of larvae tested}}\ x\ 100$$

$$\text{Corrected mortality \% } == \frac{\text{observed mortality rate } - \text{ control mortality rate}}{100 - \text{control mortality rate}}$$

$$\text{Observed mortality rate \%} = \frac{\text{control mortality \%} \times 100}{100 - \text{control mortality\%}}\ x\ 10$$ Eneojor,(2015)

Determination of LC_{50} and LC_{95}
Data preparation
Percentage mortality was corrected using Abbort's formular

Corrected mortality (%) = $\frac{\text{Expected} - \text{Control}}{(100 - \text{Control}} \times 100$

Probit Analysis
LC_{50} (Lather concentration for 50% mortality) and LC_{95} value was determined using probit regression analysis
Scientific note: LC_{50} is the most reliable indicator of larviciadal potency where lower LC_{50} indicate higher toxicity.
Statistical Analysis
A Two-way Analysis of variance (Two-Way ANOVA) was used
Factors:
Factor A: Extract concentration 250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm
Factor B: Exposure time: (24, 48,72hours)
Dependent Variable:
Larvae Mortality percentage

Model Structure
The ANOVA model
$Y_{ijk} = \mu + C_1 + T_j + (CT)_{ij} + \Sigma_{ijk}$
Y_{ijk} = Observed mortality
μ = Overall mean
C_1 = Effect of concentration
T_j = Effect of Time
$(CT)_{ij}$ = Interaction effect
Σ_{ijk} = Error term
Post Hoc Test
Turkey's HSD test was used to separate the means when the significant difference are observed at $P<0.05$)

Significant Level
Statistical significance was set at P<0.05)

Ethical and Safety consideration

Proper handling of biological specimen was ensured
Waste disposal was followed laboratory bioassay guideline
No vertebrate animal are involved hence ethical approval is minimal but institutional guideline was observed

Scientific Summary of methodology used for this study

This Methodology ensured a rigorous evaluation of the larvicidal potential of Citrus aurantifolia leave extract by intergrading phytochemical profiling dose- response bioassay and robust statistical analysis. The combined use of LD50 determination and ANOVA ensure both toxicity quantification and statistical validation of efficacy trends foster the reliability of findings

3. Results

Table 1: Summary of detected Phytochemicals

Photochemical Groups	Presence
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenol	+
Glycoside	-
Terpenoids	+

Interpretation of phytochemicals result

The quantitative phytochemical screening of Citrus aurantifolia leave extract revealed the presence of several biological active compounds including Alkaloids, Flavonoid, Tannins, saponins, Phenol, and terpenoids while Glycoside was not detected. The presence of Flavonoids, Phenols, and Tannin suggests strong antioxidant and insecticidal potential as these compounds are known to interfere with insect metabolic and neurological functions

Citrus aurantifolia leave Extract effect on Musca domestica larvae

Table 2: Larvae mortality rate at different concentrations of leave extract of Citrus aurantifolia with time rate of exposure

Citrus aurantifolia leave extract Concentration (ppm)	**No of larvae of Musca domestica**	**Mortality rate % at 24hrs**	**Mortality rate % at 48hrs**	**Mortality rate % at 72hrs**	**No(%) of alive larvae**
250 ppm	25	0(0.00%)	0(0.00%)	0(0.00%)	25(100%)
500 ppm	25	1(4%)	2(8%)	3(12%)	19(76%)
750 ppm	25	7(36%)	4(16%)	2(8%)	12(48%)
1000 ppm	25	15(60%)	6(24%)	4(16%)	0(0.00%)
1250 ppm	25	18(72%)	6(24%)	1(4%)	0(0.00%)
MEAN	25.00	8.2(32.8%)	3.6(14.4%)	2(8%)	11.2(44.8%)
CONTROL 0.00mg/L	25.00	0.00	0.00	0.00	25.00(100%)

3: Concentration response larvicidal bioassay of Citrus aurantifolia leave extract against larvae of Musca domestica

Citrus aurantifolia bark	Time	%mortality	LC50(ppm)	LC95(ppm)
Aqueous extract	24hrs	8.2(32.8%)	896	1729
	48hrs	3.6(14.4%)	1812	2250
	72hrs	2(8%)	5250	7875

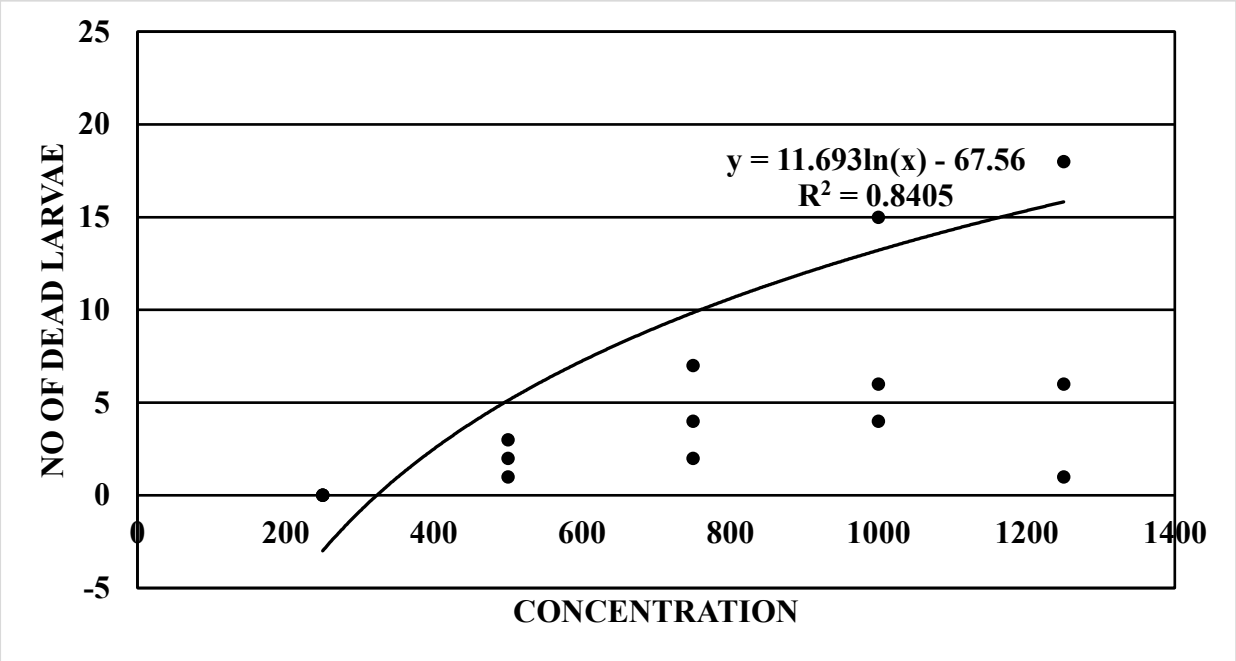


Figure 1: Mortality rates of larvae against the concentration (ppm)of aqueous extract of citrus aurantifolia leave and time period

4. Discussion

Overview of findings

This study evaluated the larvicidal activity of Citrus aurantifolia leave extract against Musca domesica larvae across different concentrations and exposure times, The results are discussed in relation to mortality trends, dose-dependent response relationship, LD50 values.

Table 1: Phytochemical Composition and Bioactivity Implications

- The qualitative phytochemical screening indicated the presence of key bioactive compounds such as alkanoids, Flavonoids, tannins, saponins, terpenoid and phenolic compounds these secondary metabolites are widely associated with insecticidal and larvicidal effect
- The observed Larvicidal activity can be attributed to these compounds through several mechanism
- Alkanoids may interfere with nervous system signaling in larvse
- Flavonoids and Phenols can distrust cellular metabolism and induce oxidative stress
- Ternnims may bind to protein and digestive enzyme reducing nutrient absorption
- Terpenoids are known to affect insect hormonal regulation and cuticle integrity

These findings support the hypothesis that Citrus aurantifolia bark contains biological active compound capable of inducing larval mortality

Table 2: Effect of Concentration on Larval Mortality and exposure time

A clear dose –dependent increase in larval mortality was observed across all exposure periods. Higher concentrations consistently produced greater mortality rates compared to lower concentration and control. This trend suggests that the extract exhibits strong concentration-dependent toxicity increasing concentration likely increase the availability of active phytochemicals interaction with larval physiological systems. The control group showed no mortality, which confirmed that mortality was due to the extract rather than environmental factors.

Furthermore, on the effect of exposure time rate on mortality increased progressively from 24 to 72 hours across all concentrations this signified a time-dependent lavicidal effect of the extract, where prolonged exposure enhances toxicity. The possible explanations include: gradual absorption of bioactive compounds through the larval cuticle Accumulation of toxic metabolites within the larval tissues over time. and delayed physiological disruption leading to eventual death.

The interaction effect of time reveled a significant interaction between concentration and exposure time ($p=<0.05$), indicating that the effect of concentration depends on the duration of exposure. This suggests that higher concentration is more effective when exposure time is extended. Lower concentrations may required longer exposure to achieve significant mortality, the combined effect of both factors enhance overall larvicidal efficacy

Table 3 and Figure 1, LC_{50} and LC_{95} interpretation, the probit analysis produced LC_{50} and LC_{95} values that reflect the toxicity level of the extract. The LC_{50} value indicates the concentration required to kill 50% of the larvae. A lower LC_{50} value demonstrates higher potency of the extract. The LC_{95} value provides insight into near complete population control efficiency. The regression line between log concentration and probit mortality showed a strong positive correlation, confirming a reliable dose response relationship

5. Comparison with Previous Studies

The larvicidal activity observed in the study is consistent with previous research on plant based insecticides. Many citrus species have demonstrated insecticidal properties due to their rich phytochemical composition, particularly terpernoids and flavonoids. When compared to similar botanical extract, Citrus aurantifolia leave extract shows a promising potential as a natural larvicide, although variation in the potency may deepen on method of extraction, environmental factors and solvent type.

6. Summary of Discussion

Overall, the results demonstrated Citrus aurantifolia leave extract exhibited a significant larvicidal activity that was characteristized by strong dose-dependent and time dependent effect. With statistically significant mortality difference and a reliable LC_{50} and LC_{95} toxicity indicator, bioactivity also supported by phytochemical constituents, these findings, support the potential use of the extract as a natural bio- insecticide vector control strategies.

Conclusion: This study evaluated the larvicidal activity of Citrus aurantifolia leave extract with the adoption of laboratory-based experimental design; the findings demonstrated that the extract has a significant larvicidal potential, with mortality rates increasing in a dose-dependent and time dependent manner across all treatment groups. Phytochemical screening also revealed the presence of important bioactive compounds including alkaloids, flavonoids, saponnin, tannins, phenolic compounds and terpenoids. These secondary metabolites are likely responsible for the observed lrvicidal activity through mechanisms such as enzyme inhibition, metabolic disruption, and interference with larval physiological processes. Also, probit analysis confirmed the effectiveness of the extract through LC_{50} and LC_{95} values, indicating measurable toxicity against the larvae.

It can be concluded from the study that Citrus aurantifolia leave extract is a well promising natural larvicidal agent that could serve as an alternative to synthetic insecticide in control of Musca domestica population.

Recommendations

Following the findings of this study, the following recommendations are made:

Further Isolation of Active Compounds

There is need for more research to focus on isolation and characterization of the specific bioactive compounds responsible for larvicidal effect of the Citrus aurantifolia leave extract.

Toxicological Evaluation on Non- Target Organisms
Additional studies should assess the safety of the extract on non-target organisms, beneficial insects, and humans, to ensure environmental safety.

Field Trials for Practical Application
The larvicidal effectiveness of the extract should be carried out under filed conditions to demine its real- world applicability in vector control program**.**

Formulation Development
There is need to develop the extract into stable formulations such sprays, granules, or emulssifiable concentrate to improve ease application and shelf life.

Integration into integrated Pest Management (IPM)
The leave extract should be considered as part of integrated pest management strategies to reduce more reliance on synthetic insecticides and minimize environment pollution**.**

Does Standardization Studies
There is need for further studies to established standardized effective dosages for large scale application to ensure consistent larvicidal performance

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A comparative Study on the Canal Transportation of Two Heat Treated Rotary NiTi Instruments; Orodeka Plex-V and AF F-One Files in Sever Curved Simulated Canals (A comparative *in vitro* Study)

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Abstract
Aim: to evaluate the shaping ability Orodeka Plex-V and Fanta AF F-One rotary nickle-titanium systems in simulated curved canals.
Materials and Methods: twenty artificial curved resin canals were divided into two groups according to the instruments used for canal preparation. All canals were instrumented to master apical file of 25/04. Outer and inner transportation were evaluated. Measurements were carried out at five different levels. Pre-and postoperative images of the canals were taken at 40X magnification. An assessment of the canal shape was determined using Photoshop CS2 software.
Results: Plex-V caused greater transportation of the outer walls of the canals than F-One files, with a statistically significant difference at all five levels examined. Similar results were observed on the inner canal walls, without statistical significance, except at the apical level, where the difference was significant. In general, the transportation on the outer walls was greater than on the inner walls along the canals.
Conclusions: Within the limitations of the study, F-One instruments maintained the original curvature significantly better than Plex-V.

Keywords: Transportation, Fanta AF F-One, Orodeka Plex-V.

1. Introduction

Root canal treatment involves several steps; canal shaping is one of the most important (1). A fundamental aspect of biomechanical preparation is shaping the canal into a tapering form without altering the original shape (2). This phase is crucial for successful canal preparation, especially in the apical third, as uneven cutting of the canal walls may lead to ledge formation or even perforation. Therefore, instruments must be used to cut the dentin evenly from all sides (3). Glossary of Endodontic Terms defined the transportation as “an excessive removal of the outer wall of the apical half of the canal due to the instrument's tendency to return to its original straight shape during instrumentation, which may end with the ledge formation and possibly perforation” (4). Teeth with narrow and curved canals are more prone to these aberrations (5). Hence, degree of transportation of root canal can be used to measure the performance of various endodontic instruments and techniques used for canal preparation (6).

The introduction of NiTi to the world of endodontics, significant advancements occurred in root canal instruments, positively impacting the time required to complete the treatment, enhancing dentist comfort, and reducing the error rate (7). Since these tools were introduced to the market, updates have been ongoing in terms of engineering design and heat treatment (8).
One of the new NiTi rotary systems that was introduced to the field of root canal treatment is the Orodeka Plex-V files. These instruments are manufactured using a special thermal process called CM wire, which aims to increase

flexibility and high resistance to cyclic fatigue. These files feature a triangular cross-section to minimize the friction with canal walls and a non-cutting tip used for canal searching and negotiation (8).

Recently, Fanta introduced a new type of files termed AF F-One system using a special heat treatment called AF Wire. A notable feature of this design is the flat surface along the cutting edge of the file, which makes the file more flexible and reduces friction with the canal walls, thus increasing the file's resistance to breakage and providing a larger space for evacuation debris coronally. In addition, the cross-section is S-shaped, which increases the cutting performance of the file (8).
According to the investigation oversaw on scientific websites, there is no research comparing these modern endodontic rotary systems in terms of their ability to prepare canals without deviating from the original paths of the canals. Therefore, this research was conducted in this experiment to find out which of these files is more capable of preparing the sever curved simulated canals while preserving the original shape of the canals in term of transportation.

2. Materials and Methods

2.1 Artificial Root Canals

Twenty artificial curved root canals (Dentspiy Maillefer, Ballaigues, Switzerland) were used in this study. These canals were identical to the geometry of ISO K-file #10. The straight part was 11 mm long, while the curved part was 5 mm long, making the total length of the canal 16 mm. the angle of curvature was 45° according to Schneider (9), and radius with 5.5mm along with Pruett et al., (10).

2.2. Canals Photography

Before starting the instrumentation, and to increase the contrast of the images, each artificial canal was filled with writing ink (Hero, China) using a 27-gauge syringe (Seoul, Korea). Each sample was magnified 40 times by a stereomicroscope (Keyence VH8000, Osaka, OSK, Japan), and the image was taken under this magnification by a digital camera (Cyber-shot, Sony, Japan). The camera was mounted on the microscope lens in a way that allowed the model to be repositioned in the exact same location to take before-and-after photos of sample preparation. After the photography, the simulated canals were washed of ink residue with tap water.

2.3. Instrumentation of Simulated Root Canals

The samples were randomly divided into two groups (n=10) based on the type of rotary system used in the preparation. Canals patency was verified by ISO K file #10 (Dentsply, Maillefer, Ballaigues, Switzerland). The canals were prepared by a root canal specialist experienced with these rotary systems. The rotary files were operated with X-Smart Plus iQ electric motor (Dentsply, maillefer, Ballaigues, Switzerland).

Group 1:
In this group, the Orodeka Plex-V system (Shandong Province, China) was used by X-Smart motor, according to the manufacturer's recommendations for this system in terms of speed and torque as follow:
Orifice Opener (15/08), WL:5mm, speed:300rpm, torque: 2.5N.
Glide Path (15/03), WL:15mm, speed:300rpm, torque:1.5N.
Shaping file (yellow ring, 20/04), WL:15mm, speed:500rpm, torque:2.5N.
Shaping file (red ring, 25/04), WL:15mm, speed:500 rpm, torque: 2.5N.

Group 2:

AF F-One (Fanta, Shanghai, China) files were used in preparing the models in this group using X-Smart Plus electronic motor, according to the program designed for this system as follow:
Orifice Opener (17/12), WL: 5mm, speed:500 rpm, torque: 2.5 N.
Path finder (16/04), WL: 15 mm, speed: 500 rpm, torque: 2 N.
Shaping file (yellow ring, 20/04), WL: 15 mm, speed: 500 rpm, torque: 2.5 N.
Finishing file (red ring, 25/04), WL: 15 mm, speed: 500 rpm, torque: 2.5 N.

The movement of the files during preparation was slow in and out pecking motion. Distilled water (Baghdad, Iraq) was used as the irrigating fluid using endodontic irrigating syringes (Ultradent Products, Inc., South Jordan, UT, USA) with NaviTip 21 mm (yellow) 30 gauge. The canal was rinsed with 2 ml of distilled water before preparation, and after each file, the canal was rinsed with another 2 ml, resulting in a total of 10 ml of irrigating fluid per canal. Once the file reaches the length of the canal and rotates freely, it is withdrawn. This process takes no more than 5 seconds. The file was used to prepare only 5 canals. After each use, the file was inspected to ensure it was free of cracks and distortion. If these defects were found, the file would be replaced immediately.

2.4. Assessment of Canal Transportation

After completing the canal preparation process, ink is injected into the canal, and the canal was imaged in the same way it was imaged before preparation. Digital images of the canals before and after preparation were superimposed and measured. Here, canal transportation was determined by comparing the digital image before and after preparation and was done by measuring the change from the original canal boundaries from the inner and outer sides of the canal at 5 reference points using Adobe Photoshop CS2 software program (fig.1). The measurements were at right angles to the canals (fig.2).

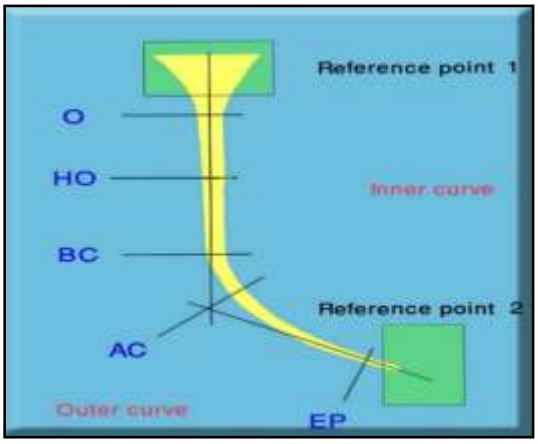


Fig. (1): *The five levels of measurement.*

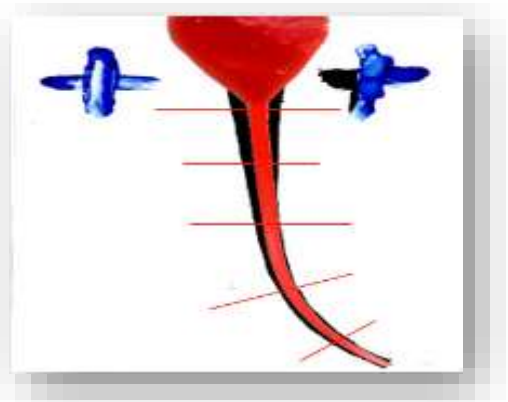


Fig. (2): *superimposed and treated images of unprepared and prepared canal with computerized program Adobe Photoshop (C).*

Level 1 (O): canal orifice.
Level 2 (HO): the point mid-way from the beginning of the curvature to the orifice.
Level 3 (BC): the point where the canal departs from the long axis of its coronal portion (beginning of the curvature).
Level 4 (AC): the point where the long axes of the coronal and the apical portions of the canal intersect (apex of the curve).
Level 5 (EP): the end point of preparation.

3. Results

3.1. Outer Transportation.

The mean values and the standard deviations of outer canal transportation after instrumentation at the five measuring levels are given in Table 1.

Table 1: Mean and standard deviation of outer transportation (mm) for the two groups.

System		O	HO	BC	AC	EP
Plex-V	*Mean*	7.08	6.69	5.80	3.94	2.37
	SD ±	0.47796	0.57629	0.71181	0.51467	0.27101
F-One	*Mean*	6.52	5.24	4.27	2.90	1.64
	SD ±	0.53914	1.13940	1.34582	0.86923	0.38064
t-test		2.458	3.591	3.178	3.256	4.940
P-value		0.004 *S*	0.002 *S*	0.005 *S*	0.004 *S*	0.000 *S*

Based on the table above, it can be observed that Plex-V removed more material from the outer surface than F-One at all levels. Statistical analysis by using the student t-test showed significant differences between the two systems at all levels.

3.2. Inner Transportation

The mean values and the standard deviations of inner transportation after instrumentation at the five different levels examined are listed in Table 2.

Table 2: Mean and standard deviation of inner transportation (mm) for the two groups.

System		O	HO	BC	AC	EP
Plex-V	*Mean*	5.780	5.080	3.640	2.390	1.590
	SD ±	0.70679	1.00973	0.57194	0.53009	0.37253
F-One	*Mean*	6.180	4.740	3.920	2.250	1.200
	SD ±	0.65963	1.08341	1.18584	0.51045	0.24944
t-test		1.308	0.726	0.673	0.602	2.751
P-value		0.207 *NS*	0.477 *NS*	0.510 *NS*	0.555 *NS*	0.013 *S*

At the orifice of the canals F-One showed the higher means of inner transportation (6.180) than Plex-V (5.780). Next to the second measuring level, Plex-V got the maximum inner transportation (5.080) and F-One showed the minimum mean (4.740). On the third level the results were as follows: F-One (3.920), Plex-V (3.640). On the fourth plane the view was completely inversed as the higher means of inner transportation was with Plex-V (2.390) than F-One (2.250). By the side of the end level of preparation, Plex-V removed more resin from the inner sides (1.590) followed by F-One (1.200).
By using Student t-Test (Table 2), in inner transportation, showed no significant difference between the two systems at all levels, but not at the apical level where the difference was significant.

4. Discussion

Canal preparation aims to create a conical shape—with the smallest diameter at the apex and the largest towards the crown—while strictly maintaining the original shape of the canal. One of the most common problems encountered during canal instrumentation is the straightening of the canal; this classically occurs in curved roots, specifically affecting the outer wall in the apical third (11).

According to the American Association of Endodontists (2016), the term "transportation" is defined as the cutting away of the outer wall of the root canal—specifically in the apical half—caused by the instrument's tendency to straighten within the canal during instrumentation; this leads to complications such as root perforation or ledge formation (4).

The degree of transportation is measured by the distance between the edge of the unprepared canal and the corresponding edge after canal preparation (12). The closer the transportation value is to zero, the better the performance of the instruments in canal preparation (13).

Apical transportation plays a crucial role in ensuring the feat of root canal treatment; the degree of curvature throughout preparation affects the seal of the filling materials, thereby determining the success or failure of the root canal management (14).

Several researchers stated that an apical transportation of 0.15 mm is acceptable; however, if it exceeds 0.30 mm, it compromises the apical seal, allowing bacteria or their byproducts to enter the root canal and leading to endodontic treatment failure. Excessive apical transportation leads to several complications; it may cause zipping and the subsequent elbow formation —which hinders the smooth insertion of accessory points of gutta-percha. This prevents a tight apical seal, ultimately resulting in endodontic treatment failure (14, 15).

Reviewing the results of this study reveals that neither of the systems used was able to produce an exact replica of the canal shapes present prior to preparation; in other words, all the instruments caused the original canals to transport to varying degrees. Under conditions of the study, no system exhibited apical transportation exceeding than 0.3 mm, indicating that preparation with these two systems falls within the ideal standards for root canal preparation.

The research by hand was conducted using transparent resin blocks that simulate natural root canals, ensuring standardized dimensional parameters—such as length, diameter, taper, and curvature—while also facilitating imaging before and after preparation. Artificial canals do not replicate the actual anatomy of root canals, but they allow for comparisons regarding canal preparation using various rotary root canal preparation systems. At the same time, it must be noted that the cutting process in these resin blocks differs from that of natural dentin; cutting these canals with rotary systems causes the resin temperature to rise and leads to the accumulation of resin debris in the instrument flutes, which can ultimately result in file breakage (11).

Thus, the results derived from using these blocks must be interpreted with caution when evaluating the performance of preparation systems (16). In the same context, Adobe Photoshop was utilized to analyze images of the canals taken before and after preparation, aided by ink injected into the artificial canals; the software allowed for image enhancement and colorization, facilitating computer-based measurements (11).

Curved canals are ideally used to evaluate root canal instrumentation, as they reflect instrument efficiency—unlike straight canals (17). Hence, rotary instruments are evaluated using curved artificial canals, regardless of whether they maintain the original curvature of the prepared canals or cause them to transport (11).

It is important to note that when comparing rotary root canal systems, the standardization of master apical files size and taper must be considered; in this study, the instruments used were size 25 with a taper of 04. Furthermore, all samples were prepared by a single operator to eliminate operator-related variables (8).

4. 1. Outer Transportation

Regarding the outer canal wall, Table (1) clearly shows that, across all five canal levels, group Plex-V instruments removed significantly more resin than group F-One files. Accordingly, the canals prepared using F-One instruments were closer to the original shape, as these instruments removed less of the outer canal wall; consequently, there was less transportation, straightening, and distortion of the canals across the five examined levels. These results can be explained by once recognizing that the mechanical and geometric properties of the files significantly influence their performance during rotation within the canals (18, 19).

Tasdemir et al. (2005) stated that several factors directly influence the degree of canal transportation; foremost among these are the flexibility of the instrument used for preparation (20). As well as the cross section of the instrument (21). In the same context, other studies have stated that the flexibility of instruments may be the main factor that allows the

instruments to plane the canal walls rather than engaging and screwing into them and to cut of dentin evenly along the canal wall (22).

According to the conclusions of a study conducted by Saad and Hamed in 2026, the F-One type files are more flexible than the Plex-V files. This high flexibility is due to the unique engineering design of the F-One files, which feature flat side surface; this reduces stress on the file as it rotates and contacts the outer surface of the canal (8).

The cross-section of the file is an important factor in determining the instrument's flexibility. According to the manufacturer, the cross-section of the F system is S-shaped, whereas it is a triangular shape for Plex-V system (8). Some studies have stated that the lower metal mass of S-shaped cross section file gives them greater flexibility (23, 19).

As stated by Di Nardo (2020), the core of a file determines its flexibility. A file with an S-shaped cross-section is more flexible than a triangular cross-section file due to its lower core. Consequently, file F-one is more flexible compared to file Plex-V (24).

Another factor that could explain the results of this study is the cutting efficiency of the file. The unique geometric design of F-one file—featuring a flat side and an S-shaped cross-section— This means there are fewer spaces along the file, causing them to fill up quickly with debris, thereby reducing its cutting efficiency (8).

4. 2. Inner Transportation

Tables (1, 2) show that the direction of transportation was toward the outer wall of the canals; that is, the amount of material removed was greater on the outer aspect compared to the inner aspect of the root canals. This outward transportation has also been confirmed in other studies (25, 26).

These results are attributed to the forces tending to restore the file to its straight shape within the curved canal; this causes friction and cutting against the outer canal wall—with less force exerted on the inner wall—resulting in greater outward transportation compared to inner one (27).

The results of inner transportations indicate that instruments Plex-V removed a greater amount of resin from the inner canal walls compared to instruments F-One; however, the difference was not statistically significant along the length of the canal, except at the apical level. Concerning the original root curvatures, the results of this study reveal that F-One systems obtained better canal geometry, demonstrated less canal transportation and straightening at the five tested levels of the simulated canals and better maintained the original shape of the curved canals compared with Plex-V instruments. Once again, these results can be explained by the differences between the two systems in terms of flexibility and cutting efficiency (8).

It is worth noting a crucial factor determining the shaping capability of root canal instruments: the heat treatment of the metal from which they are manufactured; however, this topic lies beyond the scope of the current study.

5. Conclusions

Despite the limitations of this *in vitro* study, both systems caused some transportation in the root canals along the working length, but these transportations were within acceptable limits and did not cause problems with root canal sealing. Considering that system Plex-V caused a greater deviation than F-One system.

Conflict of interest

Authors have nothing to declare.

Funding
Nothing apart from personal funds.

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Nexus International University ISSN: 3136-6449; 12(2) 31-38

A Review of Fungal Infection on Plant

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Abstract. The production and product quality of many plants are restricted by plant diseases. The interplay between pathogens, host plants, and ecological factors influences their development. The authors of this review summarize the infection processes, diagnostic methods, and strategies for managing diseases. Efforts to contain disease severity and resistance development have focused on biological control chemical management fungicide resistance and a range of other integrated measures. By correctly diagnosing the disease and integrating biological, cultural and chemical measures, disease can be sustainably managed.

Keywords: Fungal diseases; plant pathogens; fungal infection; disease diagnosis; biological control; fungicide resistance; integrated disease management.

1. Introduction

Fungal infections are still one of the most important biological limitations of agricultural production and limitation of yield quality and stability. Plant pathogenic fungi can harm various agricultural and horticultural crops and infect the roots, stems, leaves, flowers, fruits and seeds of plants. Besides causing visible disease symptoms, they can have adverse effects on photosynthesis, water and nutrient transport, reproductive development, and the commercial quality of harvested products. As also cited by Khan and Singh, harmful fungal diseases pose as a serious concern for agriculture production and food security.
A complex interaction of susceptible host virulent pathogen and favourable environmental conditions will help in the development of fungal disease.

The effectiveness with which pathogens survive, produce spores, and cause infection and diseases is considerably affected by the temperature, moisture, rainfall, soil condition, crop density and irrigation and agricultural practices. Such interactions are gaining paramount importance due to the new climate conditions, which may alter pathogen distribution, host susceptibility, and disease severity (Singh *et al*., 2023; Méndez *et al*., 2026).

Infection with a disease begins when a viable propagule arrives at a surface of the plant, which is susceptible. Infection occurs when a pathogen penetrates into the host due to a natural opening or wound. Infecting a host involves overcoming their defenses or manipulating the host. The interaction will be the result of fungal virulence mechanisms and the ability of the plants to induce structural biochemical and molecular defense mechanisms. (Khan, 2023; Aslam *et al*., 2024)

A critical diagnosis is essential for disease management. While part one of the note does not cover this sufficiently, the conventional diagnosis based on symptomatology, microscopy and pathogen isolation is still relevant, more so in laboratories that do not have high-throughput technologies to a larger extent. Despite showing similar symptoms, various pathogens or non-infectious stresses can be at work, making the symptom-based diagnosis alone inadequate in many cases. Researchers have developed molecular techniques that allow detecting pathogens more quickly and sensitively than ever before. These methods include PCR-based assays, isothermal amplification, biosensors and sequencing approaches (Sharma *et al*., 2024; Cotter *et al*., 2024).

Chemical fungicides are still important for crop protection. Due to the persistent dependence on specific mode of action fungicides, fungicide-resistant populations have come up. Target-site mutations, enhanced efflux, metabolic alterations or other adaptive regulators may all give rise to resistant strains. As a result, modern disease regulation includes a significant aspect of resistance management (Corkley *et al* 2022; Islam *et al* 2024).

Due to the limitations of using chemicals in a more intensive manner, there is an increased emphasis on biological control, host resistance, cultural practices, natural products and other compatible strategies. Microbial competitors including various Trichoderma, Bacillus and Pseudomonas species, and Paenibacillus species can kill pathogens using several ways. They compete for space and produce hydrolytic enzymes that can induce plant defence responses. Their mycoparasitic activity is due to antibiosis. (Yao *et al*. 2023; Galli *et al*. 2024; Dobrzyński and Naziębło 2024)

We can thus consider the implementation of integrated strategies that would prevent any fungal disease from appearing and affecting plants. Together, the results of the surveys enable researchers to assess R&D spending and scope. They also provide insight into new varieties needed in the future for results that grow better (Li *et al*, 2025).

2. Fungal Pathogens and Their Importance in Crop Production

These fungi obtain their nourishment from the host plant and utilize different strategies to overcome the host plant. These organisms can live in a variety of ways. They can be biotrophic, which means that they keep the host tissue alive. On the other hand, they can be necrotrophic, which means that they kill the host cells and live on them. A pathogen that may invest in the first phase of the EARM-spectrum (disease strategy spectrum) and mutilate the host tissue later may be called a hemibiotroph (Maulenbay and Rsaliyev, 2024).

Some examples of fungi that are damaging to plants are sooty molds, smuts, and rusts. Other examples include genera of the fungi Alternaria, Botrytis, and Fusarium. There are significant variations among these pathogens regarding their hosts, infection process, means of survival, and endoparasite's environment requirements (Maulenbay and Rsaliyev, 2024; Aslam *et al*., 2024).

As capable of causing disease doesn't indicate the worth of any fungal pathogen as a whole. The epidemiological significance of an agent refers to its ability to survive, and between cropping seasons (i.e. grainless periods), to produce and disperse inoculum, to colonize susceptible plant tissues, and to adapt to variation in the environment. Plants absorb nutrients from the soil and, therefore, weeds compete for these resources. Herbicide works either by preventing the weed from absorbing nutrients and water or by killing the weed directly.

Post-harvest fungal diseases increase the loss due to diseases Pathogens such as Botrytis, Alternaria, and Colletotrichum serve as illustrations of rotting goods. It is essential that fungal disease control programs cover post-harvest protection effort as well as field production effort (Li *et al*.2025)

3. Infection Process and Disease Development

3.1 Spore Germination and Host Recognition

A fungal infection typically begins when spores or other infectious agents come into contact with a host's surface. Environmental conditions, especially temperature and moisture, are important factors for germination in addition to

chemical and nutritional signals. The plant surface contracts after germination of fungal structure. Certain pathogens develop unique structures such as appressoria, which help them to enter host cells and / or break the host organisms' tissues (Khan, 2023).

3.2 Penetration and Colonization

The skin of a plant is the first line of defence against pests and pathogens and can be described as its first physical barrier. Fungal penetration is reduced by cuticle cell wall and other antifungal compounds. Colonization is limited due to defense mechanisms A successful pathogen can penetrate the tissues of a plant and disrupt its growth or even kill the plant. When a pathogen penetrates the host tissues, the fungal hyphae will either remain localized or disseminate through the host tissues. The extent to which the above takes place differs from one pathogen to another (Aslam *et al*., 2024).

3.3 Plant Defense Responses

Plants possess different kinds of defense against fungi. The identification of pathogen-related molecules could activate signaling that leads to the production of reactive oxygen species, antimicrobial agents, defence proteins and structural barriers. Structural modifications that occur as a result of pathogen invasion in plants are appressing and cell-wall reinforcement. Hormones such as salicylic acid, jasmonic acid, ethylene, and abscisic acid regulate the plant reaction(Yao *et al*., 2023; Aslam *et al*., 2024).

4. Symptoms and Consequences of Fungal Diseases

Symptom Presentation of Fungal Diseases Depending upon the pathogen and host infected, the symptoms caused by fungal diseases may vary. The symptoms of the plant diseases include leaf spots and blights, chlorosis and yellowing, necrosis, wilting, stem and branch cankers, damping-off, root and crown rot, vascular discoloration, fruit and seed rot, early defoliation and reduced growth and reproductive performance (Maulenbay and Rsaliyev, 2024).

Leaf diseases which damage functional leaf tissue can reduce photosynthetic capacity, whereas root and vascular tissue damage impairs water and nutrient acquisition. Diseases can reduce yields, affect quality, raise costs of production and reduce post-harvest shelf life with serious economic consequences.

Some fungal pathogens can also produce mycotoxins that cause food and feed safety problems (Singh *et al*., 2023; Li *et al*., 2025).

5. Environmental and Agricultural Factors Affecting Disease Development

The environmental factors and crop-management practices influence disease development.
Fungi's temperature alters how seed germinate and disease infects. All pathogens have a temperature range of optimum infection and any deviation from this temperature range may alter the disease development. (Singh *et al*., 2023)

Many leaf pathogens require moisture to thrive. Extended periods of leaf wetness can facilitate spore germination and penetration. High relative humidity may set the stage for sporulation.

Rain can disperse pathogenic through splash dispersal and run off, while overhead irrigation can increase wetness duration of leaves possibly increasing the risk of disease in susceptible crops. Densely packed plants can make a great environment for fungal growth, due to humid conditions (Khan, 2023).

By minimizing the consistent presence of susceptible hosts, crop rotation has the ability to interrupt pathogen life cycles. Appropriate management of infected residues may reduce pathogen survival between years growing (Khan, 2023).

Changing climate conditions have been complicating disease epidemiology through changes in temperature precipitation water availability pathogen distribution and host physiology. A systematic review of 106 studies found that 61% of the studies showed an increase in disease severity under the climate-related conditions investigated. However, the responses varied among different crops and pathogens (Méndez *et al*. 2026).

6. Diagnosis of Fungal Plant Diseases

An accurate diagnosis forms the basis of rational disease management.

6.1 Symptom-Based Diagnosis

Spotting a Disease Is the First Sign of Illness The lesion structure and symptoms and appearance of organ involvement and visible fungal element can signal useful preliminary information. Symptoms can be misleading, since they cannot help to distinguish pathogens, or infectious and non-infectious disorder (Sharma *et al*., 2024).

6.2 Microscopy and Culture

Observation under microscope can show fungal hyphae. Culturing the pathogen in proper media allows us to identify them further. These methods are useful but take time and expert knowledge (Sharma *et al*., 2024).

6.3 Molecular Diagnosis

The detection of pathogens has benefited from the improved sensitivity and specificity made possible by PCR, quantitative PCR, loop-mediated isothermal amplification, recombinase polymerase amplification, sequencing, and biosensor-based technologies. A review of diagnostics earlier has identified novel molecular methods that can potentially be very useful for detecting fungal and oomycete plant pathogens before disease establishment (Sharma *et al*., 2024; Cotter *et al*., 2024).

6.4 Imaging and Remote Sensing

More and more researchers are looking into optical hyperspectral thermal and other type of s en s ing for early detection of diseases. These methods allow for non-destructive information that can monitor large crop areas. Nonetheless, the necessity for assessment under changing field conditions will always be there as measured in controlled conditions may not be the same in farming time (Pereira *et al*., 2025; George *et al*., 2025).

7. Chemical Control and Fungicide Resistance

Fungicides are important for protecting crops from disease. The effectiveness of fungicides relies on factors like chemical composition, mode of action, timing, and dose sensitivity (Islam *et al*, 2024).

Using several fungicides with similar action modes will impose selection pressure on the pathogen population. Strains that develop during treatment can propagate and spread resistance. Resistance to efflux target-site mutations can involve altered metabolism and other mechanism. As a result, it is apt for current and future-resistant management, to enhance the diversification of modes of action, limit unnecessary applications, offer sound dose, and integrate with non-chemical means (Corkley *et al*., 2022; Naqvi *et al*., 2024).

8. Biological Control

Biological control refers to the strategy of employing beneficial microorganisms in inhibiting plant pathogens. The method is increasingly used in pest control, as there are concerns of chemical residues, environmental pollution, resistance development and sustainability (of the method) (Galli *et al*., 2024; Madlhophe *et al*., 2025).

Microbes employed for biocontrol can assist with competition for nutrients and niches, antibiosis, mycoparasitism, production of hydrolytic enzymes, production of antimicrobial metabolites, competition for iron and other elements, as well as stimulation of plant defence mechanisms (Yao *et al*., 2023; Dobrzyński and Naziębło, 2024).

8.1 Trichoderma spp.

Trichoderma species have been thoroughly studied and shown to be effective fungal biocontrol agents. Yao et al., 2023, reported that this activity can also be mycoparasitism, competition antibiosis, enzyme production and induction of systemic resistance in plants. Recent studies on formulations based on Trichoderma are being used against soil-borne and foliar and post-harvest diseases. Despite being efficacious, field performance may differ with strains, environmental conditions, formulation stability and application method (Li et al, 2025; Pérez-Pizá et al, 2025).

8.2 Bacterial Biocontrol Agents

The fungal pathogens have been proved to be controlled biologically by Bacillus, Pseudomonas and Paenibacillus species. Mechanisms may involve the production secretion of enzymes activation of host defenses biofilm formation and more. The genus Paenibacillus is of special interest as a potential biocontrol agent other than P. polymyxa (Dobrzyński and Naziębło, 2024).

8.3 Arbuscular Mycorrhizal Fungi

Mycorrhizal fungi can prevent plant diseases and help plants obtain nutrients and water. They may change plant nutrition, hormonal signaling, root structure, defence responses, and so on. Scientists have recently found out that they can help manage disease and promote vigour(Galli *et al*., 2024).

9. Botanical and Natural Products

Another potential group of substitutes for synthetic fungicides are plant. The activity of phenolics, flavonoids, terpenoids, alkaloids, and essential-oil components in inhibiting the growth of fungi can be attributed to their action on membrane integrity, enzyme activity, and spore germination (Calefi *et al*., 2025).
Recent reviews indicate that plant-based products can be useful against many pathogens, especially in organic systems. But this is a serious hindrance because of the variability of chemical composition stability formulation phytotoxicity and field efficacy. For real-life use in agriculture, promising compounds will need to be formulated in standard way and field validated (Calefi *et al*., 2025; Monteiro *et al*., 2026).

10. Emerging and Advanced Approaches

Advancements in plant pathology have created a whole new range of disease-management choices. Researchers are evaluating approaches like genomic editing, molecular diagnostics, and nanotechnology to enhance disease management. Despite the regulatory, ecological, economic, and technical constraints which still exist, Ogwu and Izah (2025) and Monteiro *et al*. (2026) concluded that these technologies have the potential of improving pathogen specificity and minimizing environmental exposure.
These methods are the additions to the already-established disease-management principles (Keykhasaber *et al*., 2026).
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11. Integrated Disease Management

The different methods for integrated disease management are compatible and among them reduce diseases' intensity. The aim is to avoid heavy dependence on any one method. A practical program can employ resistant or tolerant cultivars, pathogen-free planting material, incorporate crop rotation, ensure proper irrigation and drainage, include handling sanitation and residues, monitor the crop, make an early and accurate diagnosis, practice biological control,

use validated botanicals, use fungicide judiciously and rotate the mode of action of fungicide (Khan, 2023; Galli *et al*., 2024).

The main advantage of integration is that various components act via various means. This technique can help in improving disease suppression and decreasing the selection pressure for fungicide resistance. As per the recent reviews, it has endorsed integrated frameworks which combine cultural practices, host resistance, bio-control, use of natural products, and targeted application of chemicals. (Galli *et al*. 2024 Monteiro *et al*. 2026).

12. Current Challenges and Future Perspectives

Fungal diseases face many challenges that hinder their effective management. Populations of pathogens are genetically varied and adaptable. Fungicide resistance threatens the long-term effectiveness of chemical control. Variable performance shown in the field despite positive laboratory results experienced by a range of biological control agents (Corkley *et al*., 2022; Pérez-Pizá *et al*., 2025).

The uncertainty added by climate change stems from alterations in the distribution of pathogens, environmental suitability, host physiology and disease outcomes. The effects are not uniform across the pathosystems indicating that we require disease-specific forecasts making them more specialised than generalised assumptions (Singh *et al*., 2023; Méndez *et al*., 2026).
As a result of this, future research focus needs to be on field validation, better formulation of biological agents, standardization of diagnostics, resistance monitoring and combination of complementary disease-management strategies (Keykhasaber *et al*., 2026; Monteiro *et al*. 2026).
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Table 1: Major fungal pathogens, associated diseases, symptoms, and main affected plant organs.

Fungal pathogen/group	Representative disease	Major symptoms	Main affected organs
Fusarium spp.	Fusarium wilt/root rot	Wilting, vascular discoloration, root decay	Roots, stems
Alternaria spp.	Leaf spot/blight	Necrotic spots, concentric lesions	Leaves, fruits
Botrytis cinerea	Gray mold	Soft rot, gray sporulation	Flowers, fruits
Colletotrichum spp.	Anthracnose	Sunken necrotic lesions	Leaves, stems, fruits
Rhizoctonia spp.	Root/crown rot	Damping-off, root and stem lesions	Roots, stems
Sclerotinia spp.	White mold	Soft rot, white mycelium	Stems, fruits
Verticillium spp.	Vascular wilt	Wilting, vascular browning	Roots, vascular tissues
Magnaporthe spp.	Blast disease	Lesions and tissue necrosis	Leaves, stems, reproductive tissues

Table 2: Comparison of major fungal disease-management strategies.

Strategy	Main mechanism	Major advantage	Major limitation	Sustainability
Chemical control	Direct inhibition of pathogen growth	Rapid and often effective	Resistance and environmental concerns	Moderate
Host resistance	Restricts infection or disease development	Long-term and relatively low input	Pathogen adaptation may overcome resistance	High
Crop rotation	Interrupts pathogen life cycle	Low cost and preventive	Less effective against broad-host pathogens	High
Biological control	Competition, antibiosis, mycoparasitism, induced resistance	Environmentally compatible	Variable field performance	High
Botanical products	Antifungal natural compounds	Potentially biodegradable	Stability and standardization problems	Potentially high
Integrated management	Combines complementary methods	More robust disease suppression	Requires monitoring and planning	Very high

Figure 1: *Conceptual framework for fungal disease development and integrated management.*
Pathogen survival and dissemination
↓

Spore deposition / pathogen contact with host
↓
Germination and host recognition
↓
Penetration and colonization
↓
Host defense–pathogen interaction
↓
Disease development and symptom expression
↓
Early diagnosis and disease monitoring
↓
Integrated management: Cultural practices | Host resistance | Biological control | Botanical products | Judicious chemical control | Resistance management

13. Conclusion

Fungal diseases are a huge challenge for sustainable crop production and food security. The development of pathogens happens as a result of many factors including pathogen biology. Accurate diagnosis and early intervention are essential for effective management of diseases (Khan, 2023; Singh *et al*., 2023).

Even with the availability of chemical fungicides, the rising resistance and increasing environmental concerns necessitate the incorporation of host resistance, cultural practices, biological control, natural products and use of chemical fungicides options. Future studies need to increase the reliability of biological and diagnostic technologies under field conditions and explore complementary strategies for effective disease suppression that mitigates environmental impacts (Corkley *et al*., 2022; Galli *et al*., 2024; Monteiro *et al*., 2026).

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 39-46

Knowledge and Beliefs Regarding Harmful Traditional Health Practices among University Staff: A Cross-Sectional Study

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Abstract

Background: Certain customs have their roots in cultures, religions, or the counseling of new mothers by family members.

Methods: A descriptive cross-sectional study was conducted from December 1st, 2025 to March 30th, 2026. The study included a convenience sample of 200 male and female staff members from different colleges at the University of Basrah, Bab Al-Zubair campus. Data were collected using a closed-ended questionnaire consisting of two parts: socio-demographic characteristics and questions related to the staff's opinions about several traditional health practices. The Statistical Package for Social Sciences (SPSS) version 26 was used to analyze the data using both descriptive and inferential statistics.

Results: The results showed that most participants were university graduates (58.5%), while 23% had postgraduate degrees. The Participants demonstrated variable levels of knowledge, with persistence of several misconceptions. However, some incorrect beliefs were still present among the participants, such as using ash or flour to stop wound bleeding, hanging beads for neonatal jaundice, and using kohl to protect infants' eyes. Significant correlations were found between knowledge scores and some demographic variables, including age, gender, and marital status.

Conclusion: The study concluded that despite relatively high educational levels among participants, some harmful traditional health practices are still believed and practiced. Increasing awareness and providing accurate health education are essential to reduce the spread of these misconceptions.

1. Introduction

Due to poverty and poor levels of education, harmful traditional behaviors are carried out in many civilizations, particularly in developing nations. The link between the environment and human views toward nature is typically the source of traditional behaviors. Certain customs have their roots in nations, religions, or family members counseling new mothers (1). Since prehistoric times, people have utilized natural products, such as plants, animals, and marine organisms, to treat and alleviate illnesses (2).

For early people, using natural things as medicines surely posed a significant problem. When looking for food, that is highly likely. Poisonous plants were frequently eaten by early humans, who may have died as a result of vomiting, diarrhea, stupor, or other toxic effects. However, they were able to expand their understanding of natural remedies and edible ingredients in this way (3). Humans then discovered how to create new medications. Natural items are used in traditional remedies, which are quite significant. Natural goods are used in traditional medicine and folk medicine, which have been used for hundreds or even thousands of years worldwide. They have consistently thrived in official medicine, which continues to be a storehouse of human knowledge (4).

Arabs are credited with creating and establishing the principles and guidelines of traditional medicine, particularly those who specialized in traditional and folk Arab medicine (5). Traditional medicine includes procedures and manual exercises used alone or in combination to cure, diagnose, prevent, or maintain happiness, as well as plant, animal, and mineral remedies, spiritual therapies, magic, and superstition. It also refers to all of the knowledge, skills, and approaches that are employed to maintain health, prevent, diagnose, improve, or treat physical, mental, and other ailments and are taken from indigenous theories, beliefs, and experiences of other cultures, whether or not they can be explained (6).

A yellowish or, less commonly, greenish coloring of the skin and sclera caused by elevated bilirubin levels is called jaundice, or icterus. Adult cases of jaundice are usually indicative of underlying conditions including aberrant heme metabolism, liver malfunction, or obstruction of the biliary tract (7, 8). While jaundice is uncommon in adults, it is frequent in babies, affecting an estimated 80% of them during the first week of life. The most typical signs of jaundice include dark urine, pale feces, and itching (9). Excessive disintegration of red blood cells may be the cause of elevated unconjugated bilirubin. The underlying cause of jaundice usually determines the course of treatment (10, 11).

The process during which a baby's first teeth—the deciduous teeth—emerge through their gums, usually in pairs, is known as teething. The infant experiences discomfort and anguish as the mandibular central incisors, the first primary teeth to erupt, typically appear between the ages of six and ten months (12). The eruption of all 20 teeth may take several years. The body releases hormones that lead to the death and separation of some gum cells, allowing the teeth to erupt. Teething does not appear to be a major cause of vomiting, diarrhea, or fever (13,14).

Every newborn has a varied threshold for pain. Usually starting three to five days before the tooth erupts, the sensations go away as soon as the tooth penetrates the skin (15, 16). Some people think that teething causes other symptoms, such as diarrhea, but there is no evidence to support this (17).

Measles is an airborne disease that spreads quickly from person to person through the coughs and sneezes of infected people. Symptoms usually start 10–12 days after interaction with an infected person and continue for 7–10 days. Common initial symptoms include fever, often more than 40 °C (104 °F), cough, runny nose, and itchy eyes (18). Koplik spots are little white patches that might develop inside the mouth two or three days after the onset of symptoms (19). A red, flat rash typically develops on the face three to five days after symptoms start, then spreads to the rest of the body (20).

Pneumonia (6%), middle ear infection (7%), and diarrhea (8% of cases) are common consequences (21). Measles-induced immunosuppression contributes to these (22). Seizures, blindness, or brain inflammation are less prevalent (23, 24). While there are historical reports from the early 20th century suggesting that red light therapy could "abort" or speed up the recovery of measles, there is no modern scientific evidence or clinical guidelines recommending "red dressing" (red clothing) to treat or relieve measles symptoms (25, 26).

A collection of middle ear inflammatory conditions is known as otitis media. Acute otitis media (AOM), a fast-onset illness that typically manifests as ear pain, is one of the two primary forms (27, 28). Otitis media with effusion (OME) is the other primary form (29). Middle ear inflammation that causes a tympanic membrane perforation and ear discharge is known as chronic suppurative otitis media (CSOM) (30, 31).

Inflammation of the conjunctiva, the thin, transparent membrane covering the inner eyelid and the white surface of the eye, is referred to as conjunctivitis, or pink eye (32, 33). While bacterial causes are more common in children, viruses are the most common infectious causes in adults (34, 35).

An injury to skin or other tissues brought on by heat, electricity, chemicals, friction, or ionizing radiation (like sunburn, which is produced by UV radiation) is referred to as a burn (36). Burns mostly happen at home or at work (36, 37). Superficial or first-degree burns are those that only damage the outermost layers of skin. The pain usually lasts around three days, and they appear red without blisters. A partial-thickness or second-degree burn occurs when the damage penetrates part of the underlying skin layer. Blisters are common and frequently cause excruciating agony. Scarring may result, and healing may take up to eight weeks (38). All of the skin's layers are damaged in a full-thickness or third-degree burn. The burned area is often stiff and painless. Usually, healing doesn't happen on its own (39).

2. Research Methodology

Study's Design: At the University of Basrah Bab Al-Zubair campus, a descriptive cross-sectional study approach was used, involving a sample of 200 participants (male and female). The study started from December 1st, 2025, up to 30th March, 2026, in order to know the University of Basrah staff's opinions on some wrong health traditional practice at Bab Al-Zubair campus.

Setting of the Study: The current study was conducted at the Bab Al-Zubair campus of the University of Basrah.
The Sample of the Study: A convenience sample from the university staff, which consisted of 200 participants at Basrah University.

Study's Instrument: Data were collected using a closed-ended questionnaire. The questionnaire is divided into two sections. The first portion includes six questions about the population's sociodemographic characteristics, such as age, gender, college department, education level, marital status, and place of residence. The population's opinions toward several health customs are the subject of 15 questions in the second section of the survey. For the study, a standardized Likert scale with three points—agree, neutral, and disagree—was employed. Good (2.34-3), middling (1.67-2.33), and poor (1-1.66). 200 samples were given the pre-made questionnaire, which they read and completed. The researchers then collected the completed forms. Every form was examined and given a percentage score based on the correct usual response.

Statistical Analysis: Analysis was conducted using SPSS 26, so we measured: frequency, Percentage (%), Spearman correlation, and means.

Ethical Approval: In addition to receiving official approval from the Council of the College of Nursing at the University of Basrah, the study also received ethical approval from the committee overseeing the college's ethical research and was permitted to proceed. The researcher explained the purpose of the study to each participant. Oral consent was obtained from each participant before data collection.

3. Results

Table 1: Distribution of the study sample according to educational levels

Educational levels	**Frequencies**	**Percentages**
Primary school graduation	7	3.5
Secondary school graduation	16	8
University graduation	117	58.5
Institute education	14	7
Master degree	35	17.5
Doctorate	11	5.5
Total	**200**	**100**

The table shows the distribution of the study sample according to educational levels. We found that 3.5% had primary school education, 8% had secondary school education, 7% had institution education, 58.5% had university education, 17.5 % were having Master graduation, and 5.5% had doctorate graduation.

Table 2: Distribution of the study sample according to workplace location

Workplace	**Frequencies**	**Percentages**
College of administration and economy	48	24
College of education for girls	34	17
College of Nursing	34	17
College of Arts	32	16
College of Fine Arts	19	9.5
College of Law	10	5
Others	23	11.5
Total	**200**	**100**

The table showed the distribution of the study sample according to workplace; 24% of the sample were from the College of Administration and Economy, 17% of the sample were from the College of Education for Girls, 17% were from the College of Nursing, 16% were from the College of Art, 9.5% were from Fine Arts, 5% were from the College of Law, and 11.5% were from other places in the Bab Al-Zubair campus at the University of Basrah.

Table 3: Distribution of the study sample according to Age intervals

Age intervals in years	**Frequencies**	**Percentages**
20-29	38	19
30-39	68	34
40-49	57	28.5
50-59	37	18.5
Total	**200**	**100**

The table showed that 19% of the sample were within the age interval 20-29 years, 34% of the sample were within the age interval 30-39 years, 28.5% of the sample were within the age interval 40-49 years, and 18.5% of the sample were within the age interval 50-59 years.

Table 4: Distribution of the study sample according to Gender

Gender	**Frequencies**	**Percentages**
Males	89	44.5
Females	111	55.5
Total	**200**	**100**

The table shows the distribution of the study samples according to gender; 44.5% were males and 55.5% were females.

Table 5: Distribution of the study sample according to Residencies

Residencies	**Frequencies**	**Percentages**
Urban	150	75
Rural	50	25
Total	**200**	**100**

The table shows the distribution of the study samples according to residency; 75% were urban, and 25% were rural.

Table 6: Distribution of the study sample according to Marital Status

Marital status	**Frequencies**	**Percentages**
Single	77	38.5
Married	123	61.5
Total	**200**	**100**

The table shows the distribution of the study samples according to marital status; 38.5% were single, and 61.5% were married.

Table 7: Distribution of the study sample according to Scores

Scores	Frequencies	Percentages
Less than 50 marks	6	3
50-59 marks	31	15.5
60-69 marks	53	26.5
70-79 marks	45	22.5
80-89 marks	32	16
90 and above	33	16.5
Total	**200**	**100**

The table showed the distribution of the study sample according to scores they get after corresponding the typical answers with staff answers to measure their levels of knowledge using the classical way in classifying the scores (marks), 3% were having less than 50 scores (fail to answer the questionnaire items) 15.5% were having 50-59 score (low level of knowledge), 26.5% were having 60-69 score (moderate level of knowledge), 22.5% were having 70-79 scores (good level of knowledge), 16% were having 80-89 scores (very good level of knowledge) and 16.5 % were having 90 and above (excellent level of knowledge).

Table 8: Distribution of the Study Sample according to questionnaire answers

No	Item	AG (1)	Neut. (2)	DA (3)	Means
1	Ear dissection for a patient with Jaundice is an acceptable way of treating babies and adults	44	60	96	2.26
2	Hanging Garlic pieces on the body of neonates is beneficial in decreasing neonatal Jaundice	64	46	90	2.13
3	Hanging yellow jaundice beadle on the body of neonate had a role in decreasing the level of jaundice	153	31	16	1.31
4	Teething process in infants can cause some symptoms like diarrhea, vomiting, fever, and loss of appetite	50	56	94	2.22
5	Red dressing for measles patients can relieve the disease symptoms	71	65	64	1.96
6	Introduction of onion pieces on ear for managing ear diseases	80	53	67	1.93
7	Using a pack of tea to treat eye infection	31	49	120	2.44
8	Skin cauterization for management of headache	22	21	157	2.67
9	Putting animal feces on umbilical cord stump in neonate to enhance healing process	23	30	147	2.62
10	Using animal urine in the treatment of skin diseases	60	53	87	2.13
11	Using tomato paste for managing burns at home	50	52	98	2.24
12	Using ash or flour to stop wound bleeding	122	54	24	1.51
13	Baby swaddling is useful for future body alignment	118	49	33	1.57
14	Using rice water in feeding infants	87	41	72	1.92
15	Using Kohl for protecting infants' eyes from infections	115	45	40	1.62

AG= agree, Neut.= neutral, DA = Disagree

The table showed the distribution of the study sample according to the questionnaire answers. Regarding the answer to question (1-Ear dissection for a patient with Jaundice is an acceptable way for treating babies and adults), 22% agreed with such a wrong tradition, 30% had a neutral answer, while 48% only disagreed. Question (2-Hanging Garlic pieces on the body of neonates is beneficial in decreasing neonatal Jaundice): 32% agreed with such a wrong tradition, 23% had a neutral answer, while 45% only disagreed. Regarding question (3-Hanging yellow jaundice beadle on the body of neonate had a role in decreasing level of jaundice), 76.5% agreed with such a wrong tradition, 15.5% had a neutral answer, while 8% only disagreed.

Question (4- Teething process in infants can cause some symptoms like diarrhea, vomiting, fever, and loss of appetite), 25% agreed with such a wrong tradition, 28% had a neutral answer, while 47% only disagreed. Question (5-Red dressing for measles patients can relieve the disease symptoms): 35.5% agreed with such a wrong tradition, 32.5% had a neutral answer, while 32% only disagreed. Question (6-Introduction of onion pieces on ear for managing ear diseases): 40% agreed with such a wrong tradition, 26.5% had a neutral answer, while 33.5% only disagreed. Question (7-Using a pack of tea to treat eye infection): 15.5% agreed with such a wrong tradition, 24.5% had a neutral answer, while 60% only disagreed.

Question (8-Skin cauterization for management of headache): 11% agreed with such a wrong tradition, 10.5% had a neutral answer, while 78.5% only disagreed. Question (9-Putting animal feces on umbilical cord stump in neonate to

enhance healing process): 11.5% agreed with such a wrong tradition, 15% had a neutral answer, while 73.5% only disagreed. Question (10-Using animal urine in the treatment of skin diseases): 30% agreed with such a wrong tradition, 26.5% had a neutral answer, while 43.5% only disagreed.

Question (11-Using tomato paste for managing burns at home): 25% agreed with such a wrong tradition, 26% had a neutral answer, while 49% only disagreed. Question (12-Using ash or flour to stop wound bleeding): 61% agreed with such a wrong tradition, 27% had a neutral answer, while 12% only disagreed. Question (13-Baby swaddling is useful for future body alignment) 59%were agree with such a wrong tradition, 24.5% had a neutral answer, while 16.5% only disagreed. (14-Using rice water in feeding infants) Question 43.5% agreed with such a wrong tradition, 20.5% had a neutral answer, while 36% only disagreed. Question (15-Using Kohl for protecting infants' eyes from infections): 57.5% agreed with such a wrong tradition, 22.5% had a neutral answer, while 20% only disagreed.

Table 9: The Correlations of the scores with some variables

	Scores	**Age**	**Gender**	**Level**	**Residence**	**Marital status**
Spearman Correlation	1	-0.181*	0.222**	-0.105	-0.026	0.526**
Sig. (2-tailed)		0.010	0.002	0.137	0.713	0.000
N	201	201	194	201	201	201

The table showed the correlation of scores with demographic variables, using Spearman correlation. There was a significant correlation between scores and age at the 0.05 level, and there was high significant correlation between scores and both gender and marital status at the 0.01 level.

Table 10: Distribution of the study sample according to knowledge scores (No. 15 question)

Score	**Classes**	**Frequencies**	**Percentages**
1-1.66	Poor	4	26.6
1.67-2.33	Moderate	7	46.7
2.34-3	Good	4	26.7

The table showed the distribution of the study sample according to knowledge scores; 26.6% had poor knowledge, 46.7 % had moderate knowledge, and 26.6 had good knowledge, so 73.3 % were having high level of knowledge.

4. Discussion

The present study aimed to explore the opinions of University of Basrah staff regarding some common traditional health practices that may negatively affect health. Although the majority of the participants had relatively high educational levels, the findings showed that several incorrect health beliefs are still present.

According to the results, most of the participants were university graduates or held postgraduate degrees. Despite this, a noticeable proportion of the respondents agreed with some traditional practices that are not supported by scientific evidence. This suggests that educational level alone may not be sufficient to eliminate beliefs that are deeply rooted in cultural traditions.

The results of the knowledge scores indicated that most participants had moderate to good levels of knowledge about these practices. However, a smaller proportion of the sample showed low knowledge levels, which indicates that some misconceptions still exist among university staff.

The responses to the questionnaire also showed that certain traditional practices are still widely accepted. For example, a high percentage of participants believed in practices such as hanging yellow beads on neonates to reduce jaundice, using ash or flour to stop bleeding, and using kohl to protect infants' eyes. These practices are often inherited through generations and may be considered normal in some communities despite the lack of scientific evidence supporting them.

On the other hand, some practices such as skin cauterization for headache and placing animal feces on the umbilical cord stump were rejected by the majority of participants. This may indicate that awareness about some harmful practices has improved. The correlation analysis revealed significant relationships between knowledge scores and some demographic variables. Age showed a significant association with knowledge level, which may suggest that experience and exposure to information influence health awareness. Gender and marital status also showed significant relationships with the knowledge scores.

5. Conclusions

More than half of the sample had university graduation, two-thirds of the sample were from urban areas, more than half were married, and more than half of the sample were within the ages between 30-49 years. About half of the sample answered the questionnaire with 60 and 79 evaluation marks, as with standard answers. Some wrong traditional health practices are still present among University of Basrah staff. Most participants demonstrated moderate to good knowledge about these practices. A noticeable proportion still believes in some harmful practices of the population. Demographic factors such as age, gender, and marital status were significantly associated with knowledge level.

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 47-52

Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) among admitted patients at Baqubah Teaching Hospital in Diyala

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Abstract

Background: Antibiotic resistance in *Staphylococcus aureus* has emerged as a critical global public health threat, driven primarily by the rising prevalence of methicillin-resistant strains (MRSA) and their diminishing susceptibility to conventional antimicrobials.

Objective: To determine the prevalence of methicillin-resistant (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) and to evaluate their antimicrobial susceptibility patterns across various clinical isolates.

Patients and Methods: A cross-sectional study was conducted at Baqubah Teaching Hospital in Diyala, Iraq, from March 2025 to April 2026. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include 110 *S. aureus* isolates were consecutively Antimicrobial susceptibility testing was performed using standard methods. Data were analyzed using SPSS version 24, and Chi-square tests were applied to assess the associations between resistance patterns and specimen sources. P-values less than 0.05 were considered statistically significant.

Result: The isolated *S. aureus* strains exhibited substantial resistance to several commonly used antibiotics. The most prominent resistance rates were observed against benzylpenicillin (95.45%) and oxacillin (80.91%), indicating a high phenotypic prevalence of methicillin-resistant *S. aureus* (MRSA). Moderate resistance profiles were recorded for moxifloxacin (65.45%), erythromycin (66.36%), gentamicin (51.82%), levofloxacin (51.82%), tobramycin (50.91%), and rifampicin (50.00%). Conversely, the isolates retained relatively higher susceptibility to teicoplanin (73.64%), trimethoprim/sulfamethoxazole (62.73%), vancomycin (56.36%), and tetracycline (56.36%). Notably, the distribution of antimicrobial resistance varied significantly based on the specimen source; isolates recovered from urine samples demonstrated the highest resistance rates, followed by wound and vaginal swabs, whereas ear swabs exhibited the lowest. Statistical analysis confirmed significant variations in susceptibility patterns ($P \leq 0.01$), underscoring a non-random distribution of resistance among the tested pathogens.

Conclusion: In conclusion, this study demonstrates a high prevalence of multidrug-resistant *S. aureus*, including a substantial proportion of methicillin-resistant (MRSA) strains, at Baqubah Teaching Hospital. The significant variations in susceptibility patterns across different antibiotics and anatomical infection sites emphasize the necessity of tailored empirical therapy. These alarming resistance rates warrant the immediate implementation of strict antimicrobial stewardship programs and regular local surveillance to curb the dissemination of these refractory pathogens.

Keywords: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Antibiotic resistance, Antimicrobial susceptibility.

1. Introduction

S. aureus is a gram-positive bacterium that colonizes the skin and nasal passages of humans and animals, acting as both a commensal organism and a potent pathogen capable of causing a wide range of diseases [1]. The high adaptability of *S. aureus,* coupled with its ability to form biofilms on medical devices and persist in hospital environments, further complicates eradication efforts and contributes to recurrent infections [2]. The emergence of methicillin resistance in *S. aureus* strains is primarily mediated by the acquisition of the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with reduced affinity for beta-lactam antibiotics, rendering standard methicillin and related beta-lactam therapies ineffective [3]. Consequently, MRSA infections pose substantial clinical and economic burdens due to prolonged hospital stays, increased morbidity and mortality rates, and higher healthcare costs associated with the use of advanced antimicrobial agents [4]. In parallel, the emergence of vancomycin-resistant *S. aureus* (VRSA) has raised additional concerns regarding therapeutic options, as vancomycin has long been considered the antibiotic of last resort for severe MRSA infections. Vancomycin resistance in *S. aureus* is typically mediated by the acquisition of *vanA* gene clusters from vancomycin-resistant enterococci (VRE), resulting in altered peptidoglycan synthesis that reduces vancomycin binding and efficacy [5,6]. Although VRSA remains relatively rare compared to MRSA, documented cases have been reported in multiple countries, underscoring the potential for rapid dissemination in healthcare settings and the urgent need for robust surveillance systems. VRSA infections often arise in patients with chronic medical conditions, prior exposure to vancomycin, or prolonged hospitalization, highlighting the interplay between antimicrobial pressure and bacterial evolution [7]. Infection control measures, including hand hygiene, environmental cleaning, contact precautions, and screening of high-risk patients, have demonstrated variable efficacy in limiting the spread of resistant strains [8]. In this context, antimicrobial stewardship programs play a pivotal role in mitigating selective pressure by optimizing the use of antibiotics, reducing inappropriate prescriptions, and promoting adherence to evidence-based guidelines. Moreover, public health education and awareness campaigns targeting healthcare workers, patients, and the general population are essential to curtail the dissemination of resistant *S. aureus* strains and reduce the burden of infections [9]. This study aimed to determine the prevalence of methicillin- and vancomycin-resistant *S. aureus* (MRSA and VRSA) and to evaluate the antibiotic susceptibility patterns of these isolates obtained from various clinical specimens.

2. Patients and Methods

2.1 Study Design and Setting

This cross-sectional analytical study was conducted to evaluate the prevalence and antimicrobial resistance patterns of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) at Baqubah Teaching Hospital in Diyala Governorate, Iraq. The study was carried from March 2025 to April 2026 in Baqubah Teaching Hospital was selected as the study site due to its high patient turnover and diverse clinical presentations, and because it features a fully equipped microbiology laboratory staffed by trained personnel capable of performing standardized bacterial isolation and phenotypic characterization. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include only 110 *S. aureus.*

2.2 Study Population and Sampling Strategy

Non-probability consecutive sampling was employed, recruiting all patients who presented with suspected bacterial infections and met the selection criteria until the target sample size was achieved.

Inclusion Criteria: Patients of all age groups and both sexes with microbiologically confirmed *S. aureus* infections.
Exclusion Criteria: Patients who had received antibiotic therapy within 72 hours prior to sample collection, individuals with mixed bacterial infections, immunocompromised status with systemic infections, or those with incomplete medical and clinical records.

2.3 Ethical Considerations

The study protocol was reviewed and officially approved by the Research Ethics Committee of the College of Medicine, University of Diyala. Prior to specimen collection, informed written consent was obtained from all adult participants or from the legal guardians of minors. Participants were briefed on the study objectives, procedures, and their right to withdraw at any stage without compromising their medical care. Data confidentiality was strictly maintained by converting all personal data into anonymized alphanumeric codes. All procedures aligned with the ethical standards established in the Declaration of Helsinki.

2.4 Data Collection and Specimen Processing

Demographic information and clinical data were documented using a structured data collection sheet. Clinical specimens were gathered under strict aseptic conditions by trained healthcare professionals following standardized institutional protocols. All specimens were immediately placed in appropriate transport media and transferred to the microbiology laboratory to ensure bacterial viability and minimize pre-analytical errors.

2.5 Bacterial Isolation and Phenotypic Identification

Specimens were inoculated onto selective and differential media, including Mannitol Salt Agar and Blood Agar plates, and incubated aerobically at 37°C for 24–48 hours [10]. Golden-yellow colonies exhibiting β-hemolysis on blood agar and mannitol fermentation on salt agar were provisionally identified as *S. aureus* [11]. Microscopic confirmation was performed using Gram staining to observe Gram-positive cocci in clusters. Biochemical confirmation was achieved via positive catalase and coagulase (both slide and tube methods) tests. Verified isolates were preserved in nutrient broth supplemented with 15% glycerol and stored at −20°C for subsequent testing [12].

2.6 Antimicrobial Susceptibility Testing (AST)

In vitro phenotypic susceptibility testing was performed utilizing the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, strictly adhering to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Bacterial inocula were adjusted to a 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) to ensure uniform lawn growth. The panel of antibiotic disks tested included: Benzylpenicillin, Oxacillin, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Fusidic acid, Rifampicin, and Trimethoprim/Sulfamethoxazole. Following incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters. Isolates were categorized as Susceptible (S), Intermediate (I), or Resistant (R) based on the specific CLSI interpretive breakpoints. Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial categories.

2.7 Quality Control

To guarantee the accuracy, reliability, and reproducibility of the laboratory procedures, *S. aureus* ATCC 25923 was utilized as a reference quality control strain for all phenotypic and susceptibility assays. All prepared culture media and chemical reagents underwent routine sterility and performance checks prior to use.

2.8 Statistical Analysis

Data management and statistical evaluations were performed using SPSS software (version 24.0, IBM Corp., Armonk, NY, USA). Categorical variables (frequencies and percentages of susceptibility profiles across different specimen sources) were summarized in descriptive tables. The Chi-square (χ^2) test (or Fisher's exact test where applicable) was utilized to determine the statistical significance of associations between antibiotic resistance patterns and clinical specimen sources. Continuous variables were expressed as mean $\pm$ standard deviation. A p-value less than 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Antibiotic Susceptibility and Resistance Profiling

The antimicrobial susceptibility profiles of the *S. aureus* isolates (n=110) revealed high resistance rates toward several conventional antibiotics. The highest resistance rate was observed against Benzylpenicillin (95.45%, n=105), then Oxacillin (80.91%, n=89). Moderate resistance patterns were observed for Levofloxacin (51.82%), Gentamicin (51.82%), and Tobramycin (50.91%). Furthermore, resistance to Moxifloxacin and Erythromycin remained relatively high at 65.45% and 66.36%, respectively. Conversely, comparatively lower resistance rates—and thus better sensitivity profiles—were exhibited toward Nitrofurantoin (46.36%), Tigecycline (45.45%), Fusidic acid (45.45%), Clindamycin (44.55%), Vancomycin (43.64%), and Tetracycline (43.64%). Remarkably, Teicoplanin emerged as the most effective agent, demonstrating the lowest resistance rate (26.36%) and the highest susceptibility rate (73.64%). Trimethoprim/Sulfamethoxazole also maintained favorable clinical utility with a sensitivity rate of 62.73%. Statistical evaluation demonstrated a significant association between the specific antibiotic type and the resulting susceptibility patterns (Chi-square = 9.71, P=0.001), as showed in table (1).

Table (1): Antibiotic Susceptibility Profile of *S. aureus* Isolates

Antibiotics	Resistance No.	%	Sensitive No.	%	P. value & Chi-square
Benzylpenicillin	105	95.45	5	4.55	
Oxacillin	89	80.91	21	19.09	
Gentamicin	57	51.82	53	48.18	P. value = 0.001*
Tobramycin	56	50.91	54	49.09	
Levofloxacin	57	51.82	53	48.18	
Moxifloxacin	72	65.45	38	34.55	Chi-square = 9.71
Erythromycin	73	66.36	37	33.64	
Clindamycin	49	44.55	61	55.45	
Teicoplanin	29	26.36	81	73.64	
Vancomycin	48	43.64	62	56.36	
Tetracycline	48	43.64	62	56.36	
Tigecycline	50	45.45	60	54.55	
Nitrofurantoin	51	46.36	59	53.64	
Fusidic acid	50	45.45	60	54.55	
Rifampicin	55	50.00	50	45.45	
Trimethoprim/Sulfamethoxazole	41	37.27	69	62.73	

*Statistically significant ($p < 0.05$).

3.2. Distribution of Resistance Profiles Across Clinical Specimen Sources

The distribution of *S. aureus* isolates varied significantly based on the anatomical site of the clinical sample. Overall, urine samples were the primary source of *S. aureus* strains, yielding 53 isolates (48.2%), followed by wound swabs (38 isolates; 34.5%) and ear swabs (17 isolates; 15.5%), whereas vaginal swabs yielded only two isolates (1.8%). Statistical analysis revealed a significant association between the clinical sample source and the number of *S. aureus* positive isolates (P. value = < 0.0001), indicating that the clinical source of the isolate is a critical factor, as shown in Table (2).

Table (2): Distribution of *S. aureus* isolates according to clinical specimen source

Specimen source	Positive for *S. aureus* NO.	(%)	P. value
Ear swab	17	(15.5%)	

Wounds	38	(34.5%)	< 0.0001
Vaginal swab	2	(1.8%)	
Urine	53	(48.2%)	
Total	110	(100%)	

*Statistically significant ($p < 0.05$).

4. Discussion

The emergence of antibiotic-resistant *S. aureus* poses a significant challenge to public health worldwide. The increasing prevalence of methicillin-resistant and multidrug-resistant (MDR) strains complicates treatment strategies, particularly in hospital and community settings. Understanding the patterns of antimicrobial susceptibility and resistance is essential to guide rational antibiotic use and improve clinical outcomes. The analysis of antimicrobial susceptibility in this study revealed that β-lactam antibiotics, particularly Benzylpenicillin and Oxacillin, were largely ineffective against the isolates, indicating a high prevalence of methicillin-resistant *S. aureus* (MRSA) strains. These findings align with a previous study reporting high beta-lactam resistance among nasal *S. aureus* isolates [14], attributing this resistance to β-lactamase production or the presence of the $mecA$ gene. In contrast, another study observed greater susceptibility to fluoroquinolones in isolates from medical students—a variation likely reflecting differences in prior antibiotic exposure and the traditional epidemiological distinction between community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA) methicillin-resistant *S. aureus* strains [15]. This comparison emphasizes how population characteristics and local antibiotic prescribing patterns heavily influence contemporary resistance trends. Furthermore, the statistical evaluation of resistance patterns revealed significant variation among the tested antibiotics. This variation aligns with a 2021 study that documented significant differences in antimicrobial activity between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains, underscoring the need for susceptibility-guided therapy rather than empirical protocols [16].

These differences also reflect the complex biological mechanisms driving resistance—including genetic diversity, horizontal gene transfer, and divergent selective pressures. This aligns with molecular insights from previous studies demonstrating that community-associated and hospital-associated strains harbor distinct resistance determinants; this directly contributes to variations in treatment outcomes and confirms that the clinical source and strain type dictate susceptibility patterns [17].

Analysis based on sample source revealed a critical epidemiological pattern: urine samples accounted for the majority of resistant bacterial isolates, whereas ear and vaginal swabs showed the lowest levels of resistance (reflected in a smaller number of isolates) suggesting that the specific anatomical site, local microbial environment, and environment-specific antibiotic pressures accelerate the development of resistance [18]. In contrast, Babić et al. (2016) [19] indicated that beta-lactam resistance operates largely independently of sample type. This discrepancy highlights the complex interplay between systemic clonal expansion and local antibiotic selective pressure, underscoring the urgent need for source-specific surveillance to improve infection control and the rational use of antibiotics.

5. Conclusion

This study demonstrates a widespread prevalence of antibiotic resistance among clinical *S. aureus* isolates in Baqubah. The observed resistance to beta-lactams—specifically benzylpenicillin and oxacillin—highlights a significant burden of methicillin-resistant *S. aureus* (MRSA) in the region. Furthermore, the resistance rates observed against fluoroquinolones and macrolides underscore the emergence of multidrug-resistant (MDR) strains. In contrast, vancomycin, teicoplanin, trimethoprim/sulfamethoxazole, and tetracycline showed high efficacy, making them more effective therapeutic alternatives. Notably, the distribution of isolates was significantly influenced by the clinical source; urine samples yielded the highest proportion of isolates, followed by wound and ear swabs, while vaginal swabs recorded the lowest. Collectively, these findings emphasize the critical need for rigorous antibiotic surveillance, rational use programs, and enhanced infection control protocols to curb the spread of resistant *S. aureus* strains in clinical settings.

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Nexus International University ISSN: 3136-6449; 12(2) 53-62

Assessment of Awareness toward Healthcare and Lifestyle Modifications among Patients with Coronary Artery Disease in Cardiac Care Units

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Abstract
Background: Coronary artery disease is a high burden cardiovascular disease and patients need basic awareness on the relevant health behaviours. Adequate awareness in patients improves self-care, enables better use of health care services and encourages effective long-term management of diseases.
Objective: To assess the level of awareness regarding health and lifestyle modifications among patients suffering coronary artery disease (CAD) admitted in a Cardiac Care Unit, and to correlate the awareness level between patient demographics and clinical characteristics.
Methods: A descriptive cross-sectional study in Al Hussein Teaching Hospital, Al Samawa City, Al-Muthanna Governorate, Iraq from April 1-June 30 2026. A carefully curated sample of 50 adult patients with documented coronary artery disease was collected. Data were collected using a structured questionnaire developed by the investigator. The questionnaire included two parts; socio-demographic and clinical characteristics and a 10-questions awareness test on a domain of health care and lifestyle modifications. Content validity was assessed by a panel of 15 experts in the fields of research and clinical practice. The test-retest reliability was good ($r = 0.83$). Data were analyzed with the SPSS version 26 using both descriptive and inferential statistical methods. A p-value less than 0.05 was considered statiscally significant.
Results: The study results showed that there was a limited level of awareness in the top patients regarding healthcare and lifestyle changes in heart disease. Aware was analysed in relation to several socio-demographic and clinical characteristics. The assessment identified the characteristics associated with patient awareness and highlighted areas for additional patient education and nursing support.
Conclusion: Firstly, evaluating the perception of healthcare and lifestyle changes among patients with coronary artery disease is vital for understanding education needs. Education within the cardiac setting, lifestyle advice, support for self-monitoring and self-care should be further developed as this improves patients understanding of their condition and promotes effective long-term management.

Keywords: Awareness; Coronary Artery Disease; Lifestyle Modifications.

1. Introduction

At its core, cardiovascular illness arises from arterial disease, where both adaptive and maladaptive remodelling of the arterial wall are central mechanisms upon which these diseases depend and therefore are targeted by therapies. It is an active structural-restructuring process entailing cellular proliferation, apoptosis and migration in/throughout or from the tissue as well as signs of degradation and remodeling of the extracellular matrix (ECM) components with minimal secreted soluble factors within the arterial wall. Normally, this dynamic mechanism is essential for maintaining blood pressure homeostasis; however, in pathological conditions), these adaptive modifications become maladaptive vascular remodelling (Lyle & Taylor 2019).

Age-related changes to the cardiovascular system, which is relatively characteristic for any physiological element. Changes such as decreased vascular awareness, left ventricular hypertrophy (LVH), and increased stiffness (increased

arterial awareness) contribute to a greater hemodynamic workload in elderly patients. Consequently, patients suffering from heart diseases which require cardiac procedures especially cardiac catheterisation are more prone toward anxiety. This indicates that effects may become exacerbated including further heart strain, impairment haemodynamic stability and functioning. Apprently, a nonpharmacological method such as hand massage must be used to relieve nonsurgical anxiety before the procedure (Capriotti and Frizzell 2016 in Hinkle, Cheever & Overbaugh 2022)

The circulatory system is indispensable in transporting oxygen and nutrients from blood to organs and tissues, as well as in extracting toxins and carbon dioxide (CO2) from peripheral tissues of healthy persons (Commisso et al., 2018). Nurses play the roles of educating patient, counseling, and monitoring adherence to medication. Ruswati 2024 noted that their treatments especially focused on adherence problems, such as the adverse effects of prescription or lifestyle influences, and aimed to better health literacy to empower patients (Ruswati 2024).

Patient nonawareness is frequently multifactorial and may involve personal factors including fear of side effects (actual or anticipated), perception that guidelines underplay the diet effect, real or perceived ineffectiveness of treatment, forgetfulness, complex dosing schedules or polypharmacy. In addition, factors such as physician inertia (defined as reluctance to start or increase therapy for patients with high blood pressure) and poor patient-physician communication may contribute to an increased number of unmet therapeutic goals (Hamrahian et al., 2022).
Through 2019, cardiovascular diseases ranked first in the causes of deaths worldwide and were associated with an estimated 18.6 million deaths [1]. According to the report, cardiovascular disease accounted for more than 868,000 deaths in the United States during 2017. (Concistre, 2023).

Traditionally in developed countries, criminal heart disease (IHD), as well as other cardiovascular diseases (CVDs), has been the leading cause of death; indeed "heart disease in all its manifestations has headed the mortality tables of the u.s. Centers for Disease Control and Prevention since 1921. (Lemos and Omland, 2018). Ischaemic heart disease (IHD) now constitutes the leading cause of morbidity and mortality in many countries worldwide [1, 2]. Coronary artery disease, also known as ischaemic heart disease, is the leading type of heart disease that affects humans. Another name for this ailment as coronary heart disease (Morton and Thurman, 2023).

Coronary heart disease (CHD) is a major contributor to the world's mortality, killing more people annually than any other cause of death combined (World Health Organization (WHO), 2017). Secondary prevention, on the other hand, focuses on those with manifestations of cardiovascular disease and is expressed in advances to change risk factors due to dietary interventions (Saleh et al., 2021) and smoking cessation (Ralston et al., 2018). Those without an understanding of the relationship among diseases and risk factors may be inclined to refrain from acting in accordance with behavioural modification or disease treatment guidelines. On the other hand, those who understand this concept usually strive to change their lifestyle in order to prevent further cardiac events (Arnett et al., 2019).

Coronary artery disease is defined by the accumulation of exudate (mostly cholesterol) within the endothelial walls that nourish coronary vessels, causing vascular illness or loss. These substances build up and fills, then squeezes the vessels out of the arteries. Atherosclerosis (Del Buono et al., 2021) is the main cause of this ailment. Prompted by the goal to give consumers greater control over their health, the World Health Organization introduced its Global Action Plan for the Prevention and Control of Cardiovascular Diseases, among other noncommunicable diseases. Advocate through patient record data and care pathways; through health behaviour education, assistance and resources; build a patient management team (Mechanic et al., 2021).

Despite the well-known importance of lifestyle modification in coronary artery disease management and guideline-developed recommendations, there has been limited focus on the awareness of patients regarding these guidelines in cardiac care settings. Thus, the investigator conducted this study to examine the awareness of healthcare or lifestyle changes in patients with coronary artery disease admitted in cardiac care units and if that awareness is associated with certain socio-demographic and clinical characteristics.

2. Methodology

2.1 Research Framework

In Cardiac Care Units, patients with coronary artery disease were evaluated for awareness and the effectiveness of health care changes using a cross-sectional study design. The cross-sectional design was appropriate to assess patient awareness and its relationship with certain socio-demographic and clinical characteristics within a defined period of time.

2.2 Research Environment

For this study, we conducted a research prototype in Al-Hussein Teaching Hospital -Al-Samawa City, Al-Muthanna Governorate, Iraq. Medical clinic that serves people with various medical problems, including cardiovascular disease, providing healthcare and nursing assistance. Cardiac Care Unit. The Cardiac Care Unit provides special cardiac monitoring, medical treatment with nursing support and patient education for individuals suffering from cardiovascular disease.

The specific location is ideal given that it will have direct access to patients who are receiving cardiac treatment and health advice for coronary artery disease.

2.3 Research Duration

The conducted research is from the 2nd quarter of 2026, between Apr-1 and Jun-30, 2026.

2.4 Research Cohort

The study cohort included adults with a diagnosis of coronary artery disease (CAD) following the treatment at Al-Hussein Teaching Hospital in Al-Muthanna Governorate, Iraq.

2.5 Sample Size and Sampling Method

Sample of the study: The research cohort included 50 adult patients with confirmed coronary artery disease. Participants were recruited using a purposeful non-probability sampling approach by selecting individuals who met the defined eligibility criteria. Sample size calculator using Raosoft Sample Size Calculator Module based on expected population size, chosen confidence level and margin of error as well as response distribution.

2.6 Standards for Sample Selection

Eligibility Requirements

Individuals were incorporated if they:

- People aged 18 and over.
- Diagnosed with coronary artery disease.
- Patients were being treated in the Cardiac Care Unit.
- The patients were receiving some form of medical treatment and health state advice for coronary artery disease.
- Understood the survey and solved it exactly.
- Voluntarily agreed to take part in the research.

Criteria for Exclusion

Exclusion Criteria

Participants were omitted if they:

- Data gathering process was not able to communicate the information properly
- Cognitive weaknesses that made it difficult for them to understand or answer to the questionnaire.
- They were clinically unstable and unable to participate at the time of data collection.
  Declined to participate in this research.

Surveys returned with large omissions making it impossible to calculate the awareness score.

2.7 Research Tool

The study instrument was a systematic questionnaire designed by the researcher and supervised by the research supervisor, following a review of relevant literature on coronary artery disease, healthcare guidelines, disease management, lifestyle modifications, medication administration, follow-up treatment and self-care.

The survey included of two main sections.

Section 1: Socio-Demographic and Clinical Characteristics

This aspect included some socio-demographic and clinical features, including age, gender, level of education, income per month and the period of coronary artery disease.

The second section: Awareness of professional care and life changes

This part consisted of 10 awareness -based questions evaluating the patient's awareness about healthcare and life style changes that are relevant to coronary artery disease. The ten points together expressed one single, large area: awareness of Health and Lifestyle Changes. Topics among them were drug purchase, health surveillance, dietary change, exercise practice, no smoking request, controlling weight gain and its loss, lowering distress level/diminishing stress-related condition taking care of themselves; risk perception and detection of alarms requiring medical treatment.

Alternatives for Responses and Evaluation Framework

- Each was answered with a simple Yes, No.
- For each correct answer the score was 1 and for incorrect answers and "Don’t Know" gave a score of 0.

The total awareness score was between 0–10, with higher scores representing an increased level of awareness. Awareness level was classified into: • Low: 0–3 • Moderate: 4–6 • High: 7–10

Validity of the Tool

A panel of 15 academic and clinical experts in nursing, cardiovascular health and patient education and other health disciplines evaluated the content validity of the questionnaire. The scale items were rated by specialists in terms of applicability, clarity, consistency with study objectives, and comprehensiveness. As per the recommendations of experts, appropriate changes were made in selected items to increase their clarity, relevance and applicability.

Preliminary Investigation

A pilot study of 10 subjects demonstrating characteristics similar to the research population had been performed. The pilot study was conducted prior to the actual data collection to test the clarity, comprehensibility, feasibility and estimated time for completing the questionnaire. The original volunteers were excluded from the final study population.

Reliability of the Tool

The internal consistency and stability of the 10-item awareness questionnaire were assessed to check its reliability.

Test-retest reliability was analyzed by having the questionnaire administered to the same subjects on two different occasions, and the relationship between both assessments was evaluated using Pearson's correlation coefficient.

Validity was assessed against a comparable self-report measure and identified high concurrent validity ($r = 0.87$).
The 10-item awareness questionnaire was tested for reliability measured by Cronbach's alpha coefficient. Finally, the observed Cronbach's alpha must be calculated from the research data acquired and reported in the final publication.

Data Collection

Data were collected from April 1 to June 30, 2026.

Patients were identified who met the inclusion and exclusion criteria. The investigator explained the purpose and procedures of the research to each participant.

The questionnaire was administered once signed informed consent was obtained. If they were able to complete the questionnaire alone, this was allowed. The questionnaire was conducted as structured interviews with standardized wording for participants who required assistance.

The survey took about 15 to 20 minutes to complete.

Analysis of Data

The collected data were coded, entered and analysed using the Statistical Package for the Social Sciences (SPSS) version 26.

Statistical Analysis Techniques: Descriptive and Inferential

This analysis is descriptive in nature; it contains the occurrence and proportion for categorical variables. For continuous variables and awareness metrics, mean + s.d. Frequency, coverage and mean for awareness metrics.
Statistical analyses were performed as appropriate:

An independent samples t-test to compare the means of two different groups on some awareness rating.
Awareness Scores: One-way ANOVA to compare mean awareness scores across three or more independent groups
Use Pearson's correlation coefficient to investigate relation between awareness scores and continuous variables like age, duration of coronary artery disease.

Chi-square analysis to explore associations between category awareness levels and selected socio-demographic/clinical characteristics where applicable.
All p-values less than 0.05 were defined as significant.

Moral Implications

The necessary official approvals were obtained prior to commencing data collection.
The Council of the College of Nursing at the University of Al-Muthanna granted consent, while internal ethical approval from the Al-Muthanna Directorate of Health was also given to conduct research in Al-Hussein Teaching Hospital.
The study protocol was sanctioned by the ethical committee of the University of Al-Muthanna.
The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Limitation of the Research

Limitations of this research were it method, singular institution, and relied on participant feedback. Such elements may limit the generalizability of the findings to patients with coronary artery disease in other clinical settings.

3. Results

The results of the data analysis are presented in tables organized directly according to the objectives of the study as follows:

Table (1): Disposition of Study Subjects by Socio-Demographic Characteristics

Socio-Demographic characteristics		Frequency	Percentage
Age	<= 29	6	12
	30-53	12	24
	54-76	21	42
	77-90	11	22
	Mean= 65.84	SD= 16.568	
Sex	Male	19	38
	Female	31	62
Educational Level	Primary	30	60
	Secondary	11	22
	Tertiary	6	12
	College	3	6
Monthly Income	Enough	12	24
	Somewhat enough	18	36
	Not enough	20	40

According to the table (1), most of patients were aged between 54 years up to 76 years with average age was 65.84 ± 16.568 years, which is the mean [42%]. The sample consisted of 62% females, with 60% having primary education, and an inadequate monthly income reported by 40%.

Table (2): Distribution of Patients according to Awareness Questions

NO	Questions	Yes		NO		Mean	Assessment
		F	%	F	%		
1	Did you know that you must stick to the recommended schedule for heart medications to prevent future cardiac arrests?	26	52	24	48	1.54	Good
2	Discontinuing cardiac medicine without the help of your cardiologist cold turkey could lead to major health problems, did you know?	30	60	20	40	1.42	Poor
3	Did you have any idea that regular heart follow-up appointments are important, even when you feel completely healthy and asymptomatic?	14	28	35	70	1.30	Poor
4	Did you know that before you're going to stop any medicine, it is a must to tell your healthcare staff particularly if having some new or unusual side effects?	7	14	42	84	1.13	Poor
5	This can be monitoring your blood pressure, and monitoring pulse rate at home.	4	8	45	90	1.08	Poor
6	Did know that reducing routine usage of sodium (salt) and harmful fats assists in lessening blood pressure and protecting coronary arteries from blocking.	27	54	22	44	1.53	Good
7	Are you aware that smoking and passive cigarette smoke also have an immediate influence on the narrowing and blockage of coronary arteries?	5	10	45	90	1.02	Poor
8	Did you know moderate exercise as prescribed by your doctor strengthens the heart muscle?	31	62	18	36	1.63	Good
9	Do you know that habitual mental stress and anxiety may negatively impact both your heartbeat as well as blood pressure?	16	32	34	68	1.32	Poor
10	Did you know that maintaining a healthy weight reduces the overall pressure on your heart?	27	54	23	46	1.55	Poor

F-frequency, % - percentage, Assessment: Good> 1.5, Poor less than or equal 1.5
According to table (2), it shows that patients' general awareness regarding health care and lifestyle changes was insufficient as most of the items under awareness categories were marked as poor.

Table (3): Overall awareness levels regarding Health care and Life style modifications in Patients of CAD

Variables	Classification	F	%
Overall awareness	Poor	42	88
	Good	8	16
	Total	50	100

As shown in Table (3), most of the patients (88%) had insufficient overall awareness: on the contrary, only 16% of them had good awareness regarding both healthcare and lifestyle changes.

Table (4): Association between Patients Awareness and Socio-Demographic Variables

Socio-Demographical Characteristics	Significance
Age	Chi-square=2.213[a] df=3 P. value=0.563 Non-significant
Sex	Chi-square= 0.021[a] df=1 P. value=0.900 Non-significant
Educational level	Chi-square= 1.410[a] df=3 P. value=0.842 Non-significant
Monthly Income	Chi-square=1.542[a] df=2 P. value=0.394 Non-significant

HS: Highly Significant at P<0.01; S: Significant at P<0.05; NS: Non-Significant at P>0.05; significant.
Results show no significant association between awareness and age, gender, education level and monthly income (P > 0.05).

4. Discussion of Results

Results Analysis of the Demographics Related Outcomes:

Patient's Age

A total of 42% were classified in the age range between 54–76 years, with an average age of 65.84 ± 16.568 years..
As indicated by Tengiz Verulava and Giorgi Mikiashvili (2021), more than 50% of the patients were aged ≥50 years, but only 27% was younger than 39 years old.

This is attributed to the increased risk of coronary artery disease due to aging, an outcome related to a lifetime accumulation of cardiovascular risk factors and/or age-related vascular changes.

Sex

Results indicated females were a larger portion of the study at 62% compared to males=38%.
Sex analysis by Liza et al. (2023) observed that 67.5% of the participants were female, indicating that a large proportion of the study sample consisted of this sex.

This result is in conflict with the results of Samer Alwey et al 2018, who reported a female proportion of 40% and male proportion of our paper.

These could be linked to differences in etiopathogenesis and clinical expression of cardiovascular disease in men versus women, especially among the elderly.

Educational Attainment

Results revealed simple training for many participants (60%), secondary schooling was retained by 22%.
Kelli et al, support this conclusion. in patients with coronary artery disease, (2019) reported a relationship between lower educational achievement and poorer cardiovascular outcomes and increased rates of adverse events.
It might be attributed to differences in human levels of health literacy and the ability to define, comprehend (eg. using medical terminologies) and perform recommended behaviour changes related to disease management.

Monthly Income

Regarding monthly incomes, most patients (40%) rated their income as insufficient.
This conclusion matches the findings of Ahmad Homoud et al. (2025). Around eighty-two point eight % earned above seven thousand SAR and a bulk assured from 81.4% on their responses.
A reason behind this may be the financial stress associated with long-term treatment, medications, medical care and several health prices.

Analysis Finding Awareness levels related to the Healthcare and Lifestyle

Health consciousness is an important factor in the effective management of coronary artery disease. The findings showed that the majority of patients had general ignorance most likely due to a lack of health education and inadequate understanding of appropriate recommendations for healthcare and lifestyle changes.

Tengiz and Giorgi (2021) Research result indicated that some areas in understanding have been improved.
This finding contradicts the findings of Ahmad Homoud et al. (2025). The outcome presented moderate awareness of patients 66.5%.

The researchers argue that the lower awareness levels stem from how long patients have been sick and the medical mechanisms of the treatment, but they add that it is important to examine patients' mindsets as these may potentially affect adherence to the therapy.

Study of the association of general Patient Awareness with Demographic Variables

It shows that the Analysis of association between awareness and socio-demography helps to find out factors associated with health consciousness among patients. Results showed that awareness was not significantly associated with the patients' socio-demographic characteristics ($P>0.05$). This may indicate that the improvement of patients' awareness was not solely due to demographic characteristics such as age and sex, but also by other factors including quality and consistency of health education.

The results contradict the conclusions of Badr and Ahmed (2014) regarding patient awareness and age, $P = 0.020$), however are consistent with respect to gender ($P = 0.165$) and monthly income ($P = 0.217$).

Kaur and Aggarwal (2022) backed this study and showed that age P. Value=16.92, sex P. value 7.82, educational status P. value 16.92 and occupations P. value=16.92 is equal to the awareness levels of coronary artery disease which was not statistically significant compared to socio-demographic variables but were found to be significant at 0.05 level of confidence.

The limited level of awareness may be due to inadequate health education, limited access to organized and continuous advice, and insufficient support for adherence to recommended management and lifestyle change in patients at high risk for coronary artery disease.

5. Conclusion

The results showed that people with coronary artery disease had an inadequate general awareness of health, especially on topics related to lifestyle change. Patients did not perform significantly better in expected domains; however, major gaps were present in many aspects of cardiac care and healthy living habits. No significant relationship was established between the overall awareness and socio-demographic characteristics of the study subjects. These findings highlight the need for continuous and structured health education to improve patient awareness and self-management of coronary artery disease.

6. Recommendations

- Implement structured and continuous health education programmes targeting coronary artery disease patients on aspects of medications adherence, heart surveillance, as well as lifestyle modifications.
- Improve the role of nurses in teaching clients through appropriate diet, activities such as exercise, quitting smoking, reducing stress and home monitoring of BP/pulse rate.
- Provide customized and simple educational materials grounded on patients' information needs for their understanding of coronary artery disease and self-management.

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Nexus International University ISSN: 3136-6449; 12(2) 63-70

Association between DNA Damage and Glycemic Control in Iraqi Patients with Type 2 Diabetes Mellitus

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Abstract.
Background: Type 2 diabetes (T2DM) is defined as the chronic disorder associated with metabolic issues, having hyperglycemia and insulin resistance as the main drivers. It appears that oxidative stress and damage to DNA may contribute significantly to the emergence of diabetes and its complications. The present study aims to analyze the interrelation between the markers of DNA damage and glycemic control in Iraqi individuals suffering from T2DM.
Objectives: The aim of the research is to reveal the existence of any interrelationship between DNA damage (8-hydroxy-2'-deoxyguanosine [8-OHdG] and parameters obtained from comet assay) and glycemic control (HbA1c levels) among the individuals suffering from T2DM in Iraq.
Methods: This research is based on the data collected about 180 diabetes patients and 60 healthy individuals. The measurements of fasting blood glucose (FBG), HbA1c, 8-OHdG, and DNA damage were taken. The assessment of DNA damage was performed using comet assay and statistical procedure.
Results: Diabetes patients demonstrated a higher level of 8-OHdG in comparison with healthy individuals ($p < 0.001$) and show an increase in DNA damage. DNA damage has exhibited a positive correlation with HbA1c ($r = 0.742$, $p < 0.001$) and FBG ($r = 0.698$, $p < 0.001$). Multiple regression showed that an independent predictor of DNA damage was HbA1c ($\beta = 0.521$, $p < 0.001$).
Conclusions: The findings indicate that there exists a close association between glycemic control and DNA damage in patients in Iraq. Low glycemic control influences the increase in oxidative and DNA damage stressing the necessity of intensive glycemic control in diabetes treatment.

Keywords: Type 2 diabetes mellitus, DNA damage, glycemic control, 8-OHdG, comet assay, oxidative stress, HbA1c, Iraq.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the biggest public health crises of our time, with around 537 million adults suffering from it worldwide in 2021, and this number may reach 783 million by 2045 [1]. The rates of T2DM in the Middle East and North Africa, including Iraq, show significant growth over the last 20 years due to rapid urbanization, changes in lifestyle, decreased activity levels, and increase in high-caloric food consumption [2]. Iraq is undergoing a diabetes crisis, with current estimates showing about 4 million to 5 million people suffering from T2DM, although the exact figures are unclear due to a lack of proper surveillance systems [3]. T2DM is a metabolic disease marked by chronic elevation of blood sugar, which may be caused by a problem with insulin secretion, insulin action, or both, giving rise to serious vascular and non-vascular complications [4].
The pathophysiology of T2DM involves an intricate combination of factors such as insulin resistance, pancreatic beta-cell dysfunction, diseases of liver metabolism of glucose, and chronic low-grade inflammation [5]. The researched traditional risk factors include obesity, lack of physical activity, and genetics, yet more studies indicate the role of oxidative stress in T2DM and its complications [6]. Oxidative stress is a state in which there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the body. In terms of diabetes,

chronic hyperglycemia results in too much production of ROS through various mechanisms such as auto-oxidation of glucose, increased mitochondrial respiration, and activation of NADPH oxidase [8]. Excessive accumulation of ROS in diabetic patients can certainly lead to damage of cellular macromolecules such as proteins, lipids, and DNA, and hence more development of diabetic complications [9].
DNA damage is one of the important consequences of oxidative stress, since damaged DNA is sensitive to oxidative modification due to the effect of ROS on DNA [10].

About 70% of spontaneous DNA lesions is caused by oxidative damage whereby the most common and stable oxidatively damaged DNA lesion at the same time is 8-hydroxy-2'-deoxyguanosine (8-OHdG) [11]. The damage occurs when ROS attack guanine DNA base. This results in mutagenic lesion which must be repaired in order to avoid GC to AT transversions [12]. High levels of urinary and serum 8-OHdG are recorded in multiple conditions and can serve as a marker of oxidative stress and DNA damage [13]. Several works show elevated amounts of 8-OHdG in diabetic patients in relation to healthy controls and this increase is related to uncontrolled glycemia and diabetes complications [14, 15]. This method involves it measuring and besides 8-OHdG, single-cell gel electrophoresis, or the comet test may be considered a good method of assessment of DNA damage in cells [16].The comet assay is a technique that allows the quantitative measurement of DNA damage and repair capability in individual cells, giving a better representation of genotype [17]. There has been an increased application of this technique in research to study metabolic disorders impacting genome stability [18]. The link between glycaemic control and damage to DNA has been previously shown in various studies and populations [19, 20]. Many studies have shown that poor glycaemic control, defined by high levels of hemoglobin A1c (HbA1c), contributes to an increase in DNA damages and oxidative stress [21]. HbA1c is considered a 'gold standard' method of assessing long term glycaemic control with its proven correlation to the degree of vascular damage and complications due to diabetes [22].

The literature investigating the association between glycaemic control and DNA damage in Middle Eastern countries, in particular Iraq, is lacking. Iraq is dealing with many specific issues regarding treatment of diabetes, such as low availability of medical facilities, inadequate glycaemic monitoring centers, high incidence of complications due to diabetes, and lack of educational programs aimed at patients [23]. The treatment of diabetes is aggravated by the consequences of prolonged military conflicts reducing access to healthcare services and creating difficulties in the establishment of high-quality medical service [24]. Understanding the mechanisms linking poor glycaemic control to different destructive processes, such as DNA damage, is essential for selection correct strategies leading to positive results in diabetes treatment in Iraq.

There is extensive evidence that chronic hyperglycemia leads to development of diabetic complications; however, the exact mechanisms are yet to be determined. Recently, it was found that the connection between hyperglycemia and occurrence of diabetes complications is linked to the inducing of DNA damage due to oxidative stress [25].

Our study aimed at finding the relationship between DNA markers and glycemic control in Iraqi patients. Our primary goal was to define the link between the DNA damage markers (8-OHdG and comet assay parameters) and glycemic control (HbA1c and FBG) in patients suffering from T2DM in Iraq. The second goal was to find out the relationship between DNA damage and duration of diabetes, and the third goal was to study the impact of different types of glycemic control on DNA damage. We assumed that poor glycemic control will lead to higher levels of DNA damage.

2. Methodology

2.1 Study Design and Setting

This was a cross-sectional comparative study conducted at Aldiwania Teaching Hospital. The study was conducted over a 12-month period from January 2023 to December 2023.

2.2 Study Population

The study population comprised of two groups: (1) patients with T2DM (n=180) and (2) age-matched healthy controls who were free of diabetes (n=60). T2DM patients were recruited from both the outpatient diabetes clinic and inpatient

services at Aldiwania Teaching Hospital. Inclusion criteria for T2DM patients included: being aged 30 years or above, being diagnosed as having T2DM as per the criteria of American Diabetes Association (i.e. FBG 126 mg/dL or more, 2-hour glucose equal to or greater than 200 mg/dL in an oral glucose tolerance test, or HbA1c of ≥6.5%), illness duration of over one year, and ability to give informed consent. Exclusion criteria comprised: type 1 diabetes, secondary diabetes, acute diabetic complications (diabetic ketoacidosis/hyperosmolar hyperglycemic state), acute infection or sepsis, recent surgery (within prior 3 months), malignant illness, chronic kidney disease stage 4-5, liver cirrhosis, autoimmune disorders and being on immunosuppressive drugs. Healthy controls were recruited from hospital staff and relatives of such patients having no history of diabetes in themselves and their families and having no chronic illness, with FBG <100 mg/dL and HbA1c <5.7%.

2.3 Data Collection

A standardized questionnaire was used by trained researchers to gather demographic and clinical information. Collected information consisted of age, sex, body mass index (BMI), duration of diabetes, medications taken, smoking status, and complications due to diabetes (i.e. retinopathy, nephropathy, neuropathy). Medical checks were carried out to measure blood pressure, height, and weight. Blood was acquired (10 mL) after an overnight fast (8-12 hours) from all participants. Collected blood was split into three parts: 1) 2mL in EDTA tube to be processed immediately for the comet assay analysis, 2) 3mL in serum separator tube for biochemical analyses.

2.4 Biochemical Measurements

The hexokinase method was used to test FBG levels through an automated analyzer (Roche Cobas c702 Chemistry Analyzer) while determining HbA1c through HPLC (high-performance liquid chromatography), using a Bio-Rad Variant II analyzer with coefficient of variation matches of less than 2%. Serum creatinine, blood urea nitrogen (BUN), liver function tests, and biochemical analysis were done with standard enzymatic automated analyzers. The 8-OHdG levels in serum were tested through the ELISA (enzyme-linked immunosorbent assay) method. The manufacturer's instructions by Cayman Chemical Company, Ann Arbor, MI, USA specifies a minimum detection limit of 0.15 ng/mL for 8-OHdG construction and the coefficient of variation is 4.6% for the intra-assay and 6.8% for the inter-assay respectively.

2.5 Comet Assay (Single-Cell Gel Electrophoresis)

DNA damage was evaluated using the alkaline comet assay techniques as previously described [26]. In brief lymphocytes were prepared from blood using Ficoll-Paque density gradient centrifugation. The cells obtained (1×10^5 cells/mL) were then embedded in the 0.5% low melting-point agarose in microscope slides previously prepared with 1% normal melting-point agarose. On completion of cell lysis in the high salt buffer (pH 10) for 1 hour at 4°C and cell unwinding in alkaline buffer (pH >13) for 20 minutes, results of electrophoresis were recorded at 25 V and 300 mA for 25 minutes. After neutralization and ethanol precipitation slides were stained with ethidium bromide. Fifty cells from each slide (totaling 100 cells from 2 slides) were evaluated using the fluorescence microscopy with the CometScore software set to 400× magnification. DNA damages were assessed using the following measurements: (1) tail length (in micrometers) – the distance from the middle of comet blob till the end of comet tail, (2) tail intensity – the content of total DNA in the tail, (3) Olive moment – the overall index simultaneously considering tail length and tail intensity. All measurements of comet assay were done by one person that was blinded in participant's group assignment.

2.6 Glycemic Control Categories

Based on the levels of HbA1c, participants were divided into three groups: good glycemic control (HbA1c <7%), moderate glycemic control (HbA1c 7-8.9%), and poor glycemic control (HbA1c ≥9%). The groups were determined according to American Diabetes Association recommendations for personalized glycemic targets in diabetes management.

2.7 Statistical Analysis

SPSS version 26.0 (IBM Corporation, Chicago, IL) was used to execute all statistical evaluations. All of the parameters were evaluated descriptively, thus we present continuous variables in terms of means ± standard deviations (SDs) and the categorical ones in terms of frequencies and percentages. The normality of the data distribution was evaluated by means of Shapiro-Wilk test. Diabetic patients were compared to control using independent samples t test for continuous normally distributed and Mann-Whitney U test for continuous non-normally distributed variables. Chi-square test was applied to compare categorical data. The correlations of DNA damage variables and markers of glycemic control were evaluated using Pearson's and Spearman's coefficients. Multiple linear regression analysis was utilized for evaluation of DNA damage variables (tail length and olive moment) with respect to HbA1c value, FBG value, age, BMI and diabetes duration employing them as predictors. Multiple analysis of variance (MANOVA) has been performed for comparison of DNA damage values.

3. Results

3.1 Demographic and Clinical Characteristics

The T2DM and control groups were comparable in terms of age, gender, and smoking status since there was no statistically significant difference noted ($p > 0.05$). On the other hand, the groups were different from each other with respect to different clinical parameters. The T2DM subjects had the mean value of BMI to be much higher than the controls (28.9 vs. 23.4 kg/m²). Moreover, both systolic and diastolic blood pressures were found to be higher in the T2DM group compared to the controls (SBP: 135.4 vs. 118.6 mmHg; DBP: 82.3 vs. 74.2 mmHg), the differences were shown to be highly statistically significant ($p < 0.001$).

Table 1: Demographic and Clinical Characteristics of Study Participants. Data presented as mean ± SD for continuous variables and frequencies (%) for categorical variables. SBP, systolic blood pressure; DBP, diastolic blood pressure. *P < 0.001 indicates statistical significance.

Characteristic	T2DM Patients (n=180)	Controls (n=60)	P-value
Age (years)	52.3 ± 9.8	51.8 ± 10.2	0.671
Sex (M/F), n (%)	96/84 (53.3%/46.7%)	32/28 (53.3%/46.7%)	1.000
BMI (kg/m²)	28.9 ± 4.6	23.4 ± 2.8	<0.001*
Diabetes duration (years)	8.2 ± 6.4	—	—
Smoking, n (%)	54 (30.0%)	12 (20.0%)	0.112
SBP (mmHg)	135.4 ± 15.2	118.6 ± 8.4	<0.001*
DBP (mmHg)	82.3 ± 10.6	74.2 ± 7.3	<0.001*

The values of all considered biochemical indexes in patients with T2DM were notably higher than in the rest of the subjects of the research. Poor glycemic control seen in T2DM patients was demonstrated by rather elevated fasting blood glucose (172.6 vs. 89.4 mg/dL) as well as HbA1c levels (8.4% vs. 5.2%) in comparison with healthy individuals. In addition, T2DM patients presented worse lipid profile having much higher levels of total cholesterol as well as LDL-cholesterol. Most importantly, the urinary marker of oxidative DNA injury 8-hydroxy-2-deoxyguanosine (8-OHdG) was recorded to be four times higher in T2DM patients (4.8 ng/mL) compared to its concentration in healthy subjects (1.2 ng/mL), thus proving the much higher level of systemic oxidative stress in diabetic patients ($p < 0.001$ for all).

Table 2: Biochemical Parameters and 8-OHdG Levels in Study Groups. FBG, fasting blood glucose; 8-OHdG, 8-hydroxy-2'-deoxyguanosine. *P < 0.001 indicates statistical significance.

Biochemical Parameter	T2DM Patients (n=180)	Controls (n=60)	P-value
FBG (mg/dL)	172.6 ± 54.2	89.4 ± 8.6	<0.001*
HbA1c (%)	8.4 ± 1.8	5.2 ± 0.4	<0.001*
Total Cholesterol (mg/dL)	218.4 ± 48.6	182.3 ± 32.4	<0.001*
LDL-C (mg/dL)	142.8 ± 38.6	108.4 ± 28.3	<0.001*
8-OHdG (ng/mL)	4.8 ± 2.1	1.2 ± 0.6	<0.001*

Compared to those in the control group, diabetes patients showed generally higher values for comet assay parameters, which directly indicate the presence of DNA breaks. The average tail length was noticed to be 28.6 µm among the diabetes patients, as opposed to the control group's value of 8.2 µm. The tail intensity was three times as high as that recorded in the control group (35.8% vs. 9.4%). Moreover, the Olive moment measure of the T2DM group (82.4) is significantly higher than that of control (14.2) as well.

Table 3: Comet Assay Parameters Measuring DNA Damage. Olive moment is a composite measure combining tail length and tail intensity. All differences between groups are statistically significant at $P < 0.001$.

Comet Assay Parameter	T2DM Patients (n=180)	Controls (n=60)	P-value
Tail Length (µm)	28.6 ± 12.4	8.2 ± 3.6	<0.001*
Tail Intensity (%)	35.8 ± 14.2	9.4 ± 4.8	<0.001*
Olive Moment	82.4 ± 38.6	14.2 ± 7.8	<0.001*

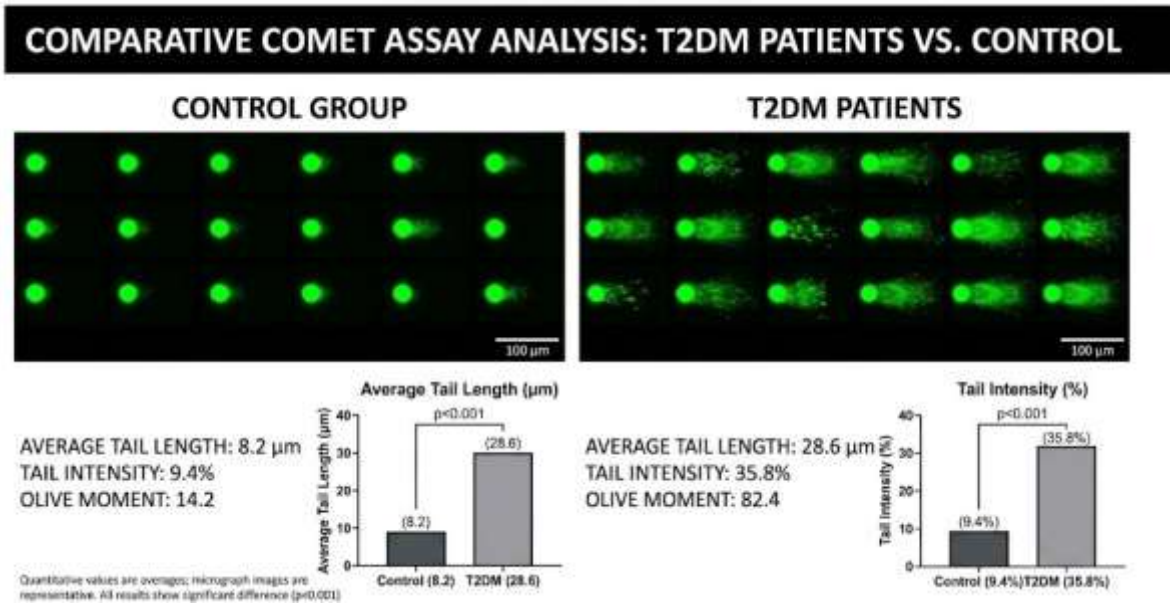


Figure 1: *Comparative comet assay analysis*

3.2 Association between DNA Damage and Glycemic Control

The examination of Pearson correlation tests showed considerable positive relations between DNA damage indicators and glycemic markers. Tail length had direct relation with HbA1c ($r = 0.742$, $p < 0.001$) and FBG ($r = 0.698$, $p < 0.001$) values. Furthermore, olive moment positively correlated with the values of HbA1c ($r = 0.718$, $p < 0.001$) and FBG ($r = 0.672$, $p < 0.001$). Serum 8-OHdG levels also positively correlated with HbA1c ($r = 0.704$, $p < 0.001$) and FBG ($r = 0.641$, $p < 0.001$) respectively. It was revealed that HbA1c was independently significant for predicting tail length from multiple linear regression analysis ($\beta = 0.521$, $p < 0.001$, 95% CI: 2.18-3.64) and olive moment ($\beta = 0.489$, $p < 0.001$, 95% CI: 8.96-14.32). There were significant results of differences between DNA damage measurements categories for different three groups when splitting the participants by glycemic control categories ($p < 0.001$). Patients with poor glycemic profile (HbA1c ≥9%) showed 3.2 times increased tail length (34.8 ± 13.2 µm) in comparison to individuals with good glycemic profile (10.8 ± 5.4 µm).

4. Discussion

Research indicates a notable link between measurements of glycemic control and DNA damage among Iraqi patients suffering from T2DM. Results indicate that T2DM patients exhibit a remarkable increase in markers of DNA damage than normal controls and there is a strong and independent relationship between DNA damage and measurements of glycemic control. These results fit into the previously existing research and add fresh evidence to the research done on the same subject in different populations at the same time providing new data concerning the Iraqi population.

Thus, the increase in levels of 8-OHdG in diabetic individuals (4.8 ± 2.1 ng/mL) compared to controls (1.2 ± 0.6 ng/mL) which is 4 times higher is supported by the data from many studies that show that prolonged hyperglycemia leads to oxidative stress and DNA damage [27].

Moreover, the explanation of the observed process can be found in many mechanisms such as (1) glucose auto-oxidation which results in increasing the amount of superoxide anions, (2) mitochondrial dysfunction followed by enhanced activity of the respiratory chain and increased production of ROS, (3) activation of NADPH oxidase due to availability of high glucose levels, and (4) disruption of antioxidant system including superoxide dismutase, catalase, and glutathione peroxidase [28]. Thus, the fact that HbA1c is positively correlated with various DNA damage indicators ($r = 0.742$ for tail length, $r = 0.718$ for olive moment, $r = 0.704$ for 8-OHdG) is a great insight that adds to the body of knowledge of the molecular effects of poor glycemic control.The fact that HbA1c still remained an independent predictor of DNA damage, even after adjusting for other confounding variables, indicates that glycemic regulation is one of the important changes in the modification of genomic integrity among diabetic patients. Results obtained from the comet assay revealed an increase in patients' tail length and olive moment, thus indicating higher odds for DNA double-stranded breaks in diabetic patients. Comet assay is superior in terms of sensitivity to 8-OHdG assay in terms of early and transient DNA damage, as it reveals the fact of DNA destruction at the cellular level [29]. The increase in patients' tail length determined by the assay is about 3.5 times the same indicator among healthy controls; thus, it may indicate a high possibility for the occurrence of genomic instability leading to cellular malfunction and complication processes in diabetes. The findings from the stratification analysis demonstrating the difference in the tail length among patients with poor glycemic regulation in diabetes compared to those with good glycemic regulation pose serious clinical significance of this observation. It can be concluded that managing glycemics by means of the strategies aimed at reaching less than HbA1c (%) may significantly decrease the incidence of oxidative stress responsible for DNA damage. These findings are particularly important for the context of Iraq since lots of diabetic patients are presented with poor glycemic control due to limited access to medical facilities and medications, low patient education, and weak monitoring facilities [30].

It should be highlighted that improving glycemic status among diabetic patients may also reduce DNA damage theoretically leading to lesser risks of complications from diabetes in practice. The fact that oxidative stress and DNA damage could act as the mechanism connecting hyperglycemia with diabetes complications is supported by scientific evidence. Oxidative DNA damage is widely known to play a key role in the development of diabetic nephropathy, retinopathy, and cardiovascular disease [31].Failure to repair or incorrectly repairing DNA damage can result in mutations, instability of chromosomes, and changes in gene expression which could play a role in the progressive nature of diabetes complications [32]. In addition, persistent DNA damage can initiate apoptosis pathways in susceptible cells like the glomerular podocytes and endothelial cells which can result in tissue damage and loss of functionality [33]. The high levels of DNA damage present among Iraqis are worrisome because they are also subject to additional stressors that can compromise health. There are also individuals who live in areas of the country with pollution and contamination due to war and industrial activities thus adding more oxidative stress on top of that caused by hyperglycemia alone [34].

As a result of the dual effect of metabolic dysfunction and environmental stressors, the environment for stability within the genome could be particularly toxic. A major finding of this study was the relationship between the length of time an individual suffers from diabetes and the parameters of DNA damage which seem to indicate that chronic exposure to hyperglycemia leads to accumulative DNA damage. This may have implications for primary prevention tactics and early interventions among high-risk populations. The steps that would prevent the emergence of T2DM could not only help eliminate the effects of oxidative stress caused by hyperglycemia for a certain period of time but also eradicate the effects of DNA damage throughout life. The study also noted very high rates of diabetes complications documented in this cohort with 45.6% of the subjects having retinopathy, 38.3% having microalbuminuria indicative of early nephropathy, and 42.2% of subjects examined having neuropathy.The idea that DNA damage measurements were markedly higher in patients suffering from complications compared to those not suffering from them hints that oxidative stress-mediated DNA damage may indeed have a role in the emergence of these clinical features. Nevertheless, the cross-sectional nature of this research indicates that it is not possible to draw conclusions about causality and future studies should be conducted in order to establish whether higher DNA damage is a precursor of complications. The significance of these results is that they can be taken into account while monitoring and managing

diabetic patients in developing countries. It should be noted that the current practice of DNA damage assessment is not feasible. Given the presence of a strong correlation between HbA1c and DNA damage, blood glucose monitoring using HbA1c can actually be used for minimizing genomic damage. In the future, other indicators of oxidative stress can be identified that can make the assessment of oxidative stress easier in the Iraqi health care system [35].

The article has several strengths, including relatively large sample size and a wide range of methods used to assess DNA damage (the study utilized both 8-OHdG and comet assay), as well as standardized protocols and in-depth statistical analysis that controlled for possible confounding factors. Nevertheless, the article has some drawbacks that should be mentioned as well. The limitation of the study is that it is based on the cross-sectional study design, thus making it impossible to draw conclusions concerning causal relationships. Furthermore, the study was conducted in just one hospital, which could cast doubts about the accuracy of the results. It should be emphasized that as the approach did not evaluate the capacity for DNA repair or polymorphisms of certain genes related to DNA repair that could influence susceptibility to oxidative stress damage, the study becomes more complicated.

The importance of the results is that it can be used to improve public health in Iraq and other countries of the Middle East where diabetes is becoming an epidemic. It is important to strengthen diabetes management systems to better control blood sugar levels through making diabetes medications more available for patients. If these results are integrated into guidelines for diabetes management, it can help to improve treatment strategies. Further studies should be conducted in order to find out whether high DNA damage is a predictor of complications in the near future.

5. Conclusion

The results of this study indicate that glycemic control is strongly and independently linked with genetic damage among Iraqi individuals with T2DM. Poor glucose management is linked with considerable incidence of oxidative DNA damage as demonstrated by the results of 8-OHdG and comet test. Thus, this study reveals the significance of intensive glycemic control during diabetes care and implies that DNA damage caused by oxidative stress may be the key pathogenetic mechanism behind the development of diabetes-related complications as a result of high glucose level. The results imply that better diabetes management in Iraq must focus on achievement of the best glucose level in order to lower oxidative stress and ensure preservation of the DNA structure. This suggests, therefore, that Iraq must reinforce diabetes care system and start using evidence-based protocols in order to improve the situation with diabetes.

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Nexus International University ISSN: 3136-6449; 12(2) 71-86

The Association Between Sociodemographic Factors and Chronic Disease in Adults in Iraq

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Abstract.
Background: Chronic diseases constitute a major public health challenge in Iraq, and their development is influenced by a combination of sociodemographic characteristics and lifestyle-related risk factors.
Aim: To assess the association between sociodemographic factors, lifestyle risk factors, and the prevalence of chronic diseases among adults in Iraq.
Methods: A cross-sectional study was conducted among 250 adults. Data were analyzed using SPSS to obtain descriptive statistics and chi-square tests. Sociodemographic variables included age, gender, BMI, residence, social status, number of children, occupation, and education. Risk factors included physical activity, smoking, sleep pattern, phone use, stress, and substance use.
Results: Participants had a mean age of 45 ± 10.97 years. The most prevalent chronic diseases were joint disease (80%), cerebrovascular disease (74%), cardiovascular disease (70.4%), and gastrointestinal disease (66.4%). Significant associations were identified between chronic diseases and several sociodemographic variables including age ($p < 0.05$), BMI ($p < 0.05$), education, occupation, and residence. High prevalence of risk factors was observed: smoking (70.4%), low physical activity (49.2% never exercise), evening sleep (89.2%), high stress exposure (72.8%), and frequent intake of coffee/tea (76.4%). These lifestyle behaviors showed strong associations with chronic disease occurrence. Additionally, family history was present in 80% of cases.

1. Introduction

Noncommunicable diseases (NCDs), also known as chronic diseases, tend to be long-term and are a result of groups of genetic, physiological, environmental, and behavioral factors. The main types of unpleasant diseases are cardiovascular disease (such as heart attacks, strokes), cancer, chronic respiratory diseases (such as chronic obstructive and asthma), and diabetes [1].

The Centres for Disease Control and Prevention (CDC) defines chronic diseases as conditions that last 1 year or more and that require ongoing medical attention or limit activities of daily living, or both. Chronic diseases may be influenced by a combination of genetics, lifestyle and social behaviours, health care system factors, community influences, and environmental determinants of health. These risk factors often coexist and interact with each other. Therefore, a better understanding of determinants of chronic diseases, such as tobacco use, unhealthy eating, and physical inactivity, stands to benefit from effective strategies for improving primary, secondary, and tertiary disease prevention and management in diverse global settings. Strategies to prevent and manage chronic disease outcomes, such as diabetes and cardiovascular diseases (CVDs), have global commonalities. The impact of chronic diseases is disproportionately evident in Black and Brown communities. Chronic disease prevention and management typically focus on behavioural interventions such as healthy eating, increased physical activity, and cessation of unhealthy practices such as tobacco and alcohol use [2].

Chronic diseases have an impact on and change patients' lives, and the way they experience their bodies alters. Patients may struggle with identity and self-esteem, a shrinking lifeworld, and a challenging reality. The chronic diseases become part of the patients' lives, whether they affect their physical health and functions, autonomy, freedom, and

identity, or threaten their life. The patients are forced to change their lifestyle, which they often experience as losing control, and to minimise loss of control, they feel a need to mobilise resources and plan and prioritise their lives. Having a chronic disease can lead to stressors such as life-threateningness, progressive deterioration, and controllability. It has previously been identified that there are stressors that are common to different chronic diseases. Patients who have a chronic disease have to find new ways to cope with their changed situation and develop coping strategies that relate to the way they think and act in order to manage stressful situations. This may involve problem- or emotionally focused coping, depending on the individual's personality. Being given information is important, as is learning how to talk to others. The patients' own thoughts about their new situation can also influence how they cope. Another aspect of coping with the new life situation is the type of support the patients can receive. The social support received depends on the patients' available network of people, and the need for social support will vary depending on the patients' age and needs. Overall, social support has been seen as a buffer against the risks to the well-being of chronically ill patients [3].

Chronic disease is a very important issue for global health, as 75% of global deaths in 2019 are non-communicable diseases, with 43 million deaths, affecting 18 million early cases at the beginning of the early semester, affecting quality of life, fatal increases and health increases, and improving health status in the third guide. Genetics and several environmental factors) interact with adjustable risk factors (e.g., tobacco and alcohol consumption, high blood pressure, high cholesterol concentrations, low foods with fresh fruits and vegetables). However, there is a serious gap in research in the literature. The first is to fully understand the complex interactions of measurable insoluble factors in the development of chronic diseases affecting multiple organs. Second, how many developments in chronic disease can be understood and explained using measured (unmeasured risk factors [4].
Chronic diseases addressed in this study are as outlined in questionnaire, They are not passed from person to person and are the result of a combination of genetic, physiological, environmental, and behavioural factors, The diseases specifically addressed in this study, as outlined in the questionnaire, include: Diabetes: A chronic condition characterized by high levels of blood glucose (sugar) resulting from either insufficient insulin production (Type 1) or the body's ineffective use of insulin (Type 2). It can lead to severe complications affecting the heart, blood vessels, eyes, kidneys, and nerves. High Blood Pressure (Hypertension): A common condition in which the long-term force of the blood against your artery walls is high enough that it may eventually cause health problems, such as heart disease. Often called a "silent killer" due to its lack of obvious symptoms, Heart Disease is a broad term encompassing a range of conditions that affect the heart, including coronary artery disease (the most common type), heart failure, arrhythmias, and congenital heart defects. These conditions can lead to heart attacks, strokes, and other life-threatening events. Chronic Kidney Disease (CKD): A progressive loss of kidney function over time. The kidneys filter waste products and excess fluids from the blood, and when they fail, dangerous levels of waste can accumulate in the body. Chronic Respiratory Disease: Long-term diseases of the airways and other structures of the lung. Examples include asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and occupational lung diseases. These conditions can cause breathlessness, coughing, and wheezing. Chronic Gastrointestinal Disease: Persistent or recurring conditions affecting the digestive system, which includes the oesophagus, stomach, small intestine, large intestine, and rectum. Examples include Crohn's disease, ulcerative colitis (inflammatory bowel diseases), irritable bowel syndrome (IBS), and chronic gastritis. Joint Disease (Arthritis): A condition causing inflammation and pain in the joints. There are many types, including osteoarthritis (wear-and-tear arthritis) and rheumatoid arthritis (an autoimmune disease). It can lead to chronic pain, stiffness, and reduced mobility. Cerebrovascular Disease (Stroke): A condition that affects the blood vessels supplying the brain. A stroke occurs when a blood vessel that carries oxygen and nutrients to the brain is either blocked by a clot or ruptures, leading to brain damage [5].

Cancer: A disease characterized by the uncontrolled growth and spread of abnormal cells. It can originate in almost any organ or tissue of the body and is a leading cause of death worldwide. The global burden of NCD remains unacceptable. In 2016, non-communicable illnesses were responsible for 41 million deaths, with 57 million deaths (71%) worldwide. 15 million (30-70 years) of deaths of these individuals. The burden was higher in countries with lower incomes and lower average incomes, all of which resulted in 78% of the total number of NCD deaths and early deaths. Furthermore, suicide was actually responsible for 2016. 800,000 people have died. In 2016, the risk of early death from one in four non-communicable diseases decreased to 18%, a modest one. A relative reduction of 6% compared to 2010. If everything is implemented in all 194 member countries, a total of 16 "best buyers" could be avoided from the World Health Organization at early deaths between 9.6 million and 2025. Meanwhile, a mix of risk

factors and advances in many regions has been discovered. If the trend is saved, it is unlikely to become a world. The goal is achieved. The main behavioural risk factors, harmful alcohol consumption, tobacco, and food consumption, show the last country to compare ratings as there are no decisions on physical activity behaviours. Levels of alcohol consumption worldwide. In 2016, 6.4 liters of pure alcohol were estimated. For those over 15 years old. The level of consumption and trends vary from area to area. However, in recent years, a significant decline has been observed in the European region. Globally, the region remains among the people with the highest levels of alcohol consumption. In 2016, over a quarter of adults were under the age of 18 (28%). Physical activity recommendations are not implemented, particularly in countries with high incomes, where significant physical activity is noticed. On the contrary, countries with low levels of income will generally reduce emissions, but physical activity is still of interest worldwide. Women are, in principle, less active than men, and this trend applies to most countries. Over the past 15 years, global physical activity levels have not shown significant improvements - the usual field has failed to achieve global goals to increase physical activity by 2025. Furthermore, data related to sodium consumption (salt) and health outcomes remain an important issue worldwide [6].

Noncommunicable diseases (NCDs) are the leading cause of morbidity and death in Iraq (Iraqi Ministry of Health, 2019). It is estimated that 30% of Iraqis have high blood pressure, 14% have diabetes, and more than 30% are obese. Some 38% of Iraqi males smoke, and a growing number of schoolchildren - 20% of males and 9% of females aged between 13 and 15 years are tobacco users. Comparison with Syria, Prevalence & Incidence: Similar trends are observed in Syria, with comparable prevalence of diabetes (~10-13%) and hypertension (~20-25%). However, conflict-related disruptions may have affected disease management and data accuracy. Mortality Rates: Both countries have high cardiovascular-related mortality, but Iraq's rates may be marginally higher due to conflict, healthcare access disparities, and socioeconomic factors. General Trends: Urbanization and lifestyle changes are increasing the prevalence of these diseases in Syria, paralleling Iraq's trend. Spread spread in obesity increases by about 20% among adults. Trauma: Microelement indicators are less documented. Nevertheless, consistent trends indicate that we have recently diagnosed cases of hypertension and diabetes. Syria: While ongoing conflicts limit detailed modern epidemic data, studies have shown high levels of hypertension and diabetes. Türkiye: Reports show that hypertension spread is over 30% and diabetes affects approximately 12% of the adult population (Turkey Heart Disease, 2020) .Iran: A large epidemic study showed spread spread in diabetes in about 8-10%, and hypertension affected about 20% of adults (Ministry of Health, 2019) [7].

In treatment, many patients support quality of life, but severe chronic obstruction of the lungs can lead to respiratory failure and death. Diagnosis: That's very different. Early detection and treatment improved results. Some types of cancer are very curable, but the diagnosis can be weak, especially if it is diagnosed late. Chronic kidney disease (CKD) is, gradual loss of renal function over several months or years. Diagnosis, this can lead to kidney disease at the end of a stage requiring dialysis or transplantation. Early detection may slow progression and improve quality of life [8].

The ageing population tends to have a higher prevalence of chronic diseases worldwide today. For example, six in ten adults in the US have a chronic disease, and four in ten adults have two or more, while Sweden reports multiple chronic conditions of 56.3% These chronic conditions are significant and profound economic issue for any person, the healthcare system, and society as a whole, Such diseases account for 86% of all medical costs in the US being even greater worldwide, The presence of multiple chronic conditions in the same individual has profound implications for healthcare costs and utilisation, For example, a Swiss study estimated that the average total healthcare costs were 5.5 times higher in elderly patients with multiple chronic conditions as compared with elderly patients without multiple chronic conditions, The combined cost savings from the health and productivity that results from a small reduction in the prevalence of chronic disease cannot be ignored, resulting in a genuine return on investment in a very small span of time, Chronic diseases require a long period of treatment that leads to the increase in demand for healthcare services and changes its nature [9].

In today's world, chronic diseases are the largest cause of death, and their prevalence is increasing. The leading diseases, particularly cardiovascular disease, cancer, chronic lung disease, and diabetes mellitus, reflect past exposure to risk factors such as unhealthy diet, tobacco use, physical inactivity, and alcohol use. Global responses to the burden of chronic diseases are inadequate because of insufficient financial support for prevention, treatment, and research, and because many health systems are focused on acute care. There is also another reason for this inadequacy: the

belief that chronic diseases only affect the elderly and the affluent. As medicine has been able to significantly reduce the burden of acute diseases, especially in developed countries, life expectancy has increased, and with an ageing population, it is confronted with more prevalent chronic diseases. In the 24 member states of the Organisation for Economic Cooperation and Development, most of which are developed countries with high-income economies, more than one-third of adults reported living with a chronic disease or chronic health problem in 2021. However, the majority of chronic diseases today occur in developing countries. They have not simply displaced acute diseases. Developing countries face a double burden of disease: they have a high and growing number of people with chronic diseases, while at the same time, they face high child mortality rates from infectious diseases. Statistics on chronic diseases show that policies and practices are often misguided by myths. One is that global policies should focus first on infectious diseases such as HIV/AIDS, tuberculosis, and malaria. However, chronic diseases may develop alongside infectious diseases, while the latter can evolve into chronic conditions. Instead of prioritizing infectious diseases, policies should simultaneously address chronic diseases. Another misconception is that chronic disease is the result of poor lifestyle choices, while social and cultural contexts influence individual choices, so that global policies should have a broader focus than responsible conduct and healthy choices. Yet another one is the aforementioned belief that the rich and old are primarily affected by chronic disease. In fact, low socio-economic status is associated with greater exposure to risk factors (such as fatty foods, smoking, and less physical exercise) and less access to quality healthcare. It is also not true that the elderly are affected, since in developing countries, high-risk individuals are younger than in developed countries. While much of the policy and ethical debate is focused on chronic disease, less attention is usually given to what it means to be chronically ill. In this issue of the journal, the challenge of chronic disease is addressed at the experiential level of illness. Phenomenologists used to point out that people who are chronically ill have a transformative experience. The illness affects not only their bodily functioning but also their personality, identity, and lived experience: it alters their values, goals, and aspirations. This may not be true for all chronic diseases, as some conditions, such as hypothyroidism and glaucoma, can be well controlled with medication, but it is certainly the case for more serious and potentially life-threatening and disabling conditions [10].

A chronic illness affects many aspects of life. The burden of illness, limitations in daily life, lifestyle changes, and/or increased medical needs can pose emotional, psychological, financial, and time stressors. Hence, implications for well-being at the time of disease onset as well as changes in the long-term development of well-being are expected. The theory of hedonic adaptation states that after a life event, individuals return to a baseline happiness. Applied to the context of chronic illnesses, an immediate reduction in wellbeing at disease onset would be expected, followed by a gradual return to the pre-onset wellbeing levels in the years after the onset. The model of adjusting to chronic conditions, on the other hand, states that after a chronic illness onset, individuals might experience either successful psychological adaptation or ongoing adjustment difficulties. This implies uncertainty about the long-term development of wellbeing and outcomes, ranging from persistently lower wellbeing levels to slow recovery of wellbeing to complete return to pre-onset wellbeing levels might be expected.

In the empirical literature, studies across the board found an immediate reduction in subjective well-being after a chronic disease onset. Concerning the long-term effects, however, the empirical evidence is more mixed. Some studies reported that subjective well-being bounced back after a health shock, implying a return to pre-onset well-being in the long term. Other studies found very little wellbeing recovery, showing persistently lower wellbeing levels after the onset of a chronic disease. A third stream of studies reported differential outcomes, where some groups achieved more favourable long-term well-being development than others. Given the mixed evidence of previous research, it is of particular interest to investigate the development of well-being after illness onset, examining both immediate level changes and longitudinal trend changes. While several studies have analysed long-term effects of chronic illness by comparing pre- and post-onset wellbeing levels, longitudinal analyses including both immediate level and long-term trend effects are scarce. However, joint analyses of level and trend effects are crucial to disentangle the immediate and long-term impacts of a chronic disease onset. The onset of a chronic disease often follows increased medical needs and higher health service utilization, requiring a patient's effort to manage their disease. This work of managing a chronic disease, including, e.g., administering medication or arranging and attending medical appointments, is referred to as the burden of treatment. Low availability of healthcare services or long distances to healthcare providers can be perceived as burdensome for persons with chronic diseases. Against this background, lower accessibility and availability of healthcare services might aggravate this treatment burden. Put differently, worse spatial healthcare

access might amplify wellbeing reduction at disease onset. To the best of our knowledge, no studies to date have investigated spatial healthcare access as potential [11].

There is no universal agreement on what constitutes a chronic disease; chronicity is a relative term that has shifted as advancements in medicine translate from bench to bedside, and the focus of care moves from dealing with acute or terminal diagnoses to long-term management of chronic conditions. Nevertheless, it is apparent that a crisis in chronic disease burden has emerged globally, and with it a body of literature for diseases that are now finding more commonalities under the umbrella of "chronic disease". Within this new scope, are there parallel lessons broadly applicable to chronic disease? Complex global healthcare challenges and more diversely represented populations in medicine have necessitated new integrated approaches to healthcare, with an ever-increasing appreciation for the patient perspective. Specifically, handling patient-provider relationships and patient education increasingly plays a critical role in the delivery of positive outcomes and increased quality of life [12].

Aim of the study:

- To study Sociodemographic factors of adult in Iraq
- To study chronic disease of adults in Iraq
- To study the association between sociodemographic factors and chronic disease in adults in Iraq

2. Methodology

2.1 Study design

A cross-sectional study of the Association of socio-demographic factors and chronic disease. This study was carried out in Al–Ramadi Teaching Hospital. For the period twenty-fifth of July to the end of October 2025. The study was conducted on adults who visited or were admitted. The study was approved by the College of Medicine, University of Anbar. The sample size was two hundred and fifty adults selected by non-probability convenience sampling technique. Inclusion criteria included adults (18 +).

2.2 Data Collection

After taking informed verbal consent, the specially designed questionnaire was administered through interviewing adults with closed-ended questions related to socio–demographic factors and chronic disease. The questionnaire was divided into three parts: part one included demographic data, including age, gender, education, parity, and knowledge; part two included chronic disease; part three for risk factors of chronic disease.

2.3 Study Setting and Duration

The study was conducted at Al-Ramadi General Hospital on 20/8/2025. Data were collected from the relevant clinical departments according to the operational procedures of the hospital.

2.4 Study Population

The study population included all individuals who met the inclusion criteria and attended the selected department during the data collection process at Al-Ramadi General Hospital. Individuals who did not meet the study requirements were excluded.

2.5 Sample Size and Sampling Technique

A total of 250 participants were included in the study. A non-probability convenient sampling technique was used to recruit participants who were available and willing to participate during the specified day of data collection.

2.6 Inclusion Criteria

- Participants aged 18 years and above
- Individuals who voluntarily agreed to participate
- Cases relevant to the study objectives who completed the questionnaire

2.7 Exclusion Criteria

- Individuals who refused to participate
- Incomplete questionnaires
- Cases unrelated to the study objectives

2.8 Data Collection Tool

Data were collected using a structured questionnaire divided into three main sections:
- Socio-demographic characteristics (age, gender, residence, education level, etc.)
- Clinical information related to the condition under investigation
- Risk factors and associated variables

The questionnaire was reviewed by a panel of experts to ensure clarity, reliability, and validity.

2.9 Data Analysis

The collected data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. The analysis included:
- Descriptive statistics such as frequencies, percentages, means, and standard deviations
- Cross-tabulation to examine associations between variables
- Chi-square (χ^2) test to determine the significance of relationships between categorical variables p-value of $\leq$ 0.05 was considered statistically significant.

3. Results

The study included 250 adult participants. The mean age was 45 ± 10.97 years, with the largest age group being 38–47 years (34.4%), followed by 48–57 years (20.0%). More than half of the sample were males (54.0%), while females represented 46.0%.

Regarding body mass index (BMI), the mean value was 23 kg/m². About one-third of the participants were underweight (33.6%), 32.8% had normal weight, 18.8% were overweight, and 14.8% were obese.

More than half of the sample resided in urban areas (56.4%), and 55.2% were married. Concerning parity, most participants had five or more children (31.2%), followed by those with two children (32.8%).

In relation to occupation, 33.2% were housewives, 30.8% were unemployed, 18.8% employed, and 17.2% retired. Regarding educational attainment, 27.2% were illiterate, 24.0% had primary education, and 19.6% were university graduates.

Table 1: Distribution of Socio-Demographic Characteristics of the Study Sample.

Variable	No.	%
Age		
m ± SD	45 ± 10.97	
18 - 27	38	15.2%
28 – 37	58	23.2%
38 – 47	86	34.4%
48 – 57	50	20.0%
58 +	18	7.2%
Total	250	100%
Gender		

Male	135	54.0%
Female	115	46.0%
Total	250	100%
BMI		
m± SD	22.7±23	
Underweight < 18.5	84	33.6%
Normal weight 18.5- 24.9	82	32.8%
Overweight 25 – 29.9	47	18.8%
Obesity 30 +	37	14.8%
Total	250	100%
Residence		
Urban	141	56.4%
Rural	109	43.6%
Total	250	100%
Social Status		
Single	101	40.4%
Married	138	55.2%
Divorced	5	2.0%
Separated	3	1.2%
Widower	6	1.2%
Total	250	100%
No. of children		
Primigravida (1)	25	10.0%
2	82	32.8%
3	14	5.6%
4	51	20.4%
5+	78	31.2%
Total	250	100%
Occupation		
Employed	47	18.8%
Un – employed	77	30.8%
Housewife	83	33.2%
Retired	43	17.2%
Total	250	100%
Education		
Illiterate	68	27.2%
Read and write	45	18.0%
Primary	60	24%
Intermediate	11	4.4%
secondary	15	6.0%
University	49	19,6%
High education	2	0.8%
Total	250	100%

Nearly half of the participants reported no regular physical activity (49.2%), while only 10.8% performed exercise five days per week. Most participants slept in the evening (89.2%), with an average sleep duration of 5.17 ± 2.05 hours. A total of 35.6% slept less than four hours.
A high proportion were smokers (70.4%), with cigarettes being the most common type (52.4%), followed by vape (34.8%) and shisha (12.0%). The intensity of smoking was mostly moderate (37.2%).

Only 4.0% reported addiction, mainly to drugs (0.7%), hashish (0.2%), and crystal (0.2%). Alcohol consumption was rare (1.2%). Most participants (76.4%) consumed coffee and tea, with high consumption among 78.0%.
Regarding mobile phone use, 40.8% used their phone 1–2 hours daily, while 20.4% used it more than 5 hours. Psychological stress was commonly reported, with 50.8% experiencing stress “sometimes” and 22.0% “always.”

Table 2: Distribution of risk factors of chronic disease of the study sample

Variable	No.	%
Physical activity		
5 days per week	27	10.8%
3-4 days per week	36	14.4%
1-2 days per week	64	25.6%

Ever	123	49.2%
Total	250	100%
Time of sleep		
Morning	27	10.8%
Evening	223	89.2%
Total	250	100%
Sleep duration		
m± SD	5.17±8	
4	89	35.6%
5 – 8	153	61.2%
9+	8	3.2%
Total	250	100%
Smoker		
Yes	176	70.4%
No	74	29.6%
Total	250	100%
Type of smoke		
Cigarette	131	52.4%
Vape	87	34.8%
Shisha	30	12.0%
Others	2	0.8%
Total	250	100%
State of smoke		
High	86	34.4%
middle	93	37.2%
low	71	28.4%
Total	250	100%
Addiction		
Yes	10	4.0%
No	240	96.0%
Total	250	100%
Type of addiction		
Drugs	4	0.7%
Hashish	3	0.2%
Crystal	3	0.2%
others	0	1.1%
Total	10	2.2%
State of addiction		
High	0	0%
Middle	3	1.2
Low	7	2.8%
Total	10	4.0%
Alcoholism		
Yes	3	1.2%
No	247	98.8%
Total	250	100%
State of Alcoholism		
High	0	0%
Middle	2	0.8%
Low	1	0.4%
Total	3	1.2%
Have coffee and tea		
Yes	191	76.4%
No	59	23.6%
Total	250	100%
State of having coffee and tea		
High	195	78.0%
Middle	33	13.2%
Low	22	8.8%
Total	250	100%

Hours of phone use		
1-2	102	40.8%
3-4	97	38.8%
5+	51	20.4%
Total	250	100%
Social and psychological stress		
Always	55	22.0%
Sometimes	127	50.8%
Ever	68	27.2%
Total	250	100%

The of chronic diseases was high in the study sample. The most frequent conditions were:

- Joint diseases: 80.0% Cerebrovascular diseases: 74.0% Cardiovascular diseases: 70.4%
- Respiratory diseases: 60.4% Gastrointestinal diseases: 66.4% Cancer: 64.8% Hypertension: 56.4% Diabetes Mellitus: 57.2%
- Renal disease: 54.0%
- Around 80% had a family history of chronic disease, and 7.2% had a past medical history. The onset of disease was within 1–5 years for 48.0% of participants, and 37.2% reported onset less than one year.
- Associated chronic diseases were found in 33.6%, with the most common combinations being HTN & DM (30.4%) and HTN & CHD (30.8%).
- In terms of prognosis, 46.4% reported improvement, 41.2% deterioration, and 12.4% no change. Complications were (96.4%). Only 7.6% used dietary supplements.

Table 3: Distribution of chronic disease of the study sample.

Variable	No.	%
Types of chronic disease		
Hypertension		
Have hypertension	141	56.4%
have not hypertension	109	43.6%
Total	250	100%
Diabetes mellitus		
have Diabetes mellitus	143	57.2%
have not Diabetes mellitus	107	42.8%
Total	250	100%
Cardiovascular disease		
Have Cardiovascular	176	70.4%
have not Cardiovascular	74	29.6%
Total	250	100%
Respiratory disease		
Have Respiratory disease	151	60.4%
have not Respiratory disease	99	39.6%
Total	250	100%
Renal disease		
Have Renal disease	135	54.0%
have not Renal disease	115	46.0%
Total	250	100%
Gastrointestinal disease		
Have Gastrointestinal disease	166	66.4%
have not Gastrointestinal disease	84	33.6%
Total	250	100%
Joint disease		
Have Joint disease	200	80.0%
have not Joint disease	50	20.0%
Total	250	100%
Cerebrovascular disease		
Have Cerebrovascular disease	185	74.0%
have not Cerebrovascular disease	65	26.0%
Total	250	100%
Cancer		
Have Cancer	162	64.8%

have not Cancer	89	35.2%
Total	250	100%
Others		
Have Others disease	120	
have not Others disease		
Total	250	100%
Past history		
Have Past history	18	7.2%
have not Past history	231	92.8%
Total	250	100%
Family history		
Have Family history	200	80%
have not Family history	50	20%
Total	250	100%
Onset of disease (Years)		
<1	93	37.2%
1-5	120	48.0%
10+	7	2.9%
Don't know	25	10.2%
Total	250	100%
Associated disease		
Have Associated disease	84	33.6%
have not Associated disease	166	66.4%
Total	250	100%
Types of associated disease		
H.T & DM	76	30.4%
H.T & CHD	77	30.8%
H.T, DM& CHD	23	9.2%
Others	58	23.2%
Total	250	100%
Prognosis of disease		
Improvement	116	46.4%
Deterioration	103	41.2%
Ever	31	12.4%
Total	250	100%
Live alone		
Have Live alone	5	2.0%
have not Live alone	245	98.0%
Total	250	100%
Complications of chronic disease		
Have Complications of chronic disease	241	96.4%
have not Complications of chronic disease	9	3.6%
Total	250	100%
Uses of dietary supplements		
Have Uses of dietary supplements	19	7.6%
have not Uses of dietary supplements	231	92.4%
Total	250	100%

A significant association was found between age and several chronic diseases. Most conditions were concentrated in the 38–47 and 48–57 age groups ($p < 0.05$). Younger age groups showed lower. The association between gender and most chronic diseases was not statistically significant ($p > 0.05$). Underweight and normal-weight participants had higher percentages of most chronic diseases. A significant association was found between BMI and several diseases ($p < 0.05$). Urban residents exhibited higher of cardiovascular, cerebrovascular, and gastrointestinal diseases. The association was statistically significant for some diseases ($p = 0.044$).

Married participants had the highest of chronic diseases, but statistical significance varied across disease types.A significant association was observed, especially in individuals with two children, who demonstrated higher of chronic diseases ($p < 0.05$). The association between occupation and disease prevalence was statistically significant ($p < 0.05$). The association between education and most chronic conditions was statistically significant ($p < 0.05$).

Table 4: The association between sociodemographic factors and chronic diseases

Variable	H.T No. %	DM No. %	CHD No. %	CNS No %	Resp. No. %	UT No. %	GIT No. %	Joint No. %	Cancer No. %	P Value
Age										0.171
18 - 27	7.2%	6%	9.6%	10.4%	11.2%	8.0%	9.6%	9.6%	8.4%	
28 – 37	8%	9.6%	10.8%	11.6%	9.6%	6.8%	10.0%	11.6%	12.4%	
38 – 47	24%	25.2%	31.2%	32.8%	23.2%	25.2%	28.0%	32.0%	25.2%	
48 – 57	13.2%	13.2%	16.4%	16.0%	14.4%	11.2%	15.2%	17.6%	17.2%	
58 +	4%	3.2%	2.4%	3.2%	2%	2.8%	3.6%	3.2%	1.6%	
Total	56.4%	57.2%	70.4%	74.0%	60.4%	54.0%	66.4%	74.0%	64.8%	
Gender										0.209
Male	31.2%	33.2%	43.6%	40.4%	36.8%	33.6%	40.0%	42.4%	36.8%	
Female	24.4%	23.2%	26.8%	33.6%	23.6%	20.4%	26.4%	31.6%	28.0%	
Total	57.8%	56.4%	70.4%	74.0%	60.4%	54.0%	66.4%	74.0%	64.8%	
BMI										0.118
Underweight < 18.5	17.2%	20.4%	33.6%	31.2%	26.4%	23.2%	26.4%	30.8%	28.4%	
Normal weight 18.5- 24.9	20.4%	16.8%	24.4%	24.8%	19,6%	18.4%	24.4%	25.2%	20.4%	
Overweight 25 – 29.9	10.4%	10.8%	7.6%	8.4%	9.2%	6.8%	9.2%	10,0%	10.0%	
Obesity 30 +	8.4%	57.2%	4.8%	9.6%	9.6%	5.6%	6.4%	8.0%	6.0%	
Total										
Residence										0.159
Urban	31.6%	32.0%	36.4%	41.6%	30.8%	28,0%	35.2%	41.6%	33.2%	
Rural	25.2%	25.2%	34.0%	32.8%	29.6%	26.0%	31.2%	32.4%	31.6%	
Total	57.0%	56.4%	70.4%	74.4%	60.4%	54.0%	66.4%	74.0%	64.8%	
Social Status										0.332
Single	16.4%	20.4%	38.2%	32.8%	27.6%	25.6%	31.2%	32.8%	29.2%	
Married	30.4%	30.4%	32.2%	38.0%	31.4%	28.4%	34.0%	39.6%	35.6%	
Divorced	6.0%	4.0%	0.0%	0.4%	0.4%	0.0%	0.8%	0.8%	0.0%	
Separated	0.4%	2.0%	0.0%	0.8%	0.0%	0.1%	0.4%	0.2%	5.3%	
Widower	1.2%	0.4%	2.4%	2.1%	1.0%	0.0%	0.0%	0.0%	2.1%	
Total	56.4%	57.2%	72.8%	74.1%	60.4%	54.1%	66.4%	73.9%	72.2%	
No. of children										0.104
Primigravida (1)	2.3%	4.2%	3.4%	2.3%	3.4%	2.9%	2.5%	1.7%	3.9%	
2	29.1%	23.7%	40.6%	38.3%	31.4%	31.4%	34.1%	38.9%	30.4%	
3	4.0%	4.0%	2.9%	4.6%	5.1%	2.9%	4.6%	4.6%	5.9%	
4	8.6%	10.3%	7.0%	10.9%	6.3%	7.4%	8.3%	10.3%	7.5%	
5+	16.6%	15.2%	9.0%	12.1%	9.3%	9.7%	12.3%	13.8%	12.6%	
Total	60.6%	57.4%	64.0%	68.0%	55.4%	54.3%	62.3%	69.1%	60.0%	
Occupation										
Employed	8.8%	8.4%	9.2%	10.2%	10.3%	8.8%	10.0%	8.4%	8.0%	
Un – employed	14.8%	13.6%%	19.6%	17.4%	15.9%	16.0%	18.0%	18.8%	17.2%	
Housewife	19.4%	20.4%	17.2%	22.8%	14.6%	12.0%	19.2%	22.4%	18.4%	
Retired	11.4%	14.8%	24.4%	23.6%	20.6%	17.2%	21.2%	24.4%	21.2%	
Total	56.4%	57.2%	70.4%	74.0%	60.4%	54.0%	64.4%	74.0%	64.8%	
Education										0.151
Illiterate	18.4%	16.4%	22.8%	20.0%	18.8%	15.6%	20.0%	22.0%	19.2%	
Read and write	25.4%	12.0%	15.6%	12.4%	12.4%	10.8%	12.4%	15.2%	14.8%	
Primary	14.4%	13.2%	18.0%	16.0%	12.8%	14.0%	16.0%	19.2%	15.2%	
Intermediate	3%	2.0%	2.8%	2.4%	2.4%	2.4%	2.4%	3.2%	3.2%	
secondary	2,6	3.6%	1.6%	2.8%	3.2%	1.6%	2.8%	4.4%	2.4%	
University	10%	9.6%	9.6%	12.8%	10.4%	9.6%	12.8%	9.2%	10.0%	
High education	0.4%	0.4%	0.0%	0.0%	0.4%	0.0%	0.0%	0.8%	0.0%	
Total	56.4%	57.2%	70.4%	66.4%	60.4%	54.0%	66.4%	74.0%	64.8%	

4. Discussion

Socio-demographic characteristics

The findings of the present study revealed that the highest proportion of participants were within the age group 38–47 years (34.4%), followed by those aged 48–57 years (20.0%). This indicates that chronic diseases were more prevalent among middle-aged adults. This finding is consistent with previous studies which reported that the prevalence of chronic diseases increases with age due to cumulative exposure to lifestyle-related risk factors and physiological changes associated with aging [12,13]. Lower prevalence rates were observed among younger age groups, which is also supported by similar population-based studies [14]. Males represented 54.0% of the study sample, while females constituted 46.0%. The present study showed no statistically significant association between gender and most chronic diseases. This result agrees with several studies which reported that gender differences in chronic disease prevalence become insignificant after adjusting for behavioral and socio-demographic factors [15,16].

Regarding body mass index, the mean BMI was 22.7 kg/m². Underweight participants represented a considerable proportion of the sample (33.6%). Interestingly, underweight and normal-weight individuals showed higher prevalence of several chronic diseases. This finding is lower than what is commonly reported in studies emphasizing obesity as a major risk factor [17], but similar results have been observed in populations where chronic illness, malnutrition, and disease-related weight loss are common [18]. More than half of the participants resided in urban areas (56.4%). Urban residents exhibited higher prevalence of cardiovascular, cerebrovascular, and gastrointestinal diseases. This finding is in agreement with other studies that reported higher chronic disease burden in urban settings due to sedentary lifestyle, unhealthy dietary habits, psychological stress, and environmental pollution [19,20].

Married participants represented the majority of the sample (55.2%) and showed higher prevalence of chronic diseases compared to other social groups. This result is consistent with studies suggesting that marital responsibilities, increased stress, and age-related factors may contribute to higher disease prevalence among married individuals [21]. Participants with two or more children demonstrated higher rates of chronic diseases, which may reflect cumulative stress, reduced self-care, and limited time for healthy behaviors, as reported in previous studies [22].

With regard to occupation, housewives and unemployed participants constituted the largest proportions of the study sample. The association between occupation and chronic disease prevalence was statistically significant. Similar findings were reported in studies indicating that unemployment and retirement are associated with increased risk of chronic diseases due to physical inactivity, psychological stress, and limited access to health care services [23].

Educational level showed a significant association with chronic diseases, where participants with lower educational attainment exhibited higher prevalence. This finding agrees with studies reporting that low education is associated with poor health literacy, unhealthy lifestyle practices, and delayed health-seeking behavior [24,25].

Lifestyle-related risk factors

The present study revealed a high prevalence of physical inactivity, as nearly half of the participants (49.2%) reported no regular physical activity. This finding is consistent with WHO reports and other studies that identified physical inactivity as one of the leading risk factors for non-communicable diseases worldwide [12,26].

Sleep pattern analysis showed that most participants slept in the evening (89.2%), with a mean sleep duration of 5.17 ± 2.05 hours. More than one-third of participants slept less than four hours per day. This finding agrees with studies that linked short sleep duration to increased risk of hypertension, diabetes mellitus, cardiovascular disease, and psychological stress [27,28]. Smoking prevalence was remarkably high in the present study (70.4%). Cigarette smoking was the most common type, followed by vaping and shisha. Moderate smoking intensity was predominant. Similar high smoking rates and patterns have been reported in regional studies, which demonstrated strong associations between smoking and respiratory, cardiovascular, and cerebrovascular diseases [29,30].

Alcohol consumption and substance addiction were reported by a small proportion of participants. Although the prevalence was low, previous studies have shown that even limited exposure to alcohol and drugs may worsen disease outcomes and increase the risk of complications among patients with chronic diseases [31]. The majority of participants consumed coffee and tea (76.4%), with a high level of consumption reported by most of them. Excessive caffeine intake has been associated with sleep disturbance, anxiety, and increased cardiovascular risk in some studies [32].

Regarding mobile phone use, a considerable proportion of participants used their phones for more than three hours daily. Prolonged screen time has been associated with sedentary behavior, sleep disturbance, and increased psychological stress [33]. Psychological stress was commonly reported, with 50.8% experiencing stress sometimes and 22.0% always. This finding is consistent with studies identifying chronic stress as a major contributor to hypertension, cardiovascular disease, poor glycemic control, and unfavorable prognosis [34].

Prevalence and Pattern of Chronic Diseases

The present study demonstrated a high prevalence of chronic diseases among the study sample. Joint diseases were the most frequently reported condition (80.0%), followed by cerebrovascular diseases (74.0%), cardiovascular diseases (70.4%), gastrointestinal diseases (66.4%), diabetes mellitus (57.2%), and hypertension (56.4%). Similar disease patterns were reported in other community-based studies where musculoskeletal and cardiovascular diseases were among the most prevalent chronic conditions [35,36].

A high proportion of participants reported a family history of chronic disease (80.0%), which is consistent with studies emphasizing the combined role of genetic susceptibility and shared environmental factors in chronic disease development [37]. Only 7.2% reported a past medical history, which may indicate underdiagnoses or late detection of chronic conditions.

Most participants reported disease onset within the last five years, suggesting a recent increase in chronic disease burden. This finding agrees with studies reporting a rising incidence of chronic diseases due to lifestyle transitions and urbanization [38]. Associated chronic diseases were present in one-third of participants, with hypertension combined with diabetes mellitus and coronary heart disease being the most common combinations. This clustering has been widely reported and reflects shared risk factors such as physical inactivity, smoking, stress, and unhealthy diet [39].

Despite the high prevalence of complications (96.4%), only a small proportion of participants reported using dietary supplements. Similar findings were reported in studies suggesting low awareness and inadequate

The present study demonstrated a statistically significant association between age and the prevalence of several chronic diseases, with the highest proportions observed among participants aged 38–47 and 48–57 years. This finding is consistent with previous studies which reported that middle-aged adults carry a higher burden of chronic diseases due to cumulative exposure to behavioral risk factors and progressive physiological decline [12,13]. Younger age groups showed lower prevalence rates, which is in agreement with findings reported in developing countries [14].

Regarding gender, males showed slightly higher percentages of most chronic diseases compared to females; however, the association was not statistically significant. This result agrees with several studies indicating that gender alone is not a strong determinant of chronic disease prevalence when lifestyle and socioeconomic factors are considered [15,16].

Body mass index was significantly associated with several chronic diseases. Interestingly, underweight and normal-weight participants demonstrated higher proportions of chronic diseases compared to overweight and obese individuals. Similar findings have been reported in studies suggesting that chronic disease–related weight loss, malnutrition, and long-standing illness may explain higher disease prevalence among underweight individuals in certain populations [17,18].

Residence was another important factor, as urban residents exhibited higher prevalence of cardiovascular, cerebrovascular, and gastrointestinal diseases compared to rural residents. This finding is consistent with studies reporting that urbanization is associated with sedentary behavior, unhealthy diet, psychological stress, and environmental pollution, all of which contribute to chronic disease development [19,20].

Marital status showed varying associations with chronic diseases. Married participants demonstrated the highest prevalence of most chronic conditions. This result agrees with studies suggesting that increased responsibilities, psychosocial stress, and age-related factors among married individuals may contribute to higher disease prevalence [21]. A statistically significant association was observed between the number of children and chronic diseases, particularly among participants with two children, who showed the highest prevalence. This finding is consistent with studies reporting that increasing family responsibilities may negatively affect self-care practices and increase stress levels [22].

Occupation was significantly associated with chronic disease prevalence. Housewives, unemployed, and retired participants showed higher proportions of chronic diseases compared to employed individuals. Similar findings were reported in studies indicating that unemployment and retirement are associated with reduced physical activity, increased stress, and poorer access to health care services [23].

Educational level showed a significant association with most chronic diseases. Participants with lower educational attainment had higher prevalence rates, which agrees with studies demonstrating that low education is associated with limited health literacy, unhealthy lifestyle behaviors, and delayed utilization of health services [24,25].

Lifestyle factors and chronic disease associations

Physical inactivity was significantly prevalent, with nearly half of participants reporting no regular exercise. This finding is consistent with WHO reports identifying physical inactivity as a major contributor to non-communicable diseases worldwide [12,26].

Sleep duration and sleep timing were also important factors. Short sleep duration (<4 hours) was common and has been associated with increased risk of hypertension, diabetes, cardiovascular disease, and psychological stress, as reported in previous studies [27,28].

Smoking showed a strong association with several chronic diseases. Cigarette smoking was the most common form, followed by vaping and shisha, with moderate smoking intensity being predominant. These findings are consistent with regional and international studies linking smoking to respiratory, cardiovascular, and cerebrovascular diseases [29,30]. Alcohol consumption and substance addiction were reported by a small proportion of participants; however, previous studies indicate that even low levels of alcohol and drug use may worsen disease prognosis and increase complications among patients with chronic diseases [31]. High consumption of coffee and tea was common among participants. Excessive caffeine intake has been associated with sleep disturbance, anxiety, and cardiovascular effects in some populations [32]. Prolonged mobile phone use was also prevalent and has been linked to sedentary behavior, sleep disturbance, and increased psychological stress [33]. Psychological stress was frequently reported and showed a strong association with chronic diseases, which is consistent with studies identifying stress as a key

5. Conclusions

This cross-sectional study of 250 adults attending Al-Ramadi hospital demonstrated a high burden of chronic disease within the study sample. Joint disease was the most frequently reported condition (80.0%), followed by cerebrovascular disease (74.0%), cardiovascular disease (70.4%), gastrointestinal disease (66.4%), cancer (64.8%), respiratory disease (60.4%), diabetes mellitus (57.2%), hypertension (56.4%), and renal disease (54.0%). A family history of chronic disease was reported by 80.0% of participants, while associated chronic diseases were present in 33.6%. The high proportion of reported complications further indicates the substantial clinical burden carried by this hospital-based sample.

Chronic disease prevalence varied across several sociodemographic characteristics, including age, body mass index, residence, marital and family characteristics, occupation, and educational level, whereas gender showed less consistent differences. At the same time, modifiable lifestyle risks were common: 49.2% of participants reported no regular physical activity, 70.4% were smokers, 35.6% reported sleeping less than four hours, and psychological stress was frequently reported. These findings show that the observed chronic disease burden occurred alongside a combination of social, demographic, familial, and behavioral factors rather than a single isolated determinant.

The findings support strengthening prevention and early-detection strategies that address smoking, physical inactivity, inadequate sleep, and psychological stress while also considering education, occupation, residence, and family history when planning health services. Targeted screening and health education may be particularly useful for groups with a higher observed disease burden. Because the study used a cross-sectional design and convenience sampling in a hospital setting, the results should be interpreted as associations within the study sample and should not be used to infer causality or precise population prevalence for all adults in Iraq.

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 87-96

The Awareness Study of Thyroid Diseases among Iraqi Patients

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Abstract.
Background: Thyroid diseases are more common worldwide among chronic illnesses, a clinical condition that coexists with anemia. Even though anemia and thyroid problems frequently coexist, it is still unclear what causes the two conditions. Even in the 21st century, very few people really know much about the small thyroid gland located at the back of the neck and its vitally important function. Any dysfunction of the thyroid has profound impact on health and well-bein Iron-deficient anemia adversely affects thyroid hormone status, whereas thyroid hormones directly and indirectly promote the proliferation of erythrocyte precursors through the augmentation of erythropoietin synthesis. As a result, many types of anemia may arise after thyroid failure.
Material and Method: A Descriptive cross-sectional study for awareness of thyroid diseases and anemia was carried out in Al–Ramadi city at the Al–Andalus health center from18th of November to the end of March 2023. Two hundred fifty participants at age 18- >65 years were enrolled in the study. They
were selected by non-probability convenient sampling technique. Data were analyzed using.
Results: The illiterate and secondary were (32%). The knowledge regarding thyroid gland was (30.4%). About (10%) knew that the function of thyroid hormone affects the menstrual cycle in women
Conclusion: Knowledge regarding thyroid gland was fairly worse.
The knowledge of prevention of thyroid disease regarding early thyroid testing can protect you from thyroid disease was unsatisfactory. The awareness about pregnancy and the postpartum period are risk factors for developing thyroid diseases was also fairly unsatisfactory. However, more strategies are needed to increase the knowledge
about the thyroid and thyroid disease.

Keywords: Thyroid diseases; awareness; anemia; prevention; risk factors; Iraqi patients.

1. Introduction

The thyroid gland is butterfly-shaped and the largest endocrine gland in the human body, and it is positioned in the anterior portion of the neck. It produces thyroid hormones, which have a significant impact on protein synthesis and the basal metabolic rate (BMR) (1) (2). Furthermore, these hormones are essential for the neurocognitive development of young children and adolescents, as well as for adults to maintain normal physiological functioning (3).

In the past few years, abnormal thyroid function has become a major problem in clinical practice, raising health issues among patients. Thyroid hormones are essential for growth, neuronal development, reproduction and regulation of energy metabolism. Hypothyroidism and hyperthyroidism are common conditions with potentially devastating health consequences that affect all populations worldwide. Iodine nutrition is a key determinant of thyroid disease risk; however, other factors, such as ageing, smoking status, genetic susceptibility, ethnicity, endocrine disruptors and the advent of novel therapeutics, including immune checkpoint inhibitors, also influence thyroid disease epidemiology. (4). Thyroid illness is caused by a deficiency in iodine or by autoimmune diseases. The thyroid gland plays a significant role in the metabolism and proliferation of blood cells; hematological disorders are frequently observed in patients with thyroid disorders, and the most frequent problem is anemia Thyroid dysfunction is one of the leading endocrine disorders. It represents around 30% to 40% of the patients seen in an endocrine practice. (5) (6).

Thyroid problems are mostly caused by iodine insufficiency. Over 190 million people worldwide have been estimated to suffer from iodine deficiency illnesses, and approximately one-third of all people live in areas that are iodine deficient (7) Thyroid disease is becoming increasingly common worldwide, and females are more likely to be affected than males. The prevalence of thyroid among middle-aged women was 7%. The major cause of (subclinical) hypothyroidism in Europe and is more prevalent in women with idiopathic subfertility and polycystic ovarian syndrome (PCOS) than in fertile women (8) The thyroid nodule is the most common type of the condition, accounting for 19-68% of the general population. It is more frequently seen in women. THYROID NODULES ARE A common clinical problem. Epidemiologic studies have shown the prevalence of palpable thyroid nodules to be approximately 5% in women and 1% in men living in iodine-sufficient parts of the world (9).

Thyroid diseases are one of the most under-diagnosed and neglected medical illnesses, and patients' lack of general awareness may be an important concern. In general, lack of knowledge and comprehension of the thyroid gland and its symptoms will lead patients to go undiagnosed. Some of the factors may be lack of qualified physicians treating such patients, less time spent by doctors for patient education, lack of awareness and use of available electronic media to harness information, poor knowledge about reliable sources of information. Educational needs are much greater in diseased population with poor health literacy. Any dysfunction of the thyroid has profound impact on health and well-being. Thyroid disorders are believed to be a common health issue in India, as it is worldwide. Globally, thyroid disorders continue to be common yet one of the most under- diagnosed and neglected chronic health conditions (10).

Anemia and low thyroid function are common, especially in older individuals. Thyroid function measurements are often included in the clinical examination of anemia. The symptoms of (subclinical) hypothyroidism and anemia are similar, which not only frequently presents a challenging diagnostic problem, but may also point to a causal relationship between low thyroid function and anemia (11). Both hyperthyroidism and hypothyroidism were associated with reduced hemoglobin levels in a population-based cohort in cross-sectional analyses but not in longitudinal analyses (12).

Aim of the study: To study the awareness study of thyroid diseases and anemia

2. Research Methodology

2.1 Study design

A cross-sectional study for awareness of thyroid diseases and anemia among Iraqi patients. This study was carried out in Al – Ramadi city at the Al – Andalus health center. For the period 18th of November to end of March 2023. The study was conducted on women who visit antenatal care. The study was approved by College of Medicine University of Anbar. The sample size was two hundred and fifty, selected by non-probability convenient sampling technique.

2.2 Data collection

After taking informed verbal consent, the specially designed questionnaire was administered through interviewing women with closed ended questions related to the awareness study of thyroid diseases and anemia. The questionnaire was divided into
Three parts: part one was demographic data included:
Gender, age, education, occupation...etc.; part two: Knowledge about thyroid disease. Part three for awareness about the prevention of thyroid disorders.

2.3 Statistical analysis

The statistical analysis was conducted in the form of percentages. The statistical analysis was carried out by using Statistical Analysis System (SAS) version 9.6

3. Results

The study sample is two hundred and fifty participants who were interviewed. They were from Al – Andalus health center. Table 1 shows the gender distribution; the highest frequency were females (76.4%). The age range of participants were ranges from18 to more than 65. The highest proportion were 18 – 40 years old (55.2 %), while the lowest frequency (2.8 %) at the age more than 65 years. The Table also shows education that ranges from the lowest frequency (0.8%) among post graduate
And the illiterate and secondary groups have the same highest frequency (32%).

Occupation wise, the study sample was composed of house wives that representatives (62%) and the employed (14.8%). Regarding marital status, the highest frequency (72%) was married, while the lowest frequency was divorce that representatives (2.4%). About (60.4%) of participants were having one to four number of family member. Table 1 also shows the distribution for participants having thyroid disease, the lowest proportion were hypothyroidism (11.6%) while those who didn't know about thyroid disease (49.6%). About (80.4%) of participants were haven't had thyroid test. About (27.2%) of participants were usage medication to treat thyroid problem. Only (8%) of participants have family history of throid disease

Variables	No.	%
Sex		
Male	59	23.6
Female	191	76.4
Age (years)		
(18 – 40)	138	55.2
(41 – 65)	105	42
(>65)	7	2.8
Education		
Illiterate	80	32
Primary	72	28.8
Secondary	80	32
University	16	6.4
Postgraduate	2	0.8
Occupation		
Housewife	155	62
Employee	37	14.8
Student	58	23.2
Married status		
Married	180	72
Single	64	25.6
Divorce	6	2.4
Number of family members		
(1 – 4)	151	60.4
(5 – 8)	95	38
(> 8)	4	1.6
You have thyroid disease?		
No	67	26.8
Hypothyroidism	29	11.6
Hyperthyroidism	30	12
I don't know	124	49.6
Have you had thyroid tests?		
Yes	49	19.6
No	201	80.4
Do you use medications to treat thyroid problems?		
Yes	68	27.2
No	182	72.8
You have a family history of thyroid disease?		
Yes	20	8
No	101	40.4
I don't Know	129	51.6

Table 1: The distribution of socio-demographic characteristics of the study sample

From two hundred and fifty participants interviewed, (30.4%) responded that they have heard of thyroid disease and (58.4%) didn't hear of the function of thyroid hormone affects the menstrual cycle in women. While those who knew the function of thyroid hormone affects the menstrual cycle in women have the lowest frequency (10%) as shown in Table 2.

Table 2 also shows the knowledge of hyperthyroidism, most of participants (70%) did not hear about hyperthyroidism. Only (9.6%) knew hyperthyroidism and (20.4%) didn't knew there. knowledge of hypothyroidism, most of participants (70%) did not hear about hypothyroidism. Only (8%) knew hypothyroidism and (21.6%) didn't knew there. Less than three quarter of participants (71.6%) didn't hear about unstop treatment of hypothyroidism when thyroid test is normally. More than half (66.8%) didn't hear about unstop treatment for thyroid disease when test are normal.

The participants who didn't hear that women with hypothyroidism get pregnant (57.6%), and those who knew the women with hypothyroidism can get pregnant (1.6 %). Almost (41.6%) did not know the euthyroid.

More than half (56%) didn't hear that the thyroid gland is an endocrine gland. About (68.8%), didn't hear that thyroid dysfunction affected brain. Only (2%) knew that thyroid dysfunction affects cholesterol levels and (42%) didn't knew them. More than half (55.2%) didn't hear that thyroid dysfunction affects heart disease, while less than half (42%) didn't knew that thyroid dysfunction affects heart disease. The participants who knew that exercise affects thyroid dysfunction have constituted (4%) and those who didn't hear that exercise affect thyroid function. About (6.8%) knew that iodized salt has a role in treating hypothyroidism, and (8%) know that iodine helps secrete thyroxine. Less than half (49.2%) Did not hear that the cause of thyroid disease can be hereditary, while (6%) knew them as shown in table 2.

Table 2 also shows that (10%) of study sample who were know weight gain is a symptom of hypothyroidism, (38.4%) did not know, while (51.6%) had not hear. The knowledge for presence of a lump in the neck could be a sign of thyroid disease or thyroid cancer (10%), while difficulty swallowing may be a sign of thyroid cancer (6.8%). More than half (52%) did not hear that skin changes, nails or hair loss could be signs of thyroid disease, and only (2%) knew them. Most of participants (56%) did not hear that stomach pain could be a symptom of thyroid disease. The knowledge for Feeling hot, losing weight despite a good appetite, insomnia, and lack of sleep are symptoms of hyperthyroidism that (2%). Almost (7.6%) knew that increased heart rate symptoms of hyperthyroidism, the knowledge of interruption of the menstrual cycle is a symptom of hyperthyroidism (3.2%). Only (6.8%, 2%) knew hyperthyroidism and hypothyroidism are accompanied by anemia respectively. More than half (56%) did not hear that feeling cold is a symptom of hypothyroidism, while (41.6%) did not know them. About (6%) of participants knew that miscarriage is a symptom of hypothyroidism, while (41.6%) did not know. Almost (2%) knew that bulging eyes could be a sign of thyroid disease. Medical heath center considered the major source of knowledge of thyroid disease (46.8%), while the lowest source included learning (3.2%) as shown in Table 2.

Table 2: The distribution of knowledge about thyroid diseases among study sample

Variable	No.	%
Have you heard of the thyroid gland?		
I know	76	30.4
I don't know	62	24.8
I don't hear	112	44.8
Did you know that the function of thyroid hormone affects the menstrual cycle in women?		
I know	25	10
I don't know	79	31.6
I don't hear	146	58.4
Do you know what hyperthyroidism is?		
I know	24	9.6
I don't know	51	20.4
I don't hear	175	70

Do you know what hypothyroidism is?		
I know	20	8
I don't know	54	21.6
I don't hear	175	70
Can you stop treating hypothyroidism when the tests are normal?		
I know	3	1.2
I don't know	68	27.2
I don't hear	179	71.6
Can you stop treating your thyroid when tests are normal?		
I know	6	2.4
I don't know	77	30.8
I don't hear	167	66.8
Can women with hypothyroidism get pregnant normally?		
I know	4	1.6
I don't know	102	40.8
I don't hear	144	57.6
Do you know what the euthyroid activity?		
I know	46	18.4
I don't know	104	41.6
I don't hear	100	40
Did you know that the thyroid gland is an endocrine gland?		
I know	5	2
I don't know	105	42
I don't hear	140	56
Did you know that thyroid dysfunction affects the brain?		
I know	15	6
I don't know	63	25.2
I don't hear	172	68.8
Did you know that thyroid dysfunction affects cholesterol levels?		
I know	5	2
I don't know	105	42
I don't hear	140	56
Did you know that thyroid dysfunction affects heart disease?		
I know	7	2.8
I don't know	105	42
I don't hear	138	55.2
Did you know that exercise affects thyroid dysfunction?		
I know	10	4
I don't know	105	42
I don't hear	135	54
Does iodized salt have a role in treating hypothyroidism?		
I know	17	6.8
I don't know	105	42
I don't hear	128	51.2
Did you know that iodine helps secrete the hormone thyroxine?		
I know	20	8
I don't know	104	41.6
I don't hear	126	50.4
Did you know that the cause of thyroid disease can be hereditary?		
I know	15	6
I don't know	112	44.8
I don't hear	123	49.2
Did you know that weight gain is a symptom of hypothyroidism?		
I know	25	10
I don't know	96	38.4
I don't hear	129	51.6
Do you think that a lump in the neck could be a sign of thyroid disease or thyroid cancer?		
I know	25	10
I don't know	96	38.4
I don't hear	129	51.6

Did you know that persistent difficulty swallowing may be a sign of thyroid cancer?		
I know	17	6.8
I don't know	104	41.6
I don't hear	129	51.6
Do you think that skin changes, nails or hair loss could be signs of thyroid disease?		
I know		
I don't know	5	2
I don't hear	115	46
	130	52
Do you believe that stomach pain could be a symptom of thyroid disease?		
I know	5	2
I don't know	105	42
I don't hear	140	56
Did you know that feeling hot and losing weight despite a good appetite is a symptom of hyperthyroidism?		
I know	5	2
I don't know	106	42.4
I don't hear	139	55.6
Did you know that insomnia and lack of sleep are symptoms of hyperthyroidism?		
I know		
I don't know	5	2
I don't hear	108	43.2
	137	54.8
Did you know that increased heart rate is a symptom of hyperthyroidism?		
I know		
I don't know	19	7.6
I don't hear	115	46
	116	46.4
Did you know that interruption of the menstrual cycle is a symptom of hyperthyroidism?		
I know	8	3.2
I don't know	110	44
I don't hear	132	52.8
Did you know that hyperthyroidism is accompanied by anemia?		
I know	17	6.8
I don't know	113	45.2
I don't hear	120	48
Did you know that hypothyroidism is accompanied by anemia?		
I know	5	2
I don't know	127	50.8
I don't hear	123	49.2
Did you know that feeling cold is a symptom of hypothyroidism?		
I know	16	6.4
I don't know	94	37.6
I don't hear	140	56
You know that miscarriage is a symptom of hypothyroidism?		
I know	15	6
I don't know	104	41.6
I don't hear	131	52.4
You think that bulging eyes could be a sign of thyroid disease?		
I know	5	2
I don't know	105	42
I don't hear	140	56
Sources of knowledge		
TV	30	12
Social media	45	18
Friends	50	20
Medical health center	117	46.8
Learning	8	3.2

Regarding the awareness about the prevention of thyroid disorders, that a balanced diet protects you from thyroid disease, don't hear by 130 (52%). 35 (14%) of participants knew that early thyroid testing and avoiding smoking and stimulants protected them from thyroid demands as shown in Table 3.

Table 3: Awareness regarding preventive behaviors of participants

Variable	No.	%
Did you know that a balanced diet protects you from thyroid disease?		
I know	16	6.4
I don't know	104	41.6
I don't heard	130	52
Did you know that early thyroid testing can protect you from thyroid disease?		
I know		
I don't know	35	14
I don't heard	77	30.8
	138	55.2
Did you know that reducing foods containing soy protein protects you from thyroid demands?		
I know		
I don't know	5	2
I don't heard	77	30.8
	168	67.2
Did you know that avoiding large amounts of iodine in foods and medications protects you from thyroid demands?		
I know		
I don't know	10	4
I don't heard	85	34
	155	62
Did you know that avoiding smoking and stimulants protects you from thyroid disorders?		
I know		
I don't know	35	14
I don't heard	87	34.8
	128	51.2

Awareness about the risk factors for thyroid disorders 125 (50%), 130 (52%), 120 (48%), and 104 (41.6%) of individuals don't hear about smoking & women, exposure to radiation, insufficient or excess iodine intake, pregnancy, or the postpartum period, respectively as shown in Table 4.

Table 4: Displays the participants of their risk factor.

Variable	No.	%
Do you think that smoking is a risk factor for thyroid disease?		
I know	35	14
I don't know	90	36
I don't heard	125	50
Do you believe that exposure to radiation is a risk factor for developing thyroid diseases?		
I know		
I don't know	15	6
I don't heard	105	42
	130	52
Do you think that insufficient or excess iodine intake is a risk factor for thyroid disease?		
I know		
I don't know	20	8
I don't heard	110	44
	120	48
Do you think that women are more susceptible to thyroid diseases?		
I know	20	8
I don't know	105	42
I don't heard	125	50
Do you think that pregnancy and the postpartum period are risk factors for developing thyroid diseases?		
I know		
I don't know	41	16.4
I don't heard	105	42
	104	41.6

4. Discussion

The current study has focused on the awareness study of thyroid diseases and anemia. The findings of this study revealed a highest proportion at age 18- 40 years old (55.2%), this result higher than that reported by other studies (13). Most participants were female, this finding agree with (13).

The present study showed that (44.8%) of participants don't hear the thyroid gland, less than (1.6%) know women with hypothyroidism can get pregnant normally, according to a study done in Delhi that shows similar results to this study (13) but in a study by Singh *et al*., 60% patients knew the correct meaning of the term "thyroid." (14) .

Weight gain is a symptom of hypothyroidism but don't know by 38.4%; the presence of a lump in the neck could be a sign of thyroid disease or thyroid cancer; and difficulty swallowing may be a sign of thyroid cancer (51.6%) of individuals don't hear that and that similar study in Saudi Arabia that found (39.4%) had no idea about thyroid cancer (15).

The current study demonstrates that there is no significant difference between genders in the level of awareness regarding thyroid diseases and that, similar to the study by Almuzaini A et al., there is no significant difference in knowledge levels among genders in the level of knowledge (16).

Knowledge the risk factors associated with thyroid diseases is an essential aspect, smoking is thought to increase the risk of thyroid disorders in a variety of physiological and pathological contexts (17).

According to the present study, just 8% of respondents thought that being female increased the possibility of developing thyroid disease, that's acceptable, according to a study done in Saudi Arabia, 21% of participants strongly agreed that having a female partner is a risk factor. These findings suggest that most people are unaware of this, which could delay getting a diagnosis (18).

Past medical history and past family history were both significantly correlated with better knowledge regarding thyroid disorders. This study shows that 8% thought that past family history was correlated with thyroid dysfunction, and only about 8.8% thought that thyroid dysfunction was associated with anemia. This corresponds with what Canaris et al. described in their article, which showed that a higher prevalence of thyroid diseases is associated with an increase in the level of knowledge about the manifestation of these conditions and a higher willingness to participate in thyroid screening efforts (19).

Consistent with the present findings, that smoking is a risk factor for thyroid disease (14%). This result disagree with other study (20). The findings of exposure to radiation is a risk factor for developing thyroid diseases revealed a lowest proportion (6%), this result lowest than that reported by (20).

5. Conclusion

- The study revealed that the knowledge of participants regarding heard of thyroid disease is fairly worse 30 .4%.
- The knowledge of prevention of thyroid disease regarding early thyroid testing can protect you from thyroid disease was unsatisfactory as 14%.
- The awareness about pregnancy and the postpartum period are risk factors for developing thyroid diseases was also fairly unsatisfactory as 16.4%.
- Most participants had not heard of either hyperthyroidism or hypothyroidism (70% for each), indicating limited awareness of the main thyroid disorders.
- Awareness of the relationship between thyroid dysfunction and anemia was very low: only 6.8% recognized an association with hyperthyroidism and 2% with hypothyroidism.
- Most participants (80.4%) had never undergone thyroid testing, while medical health centers were the leading source of information about thyroid disease (46.8%).

6. Recommendations

- Further studies are needed including follow up studies for non-heard of thyroid disease.
- Government involvement in fornication of food like our, salt with iodine is the need of the day.
- There is a need for a public health education initiative

And more strategies to increase the awareness of thyroid disease.

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 97-103

Maternal-Infant Bonding in the Postpartum Period

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Abstract
Background: One of the most important predictors of a child's emotional, cognitive, and social development is the relationship between mother and child during the postnatal period; any disruptions in this process may cause long-term bonding issues for both the mother and their child.
Aim: To determine the level of maternal-infant bonding among Iraqi postpartum mothers and to look at the correlations between bonding and specific demographic characteristics such as age, education, economic position, and birth order.
Method: Study was a descriptive cross-sectional, which carried out at Bint Al-Huda Teaching Hospital in Nasiriyah, Iraq, extended from July to December 2025. The Postpartum Bonding Questionnaire (PBQ), translated into Arabic, was administered to the carefully chosen sample of 108 postpartum women in Nasiriyah. SPSS version 26 was used to analyze the data by using descriptive and inferential statistics.
Results: The study's findings which related to mothers' bonding, showed that 74.1% had excellent, 22.2% were moderate, and 3.7% of mothers had low attachment. In addition, the economic status (P = 0.04), maternal age (P=0.02), the child's birth order (P = 0.01), and were all statistically substantially correlated with bonding levels. There were no significant correlations with residence or education.
Conclusion: The current study referred that the level of Maternal-Infant Bonding was generally adequate, a small percentage of mothers exhibited low levels of this bond, attributed to young age, first birth, and economic status. These findings suggest the need for early screening and tailored psychological interventions in postpartum care to maintain healthy mother-infant bonds and prevent any potential problems in this bond.

Keywords: Infant, Bonding, Nursing, Maternity, Postpartum, Pediatric.

1. Introduction

The mother and infant's bond developed throughout the postpartum phase is arguably one of the most crucial foundations of early-age child development. The mother-infant bond is one part of the more general attachment system. It forms early within hours or days after delivery and helps the infant securely attach, manage emotions, and develop both socially and cognitively (Liu et al., 2023; Keller and Kärtner, 2021). During this sensitive period, the elevation of the maternal hormone oxytocin triggers soothing and calming behaviors such as gentle stroking and soft vocalizations, which strengthen the mother's and infant's emotional attachment (Scatliffe et al., 2022; Vittner and McGrath, 2021).

Furthermore, the bond between mother and infant influences the infant's development as well as the mother's psychological health. Emotional connection between the mother and child improves the intimacy and sensitivity of the mother's caregiving (Dubber et al., 2022; Prevatt and Desmarais, 2023). On the other hand, bonding disruptions due to trauma experienced during birth, postpartum mental health issues, hormonal dysregulations, or social support deficits can lead to postpartum bonding disorder. The mother may in these cases feel overwhelmed, emotionally and

socially distant, which deteriorates the quality of caregiving and infant development (Wang et al., 2022; Pilkington et al., 2021).

The advanced technique in neuro-imaging have showed the brain regions involved in maternal attachment such as the amygdala, prefrontal cortex, and striatum. These findings suggest that maternal-infant attachment is not simply a behavioral-emotional process but is also neurobiological and influenced by intrinsic and extrinsic factors (Feldman et al., 2022; Swain and Ho, 2021). Understanding these neural pathways could offer valuable insight into the enormous effect of maternal bonding on the mother's and child's brain development.

Maternal-infant bonding does not have the same quality in all societies and even differs within the same society depending on healthcare access. Iraq and many other low- to middle-income countries (LMICs) have restricted or unequal access to perinatal care. Mental health stigma, culture, and barriers to healthcare all shape the maternal experience (Ahmed and Al-Jawadi, 2023; Al-Hamad and Al-Nashmi, 2021). Understanding these barriers is vital for formatting the bonding and maternal mental health-focused interventions that are sensitive to the culture.

2. Methodology

2.1 Study Design

A descriptive cross-sectional approach used in the current study to assess postpartum maternal-infant bonding and evaluate the presence of bonding disorders. Data collection was conducted at Bint Al-Huda Teaching Hospital in Al-Nasiriyah City, Iraq, during the period extended from 1st July to 31st December 2025. This extended duration allowed for a more varied sample and reduced the potential for bias, thus enhancing the generalizability of the outcomes.

2.2 Study Sample

The researchers used to select 108 postpartum mothers, a non-probability purposive sampling technique. Participants were included according to the following criteria:

- Mothers who had recently given birth (within 6 weeks postpartum).
- Mothers who consented to participate voluntarily.

Study Instrument

The main tool used in this study was a structured, then Arabic-language questionnaire developed after an extensive literature review. The questionnaire consisted of two parts:
Part I: The demographic characteristics include (e.g., age, education, economic status, number and order of children, residence).
Part II: Items which used to assess the bonding between mothers and their infants were adopted from the Postpartum Bonding Questionnaire (PBQ) by Brockington et al. (2006), adapted and validated for the current study.
The scoring items was interpreted as follows:
Low bonding: Mean score less than 1.66.
Modest bonding: Mean score between 1.67 and 2.33.
High bonding: Mean score more or equal to 2.34.

Pilot Study:

To evaluate the feasibility, clarity, timing, and reliability of the questionnaire, ten individuals were included in a pilot study. Some modifications were made to ensure content adequacy and ease of administration, and based on the results and expert feedback.

Validity and Reliability:

A committee from the College of Nursing at Thi-Qar University, consist of seven specialists, was evaluating the content to determine its validity. Cronbach's alpha coefficient was used to confirm the instrument's reliability, the result was a coefficient of 0.87, representative a strong internal consistency.

Data Collection Procedure:

The data was collected via face-to-face interviews directed by the researcher. Each interview taken approximately 20-25 minutes in each interview, and written consent was obtained from all participants. The researcher clarified the study's objective and assured participants of data confidentiality before distributing the questionnaire.

Statistical Analysis

By using SPSS version 26, the data were analyzed. The analysis included:
Descriptive statistics: mean, standard deviation, frequencies, and percentages.

Inferential statistics:

To examine associations between bonding levels and nominal demographic variables; the Chi-square test was used.
To compare bonding scores amongst more than two groups, ANOVA test was used.
While the independent t-test used to assess differences between two independent groups.

Ethical Consideration:

This work received ethical approval from the Research Ethics Committee at Middle Euphrates Technical University (Ref. No. AATUCMHTLAB-June. 6225). After participants were fully informed of the study's objectives and methodology and their right to withdraw at any time without affecting their care, each participant provided written informed consent. Privacy and confidentiality were maintained throughout the study.
Study Results:

Table 1: Participants' Demographic Characteristics

Variables	Category	Frequency	Percentage
Age Group	17 - 22 years	28	25.9
	23 - 28 years	42	38.9
	29 - 34 years	26	24.1
	35 years and above	12	11.1
	Mean ± SD: 26.46 ± 4.76		
	Range: 17 - 43 years		
Education Level	Not reading & writing	15	13.9
	Primary school	37	34.3
	High school	39	36.1
	College / University	17	15.7
Residence	Urban	84	77.8
	Rural	24	22.2
Economic Status	Not enough	12	11.1
	Enough	57	52.8
	Very enough	39	36.1
Birth Order of Child	First	36	33.3
	Second	32	29.6
	Third	21	19.4
	Fourth or more	19	17.6

The table overhead shows the demographic characteristics of the participating mothers. where 38.9% of them were 23–28 years old, with the mean age of 26.46 years (standard deviation = 4.76), and ages arranged between 17 and 43 years. 77.8% of the sample lived in urban areas, and 70.4% had completed at least one primary or secondary education. The majority of the participants were of the economic status of "acceptable" (52.8%). For birth order, the first-time mothers had the largest percent by birth order (33.3%).

Table 2: Overall Bonding Level Distribution

Bonding Level	Frequency	Percentage	Mean	SD	Result
Low	4	3.7%	2.52	0.28	High
Moderate	24	22.2%			(Overall)
High	80	74.1%			
Total	108	100%			

The table presents all classifications of bonding levels. The high bonding levels with children were demonstrated by the majority of participants (74.1%), while the moderate bonding was 22.2%, and 3.7% showed low bonding levels. In general, this distribution indicates a strong mother-child bond, while approving that a minority may experience bonding difficulties.

Table 3: The relation between Bonding Level and all Demographic Variables:

Variables	Chi-Square Value	df	Asymptotic Significance (2-sided)
Age Group	32.45	18	0.02
Education Level	15.78	15	0.47
Economic Status	20.34	12	0.04
Birth Order of Child	28.67	15	0.01
Residence	8.92	6	0.18

This table shows the results of the chi-square test, which explores the relationships between levels of correlation and demographic variables. Significant associations were found for the age group ($P = 0.02$), economic status ($P = 0.04$), and birth order of child ($P = 0.01$). This indicates that the younger mothers, with difficulties in economic status, and mothers with a first-born child were more likely to experience bonding challenges. In addition, there is no significant association between the other demographic variables and bonding levels.

3. Discussion

The current study aimed to assess the levels of bonding between mothers and their children, as well as the relations between demographic factors and the bonding quality. The results highlighted the pivotal role of maternal age, socio-economic status, and birth order in forming emotional bonds.

In alignment with previous studies, the maternal age was significantly associated with the correlation ($P=0.02$); particularly, those younger mothers aged 17-22 years appeared to have greater difficulty building close emotional relationships with their children. This may be related to the lower emotional maturity and higher stress levels, in addition to a few life experiences that can interfere with emotional availability and supple caregiving. often more emotionally mature and financially secure, founded in the older mothers, may hold caregiving skills and emotional self-regulation, which reflecting in facilitating a stronger connection with their infants (Aas and Nordheim, 2021; Bozzatello et al., 2021).

Remarkably, the mothers' educational level did not show a statistically significant relation to mother-child bonding ($p < 0.05$). While higher education may provide mothers with better problem-solving skills and the ability to search for information about child growth and development, the findings suggest that bonding is fundamentally an innate emotional process which is not enhanced by formal education as automatically. This finding is supported by a study which demonstrated that educational attainment alone is a weaker determinant of attachment than emotional availability and inherent nurturing behaviors (Harding and Paulsell, 2020; Tichelman et al., 2022).

The significant connotation between economic status and bonding ($P = 0.04$), was one of the most important findings of this study. The mothers who reported "not enough" for their economic status were more probable to exhibit moderate or low bonding levels. This supports by a body of literature which indicate that financial difficulties can cause a chronic stress, anxiety, and depression, which in turn reduce a mother's emotional abilities and responsiveness in caregiving (Giallo et al., 2020; Kim and Bianco, 2021). Economic stress may shift a mother's focus towards meeting basic survival needs, causing less emotional energy to nurture the subtle mother-infant's bond.

In the line of bonding, the study found a strong connection between the child's birth order and bonding (P = 0.01), where the mothers displayed a higher tendency to build a strong bond emotionally with their firstborn child. This is because first-time mothers can give over-focused and intentional attention to their first child. the mother's attention and caregiving become distributed with subsequent children, which may lead to a qualitatively different form of attachment that may be not necessarily insecure. This concurs with Baranowska and Tataj-Puzyna (2021), who found that first-time mothers experience a lot of emotional intense issues, novelty-oriented by bonding process.

Finally, the current study institute no significant association between urban vs. rural residence and bonding (p = 0.18). This result proposes that in the specific socio-cultural line of this sample, other factors, such as access to healthcare in cities or family support centers in rural areas, might counterbalance one another. It promotes the idea that proximal factors like maternal mental health and caregiving behavior are more predictive of attachment than location-based on geographic alone (Hussein et al., 2022; Li and Liu, 2020).

The distribution of attachment levels (74.1% high, 22.2% medium, and 3.7% low) is encouraging, indicating that the majority of mothers are able to establish stable attachment bonds. Nevertheless, there is a clinical significance when identifying the group with moderate attachment and a small group with low attachment. Those mothers, particularly young mothers from low socio-economic backgrounds or first-time mothers, may benefit from aimed interventions. Medium attachment may represent a suboptimal emotional connection that can be, with appropriate support, strengthened, potentially preventing the long-term relational difficulties (Tichelman et al., 2022).

4. Limitations of the Current Study

There are some restrictions to be aware of, while the study provides valuable insights into maternal-infant bonding in this population. At first, bonding is taken at a single moment in time through the cross-sectional design, causing a reduced ability to understand how bonding develops or changes through the postpartum period. Second, the results may not be applicable to all Iraqi postpartum women due to the sample technique which was used from a single hospital in Al-Nasiriyah. Third, data collection was dependent on a self-report questionnaire (PBQ), This could cause bias due to social desirability, where mothers might over-report positive bonding experiences. Fourth, the factors related to the mother's mental health status (such as depression and anxiety) and other variables which may have significantly influenced the study were not assessed, nor was the quality level of the marital rapport or the availability of social care, which mostly influences bonding. Future research is useful to employ longitudinal designs, include multiple sites across Iraq, and incorporate objective measures and assessments of mental health to build upon this study's results.

5. Nursing Role

The nurses considered as the first line of support for evaluating, fostering, and nourishing mother-child attachment, particularly in the delicate postpartum phase. Nurses are ideally positioned to conduct initial assessments using reliable tools like the PBQ (Brockington et al., 2006). In addition, it's crucial for nurses to educate mothers about typical infant behaviors and the ways to encourage responsive caregiving, which is essential to fostering secure attachment (Tichelman et al., 2022). The nurses as well provide essential emotional support, offering a compassionate care and counselling to relieve maternal worries and enhancing comfort. Furthermore, the nurses help the child bonding by connecting families with further resources, such as lactation consultants, social workers, or mental health services, ensuring a comprehensive support system (Dubber et al., 2022). At last, nurses act as guardians, which can accessible through careful assessment, skilled intervention, and a caring presence, helping to preserve the vital bond between mother and infant, in addition to supporting the children develop mentally and emotionally.

6. Conclusion

The current study proves the complex nature of the normal mother-infant bonding during the postpartum period. The findings showed that although the majority (74.1%) of participants mothers developed a good bond with their newborns, a sizeable minority (22.2% moderate, 3.7% low) struggle with bonding. Definitely, younger maternal age (P = 0.02), economic level difficulties (P = 0.04), and having a first-born child (P = 0.01) were identified as vital risk

factors associated with lower bonding levels. In addition, the maternal education and urban/rural residence did not show a significant correlation. These results underscore the need for targeted, culturally sensitive interventions. Healthcare providers should prioritize early screening for bonding difficulties, particularly for mothers who exhibit recognized risk factors. The supporting young mothers, reducing the economic stress through the convenient social support referrals, and providing extra guidance to first-time mothers, were the effective strategies to promote secure attachment and foster healthy emotional, social, and cognitive development in children.

7. Recommendations

Considering the results of the current study, which carried out in Iraq, the PBQ tool can be used for maternal-infant bonding to routinely examine young, primipara, and economically disadvantaged moms. Also, the support programmers should be initiated by healthcare centers and nursing counselling services as supported ways that enhance maternal-infant attachment. Training courses in bonding assessment and intervention should be included in nursing education. Postnatal care facilities should be provided by policymakers and funds invested in the support of maternal mental health. In addition, for future research the longitudinal study designs and an examination of the underlying psychological and cultural mechanisms influencing bonding are required.

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 105-109

Factors Affecting Young Medical Students' Choice for Future Medical Specialty

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Abstract

Background: Young doctors' choice of medical specialty is essential to maintaining a sufficient health workforce and developing a sustainable healthcare system.
Aim: The aim of this article is to explore the factors taken into consideration by young medical students in choosing their future specialty.
Materials and Methods: A cross-sectional study was conducted, for the period from 1st of September to the 31st of December /2025. The study sample were (176) medical students, (47), males and (129) females, selected randomly from the 1st, 2nd, 3rd, 4th, 5th and 6th grades of Diyala Medical College. A questionnaire was designed by the researchers included personal, educational level' variables in regard to the medical students in addition to socio-demographic variables related to the students' family. The students were asked to complete the questionnaire following the explanation of the subject and objectives of the research with each grade.
Results: Reveled that highest preference rate among males' undergraduates for Surgery (31.3%), followed by internal Medicine and Pediatrics (14.8% & 10.8%) respectively. While for females the choice for Obstetrics & Gynecology following surgery choice. There is choice' variation according to the grade. The results also revealed a relation between personal interest, social and economic factors with the choice of medical specialty.
Conclusion & Recommendations: The overall two preferred specialties were general surgery (27%), internal medicine, ophthalmology and pediatrics (8.3%). While the choice among female it was surgery, gyne and obstetrics. Deficient choice for Family and Community Medicine, this is against the term of "Tomorrow Doctor" in its emphasis on Primary health care, Family doctors and Public Health. The study recommends Career counseling should be offered to students about each specialty's challenges, /opportunities with an ultimate goal to match the country-specific demand and supply of physicians. Another points focusing on spreading the importance of the principles of primary health care, family doctor and public health to be choices for future specialty, as these are the pillars of tomorrow's doctor.

Keywords: Medical students, Undergraduates, Medical Specialties, Diyala Medical College.

1. Introduction

After graduating from medical school, medical students have to decide a career path to pursue for further specialization (1). The future career specialties chosen by medical students and the factors involved in making these choices are of great significance for balanced distribution of doctors in the different specialties and for planning of the workforce of healthcare services (1, 2). Clinical specialties were statistically rated higher than basic medical sciences specialties

(3). Therefore, there is strong evidence that students decide their future medical career during or even before medical school [4, 5]. Their choice, however, can change during the course of medical school [6-7]. Many factors influence the medical students' choice of future career [7-8]. Medical students' choices have evolved throughout time, with a growing interest in quality of life or in technological excellence (9, 10). Generally, these factors are classified into two groups; intrinsic factors, for example those related to personal attributes, interest and preferences, and extrinsic factors like those related to work environment social or economic effect (11). Although studies conducted around the world have attempted to identify the factors that affect medical students' choice of specialty, data is scarce on the real and deep factors that influence the choice of specialty (12). The impact of these factors varies from setting to setting. In United Kingdom, other peoples' perception of the job and fewer practice hours were considered the most important factors by students, whereas in Turkey, financials and prestige were more important [13, 14). Gender is another important factor that influences career choice (12, 15, 16). Specialty choice for their future medical practice (12, 15, 16). On the other hand, students in Jordan considered the intellectual content of the specialty and the individual's competencies most influential while a personal interest in the specialty was the deriving factor for students in Kingdom of Saudi Arabia, UAE, Lebanan, Kuwit, Australia, Hong Kong, Rawanda Korea and India, (2,3, 5, 6, 9, 10, 16 , 17 & 23). This study aims to determine the factors that influence medical student' choice of future medical specialty.

2. Persons & Method

Cross sectional study was conducted for the period from 1st September to the 31st of December. 2025. The study sample were medical student from Diyala Medical College. Questionnaire was designed by researcher sent to (176) medical student to collect data for the current study. The questionnaire covered demographic characteristics, specialty preferences, and factors that influenced career preferences. Demographic characteristics included the student's age, gender, grade, and residency. Students were asked to select the most preferred career preference. Specialties listed were surgery, obstetrics & gynecology, pediatrics, internal medicine, ophthalmology, dermatology, radiology, otolaryngology (ENT), and other specialties. The questionnaire influences on specialty preferences were indicated in order to assess the factors to which students attach importance when choosing their first specialty preferences. Responses to the influences were categorized to interesting subject, intellectual challenge, good income, prestige and good social life, advice from faculty practicing physician, parents or friends.

2.1 Inclusion and Exclusion Criteria

The study had to focus on medical students after their selection year and before their graduation, to ask about the detail the choice of specialty of each student, and look for factors influencing their choice. Juniors and Specialist's physicians excluded from this study.

3. Result

Table 1: Frequency of specialization preference between males and females Medical students

Medical Specialty	Males No. (%)	Females No. (%)	Total No. (%)
-Pediatric	3 (15,78%)	16 (84,21%)	19 11%
-Internal medicine	9 (34,61%)	17 (65,38%)	26 15%
Surgery	19 (34,54%)	36 (65,45%)	55 31%
Radiology	1 (9,09%)	10 (90,90%)	11 6%
Gynecology & Obstetrics	0 (0%)	18 (100%)	18 10%
Dermatology	5 (27,77%)	13 (72,22%)	18 10%
-ENT	2 (10%)	0 (0%)	2 1%
Ophthalmology	4 (33,33%)	8 (66,66%)	12 7%
- Others	4 (26,66%)	11 (73,33%)	15 9%

Table 2: Frequency distribution of medical specialists' preference among medical students according to the grade

Medical Specialty	1st grade No. (%)	2nd grade No. (%)	3rd grade No. (%)	4th grade No. (%)	5th grade No. (%)	6th grade No. (%)
1-pediatric	5,26%	21,05%	21,05%	31,57%	10,52%	(10,52%
2-internal medicin	0%	86,92%	23,07%	3,84%	19,23%	26,92%
3-surgery	23,72%	21,81%	14,54%	9,09%	10,95	10,9%

4-Radiology	9,09%	9,09%	18,18%	18,18%	36,36%	0%
5-Gyncoylogy	27,77%	0%	0%	22,2%	16,66%	33,33%
6-Dermatology	11,11%	16,66%	22,22%	22,22%	16,66%	11,11%
7-ENT	0%	0%	0%	50%	50%	0%
8-opthalmology	25%	16,66%	16,66%	25%	8,33%	8,33%
9- other	13,33	0%	20%	13,33%	20%	33,3%

Table 3: Frequency distribution of the factors affecting choice of medical specialty among Medical students according to gender

Factors affecting choice	Males No. (%)	Females No. (%)	Total No. (%)
-Interesting subject & personal interest	33 (21,29%)	122 (78,70%)	155 (88%)
-Good in come, & work-life balance	4 (80%)	1 (20%)	5 (3%)
-Prestige and social life requirements	14 (87,5%)	2 (12,5%)	16 (9%)
Total	51 (29 %)	125 (71%)	176 (100%)

4. Discussions

Career preferences made by medical students and factors influencing these preferences are of importance to medical workforce planners especially in times of over-supply or under-supply of doctors. Surgery, internal medicine, pediatrics, and obstetrics and gynecology were the most specialty preferred preferences among medical students at Diyala medical college. Factors influencing students' choices were studied depending on each discipline. The most frequent factor was their real interest in that specialty. Prestige and social life requirements second affecting factor followed by income factor and work-life balance. Clinical specialties more than basic medical sciences specialties as specialty preferences. Personal interest and an anticipated higher income were other influential factors contributing to specialty preference. (1) Saudi medical students and interns at the University of Dammam were influenced mostly by lifestyle when they considered their future specialty. Internal medicine, family medicine, general surgery, pediatrics, and emergency medicine were the preferred specialties. (3, 4).

Table (1) show the variation of specialties among student about 3(15,78%) of male chose pediatric but in female about 16(84,21%). Some of student chose internal medicine about 9(34,61%) of male and in female about 17(65,38%). While about 19(34,54%) of male and36(65,45%) of female chose the surgery. But some student chose radiology about 1(9,09%) of male and 10(90,90%) of female. About (0%) of male and 18(100) of female chose gynecology & Obestetrics. While some student about 5(27,77%) of male and 13(72,22%) of female chose the dermatology. About 2(100%) of male and 0(0%) of female chose ENT. While some student about 4(33,33%) of male and 8(66,66%) of female chose Opthalmology. Furthermore, some student chose other specialties about 4(26,66%) of male and 11(73,33%) of female.

Not only thoughtful individual factors such as interest for the discipline, work-life balance or role modeling can influence the specialization choice, but also sociobiological aspects. For example, gender can modify factors associated with the choice of several medical specialties: in the US, men-to-female ratio was 4.9:1 in obstetrics and gynecology [6], 24.6% of last-year male medical students would chose internal medicine compared with 11.8% female in Rwanda [7], or in Korea, where 2.9% of male would be interested in pediatrics for 10.7% of woman [8]. Men are more interested by technical challenges, salary, career and prestige, women by time related aspects and societal orientation [9], [10], [11]. Gender influences an important variable, A shift in the gender ratio in medical students have occurred at the end of the 20th century [12] from a male to a female over-representation, leading to difficulties for policymakers to adapt incentives, learning programs, schemes for managing physicians' flows and the specialization's issue. If many papers have investigated the gender imbalance among physicians [6], [7], [8],

Table (2) Show frequency of specialization preference among grade of students. First grade about 1(5,26%), Second grade about 4(21,05%), Third grade about 4(21,05%), Fourth grade about 6(%), Fifth grade about 2(10,52%), And

sixth stage about 2(10,52%) chose pediatric. Other chose Internal medicine about 0(0%) of first grade,7(86,92%) of second grade,6(23,07%) of third grade,1(3,84%) of fourth grade,5(19,23%) of fifth grade, And 7(26,92%) of sixth grade. While about 18 (23,72%) of first grade,12(21,81%) of second grade,8(14,54%) of third grade,5(9,09%) of fourth grade,6(10.95%) of fifth grade, and 6(10,95%) of sixth grade chose the surgery. Some student in first grade about 1(9,09%),1(9,09%) in second grade,2(18,18%) in third stage,2(18,18%) in fourth grade,4(36,36%) in fifth grade,And 0(0%) in sixth grade chose the radiology. The current and projected nationwide shortage of primary care physicians necessitates studying factors that could influence students' specialty choice for their future medical practice (11,12).

First grade about 5(27,77%), second grade about 0(0%), third grade about 0(0%), fourth grade about 4(22,22%), fifth grade about 3(16,66%), And sixth grade about 6(33,33%) chose the gyncoylogy. About 2(11,11%) of first grade, 3(16,66%) of second grade,4(22,22%) of third grade, 4(22,22%) of fourth grade ,3(16,66%) of fifth grade, and 2(11,11%) of sixth grade chose the dermatology. While some student of first grade about 0(0%) ,0(0%) of second grade,0(0%) of third grade,1(50%) of fourth grade,1(50%) of fifth grade, and 0(0%) of sixth grade chose ENT. About 3(25%) of first grade,2(16,66%) of second grade,2(16,66%) of third grade,3(25%) of fourth grade,1(8,33%) of fifth grade, and 1(8,33%) of sixth grade chose Ophthalmology. About 2(13,3%) of first grade,0(0%) of second grade,3(20%)0f third grade,2(13,33%) of fourth grade,3(20%) of fifth grade, and 5(33,3%) of sixth grade chose other. Table(3) show frequancy of factor affecting the choice of male and female students.About 33(21,29%)of male,122(78,70%) of female affecting by interesting subject .While some student about4(80%) of male and 1(20%) of female affecting by good income.Other about 14(87,5%) of male and 2(12,5%) of female affecting by prestige and good life social life.

5. Conclusions & Recommendations

The overall two preferred specialties were general surgery (27%) , internal medicine and pediatrics (8.3%) among males. While it was for surgery, gynecology and dermatology for females. Deficient choice for Family and Community Medicine, this is against the term of "Tomorrow Doctor" in its emphasis on Primary health care, Family doctors and public health. The study recommends Career counseling should be offered to students about each specialty's challenges, /opportunities with an ultimate goal to match the country-specific demand and supply of physicians. Knowledge about doctors' preferences regarding their medical specialty could support the development of targeted policies to increase the attractiveness of deficit specialties. The study recommends Focusing on spreading the importance of the principles of primary health care, family doctor and public health to be choices for future specialty, as these are the pillars of tomorrow's doctor.

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NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(2) 111-120

Pulmonary Injury Biomarkers, Oxidative Stress and Systemic Inflammation in Conventional Cigarette, Waterpipe and Electronic Cigarette Users: A Cross-Sectional Comparative Study

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Abstract

Background: Tobacco smoke injures the airway epithelium, imposes an oxidant load and activates innate immunity, but the biological footprint of waterpipe smoking and electronic cigarette (e-cigarette) use is less well characterised, particularly in Middle Eastern populations where all three modalities coexist.
Objective: To compare a panel of pulmonary injury biomarkers (CC16, KL-6, YKL-40, galectin-3, TREM-1), oxidative stress indices (malondialdehyde [MDA], superoxide dismutase-1 [SOD-1] protein) and the systemic inflammatory cytokine interleukin-6 (IL-6) among conventional cigarette smokers, waterpipe smokers, exclusive e-cigarette users and non-smokers. Methods: In this cross-sectional study, 120 apparently healthy men aged 18–45 years were classified by smoking status into four equal groups (n = 30 each): non-smokers, cigarette smokers, waterpipe smokers and exclusive e-cigarette users. All eight analytes were quantified in serum by enzyme-linked immunosorbent assay. Group differences were tested by one-way ANOVA with Tukey HSD post-hoc comparisons, and associations by Pearson correlation.
Results: Serum CC16 was significantly lower, and KL-6, YKL-40, IL-6 and MDA significantly higher, in every smoking group than in non-smokers (all $p < 0.001$; $\eta^2 = 0.64–0.74$). The largest deviations occurred in the two combustible groups, which did not differ significantly from each other, whereas e-cigarette users showed an intermediate profile lying between non-smokers and combustible smokers. Galectin-3, TREM-1 and SOD-1 protein did not differ among groups ($p > 0.20$). CC16 correlated inversely with the injury, oxidation and inflammation markers ($r = −0.63$ to $−0.71$), which were positively inter-correlated ($r = 0.69–0.73$; all $p < 0.001$).
Conclusion: A coherent signature of airway epithelial injury, lipid peroxidation and IL-6-mediated inflammation separates smokers from non-smokers. The signature is most pronounced with combustible products, including the waterpipe, and is attenuated but clearly present in e-cigarette users. CC16, KL-6, YKL-40, IL-6 and MDA were the most discriminating circulating markers in this population.

Keywords: club cell protein 16; Krebs von den Lungen-6; YKL-40; lipid peroxidation; interleukin-6; waterpipe; electronic cigarettes; tobacco harm reduction.

1. Introduction

Tobacco use remains one of the leading preventable causes of morbidity and mortality worldwide, accounting for more than seven million deaths each year from direct use and over one million further deaths attributable to second-hand smoke [1,2]. Although the age-standardised burden has declined in many high-income countries, the absolute number of tobacco-related deaths continues to rise, and the industry's marketing has shifted towards low- and middle-income countries with weaker regulatory systems [3]. The Eastern Mediterranean region has a distinctive profile: smoking prevalence among men remains high, and while cigarette smoking has fallen by roughly 7% over the past decade, waterpipe smoking has increased by almost 8% over the same period [4]. This shift is clinically relevant

because a single waterpipe session delivers substantially more carbon monoxide, particulate matter and several other toxicants than a single cigarette [5].

Three interconnected mechanisms underlie the respiratory effects of inhaled tobacco toxicants: disruption of the airway epithelial barrier, oxidative injury by reactive oxygen species, and activation of innate and cytokine-mediated immunity [6]. Several circulating proteins index these processes with increasing specificity. Club cell secretory protein-16 (CC16) is an anti-inflammatory pneumoprotein produced by non-ciliated bronchiolar epithelial cells; its serum concentration falls as club cell populations are depleted by chronic smoke exposure, and low CC16 predicts accelerated lung-function decline and incident chronic obstructive pulmonary disease (COPD) [7–10]. CC16 is also implicated in acute epithelial injury and viral lung damage, underscoring its sensitivity as a marker of barrier integrity [11,12]. Krebs von den Lungen-6 (KL-6) is a high-molecular-weight MUC1 glycoprotein released into the circulation when alveolar epithelial cells are damaged [13,14]. YKL-40 (chitinase-3-like protein 1) is secreted by epithelial cells, macrophages and neutrophils, is elevated in smokers, and correlates with airway inflammation, remodelling and neutrophilic disease phenotypes [15–17]. Galectin-3, a β-galactoside-binding lectin involved in tissue remodelling and fibrosis [18,19], and the triggering receptor expressed on myeloid cells-1 (TREM-1), an amplifier of innate immune responses [20,21], have also been proposed as lung injury markers, but their behaviour in otherwise healthy smokers is poorly defined. Large population analyses further indicate that smoking perturbs several systemic immune and inflammatory markers simultaneously, which argues for a multi-marker approach [22].

Oxidative stress is the common biochemical substrate of these processes. Malondialdehyde (MDA), an end-product of membrane lipid peroxidation, reflects the oxidant load imposed by smoke, whereas superoxide dismutase (SOD), a first-line antioxidant enzyme, may increase, decrease or remain unchanged depending on the intensity and duration of oxidant exposure [5,23]. Interleukin-6 (IL-6) links local airway events to low-grade systemic inflammation and is consistently elevated in current smokers [24,25].

Whereas the literature on conventional cigarettes is extensive, few studies have examined such a biomarker panel in cigarette, waterpipe and e-cigarette users drawn from the same demographically matched population. Comparative data are scarcer still for Middle Eastern cohorts, where waterpipe smoking is culturally entrenched and e-cigarettes are gaining popularity among younger adults.

1.1 Previous studies and research gap

Regional and international studies provide the context for the present analysis. Al-Tameemi and colleagues, in a cross-sectional study of 108 smokers and 51 non-smokers in Iraq, reported dose-dependent increases in plasma IL-6 (from 1.86 to 2.58 pg/mL) and TNF-α (from 4.93 to 6.29 pg/mL) among smokers [26]. The direction and magnitude of that effect resemble the present findings, although absolute IL-6 concentrations here are higher, a difference most plausibly attributable to the assay platform, sample matrix and population characteristics rather than to a different biological pattern. On the oxidative side, a 2024 pilot study documented a significant rise in MDA together with reduced activities of SOD, catalase and glutathione peroxidase in chronic smokers, confirming the importance of lipid peroxidation and suggesting that antioxidant enzyme activity may decline with heavier or longer exposure [23]. Most directly relevant, a 2025 Saudi study compared respiratory, haematological and inflammatory profiles across cigarette, shisha, e-cigarette and non-smoking groups and found that lung function was more impaired with combustible products than with e-cigarettes, while cigarette and shisha users shared a similar inflammatory profile [27].

This body of work exposes two gaps. First, most regional studies have relied on a conventional oxidative–inflammatory panel (MDA, SOD, catalase, glutathione peroxidase, IL-6, TNF-α, C-reactive protein) rather than on epithelium-specific markers of lung injury. Second, very few have compared cigarette, waterpipe and e-cigarette exposure within a single matched cohort. The present study addresses both gaps by adding CC16, KL-6, YKL-40, galectin-3 and TREM-1 to the panel, thereby focusing on early, subclinical airway injury by smoking modality. Specifically, we aimed to compare these markers of pulmonary injury, oxidative stress and systemic inflammation across four matched male groups, and to identify which circulating markers best discriminate smokers from non-smokers.

2. Materials and Methods

2.1 Study Design and Setting

This cross-sectional comparative study was conducted at Tikrit Teaching Hospital and its affiliated primary healthcare centres in Salah Al-Din Governorate, Iraq. Recruitment, sampling and laboratory analysis were carried out between January and April 2026.

2.2 Participants and Grouping

A total of 120 apparently healthy men aged 18–45 years were enrolled consecutively and classified according to their self-reported smoking status into four equal groups (n = 30 each): non-smokers (controls), conventional cigarette smokers, waterpipe smokers and exclusive e-cigarette users. Participants in the smoking groups were required to have used their respective product for at least one year. Only men were enrolled in order to minimise sex-related variation in IL-6 and oxidative markers [24].

Exclusion criteria were chronic respiratory disease, asthma, tuberculosis, COVID-19 within the preceding three months, autoimmune disease, malignancy, chronic kidney disease, chronic liver disease, and dual or poly-use of tobacco or nicotine products. These criteria were applied to remove the confounding influence of established pulmonary disease, altered renal clearance of the analytes, and the additive effects of multiple exposures.

2.3 Sample collection and biomarker assays

Venous blood (5 mL) was drawn from each participant, allowed to clot, and centrifuged at 3000 rpm for 10 min. Serum was separated and stored at −80 °C until analysis. Serum concentrations of the eight analytes (CC16, KL-6, YKL-40/CHI3L1, galectin-3, TREM-1, IL-6, MDA and SOD-1) were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co., Ltd, Hangzhou, China) according to the manufacturer's instructions (Table 1). Protein analytes were quantified by quantitative sandwich ELISA and MDA by competitive ELISA. Samples were diluted five-fold as specified in each kit insert, absorbance was read at 450 nm, and concentrations were interpolated from each kit's standard curve and expressed in the kit's calibrated units. It should be noted that the SOD-1 assay measures the concentration of SOD-1 protein rather than its catalytic activity.

Table 1. ELISA kits used for serum biomarker quantification (Sunlong Biotech Co., Ltd, Hangzhou, China).

Biomarker	Kit	Catalogue no.	Assay format
CC16	Human Clara cell protein (CC16) ELISA Kit	QS0462Hu	Sandwich
KL-6	Human Krebs von den Lungen-6 (KL-6) ELISA Kit	SL2082Hu	Sandwich
YKL-40 (CHI3L1)	Human Chitinase-3-like Protein 1 (YKL-40/CHI3L1) ELISA Kit	SL2399Hu	Sandwich
Galectin-3	Human Galectin-3 ELISA Kit	SL2065Hu	Sandwich
TREM-1	Human Triggering Receptor Expressed on Myeloid Cells-1 ELISA Kit	QS1742Hu	Sandwich
IL-6	Human Interleukin-6 (IL-6) ELISA Kit	SL1001Hu	Sandwich
MDA	Human Malondialdehyde (MDA) ELISA Kit	SL1135Hu	Competitive
SOD-1	Human Superoxide Dismutase 1 (SOD-1) ELISA Kit	QS2309Hu	Sandwich

Note: *All assays were performed according to the manufacturer's protocols, with absorbance read at 450 nm and concentrations interpolated from kit-specific standard curves. The SOD-1 assay quantifies SOD-1 protein concentration rather than enzymatic activity.*

2.4 Ethical considerations

The study protocol was approved by the Scientific Research Ethics Committee of Northern Technical University (approval no. 127A/26, dated 25 February 2026). All participants provided written informed consent, confidentiality was maintained at every stage, and the study was conducted in accordance with the Declaration of Helsinki.

2.5 Statistical analysis

Data were analysed with SPSS version 27 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD). Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test before parametric analysis. Between-group differences were tested by one-way analysis of variance (ANOVA), with eta-squared (η^2) reported as the effect size and Tukey's honestly significant difference (HSD) test used for post-hoc pairwise comparisons, which controls the family-wise error rate within each marker. Where variances were unequal, results were confirmed with Welch's ANOVA and Games–Howell post-hoc tests. Eight separate ANOVAs were performed (one per biomarker) and no formal correction was applied across markers; however, all significant omnibus tests had $p < 0.001$ and would remain significant after a Bonferroni correction for eight tests ($\alpha = 0.00625$). Pearson correlation coefficients were calculated among the biomarkers that showed significant group effects. No a priori sample-size calculation was performed; the fixed group size of 30 is acknowledged as a design limitation, and the observed effect sizes are reported descriptively rather than being used to compute retrospective power. A two-sided p value < 0.05 was considered statistically significant.

3. Results

3.1 Demographic comparability

The four groups did not differ significantly in age (one-way ANOVA, $F = 0.93$, $p = 0.428$). Mean ages were 33.3 ± 8.6 years in non-smokers, 30.8 ± 8.2 years in cigarette smokers, 34.2 ± 8.3 years in waterpipe smokers and 33.5 ± 8.1 years in e-cigarette users, supporting the validity of between-group comparison of the biomarker panel.

3.2 Pulmonary injury biomarkers

Serum CC16 differed markedly across groups ($F = 69.85$, $p < 0.001$, $\eta^2 = 0.644$; Table 2). The highest concentration was observed in non-smokers (15.91 ± 1.59 pg/mL). All three smoking groups had significantly lower values: waterpipe smokers showed the lowest concentration (10.97 ± 1.57 pg/mL), closely followed by cigarette smokers (11.17 ± 1.24 pg/mL), while e-cigarette users occupied an intermediate position (13.09 ± 1.59 pg/mL). Each smoking group differed from controls, and e-cigarette users differed from both combustible groups, whereas cigarette and waterpipe smokers did not differ from each other (Tukey $p = 0.955$; Table 3).

The alveolar damage marker KL-6 showed the strongest overall group effect ($F = 111.28$, $p < 0.001$, $\eta^2 = 0.742$), rising from 220.48 ± 17.68 U/mL in non-smokers to 326.70 ± 33.45 U/mL in cigarette smokers and 346.12 ± 36.65 U/mL in waterpipe smokers, with e-cigarette users again intermediate (279.35 ± 24.61 U/mL). YKL-40 followed a parallel pattern ($F = 104.69$, $p < 0.001$, $\eta^2 = 0.730$), increasing from 39.94 ± 4.74 pg/mL in non-smokers to 62.76 ± 6.10 and 59.87 ± 6.47 pg/mL in the cigarette and waterpipe groups, respectively. The e-cigarette group mean for YKL-40 (45.70 ± 6.08 pg/mL) lay closer to that of non-smokers than to either combustible group, and the non-smoker versus e-cigarette contrast was the least pronounced of the smoking comparisons ($p = 0.001$). For both KL-6 and YKL-40, the combustible groups did not differ significantly from each other, both exceeded the e-cigarette group, and the e-cigarette group exceeded non-smokers.

Neither galectin-3 ($F = 1.48$, $p = 0.223$) nor TREM-1 ($F = 0.15$, $p = 0.927$) differed among groups; mean values clustered around 8 ng/mL and 26 pg/mL, respectively, in all four groups. These two markers therefore did not distinguish smokers from non-smokers in this cohort.

Table 2: Serum pulmonary injury biomarkers, oxidative stress indices and IL-6 (mean ± SD) by study group.

Biomarker	Non-smokers	Cigarette	Waterpipe	E-cigarette	F	p	η^2
CC16 (pg/mL)	15.91 ± 1.59	11.17 ± 1.24	10.97 ± 1.57	13.09 ± 1.59	69.85	< 0.001	0.644
KL-6 (U/mL)	220.48 ± 17.68	326.70 ± 33.45	346.12 ± 36.65	279.35 ± 24.61	111.28	< 0.001	0.742
YKL-40 (pg/mL)	39.94 ± 4.74	62.76 ± 6.10	59.87 ± 6.47	45.70 ± 6.08	104.69	< 0.001	0.730
Galectin-3 (ng/mL)	7.98 ± 0.97	8.43 ± 0.95	8.49 ± 1.05	8.43 ± 1.31	1.48	0.223	0.037

Biomarker	Non-smokers	Cigarette	Waterpipe	E-cigarette	F	p	η^2
TREM-1 (pg/mL)	26.05 ± 2.76	26.41 ± 2.94	26.48 ± 2.65	26.45 ± 2.91	0.15	0.927	0.004
IL-6 (pg/mL)	2.08 ± 0.39	4.09 ± 0.78	4.54 ± 0.81	2.99 ± 0.59	83.67	< 0.001	0.684
MDA (ng/mL)	1.82 ± 0.26	2.83 ± 0.28	2.92 ± 0.40	2.26 ± 0.18	94.91	< 0.001	0.711
SOD-1 (pg/mL)	121.97 ± 8.16	120.72 ± 10.31	117.78 ± 12.54	118.29 ± 8.37	1.18	0.319	0.030

Note: *Values are mean ± SD; n = 30 per group. F and p from one-way ANOVA (df = 3, 116); η^2 = eta-squared. Units are those of each kit's calibrated standard curve with the manufacturer's five-fold dilution applied. CC16, club cell secretory protein-16; KL-6, Krebs von den Lungen-6; TREM-1, triggering receptor expressed on myeloid cells-1; IL-6, interleukin-6; MDA, malondialdehyde; SOD-1, superoxide dismutase-1.*

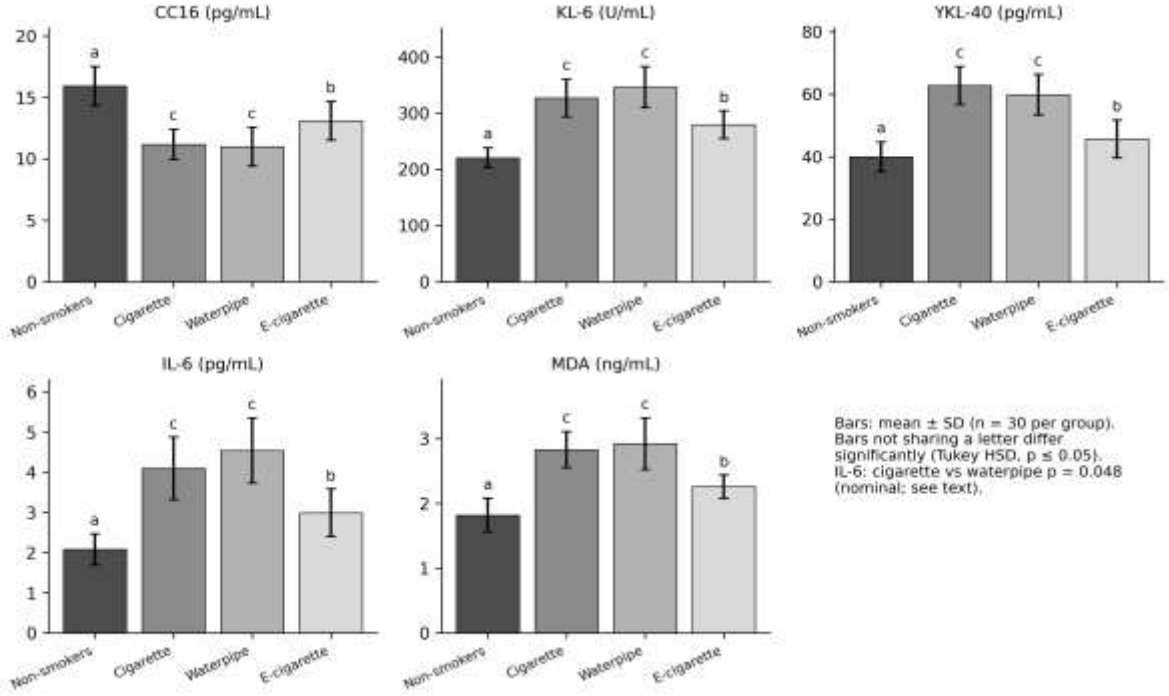


Figure 1. Mean (± SD) serum concentrations of the five discriminating biomarkers in the four study groups (n = 30 per group). Bars not sharing a common letter differ significantly by Tukey HSD ($p \leq 0.05$). Cigarette and waterpipe groups share a letter for every marker, indicating comparable measured effects of the two combustible products, with e-cigarette users occupying an intermediate position.

3.3 Oxidative stress indices and systemic inflammation

Lipid peroxidation, indexed by MDA, increased significantly across groups ($F = 94.91$, $p < 0.001$, $\eta^2 = 0.711$), from 1.82 ± 0.26 ng/mL in non-smokers to 2.83 ± 0.28 and 2.92 ± 0.40 ng/mL in cigarette and waterpipe smokers, with e-cigarette users intermediate (2.26 ± 0.18 ng/mL). Systemic IL-6 mirrored this gradient ($F = 83.67$, $p < 0.001$, $\eta^2 = 0.684$), rising from 2.08 ± 0.39 pg/mL in non-smokers to 4.09 ± 0.78 and 4.54 ± 0.81 pg/mL in cigarette and waterpipe smokers, and 2.99 ± 0.59 pg/mL in e-cigarette users. Waterpipe smokers had the numerically highest IL-6 concentration; the contrast with cigarette smokers reached nominal significance (Tukey-adjusted $p = 0.048$) but lies at the margin of the threshold and would not survive correction for the multiple markers tested, so it is interpreted cautiously rather than as evidence of a substantive difference between the two combustible products.

In contrast, SOD-1 protein concentration was closely similar across groups (117.8–122.0 pg/mL) and did not differ significantly ($F = 1.18$, $p = 0.319$). The combination of a clear, graded rise in MDA with unchanged SOD-1 protein indicates a dissociation between the oxidant load imposed by smoking and the expression of this first-line antioxidant protein in these relatively young smokers.

Table 3: Tukey HSD post-hoc pairwise comparisons (adjusted p values) for the five biomarkers with significant group effects.

Comparison	CC16	KL-6	YKL-40	IL-6	MDA
Non-smokers vs cigarette	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Non-smokers vs waterpipe	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Non-smokers vs e-cigarette	< 0.001	< 0.001	0.001	< 0.001	< 0.001
Cigarette vs waterpipe	0.955	0.053	0.233	0.048	0.603
Cigarette vs e-cigarette	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Waterpipe vs e-cigarette	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note: *Significance threshold $p \leq 0.05$. The recurrent non-significant cigarette-versus-waterpipe contrast indicates a comparable measured effect of the two combustible modalities under the present sampling; in the absence of quantitative exposure data it should not be read as proof of biological equivalence. The IL-6 cigarette-versus-waterpipe contrast ($p = 0.048$) is nominally significant only.*

3.4 Correlations among biomarkers

Across the whole sample (n = 120), the five discriminating biomarkers were strongly inter-correlated in a biologically coherent direction (Table 4, Figure 2). CC16 correlated negatively with every injury, oxidation and inflammation marker (r = −0.63 to −0.71; all p < 0.001), reflecting loss of the protective epithelial protein as injury, oxidation and inflammation increase. The remaining markers correlated positively with one another (r = 0.69–0.73; all p < 0.001), the strongest association being between KL-6 and MDA (r = 0.73). This pattern is consistent with a single underlying axis linking epithelial injury, lipid peroxidation and IL-6-mediated inflammation.

Table 4: Pearson correlation matrix among the five biomarkers with significant group effects (whole sample, n = 120).

	CC16	KL-6	YKL-40	IL-6	MDA
CC16	1.00	−0.71	−0.66	−0.63	−0.69
KL-6	−0.71	1.00	0.72	0.71	0.73
YKL-40	−0.66	0.72	1.00	0.69	0.69
IL-6	−0.63	0.71	0.69	1.00	0.71
MDA	−0.69	0.73	0.69	0.71	1.00

Note: *All correlations significant at $p < 0.001$. Negative coefficients for CC16 reflect its inverse relationship with the injury, oxidation and inflammation markers.*

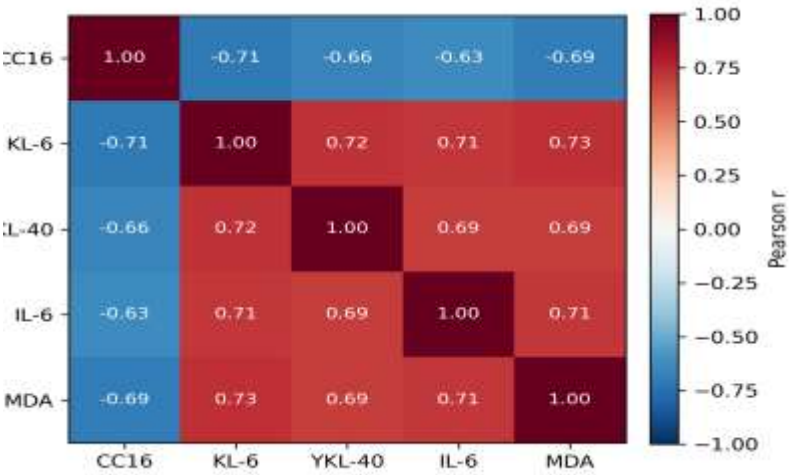


Figure 2: Pearson correlation heatmap among the five discriminating biomarkers (whole sample, n = 120). The inverse loading of CC16 against the positively co-varying injury, oxidation and inflammation markers is consistent with a single exposure-responsive axis. All correlations were significant at p < 0.001.

4. Discussion

This study compared a multidimensional biomarker panel among conventional cigarette, waterpipe and e-cigarette users within a single age-matched male cohort. Three findings stand out. First, a consistent and biologically coherent signature (reduced CC16 with elevated KL-6, YKL-40, IL-6 and MDA) discriminated all smokers from non-smokers. Second, the two combustible modalities produced statistically indistinguishable derangements on almost every measure, whereas e-cigarette use produced a consistently intermediate profile. Third, three of the markers examined (galectin-3, TREM-1 and SOD-1 protein) did not discriminate smoking status, a negative finding that carries its own interpretation.

The reduction in CC16 mirrors observations from prospective cohorts in which low circulating CC16 precedes and predicts lung-function decline and incident COPD [9,28]. Because club cells both secrete CC16 and participate in the detoxification of inhaled xenobiotics, depletion of these cells in chronic smokers offers a parsimonious explanation for the observed fall [8,10]; the partial preservation of CC16 in e-cigarette users is consistent with a smaller, though not trivial, epithelial insult. The elevation of KL-6 accords with its established role as a marker of alveolar epithelial damage [13,14,29], and the rise in YKL-40 agrees with earlier reports of increased serum chitinase-like protein in both combustible smokers and e-cigarette users [15,16,30].

It is noteworthy that waterpipe smoking produced derangements statistically similar to, and in the case of IL-6 numerically greater than, those of cigarette smoking, although the IL-6 contrast ($p = 0.048$) is inconclusive. The waterpipe is widely perceived as a benign social alternative to cigarettes, yet toxicant dosimetry shows that users may inhale greater quantities of carbon monoxide and particulate matter than cigarette smokers and are therefore exposed to comparable or greater biological hazard [5,31,32]. Experimental and human studies likewise indicate that waterpipe smoke is at least as pro-inflammatory and pro-oxidant for the lung as cigarette smoke [31,33]. An important caveat applies to this comparison: exposure was not quantified (no pack-years, cotinine or exhaled carbon monoxide data were collected), so the statistical similarity of the two combustible groups may reflect the doses actually delivered rather than the intrinsic toxicity of the products. The finding is therefore best described as a similar measured effect rather than as proof of biological equivalence.

The intermediate position of e-cigarette users is in keeping with an emerging body of evidence. A recent meta-analysis of approximately 24,000 adults found that combustible smokers had higher inflammatory marker concentrations than both non-smokers and e-cigarette users, with the latter group typically falling between the two [34]. The 2025 Saudi comparison of cigarette, shisha, e-cigarette and non-smoking groups reached a convergent conclusion, with combustible products impairing lung function more than e-cigarettes while cigarette and shisha users displayed similar inflammatory profiles [27]. Controlled comparisons of combustible and electronic hookah also show a stronger oxidative and inflammatory signal with the combustible product, yet vaping is far from inert [35]. E-cigarette aerosols induce oxidant generation, epithelial toxicity and IL-6/IL-8 release in vitro and in vivo [36–39], cause vascular oxidative stress and endothelial dysfunction after even short-term exposure [40], and have recognised cardiovascular and systemic effects [41,42]. The present data therefore support a graded interpretation: on these indicators, e-cigarettes are less harmful than combustible products but are not equivalent to non-exposure.

The unchanged galectin-3 and TREM-1 concentrations warrant cautious interpretation. Their stability in a young, clinically healthy cohort suggests that the early, subclinical injury captured by CC16 and KL-6 had not yet progressed to the fibrotic remodelling or acute innate immune amplification that these proteins index [19,21]. The stable SOD-1 value requires even greater care. The present finding is not directly comparable with studies that measured SOD enzymatic activity, such as the 2024 pilot study of chronic smokers that reported reduced activity [23], because the assay used here quantifies the concentration of SOD-1 protein. An unchanged protein concentration does not exclude a loss of function: oxidative inactivation or post-translational modification can reduce catalytic activity without altering the amount of immunoreactive protein. The dissociation between clearly elevated MDA and stable SOD-1 protein should therefore be read as evidence of unchanged antioxidant protein expression, not of preserved antioxidant capacity, and direct activity assays would be needed to distinguish the two. Differences in age and exposure duration

between cohorts may also contribute. Overall, the null result for SOD-1 protein does not contradict the literature on SOD activity but adds a methodologically distinct observation.
The strong inter-correlations among the discriminating markers further support a single exposure-responsive pathway linking epithelial barrier injury, lipid peroxidation and IL-6-mediated inflammation in the systemic circulation. The inverse loading of CC16 on this axis is expected, given its anti-inflammatory and antioxidant properties and its contribution to the resolution of inflammation and repair of the airway epithelial barrier [8,43].

4.1 Strengths and Limitations

The strengths of this study include the balanced group sizes, the age comparability across groups, the exclusion of dual users, and a panel that spans epithelial injury, oxidative stress and inflammation. Several limitations must be acknowledged. The cross-sectional design permits only associative inference, and the biomarker differences represent a single point in time. The most important limitation is the absence of a quantitative exposure measure; smoking was categorised by product only, so the statistical similarity of the combustible groups may reflect delivered dose rather than intrinsic toxicity, and no dose–response relationship could be modelled. Apart from age, potential confounders such as body mass index, socioeconomic status and the duration or intensity of use were not recorded, so no multivariable adjustment (for example by ANCOVA) was possible and residual confounding cannot be excluded. The young, single-sex, single-region sample limits generalisability, and the lack of spirometry prevents the biomarker changes from being linked to functional outcomes. Two analytical caveats also apply. First, the SOD-1 assay measured protein concentration rather than enzymatic activity, so stable SOD-1 does not imply preserved antioxidant activity. Second, all concentrations are expressed in the units of each manufacturer's calibrated standard curve, and the absolute values of some analytes lay near the nominal limits of their kits and depend on the dilution and calibration applied; the results should therefore be interpreted as relative comparisons between groups rather than as absolute reference concentrations, and confirmation on an orthogonal analytical platform would strengthen them.

5. Conclusion

In this matched male cohort, conventional cigarette, waterpipe and e-cigarette use were each associated with a clear biomarker profile of airway epithelial injury (reduced CC16, elevated KL-6 and YKL-40), lipid peroxidation (elevated MDA) and systemic inflammation (elevated IL-6). The two combustible products produced statistically similar and most severe derangements, contradicting the common perception of the waterpipe as a benign alternative, whereas e-cigarettes produced a measurable but consistently smaller effect. Because exposure intensity was not quantified, the similarity between the combustible groups should be regarded as a similarity of measured effect rather than of biological equivalence. CC16, KL-6, YKL-40, IL-6 and MDA behaved as sensitive and mutually consistent markers of early, subclinical lung injury, whereas galectin-3, TREM-1 and SOD-1 protein did not discriminate smoking status. These findings do not support the notion that e-cigarettes are a safe alternative to combustible products, while at the same time not overstating their lower biomarker burden. Longitudinal studies incorporating quantitative exposure metrics and lung-function testing are needed to establish causal pathways and clinical relevance.

5.1 Future Directions

Future work should link these biomarkers to spirometric decline, include women and older adults, quantify exposure with cotinine or exhaled carbon monoxide, and follow e-cigarette users over time. Such studies would strengthen the evidence base and help resolve whether the intermediate profile of vaping represents a real reduction in risk or a delayed pathway to the same harm.

Declarations

Ethics approval: The study was approved by the Scientific Research Ethics Committee of Northern Technical University (no. 127A/26, 25 February 2026) and conducted in accordance with the Declaration of Helsinki.
Consent to participate: All participants gave written informed consent.
Funding. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
Conflicts of interest: The author declares no conflict of interest.

Data availability: The data analysed in this study are available from the corresponding author upon reasonable request.

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Nexus International University ISSN: 3136-6449; 12(2) 121-132

Testicular Gene Expression Response to *Icacina Trichantha* Root Ethanol Extract Therapy in Paroxetine-Induced Reproductive Dysfunction in Male Wistar Rats

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Abstract. Male infertility is increasingly linked to pharmacological agents, particularly selective serotonin reuptake inhibitors (SSRIs) such as paroxetine, which may disrupt spermatogenesis through endocrine and molecular mechanisms. Paroxetine is therefore used as an agent in studying some intervention strategy in male infertility in experimental animals. This study investigated the effects of paroxetine on the expression of key spermatogenic genes, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) in male Wistar rats and evaluated the remedial potential of *Icacina trichantha* root ethanol extract (ITREE) in comparison wDith tadalafil and clomiphene citrate. Twenty-five rats were randomly assigned to five groups: control (Group A), paroxetine only (Group B), paroxetine + ITREE (Group C), paroxetine + tadalafil (Group D), and paroxetine + clomiphene (Group E). The oral treatment regimen spans through 56 days. Paroxetine significantly increased CREM (92.64 ± 2.35 vs. 28.80 ± 2.94), PRM I (58.17 ± 1.73 vs. 21.64 ± 1.29), and PRM II (66.08 ± 3.25 vs. 42.74 ± 1.91) compared with control, and elevated the PRM I/PRM II ratio from 0.51 in control to 0.88. Co-administration of ITREE partially restored expression levels (CREM 68.87 ± 1.46, PRM I 42.15 ± 1.63, PRM II 55.71 ± 3.28) and attenuated the PRM I/PRM II ratio to 0.76. Tadalafil and clomiphene produced varying degrees of modulation, with CREM expression of 66.96 ± 2.07 and 105.12 ± 3.85, respectively. These findings indicate that paroxetine disrupted spermatid differentiation and chromatin remodeling, while ITREE effectively mitigates these molecular alterations. The study highlights the therapeutic potential of *I. trichantha* roots in preventing SSRI-induced male reproductive toxicity and provides a molecular basis for plant-based interventions.

Keywords: Paroxetine, Spermatogenesis, CREM, Protamines, *Icacina trichantha*

1. Introduction

Male infertility is a growing global health concern, accounting for approximately 40–50% of infertility cases worldwide (Kumar & Singh, 2015). A wide range of factors including genetic abnormalities, endocrine disruption, environmental exposures, and pharmacological agents have been implicated in the impairment of male reproductive function (Agarwal *et al.*, 2021). Among pharmacological agents, antidepressants particularly selective serotonin reuptake inhibitors (SSRIs) have attracted increasing attention because of their potential adverse effects on reproductive physiology and sperm function. One of the most widely prescribed SSRIs is paroxetine, which is commonly used in the treatment of depression, anxiety disorders, obsessive–compulsive disorder, panic disorder, and post-traumatic stress disorder (Nevels *et al.*, 2016).

Paroxetine exerts its therapeutic action primarily through inhibition of the serotonin reuptake transporter (SERT), resulting in increased synaptic concentrations of serotonin in the central nervous system. While this mechanism is beneficial in the management of psychiatric disorders, excessive serotonergic activity has been associated with

disturbances in the hypothalamic–pituitary–gonadal (HPG) axis, leading to impaired reproductive hormone regulation (Mosbah *et al.*, 2019). Elevated serotonin levels may suppress gonadotropin-releasing hormone (GnRH) secretion and subsequently reduce follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone production, thereby disrupting spermatogenesis and sperm maturation (Schenker *et al.*, 1992; Mosbah *et al.*, 2019). Experimental and clinical studies have demonstrated that paroxetine administration can lead to reduced sperm count, impaired sperm motility, abnormal sperm morphology, and increased DNA fragmentation (Mosbah *et al.*, 2021; Yardimci *et al.*, 2019). Histopathological alterations including degeneration of seminiferous tubules, cellular disorganization, vacuolization, and reduced numbers of spermatozoa within the epididymal lumen have also been reported in animal models following paroxetine exposure (Elkelany & Kashef, 2023).

Beyond endocrine disruption and structural testicular damage, growing evidence suggests that male infertility may also result from alterations in the expression of genes essential for spermatogenesis. Spermatogenesis is a highly coordinated process involving mitotic proliferation of spermatogonia, meiotic division of spermatocytes, and differentiation of haploid spermatids into mature spermatozoa (Sánchez-Jasso *et al.*, 2023). This process is tightly regulated by transcription factors and signaling pathways that control stage-specific gene expression. Among these regulatory molecules, the cyclic adenosine monophosphate (cAMP) response element modulator (CREM) plays a central role in post-meiotic germ cell differentiation.

CREM is a cAMP-responsive transcription factor belonging to the CREB/ATF family of transcription regulators and is considered a key regulator of spermiogenesis. Through alternative promoter usage and extensive alternative splicing, the CREM gene produces multiple isoforms that function either as transcriptional activators or repressors (Behr & Weinbauer, 2001). During normal spermatogenesis, a developmental "switch" occurs in which CREM repressor isoforms expressed in pre-meiotic germ cells are replaced by activator isoforms, particularly CREMτ, in post-meiotic haploid spermatids (Peri & Serio, 2000). This switch is essential for the transcription of several spermatid-specific genes involved in sperm structural development and chromatin remodeling. Studies in CREM-deficient mice have demonstrated complete infertility characterized by arrest of germ cell maturation at the round spermatid stage, underscoring the indispensable role of CREM in spermatid differentiation (Blendy *et al.*, 1996; Nantel *et al.*, 1996). Similarly, abnormal CREM expression patterns have been observed in men with spermatogenic failure and round spermatid maturation arrest, indicating that disruption of CREM signaling contributes to male infertility (Steger, 1999; Sánchez-Jasso *et al.*, 2023).

Among the downstream targets regulated by CREM are the protamine genes, which encode sperm-specific nuclear proteins essential for chromatin condensation during the final stages of spermiogenesis. Protamines are small arginine-rich proteins that replace histones in elongating spermatids, leading to the formation of highly compacted sperm chromatin. In mammals, two major protamines, protamine 1 (PRM1) and protamine 2 (PRM2) are expressed and function cooperatively to achieve the extreme condensation of paternal DNA within the sperm head (Ren *et al.*, 2021). This histone-to-protamine transition is critical for maintaining genomic integrity, protecting sperm DNA from oxidative damage, and ensuring proper transmission of paternal genetic information during fertilization (Chioccarelli *et al.*, 2020; Kobayash *et al.*, 2021).

The expression of PRM1 and PRM2 is tightly regulated at transcriptional, translational, and post-translational levels during spermatogenesis. Any imbalance in the expression or ratio of PRM1 to PRM2 can disrupt chromatin packaging, resulting in abnormal sperm morphology, reduced motility, and increased DNA fragmentation (Pardede *et al.*, 2020; Ren *et al.*, 2021). Altered protamine expression has therefore been widely recognized as a biomarker of impaired spermatogenesis and male infertility. Environmental toxicants, pharmacological agents, and oxidative stress have all been reported to interfere with protamine expression and chromatin remodeling processes in germ cells (Bashiri *et al.*, 2021).

Given the increasing use of antidepressant medications and their potential reproductive toxicity, there is growing interest in identifying therapeutic agents capable of mitigating drug-induced reproductive dysfunction. Natural products and plant-derived bioactive compounds have been widely investigated for their protective effects on reproductive health due to their antioxidant, anti-inflammatory, and endocrine-modulating properties (Agarwal *et al.*,

2021). In this context, medicinal plants used in traditional medicine represent promising candidates for the management of male reproductive disorders.

Despite the documented reproductive toxicity of paroxetine, limited studies have explored its impact on the molecular regulators of spermatogenesis, particularly transcription factors such as CREM and downstream structural proteins such as protamines. Furthermore, the potential of phytotherapeutic agents to restore paroxetine-induced alterations in spermatogenic gene expression remains largely unexplored. Therefore, this study was designed to investigate the effects of paroxetine on the expression of CREM and protamine genes in the testes of male rats and to evaluate the remedial potential of *Icacina trichantha* root ethanolic extract in comparison with established therapeutic agents. Understanding the molecular mechanisms underlying these effects may provide valuable insight into novel strategies for the management of drug-induced male infertility.

Given the increasing incidence of antidepressant use and the growing concern about their potential reproductive side effects, there is a need to explore therapeutic strategies capable of mitigating drug-induced reproductive dysfunction. Plant-derived bioactive compounds have gained considerable attention in recent years due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, and endocrine-modulating effects that may protect against reproductive toxicity (Agarwal *et al.*, 2021; Bashiri *et al.*, 2021). Medicinal plants used in traditional medicine therefore represent promising candidates for the management of male infertility associated with xenobiotic exposure.

Icacina trichantha, a medicinal plant widely used in African ethnomedicine, has been reported to possess several biological activities including antioxidant, anti-inflammatory, and cytoprotective properties (Nwankwo *et al.*, 2022). These pharmacological properties suggest that extracts derived from *I. trichantha* may have the potential to protect or restore reproductive function following chemical or drug-induced testicular damage. However, despite the increasing interest in medicinal plants as therapeutic agents, there is limited information regarding the potential role of *I. trichantha* in modulating molecular pathways involved in spermatogenesis.

In addition to phytotherapeutic agents, pharmacological interventions such as clomiphene citrate and tadalafil are commonly used in the management of male reproductive disorders. Clomiphene citrate is a selective estrogen receptor modulator that enhances endogenous testosterone production by stimulating gonadotropin secretion through blockade of estrogen-mediated negative feedback on the hypothalamic–pituitary axis (Krzastek *et al.*, 2019). Tadalafil, a phosphodiesterase type-5 inhibitor, is widely used in the treatment of erectile dysfunction and has also been shown to improve testicular blood flow, sperm parameters, and overall reproductive function (Corona *et al.*, 2020). These agents therefore serve as useful pharmacological comparators when evaluating the potential reproductive benefits of novel therapeutic compounds.

Although the adverse effects of paroxetine on sperm quality and testicular histology have been documented, there is limited information on its influence on key molecular regulators of spermatogenesis such as the cyclic AMP response element modulator (CREM) and protamine proteins. Since CREM acts as a critical transcription factor regulating post-meiotic germ cell differentiation and protamines are essential for chromatin condensation during spermiogenesis, alterations in the expression of these genes may contribute significantly to impaired sperm development and male infertility.

Therefore, the present study was designed to investigate the effects of paroxetine-induced reproductive toxicity on the expression of CREM and protamine genes in the testes of male Wistar rats and to evaluate the potential remedial effects of *Icacina trichantha* root ethanolic extract (ITREE). In addition, the therapeutic efficacy of ITREE was compared with established pharmacological agents, tadalafil and clomiphene citrate. It was hypothesized that paroxetine exposure would disrupt the expression of genes critical for spermatogenesis, particularly CREM and protamine, and that treatment with ITREE may ameliorate these molecular alterations and restore normal spermatogenic function.

2. Materials and Methods

2.1 Preparation of *Icacina trichantha* Root Ethanol Extract (ITREE):

Roots of *Icacina trichantha* were collected and washed thoroughly to remove soil and debris. They were then air-dried at room temperature and subsequently pulverized into coarse powder using a mechanical grinder. A measured quantity of the powdered root material was macerated in 40% ethanol at a ratio of 1:5 (w/v) and agitated on a mechanical shaker at room temperature for 72 hours, as previously described by Olumese *et al.* (2015). The mixture was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further lyophilized using a freeze dryer to obtain a dry ethanol extract, which was stored in a desiccator until use.

2.2 Experimental Animals

25 adult male Wistar rats weighing 180–220 g were used for this study. The animals were obtained from the Animal House Facility of the Faculty of Life Sciences, University of Benin, Nigeria. The rats were housed in standard plastic cages under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 50–60%, and a 12 h light/dark cycle). Animals had free access to standard laboratory chow and clean drinking water *ad libitum*. The animals were acclimatized for two weeks prior to the commencement of the experiment.

2.3 Experimental Design

The experimental animals were randomly assigned into five groups (n = 5 per group). Paroxetine was administered to induce reproductive toxicity in group B which served as negative control, while *Icacina trichantha* root ethanolic extract (ITREE) was evaluated for its potential remedial effects by co-administration with paroxetine in group C. Tadalafil and clomiphene citrate served as standard reference drugs and were co-administered with paroxetine in groups D and E respectively. All treatments were administered orally using an oral gavage once daily for 56 days. The experimental design is presented in Table 1.

Table 1: Experimental design for evaluation of the effects of ITREE on paroxetine-induced alterations in gene expression.

Group	Number of rats	Treatment	Duration
A (Control)	5	Distilled water	56 days
B (Negative control)	5	Paroxetine (10 mg/kg body weight)	56 days
C	5	Paroxetine (10 mg/kg bw) + ITREE (200 mg/kg bw)	56 days
D	5	Paroxetine (10 mg/kg bw) + Tadalafil (5 mg/kg bw)	56 days
E	5	Paroxetine (10 mg/kg bw) + Clomiphene citrate (10 mg/kg bw)	56 days

2.4 Sample Collection

At the end of the experimental period (56 days), the rats were fasted overnight and sacrificed by cervical dislocation in accordance with established procedures for laboratory animals (Aguwa *et al.*, 2020). The animals were dissected and the testes were carefully excised.

2.5 Gene Expression Analysis

Gene expression of CREM, PRM I, PRM II and PDE5 in the testis of male Wistar rats was be determined via Reverse Transcriptase Polymerase Chain Reaction Analysis (Elekofehinti *et al.* 2020; Olumegbon *et al.*, 2020) in the following steps;

2.6 RNA Isolation

Total RNA was extracted from testicular tissue using the Quick-RNA MiniPrep™ Kit (Zymo Research, USA) according to the manufacturer's instructions. To remove genomic DNA contamination, RNA samples were treated

with DNase I (New England Biolabs, USA). The concentration and purity of RNA were determined spectrophotometrically by measuring absorbance at 260 nm and the A260/A280 ratio.

2.7 cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg of DNase-treated RNA using the ProtoScript® II First-Strand cDNA Synthesis Kit (New England Biolabs, USA). Reverse transcription was performed under the following conditions: 65°C for 5 min, 42°C for 60 min, and 80°C for 5 min to terminate the reaction.

2.8 Polymerase Chain Reaction (PCR) Amplification

PCR amplification of the target genes was carried out using OneTaq® 2X Master Mix (New England Biolabs, USA) and gene-specific primers synthesized by Inqaba Biotec (Pretoria, South Africa). The genes analyzed included cyclic AMP response element modulator (*Crem*), protamine 1 (*Prm1*), protamine 2 (*Prm2*), and phosphodiesterase 5A (*Pde5a*). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as the housekeeping gene. PCR reactions were performed in a final volume of 25 µL containing cDNA template, forward and reverse primers, nuclease-free water, and PCR master mix. Amplification was carried out under the following conditions:Initial denaturation: 95°C for 5 min
30 cycles of amplification: Denaturation at 95°C for 30 s, annealing for 30 s and extension at 72°C for 60 s, Final extension: 72°C for 10 min.

2.9 Agarose Gel Electrophoresis

The PCR products were separated on a 1.0% agarose gel containing ethidium bromide and visualized under ultraviolet illumination. Band intensities were quantified using ImageJ software (National Institutes of Health, USA). Relative gene expression levels were normalized to the housekeeping gene, *Gapdh* (Elekofehinti *et al.*, (Olumegbon *et al.*, 2020).

2.10 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. One-way analysis of variance (ANOVA) was used to determine differences among groups, followed by the Least Significant Difference (LSD) post hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$.

2.11 Ethical Approval

Ethical approval for the study was obtained from the Research Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria. All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals (National Research Council, 2011).

3. Results

The effects of the various treatments on the expression of key genes involved in spermatogenesis and sperm chromatin remodeling in the testes of male Wistar rats are presented in Figures 1 to 4. The genes evaluated included cyclic AMP response element modulator (CREM), protamine I (PRM I), protamine II (PRM II), and the PRM I/PRM II ratio.

The negative control group (Group B), which received paroxetine alone, showed a significant ($p < 0.05$) increase in the expression of CREM (92.64 ± 2.35), PRM I (58.17 ± 1.73), and PRM II (66.08 ± 3.25) compared with the control group (Group A) which recorded values of 28.80 ± 2.94, 21.64 ± 1.29, and 42.74 ± 1.91, respectively. This indicates that administration of paroxetine markedly altered the expression of spermatogenesis-related genes in the testes of the experimental animals.

Treatment with the plant extract and standard drugs produced varying degrees of modulation of gene expression relative to the negative control group. Co-administration of paroxetine with ITREE (Group C) resulted in significant reduction in gene expression levels among the treatment groups, with CREM, PRM I, and PRM II values of 68.87 ± 1.46, 42.15 ± 1.63, and 55.71 ± 3.28, respectively. These values were significantly different ($p < 0.05$) from both the control and negative control groups. Similarly, Groups D co-administered Tadalafil and ITREE demonstrated partial normalization of gene expression levels compared with the negative control group, although the magnitude of reduction was less pronounced than that observed in Group C particularly for PRM I. In contrast, Group E co-administered Clomiphene and ITREE showed relatively unperturbed expression of PRM I and II compared to negative control but a particularly notable increase in CREM (105.12 ± 3.85) expression.

The PRM I/PRM II ratio, an important indicator of proper sperm chromatin packaging and spermatid maturation, was also altered across the experimental groups. The negative control group exhibited an increased ratio (0.88) compared with the control group (0.51), suggesting disruption of normal chromatin condensation dynamics. Among the treatment groups, the paroxetine + ITREE group (Group C) demonstrated the greatest restoration toward the control ratio (0.76). Groups E also showed a very partial improvement with ratio of 0.84, while Group E administered clomiphene exhibited an elevated ratio (1.23), indicating a greater imbalance in protamine expression.

Overall, the findings as presented in Figures 1 to 4 demonstrate that paroxetine administration significantly altered the expression of genes involved in spermatogenesis and chromatin remodeling, while co-administration with plant extracts and standard therapeutic agents resulted in varying degrees of modulation of these molecular markers.

Figure 1: Effect of administration of ITREE on CREM gene expression

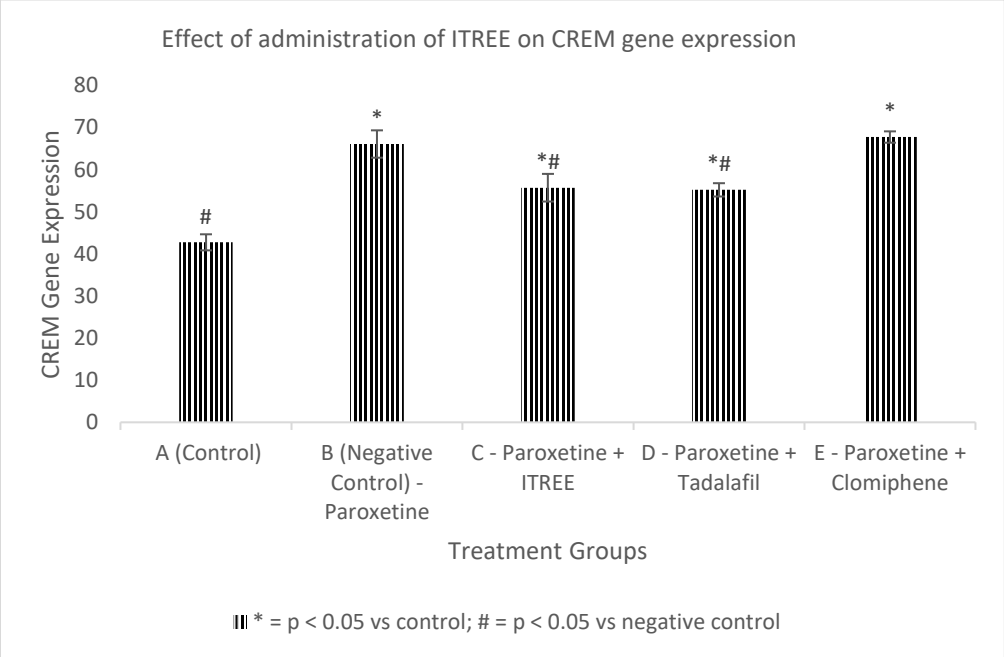


Figure 2: Effect of administration of ITREE on PRM I gene expression

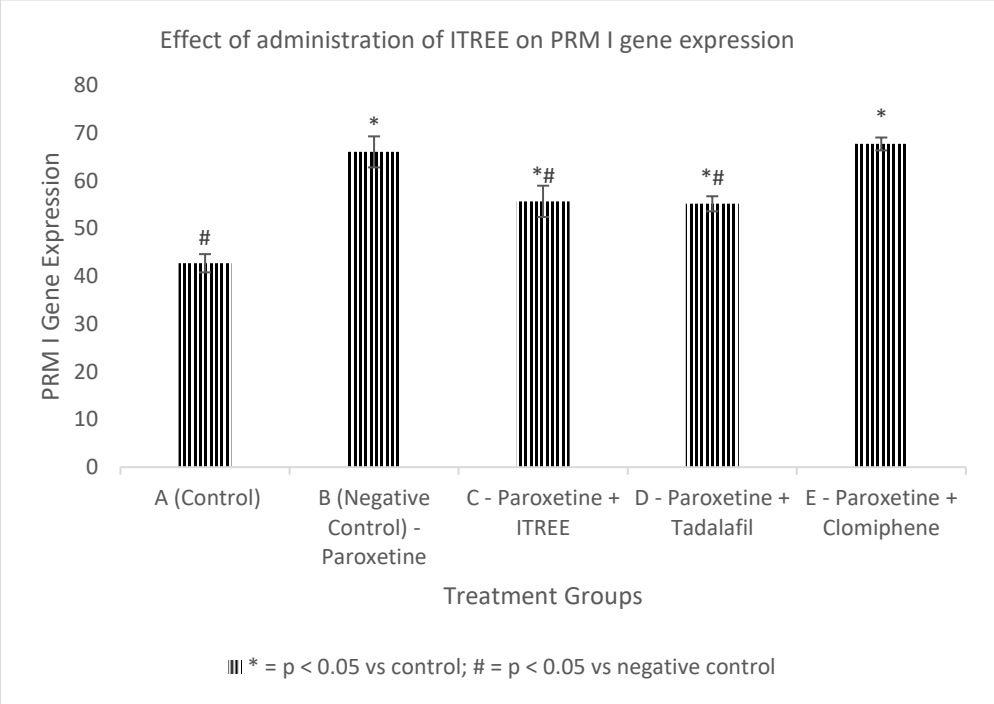


Figure 3: Effect of administration of ITREE on PRM II gene expression

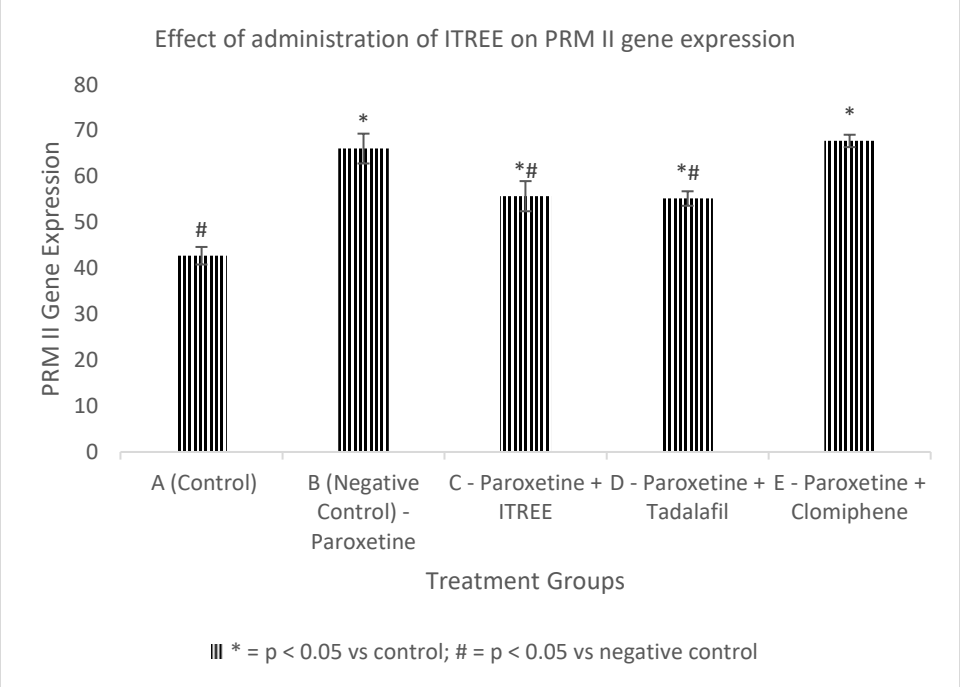
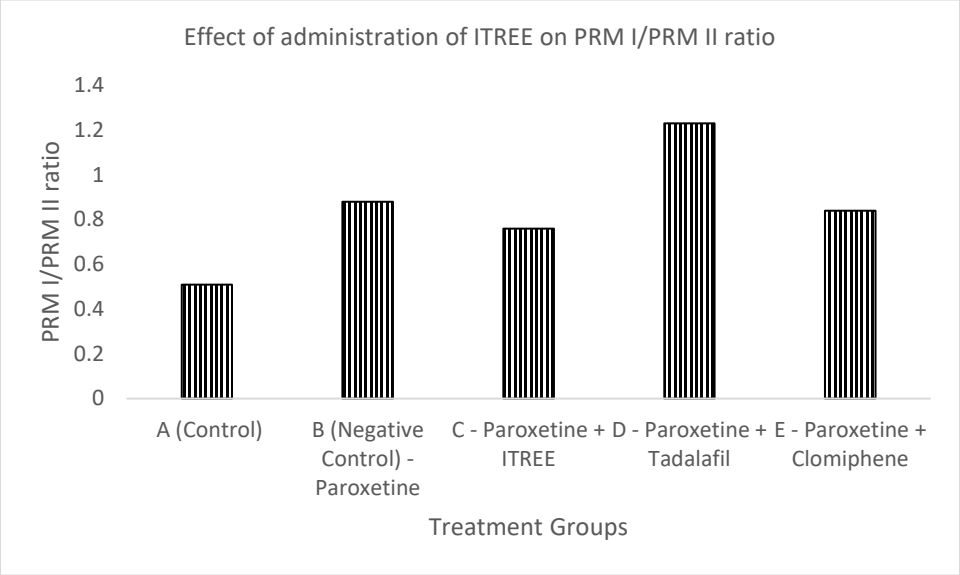


Figure 4: Effect of administration of ITREE on PRM I/PRM II ratio



4. Discussion

The present study investigated the effect of paroxetine on the testicular expression of key genes involved in spermatogenesis, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) and evaluated the potential ameliorative role of ITREE in comparison with established therapeutic agents, tadalafil and clomiphene. These genes are central regulators of post-meiotic germ cell differentiation and chromatin remodeling during spermiogenesis; therefore, alterations in their expression may provide important molecular insights into mechanisms underlying drug-induced male reproductive dysfunction.

The findings of this study demonstrated that administration of paroxetine resulted in a marked dysregulation of spermatogenesis-related gene expression in the testes of male Wistar rats. Specifically, the negative control group (Group B) exhibited a significant elevation in the expression of CREM, PRM I, and PRM II compared with the control group (Group A). This observation suggests that paroxetine exposure disrupts the molecular processes governing spermatid differentiation and sperm chromatin packaging. Increasing evidence indicates that selective serotonin reuptake inhibitors (SSRIs), including paroxetine, may negatively affect male reproductive physiology through oxidative stress, endocrine disturbances, and disruption of testicular gene expression (Tanrikut & Schlegel, 2007). The significant overexpression of the investigated genes observed in the present study therefore supports the hypothesis that paroxetine can interfere with the transcriptional regulatory networks essential for normal spermatogenesis.

CREM is a pivotal transcription factor that mediates the expression of numerous genes required for the transition of round spermatids into elongated spermatids during spermiogenesis. It functions within the cyclic AMP signaling pathway and regulates genes involved in chromatin condensation, acrosome formation, and flagellar development. Previous studies have confirmed that CREM plays a critical role in both human and murine spermatogenesis (Peri & Serio, 2000; Behr & Weinbauer, 2001). Experimental evidence from knockout models has demonstrated that mice lacking the CREM gene are infertile due to an arrest of germ cell maturation at the round spermatid stage (Blendy *et al.*, 1996; Nantel *et al.*, 1996). In such models, the absence of CREM leads to the loss of expression of several post-meiotic genes essential for spermiogenesis, ultimately resulting in impaired sperm production (Sánchez-Jasso *et al.*, 2023).

Regulation of CREM expression is highly complex and occurs at multiple levels, including transcriptional control through the use of multiple promoters (Molina *et al.*, 1993; Stehle *et al.*, 1993; Daniel *et al.*, 2000), alternative transcript splicing (Foulkes *et al.*, 1991; Foulkes *et al.*, 1992), alternative polyadenylation sites (Foulkes *et al.*, 1991),

and translational regulation (Delmas *et al.*, 1992; Gellersen *et al.*, 1997). These regulatory mechanisms give rise to various CREM isoforms that may function either as transcriptional activators or repressors depending on the physiological context. Consequently, tight regulation of CREM expression is required to maintain the proper balance of gene activity during spermatogenesis.

In the present study, paroxetine administration resulted in a marked overexpression of CREM compared with the control group. Dysregulated or excessive expression of CREM may disturb the tightly controlled transcriptional network involved in germ cell differentiation. Recent evidence suggests that aberrant CREM expression may contribute to abnormal spermatid development, defective chromatin remodeling, and impaired sperm maturation (Sánchez-Jasso *et al.*, 2023). Furthermore, altered CREM expression has been associated with abnormal sperm morphology, reduced motility, and idiopathic male infertility (Karami Hezarcheshmeh *et al.*, 2024). Environmental and xenobiotic exposures have also been implicated in the disruption of CREM-regulated pathways (Sánchez-Jasso *et al.*, 2023). Therefore, the overexpression of CREM observed following paroxetine exposure in this study may represent a compensatory or pathological response to testicular stress that ultimately interferes with normal spermatogenic progression.

Co-administration of ITREE with paroxetine resulted in a substantial reduction in CREM expression compared with the paroxetine-only group, although the levels remained higher than those of the control group. This finding suggests that ITREE possesses the capacity to partially restore the normal regulatory balance of spermatogenic transcription factors. The improvement observed may be attributed to the presence of bioactive phytochemicals in *I. trichantha*, which have been reported to exhibit antioxidant and cytoprotective activities capable of mitigating cellular damage induced by xenobiotics. Oxidative stress is widely recognized as a major mechanism through which pharmacological agents impair male reproductive function; therefore, the protective effect of ITREE on CREM expression may be related to its ability to reduce oxidative damage within the testicular microenvironment. The observation that ITREE improved CREM expression toward normal levels suggests a potential regulatory influence on the molecular pathways controlling spermiogenesis.

In comparison, treatment with tadalafil also resulted in a reduction in CREM expression relative to the paroxetine group, indicating partial restoration of transcriptional regulation. Tadalafil, a phosphodiesterase-5 inhibitor commonly used in the management of erectile dysfunction, has been reported to improve testicular perfusion and enhance reproductive function through improved blood flow and modulation of nitric oxide signaling pathways. Improved vascular supply to the testes may enhance the microenvironment required for germ cell development, thereby contributing to normalization of gene expression patterns.

Interestingly, administration of clomiphene in combination with paroxetine resulted in an even greater increase in CREM expression compared with the paroxetine-only group. Clomiphene is a selective estrogen receptor modulator that stimulates endogenous testosterone production by blocking estrogen-mediated negative feedback on the hypothalamic–pituitary–gonadal axis. While this mechanism may increase androgen levels and improve certain aspects of male reproductive function, the elevated CREM expression observed in this group suggests that clomiphene may not effectively correct the specific transcriptional imbalance induced by paroxetine. Instead, the increased gonadotropin stimulation may have further enhanced testicular transcriptional activity, thereby amplifying CREM expression.

Protamine proteins represent another essential component of spermatogenesis and are directly involved in the final stages of sperm chromatin condensation. During spermiogenesis, histones in the spermatid nucleus are progressively replaced by transition proteins and ultimately by protamines, leading to highly compacted chromatin that protects paternal DNA during fertilization. PRM I and PRM II are the two major protamines present in mammalian sperm, and their precise expression ratio is critical for maintaining proper chromatin structure and sperm function (Oliva, 2006; Jodar & Oliva, 2014).

In the present study, paroxetine administration resulted in significant increases in the expression of both PRM I and PRM II relative to the control group. This finding indicates that paroxetine may disrupt the regulatory pathways governing chromatin remodeling during spermiogenesis. Although protamines are essential for DNA condensation,

their expression must be tightly coordinated. Excessive or dysregulated expression can lead to abnormal chromatin packaging, which may compromise sperm morphology, motility, and fertilizing ability.

The PRM I/PRM II ratio is widely regarded as a sensitive biomarker of sperm chromatin integrity. In healthy individuals, this ratio remains relatively stable; however, alterations in the ratio have been associated with defective spermatogenesis and male infertility (Steger & Balhorn, 2018; Pardede *et al.*, 2020). In the current study, paroxetine increased the PRM I/PRM II ratio from 0.51 in the control group to 0.88 in the negative control group, indicating disruption of the normal balance between the two protamines. Such imbalance may impair chromatin condensation and increase susceptibility of sperm DNA to damage. Treatment with ITREE partially corrected this imbalance by reducing the PRM I/PRM II ratio to 0.76, suggesting an improvement in chromatin packaging dynamics. This observation supports the potential protective role of ITREE in maintaining the molecular processes required for proper sperm maturation. Similarly, clomiphene administration resulted in a moderate reduction of the ratio to 0.84, indicating partial normalization. In contrast, tadalafil treatment produced a pronounced increase in the PRM I/PRM II ratio (1.23), which represents a greater deviation from the normal value observed in the control group. This finding suggests that while tadalafil may improve certain physiological aspects of reproductive function, its influence on the molecular regulation of protamine expression may be limited or indirect.

Overall, the findings of this study demonstrate that paroxetine induces significant alterations in the expression of key spermatogenic genes, including CREM, PRM I, and PRM II, thereby disrupting the molecular mechanisms responsible for sperm chromatin remodeling. Co-administration of ITREE was able to partially restore normal gene expression patterns and improve the balance between protamine proteins, indicating a protective effect on spermatogenic processes. When compared with the conventional therapeutic agents evaluated in this study, ITREE demonstrated a comparable or potentially superior ability to modulate the molecular markers associated with sperm maturation.

These results suggest that *Icacina trichantha* root ethanolic extract may represent a promising natural therapeutic candidate for mitigating drug-induced reproductive toxicity. Nevertheless, further investigations involving larger experimental models, protein-level analyses, and fertility outcome studies are necessary to elucidate the precise molecular mechanisms through which ITREE exerts its protective effects on male reproductive function.

Acknowledgements

The authors wish to acknowledge Poiema Analytical Laboratory and Education Consult for its immense contribution towards the success of this study.

Declaration of Conflict of Interest

The authors declare no conflicts of interest

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Nexus International University ISSN: 3136-6449; 12(2) 133-144

Molecular Detection and Expression of the *blaZ* Gene in Clinical *Staphylococcus aureus* Isolated from Hospital Patients

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Abstract. This study aimed to investigate the presence and expression of the *blaZ* gene in clinical *Staphylococcus aureus* isolates obtained from patients attending hospitals in Iraq. A total of 140 clinical specimens were collected, including wound swabs (71), vaginal swabs (35), urine samples (20), and sputum samples (14). Bacterial isolation was performed using Mannitol Salt Agar (MSA), followed by morphological and biochemical identification and confirmation using the VITEK-2 Compact system. Among the collected specimens, 25 *S. aureus* isolates were identified. β-Lactamase production was assessed using the Nitrocefin disk test, with 20 isolates showing positive results. These β-lactamase-positive isolates were selected for molecular analysis. PCR was performed for detection of the *blaZ*, *mecA* and 16S rRNA genes, and the *blaZ* gene was detected in all 20 examined isolates. The *mecA* gene was also detected in all selected isolates. The expression of *blaZ* was subsequently analyzed by real-time quantitative PCR using 16S rRNA as the reference gene. The results revealed considerable variation in *blaZ* expression among the examined isolates, with fold-change values ranging from 0.001953 to 128 and an overall mean of 17.90. Overall, the study demonstrated the presence of *blaZ* among the selected β-lactamase-positive clinical *S. aureus* isolates and showed considerable variation in its expression between isolates. These findings provide molecular characterization of *blaZ* expression among the clinical isolates examined in this study.

Keywords: *Staphylococcus aureus*; *blaZ*; β-Lactamase; Antimicrobial resistance; *mecA*; 16S rRNA.

1. Introduction

Staphylococcus aureus is an important Gram-positive bacterial pathogen associated with a wide range of human infections, including skin and soft-tissue infections as well as more severe diseases such as bacteremia, pneumonia, endocarditis and bone and joint infections. It can colonize humans without causing disease but may cause infection when it gains access to damaged tissues or sites where host defenses are compromised (Cheung et al., 2021). The ability of *S. aureus* to adapt to different environments and acquire antimicrobial resistance has contributed to its continued clinical importance in both community and healthcare settings. Resistant *S. aureus* is of particular concern because infections caused by resistant strains may be more difficult to treat and may increase the burden on healthcare systems (WHO, 2024; Touaitia et al., 2025). Antimicrobial resistance (AMR) is a major challenge to the effective treatment of bacterial infections. *S. aureus* can develop resistance through several mechanisms, including alteration of antimicrobial targets, changes in permeability, active efflux and enzymatic inactivation of antimicrobial agents. Among these mechanisms, enzymatic inactivation is particularly important for β-lactam antibiotics. β-lactamase enzymes hydrolyze the β-lactam ring of susceptible antibiotics, reducing or eliminating their antibacterial activity. Consequently, β-lactamase production represents an important mechanism contributing to β-lactam resistance in clinical *S. aureus* isolates (An et al., 2024; Brdová et al., 2024). In *S. aureus*, the *blaZ* gene is the principal genetic determinant encoding the staphylococcal β-lactamase enzyme. Expression of *blaZ* is regulated by the blaI-blaR1 regulatory system, in which BlaR1 functions as a β-lactam sensor and BlaI acts as a transcriptional repressor. Exposure to β-lactam antibiotics can activate this regulatory pathway, resulting in derepression of *blaZ* and subsequent

production of β-lactamase (Cong et al., 2020). Therefore, investigation of *blaZ* is important for understanding the genetic basis of β-lactam resistance and the mechanisms that influence its expression in *S. aureus* (Brdová et al., 2024).

Phenotypic and molecular methods provide complementary information for investigating β-lactam resistance. Antimicrobial susceptibility testing determines the susceptibility or resistance of bacterial isolates to selected antibiotics, while the Nitrocefin disk test provides a rapid phenotypic method for detecting β-lactamase activity. At the molecular level, polymerase chain reaction (PCR) can be used to detect specific resistance-associated genes such as *blaZ*. Molecular identification using the 16S rRNA gene can also support confirmation of bacterial identity because this gene contains conserved and variable regions that are useful for bacterial characterization (Drevinek et al., 2023; López-Aladid *et al*., 2023). Although PCR detection of *blaZ* demonstrates the presence of the resistance-associated gene, it does not necessarily indicate that the gene is expressed at the same level in all isolates. Differences in gene regulation and bacterial physiological conditions may influence the transcriptional activity of resistance determinants. Therefore, assessment of gene expression can provide additional information beyond simple gene detection. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) enables quantitative assessment of specific RNA transcripts and is therefore useful for investigating the expression of bacterial resistance-associated genes (Bong et al., 2024).

The relationship between the presence of *blaZ*, its expression and the corresponding phenotypic characteristics is particularly relevant in clinical isolates method (Vang et al., 2025). An isolate may carry the *blaZ* gene but show differences in β-lactamase activity or antimicrobial susceptibility depending on the level of gene expression and other regulatory factors. Thus, combining phenotypic testing with molecular detection and expression analysis can provide a more complete characterization of β-lactam resistance than using a single method s (Leistner et al., 2022). This integrated approach can help clarify whether the detection of *blaZ* is associated with phenotypic β-lactamase production and differences in antimicrobial susceptibility among clinical *S. aureus* isolates.The clinical significance of antimicrobial-resistant *S. aureus* also extends to public health because resistant infections can limit therapeutic options, increase treatment complexity and contribute to the persistence and transmission of resistant organisms in healthcare environments (Birlutiu et al., 2026). The World Health Organization has identified methicillin-resistant *S. aureus* as a high-priority bacterial pathogen, emphasizing the importance of continued research and surveillance of antimicrobial resistance. Therefore, studying resistance-associated determinants such as *blaZ* in clinical isolates contributes not only to understanding the molecular mechanisms of resistance but also to the broader monitoring and control of AMR in healthcare settings (WHO, 2024).

Accordingly, the present study aimed to investigate β-lactam resistance among clinical *Staphylococcus aureus* isolates, with particular focus on β-lactamase production and the role of the *blaZ* gene in this resistance mechanism. The study evaluated antimicrobial susceptibility patterns, assessed β-lactamase production using the Nitrocefin disk test, detected the *blaZ* and *mecA* genes using PCR and investigated *blaZ* expression in selected isolates using RT-qPCR to characterize the involvement of *blaZ* in β-lactamase-mediated resistance.

2. Materials and Methods

2.1 Sample Collection and Bacterial Identification

A total of 140 clinical specimens were collected from patients attending Tikrit General Hospital, Salah Al-Din Governorate and Medical City Hospital, Baghdad, Iraq, during the period from January 1 to April 16, 2026. The patients ranged in age from 10 to 50 years. The collected specimens included 71 wound swabs (50.71%), 35 vaginal swabs (25.00%), 20 urine samples (14.29%) and 14 sputum samples (10.00%). Clinical specimens were collected according to the patients' clinical presentation and the physician's request for microbiological investigation. Vaginal swabs were collected from patients presenting with clinical signs and symptoms of vaginal infection, while sputum specimens were collected from patients presenting with clinical signs and symptoms of respiratory tract infection. Clinical specimens were directly inoculated onto Blood Agar (HiMedia, India) and incubated aerobically at 37°C for 18–24 h. Plates showing no growth after the initial incubation were re-incubated for an additional 48 h. Suspected colonies were subcultured onto fresh Blood Agar to obtain pure cultures.

Initial identification of suspected *Staphylococcus aureus* isolates was performed based on colony morphology, Gram staining, catalase activity, coagulase production and mannitol fermentation on Mannitol Salt Agar (Oxoid, England). The isolates were subsequently subjected to automated identification using the VITEK 2 system (bioMérieux, France). Bacterial suspensions were prepared at a turbidity of approximately 0.50–0.63 McFarland and inoculated into Gram-Positive identification cards according to the manufacturer's recommended procedure. The cards were incubated at approximately 35.5 ± 1°C and automatically analyzed by the VITEK 2 system. Amplification of the bacterial 16S rRNA gene was additionally performed as a molecular assay for bacterial identification, as shown in Table 1 (Drevinek et al., 2023).

Table 1: Primers used for PCR and RT-qPCR.

Primer	Primer sequence (5-3)	Product size (bp)	Reference
blaZ	**F AAAGAAATCGGTGGAATCAAAA 22**	**248**	**Gene Pharma (Shanghai, China).**
	R TCTTACCGAAAGCAGCAGGT 20		
mecA	**F CAAAATGGAGCTTGGGAGTC 20**	**475**	**Gene Pharma (Shanghai, China).**
	R ATCATCGAAACTTGGCAAATG 21		
16s rRNA	**F CCAGACTCCTACGGGAGGCAG 21**	**210**	**Gene Pharma (Shanghai, China).**
	R CGTATTACCGCGGCTGCTG 19		

Phenotypic Characterization and Antimicrobial Susceptibility

β-Lactamase production was evaluated using a nitrocefin-based chromogenic test. Fresh bacterial colonies obtained after 18–24 h of incubation were tested, and development of a yellow-to-pink/red color was considered indicative of β-lactamase activity. Phenotypic detection of β-lactamase activity was included to complement molecular detection of the *blaZ* gene, as the presence of a resistance gene does not necessarily indicate equivalent phenotypic enzyme activity. *BlaZ* is a major β-lactamase-associated determinant of penicillin resistance in *S. aureus* (Lade & Kim, 2023; Brdová et al., 2024).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Fifteen antimicrobial agents were tested using commercial antibiotic discs (Bioanalyse, Turkey): oxacillin (30 µg/disc), methicillin (30 µg/disc), ampicillin (10 units/disc), gentamicin (10 µg/disc), erythromycin (15 µg/disc), tetracycline (30 µg/disc), doxycycline (30 µg/disc), clindamycin (20 µg/disc), chloramphenicol (30 µg/disc), vancomycin (30 µg/disc), levofloxacin (5 µg/disc), amoxicillin, azithromycin, ceftriaxone (30 µg/disc) and norfloxacin (30 µg/disc). The disk diffusion procedure was performed in accordance with CLSI M02 recommendations, and susceptibility categorization was based on the applicable Clinical and Laboratory Standards Institute (CLSI) M100 breakpoint criteria (CLSI, 2024; CLSI, 2026).

A bacterial suspension equivalent to 0.5 McFarland was prepared from a fresh culture and uniformly inoculated onto the agar surface using a sterile swab. Antibiotic disks were then placed on the inoculated plates and the plates were incubated aerobically at 37°C for 24 h. The inhibition zones were measured in millimeters and interpreted as susceptible, intermediate or resistant according to the applicable Clinical and Laboratory Standards Institute criteria. The antimicrobial agents tested included Ampicillin, Doxycycline, Clindamycin, Chloramphenicol, Vancomycin, Gentamicin, Erythromycin and Tetracycline. The disk diffusion procedure followed the standardized recommendations of CLSI M02, while interpretation was based on the current CLSI M100 breakpoints (CLSI, 2024, 2026). Antimicrobial susceptibility testing was included because antimicrobial resistance in *S. aureus* remains an important clinical concern, particularly in healthcare-associated infections. Recent reviews have emphasized the

diversity of molecular and phenotypic resistance mechanisms in *S. aureus* and the continuing need for standardized susceptibility testing and surveillance (Silva-Santana, 2025; Brdová *et al*., 2024; Hussein *et al*., 2024).

DNA Extraction and PCR Detection

Genomic DNA was extracted from 20 selected *S. aureus* isolates. Bacterial cultures were grown in nutrient broth at 37°C for 24 h. Approximately 2 mL of each culture was transferred into a microcentrifuge tube and centrifuged at 14,000 rpm for 5 min. The supernatant was discarded and the bacterial pellet was processed for genomic DNA extraction using the Geneaid genomic DNA extraction procedure (Taiwan). The pellet was treated with 500 µL of TE buffer followed by centrifugation under the same conditions. Subsequently, 500 µL of 2× CTAB solution was added, mixed thoroughly and incubated at 65°C for 30 min with intermittent mixing. Then, 100 µL of 20% SDS and 300 µL of phenol:chloroform:isoamyl alcohol were added. Following centrifugation, the aqueous phase was transferred to a new tube and the extraction step was repeated with an equal volume of phenol:chloroform:isoamyl alcohol. DNA was precipitated using two volumes of absolute ethanol at −20°C, washed with 500 µL ethanol and centrifuged. The DNA pellet was air-dried and dissolved in 100 µL of distilled water before storage at −20°C.

DNA concentration and purity were assessed using a NanoDrop spectrophotometer by measuring absorbance at 260 and 280 nm. DNA quality was evaluated using the A260/A280 ratio before PCR amplification. Conventional PCR was performed to detect the *blaZ*, *mecA* and 16S rRNA genes. The *blaZ* gene was selected as the principal molecular target because *BlaZ*-mediated β-lactamase production represents an important mechanism of β-lactam resistance in *S. aureus* (Lade & Kim, 2023; Brdová et al., 2024). The 16S rRNA gene was included as a bacterial molecular identification target. Recent clinical microbiology literature supports the use of 16S rRNA-based molecular approaches for bacterial identification (Drevinek et al., 2023).

PCR reactions were prepared in a final volume of 25 µL containing 12.5 µL of Master Mix, 5 µL of DNA template, 1 µL of each forward and reverse primer and 5.5 µL of nuclease-free water. Amplification was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s and extension at 72°C for 45 s, followed by a final extension at 72°C for 6 min. PCR products were separated by electrophoresis on 1% agarose gel and visualized using a UV transilluminator.

RNA Extraction, cDNA Synthesis and RT-qPCR

RNA was extracted from 20 selected *S. aureus* isolates for analysis of *blaZ* gene expression. The 20 isolates were selected based on positive β-lactamase activity detected by the Nitrocefin disk test and were subsequently subjected to molecular analysis. Bacterial cultures were grown in nutrient broth at 37°C for 24 h and centrifuged at 13,000 rpm for 2 min. The bacterial pellet was treated with 200 µL of lysozyme solution for 5 min, followed by the addition of 600 µL of TransZol. The mixture was incubated for 10 min, after which 200 µL of chloroform was added. Samples were centrifuged at 12,000 rpm for 10 min at room temperature. The upper aqueous phase was carefully transferred to an RNA purification column and processed using the appropriate washing buffers. Following the washing steps, an additional dry centrifugation was performed to remove residual ethanol. RNA was eluted in 100 µL of RNA dissolving solution and stored at −20°C until further analysis.

The extracted RNA was used for complementary DNA (cDNA) synthesis using RT-PCR Master Mix (Tinzyme, China). Each 20 µL reaction contained 10 µL of total RNA, 4 µL of 5× RT-PCR Master Mix and 6 µL of RNase-free water. Reverse transcription was performed at 25°C for 10 min, followed by 42°C for 15 min and 85°C for 5 s. The resulting cDNA was used as the template for subsequent real-time PCR analysis. The RT-qPCR workflow was performed in accordance with established methodological recommendations (Bong et al., 2024; Bustin et al., 2025).

Lyophilized primers were briefly centrifuged and dissolved in nuclease-free water. Working primer solutions were prepared at a concentration of 10 pmol/µL. RT-qPCR was performed using UltraSYBR Mixture with Low ROX, 2× (Tinzyme, China). Each reaction was prepared in a final volume of 25 µL containing 12.5 µL of 2× UltraSYBR mixture, 0.5 µL of forward primer, 0.5 µL of reverse primer, 2 µL of cDNA template and 9.5 µL of nuclease-free

water. Amplification was performed using a Real-Time PCR Detection System (Applied Biosystems, USA) (Bustin et al., 2025; Bong et al., 2024).

The thermal profile consisted of an initial denaturation step at 95°C for 10 min, followed by 35–40 amplification cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The *blaZ* gene was analyzed as the target gene, whereas the 16S rRNA gene was used as the reference gene for normalization. The selection and reporting of RT-qPCR conditions were guided by current recommendations emphasizing assay design, sample handling, reference-gene selection and transparent reporting of qPCR experiments (Bustin et al., 2025; Bong et al., 2024).

Relative *blaZ* expression was determined using the comparative cycle threshold (Ct) method. The Ct values obtained for *blaZ* were normalized to those of the 16S rRNA reference gene by calculating ΔCt (ΔCt = Ct_target − Ct_reference). The ΔCt value of each tested isolate was compared with that of the designated control sample to obtain ΔΔCt (ΔΔCt = ΔCt_target − ΔCt_control). Relative *blaZ* expression was calculated as fold change using the equation 2^−ΔΔCt according to Livak and Schmittgen (2001). The analysis and reporting of relative gene expression were conducted with consideration of current qPCR recommendations (Bustin et al., 2025).

Statistical Analysis

Statistical analysis was performed using SPSS software version 26. Quantitative data were expressed as mean ± standard deviation (SD). A P-value <0.05 was considered statistically significant.

3. Results and Discussion

Isolation and Identification of *Staphylococcus aureus*

A total of 140 clinical samples were examined. Bacterial growth was observed on Mannitol Salt Agar (MSA) in 126 samples (90%), while 14 samples (10%) showed no bacterial growth. Among the growing isolates, 31 (22.14%) were mannitol fermenters and 95 (67.86%) were non-fermenters, as shown in Table (2). The presumptive *Staphylococcus aureus* isolates were identified based on their cultural characteristics and conventional biochemical tests, followed by confirmation using the VITEK-2 Compact system.

Table 2: Number and percentage of isolates on Mannitol Salt Agar

Non-mannitol fermentation on results	Mannitol fermentation results	+ve	-ve	total
95	31	126	14	140
67.86%	%22.14	%90	%10	

The samples were distributed into four age groups, the highest proportion was recorded among individuals aged 41–50 years (30%), followed by those aged 31–40 years (28.12%), 21–30 years (25%) and 10–20 years (16.87%), as shown in Table (3). The higher representation of the older age groups may be related to differences in underlying health conditions, healthcare exposure and antimicrobial use, which can influence susceptibility to *S. aureus* infections. Age-related variation in the occurrence and antimicrobial resistance of *S. aureus* has also been reported in clinical populations (Tong et al., 2015; An et al., 2024).

Table 3: Distribution of samples according to age group.

Age group	Number	Percentage
10-20 years	22	%16.87
21-30 years	35	25%
31- 40 years	40	%28.12

41-50 years	43	%30.0
Total	140	%100

Distribution of Samples According to Sex

Of the 140 examined samples, 83 (59.29%) were obtained from males and 57 (40.71%) from females. Thus, males represented a higher proportion of the examined samples. This difference may be related to biological, behavioral, occupational, and environmental factors that can influence exposure to bacterial infections. However, other studies have reported higher rates among females, indicating that sex-related differences may vary according to the infection site and study population.

Distribution of Samples According to Isolation Source

The distribution of the examined clinical samples according to isolation source showed that wound swabs accounted for the highest proportion, with 71 samples (50.71%), followed by vaginal swabs with 35 samples (25.00%), urine samples with 20 samples (14.29%) and sputum samples with 14 samples (10.00%). The higher proportion of wound specimens may be related to the susceptibility of damaged skin and soft tissues to bacterial colonization and infection by *S. aureus*, which is a common pathogen associated with skin and wound infections (Cheung et al., 2021; Touaitia et al., 2025).

Cultural, Microscopic, Biochemical Identification of *Staphylococcus aureus*.

Cultural examination showed that the suspected *S. aureus* isolates grew on Mannitol Salt Agar and produced yellow colonies with a corresponding yellow color change in the medium due to mannitol fermentation. The colonies were generally circular and medium-sized. Microscopic examination revealed Gram-positive cocci arranged predominantly in grape-like clusters, which is characteristic of the genus *Staphylococcus*. Based on the cultural and microscopic characteristics, 31 isolates were considered presumptive *S. aureus*. The 31 suspected *S. aureus* isolates were subjected to conventional biochemical tests. All isolates were Gram-positive and positive for catalase, coagulase, and mannitol fermentation, with a positivity rate of 100% for each test.

The combined cultural, microscopic, and biochemical characteristics supported the identification of the isolates as *S. aureus*. Catalase positivity distinguishes Staphylococcus from catalase-negative genera such as Streptococcus, while coagulase positivity is an important characteristic used to differentiate *S. aureus* from most coagulase-negative staphylococci (Cheung et al., 2021).

No Individual Identification Tests

The Gram stain showed purple, Gram-positive cocci arranged mainly in clusters. The catalase test produced visible bubbles following the addition of hydrogen peroxide, indicating a positive reaction. Both slide and tube coagulase tests produced positive reactions in all 31 isolates. Mannitol fermentation resulted in a yellow color change of the MSA medium, confirming the characteristic fermentation reaction.

The results of these tests were consistent with the expected phenotypic characteristics of *S. aureus* and therefore provided reliable preliminary identification before further automated and molecular confirmation.

Detection of β-Lactamase Production in *Staphylococcus aureus* Isolates Using the Nitrocefin Disk Test.

β-Lactamase production was detected in 25 *Staphylococcus aureus* isolates using the Nitrocefin disk test. The results showed that 20 isolates (80%) were positive, as indicated by a color change from yellow to red within 1–5 min. These 20 β-lactamase-positive isolates were subsequently selected for molecular analysis. The present result was comparable with that reported by Kilani et al. (2024), who detected β-lactamase production in 77.5% of *S. aureus* isolates using a Nitrocefin-based assay. Onaolapo et al. (2016) reported β-lactamase production in 64% of *S. aureus* isolates, whereas

Oliveira et al. (2016) reported a lower rate of 48%. Such differences may be related to variations in bacterial sources, geographical areas and study conditions.

Antimicrobial Susceptibility Testing.

The antimicrobial susceptibility of 20 *S. aureus* isolates was determined against 15 antibiotics using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to CLSI (2024) guidelines (Fig. 1). The antimicrobial susceptibility testing revealed variable resistance patterns among the tested isolates. The highest resistance rate was observed for Ampicillin (100%), followed by Ceftriaxone (95%), Methicillin (95%) Levofloxacin (85%), Azithromycin (75%), Oxacillin (60%), Norfloxacin (45%), Erythromycin (35%), Amoxicillin (30%), Gentamicin (20%), Tetracycline (10%), Clindamycin (5%), Chloramphenicol (5%), Vancomycin (5%) and Doxycycline (5%).

Intermediate susceptibility was observed for Tetracycline (20%), Norfloxacin (5%), Amoxicillin (5%), Azithromycin (5%) and Vancomycin (5%). The highest susceptibility rates were observed for Doxycycline (95%), Clindamycin (95%), Chloramphenicol (95%), Erythromycin (65%), Gentamicin (80%), Oxacillin (40%) and Methicillin (5%). The susceptibility rates for Tetracycline, Norfloxacin, Amoxicillin, Azithromycin and Vancomycin were 70%, 50%, 65%, 20% and 90%, respectively, while the lowest susceptibility rate was recorded for Ampicillin (0%).

The antimicrobial susceptibility pattern observed in the present study showed both similarities and differences compared with previous reports. The relatively high susceptibility to Doxycycline, Gentamicin and Vancomycin observed in the present study is comparable to findings reported by Birlutiu et al. (2026), who reported low resistance to Doxycycline (9.50%), Gentamicin (with 92.9–100% susceptibility) and Vancomycin (2.92% resistance) among clinical *S. aureus* isolates. However, the present study showed lower resistance to Clindamycin and Tetracycline than that reported in their study, indicating variation in antimicrobial susceptibility patterns between clinical populations.

In contrast, An et al. (2024) reported substantially higher resistance rates to Azithromycin (82.28%), Erythromycin (82.82%) and Clindamycin (82.32%) compared with the resistance rates observed in the present study (75%, 35% and 5%, respectively). Their study also reported higher resistance to Tetracycline (67.06%) and Gentamicin (25.87%) than the corresponding rates in the present study (10% and 20%, respectively). These differences may reflect variation in geographical location, patient populations, specimen types, hospital settings and temporal patterns of antimicrobial use and resistance. The complete resistance to Ampicillin is consistent with Odetokun et al. (2023) and may be associated with β-lactamase production, which contributes to the inactivation of β-lactam antibiotics (Aarris et al., 2025). The resistance observed to Oxacillin and Methicillin indicates reduced activity of these antibiotics against some of the studied isolates. The high susceptibility to Doxycycline, Clindamycin, Chloramphenicol and Vancomycin indicates that these antibiotics retained relatively good activity against the isolates examined. Overall, the findings demonstrate considerable resistance to some β-lactam antibiotics, particularly Ampicillin, while several other antibiotics maintained high activity against the studied *S. aureus* isolates. From a public health perspective, the observed resistance pattern highlights the importance of continuous antimicrobial susceptibility surveillance and antimicrobial stewardship in healthcare settings. The complete resistance to Ampicillin may limit the usefulness of this commonly used β-lactam agent against the clinical *S. aureus* isolates examined in the present study (Table 4).

Table 4: Results of the antimicrobial susceptibility test.

%	Sensitive No.	%	Intermediate No.	%	Resistant No.	Symbol	Antibacterial	No.
65	13	–	–	35	7	E	Erythromycin	1
70	14	20	4	10	2	ET	Tetracycline	2
95	19	–	–	5	1	DO	Doxycycline	3
95	19	–	–	5	1	DA	Clindamycin	4
80	16	–	–	20	4	CN	Gentamicin	5
95	19	–	–	5	1	C	Chloramphenicol	6
40	8	–	–	60	12	OX	Oxacillin	7
5	1	–	–	95	19	ME	Methicillin	8
0	0	–	–	100	20	AMP	Ampicillin	9
50	10	5	1	45	9	NO	Norfloxacin	10
15	3	–	–	85	17	LFX	Levofloxacin	11
65	13	5	1	30	6	AMX	Amoxicillin	12

5	1	–	–	95	19	CTR	Ceftriaxone	13
20	4	5	1	75	15	AZM	Azithromycin	14
90	18	5	1	5	1	VA	Vancomycin	15

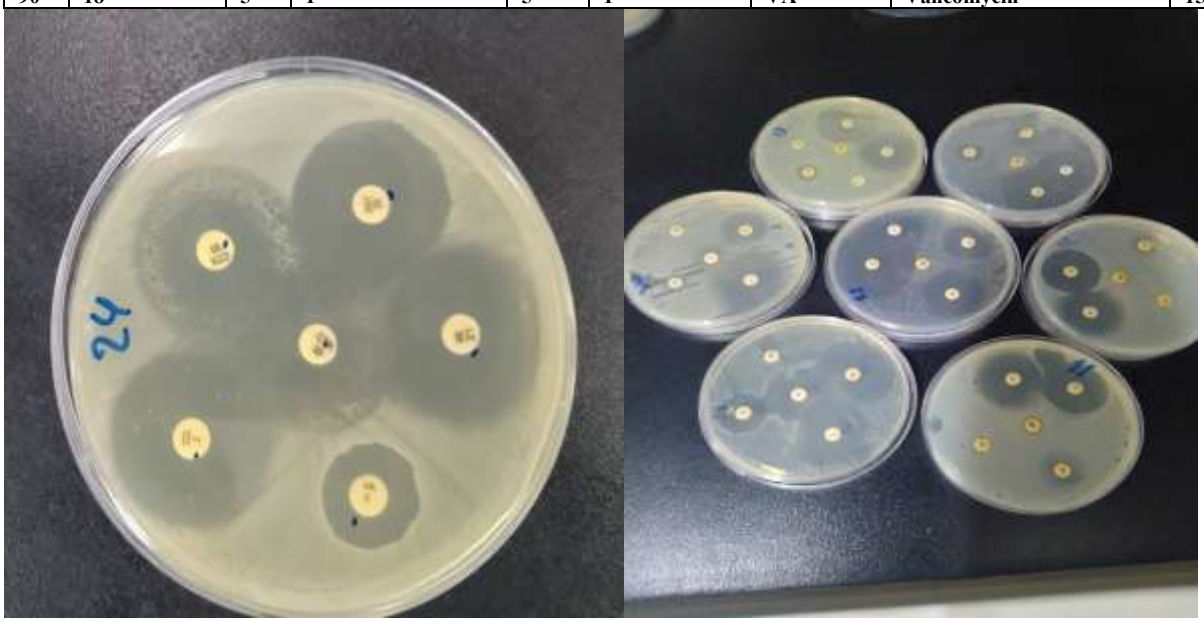


Figure 1: Selected results of the antimicrobial susceptibility test for *Staphylococcus aureus*.

PCR Amplification of the 16S rRNA Gene

The 16S rRNA gene was amplified by conventional PCR to confirm the molecular identification of *S. aureus* (Fig 2). Specific primers were used to amplify a 210-bp fragment. Agarose gel electrophoresis showed clear bands at the expected size in the examined isolates, confirming successful amplification and supporting the identification of the bacterial isolates.

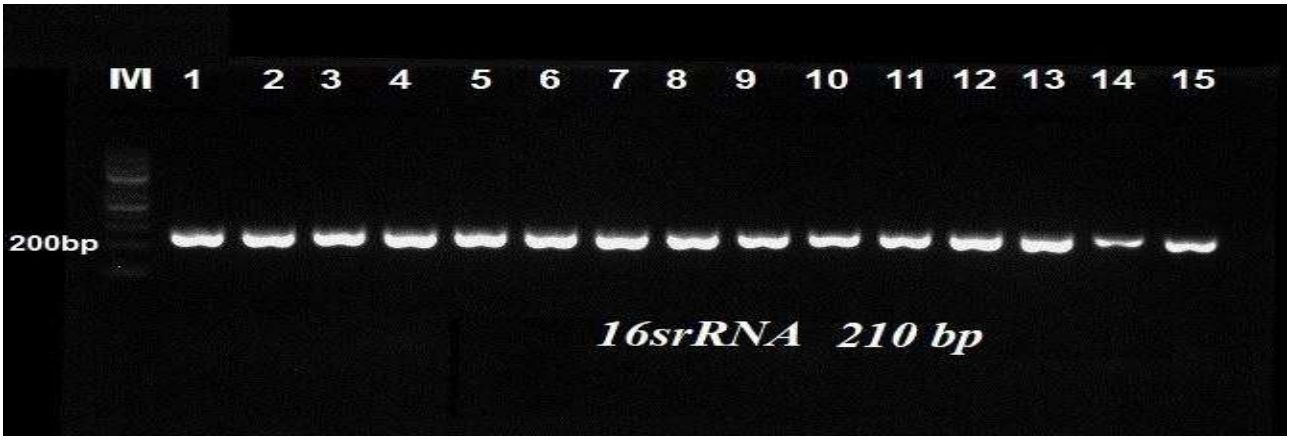


Figure 2: PCR amplification of the 16S rRNA gene, 210-bp product.

Amplification of the *mecA* Gene

The *mecA* gene was detected by PCR in 20 selected clinical *Staphylococcus aureus* isolates. A specific 475-bp PCR product was obtained in all 20 tested isolates, giving a detection rate of 100% (20/20). This result indicates the presence of the *mecA* gene among the isolates selected for molecular analysis. The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has low affinity for β-lactam antibiotics and is associated with methicillin resistance (Lade & Kim, 2023). As shown in Figure (3), electrophoresis on 1.5% agarose gel at 100 V for 60 min revealed clear bands at approximately 475 bp, confirming amplification of the *mecA* gene in the tested isolates.

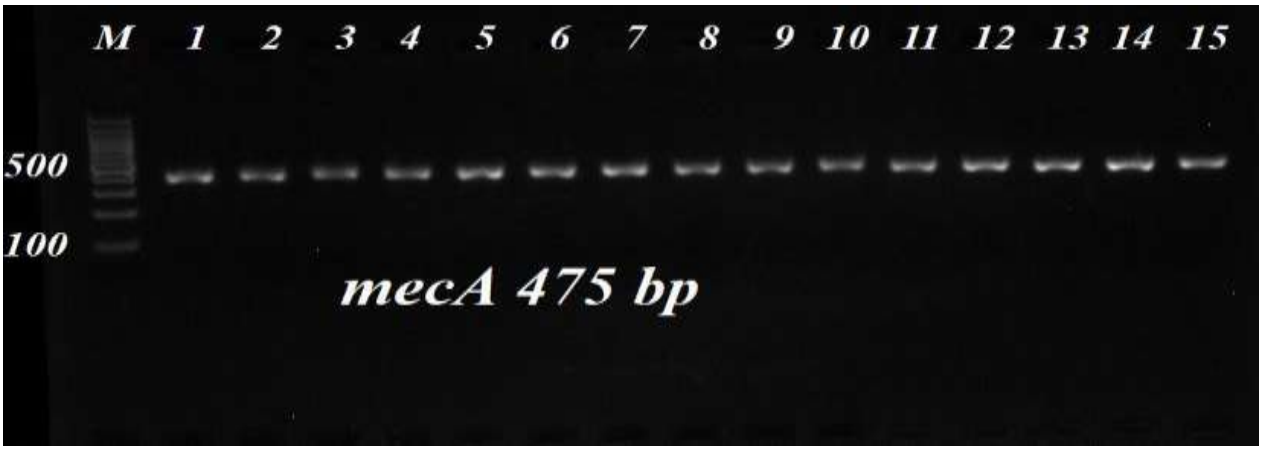


Figure 3: PCR amplification of the *mecA* gene, 475-bp product.

PCR Amplification of the *blaZ* Gene

The *blaZ* gene was detected by conventional PCR in selected *S. aureus* isolates (Fig 4). The primers amplified a specific fragment of 248 bp. Clear bands corresponding to the expected size were observed on agarose gel electrophoresis, indicating the presence of the *blaZ* gene in all examined isolates. The presence of this gene confirms the carriage of *blaZ* in the examined isolates and indicates their genetic potential for β-lactamase production (Aarris et al., 2025).



Figure 4: PCR amplification of the *blaZ* gene, 248-bp product.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from 20 selected *S. aureus* isolates using the TransZol™ Up Plus RNA Kit according to the manufacturer's instructions. The RNA was subsequently converted into complementary DNA (cDNA) using a reverse-transcription Master Mix. The resulting cDNA was used as the template for RT-qPCR analysis.

Real-Time qPCR Analysis of *blaZ* Gene Expression

The expression levels of the *blaZ* gene were determined in 20 selected clinical *S. aureus* isolates using RT-qPCR and the comparative Ct method ($2^{-}\Delta\Delta Ct$). Five replicates were performed for each isolate, and the reported fold-change value represents the mean of the five replicates. The results showed marked variation in *blaZ* expression among the examined isolates, with fold-change values ranging from 0.001953 to 128 and an overall mean of 17.90. Some isolates exhibited very low expression levels, whereas others showed markedly higher expression levels (Table 5).

Table 5: Relative expression (fold-change) of the *blaZ* gene in isolates.

Fold change (Mean ± SD)	Sample
0.000195 ± 0.001953 [a]	**1**
0.05 ± 0.50 [b]	**2**
1.60 ± 16.00 [d]	**3**
3.20 ± 32.00 [f]	**4**
6.40 ± 64.00 [g]	**5**
0.05 ± 0.50 [b]	**6**
0.000781 ± 0.007813 [a]	**7**
0.40 ± 4.00 [c]	**8**
6.40 ± 64.00 [g]	**9**
0.80 ± 8.00 [d]	**10**
1.60 ± 16.00 [e]	**11**
0.025 ± 0.25 [b]	**12**
0.20 ± 2.00 [c]	**13**
0.40 ± 4.00 [c]	**14**
0.20 ± 2.00 [c]	**15**
0.05 ± 0.50 [b]	**16**
0.001563 ± 0.015625 [a]	**17**
1.60 ± 16.00 [e]	**18**
0.025 ± 0.25 [b]	**19**
12.80 ± 128.00 [h]	**20**
17.90 ±32.90	**Mean**

Different superscript letters indicate statistically significant differences among the fold-change expression levels of the examined *S. aureus* isolates ($P < 0.05$).

The present study demonstrated considerable variation in relative *blaZ* expression among the examined clinical *Staphylococcus aureus* isolates, with fold-change values ranging from 0.001953 to 128. Values below 1 indicate lower *blaZ* expression relative to the designated control, whereas values above 1 indicate higher relative expression. This wide range suggests substantial isolate-to-isolate variation in *blaZ* transcription. The overall mean fold-change of 17.90 represents the arithmetic mean of the relative expression values and should not be interpreted as a uniform 17.90-fold increase in *blaZ* expression across all isolates. Such variation may reflect differences in the genetic and regulatory characteristics of the *blaZ* system among individual isolates. These findings are consistent with Aarris et al. (2025), who reported substantial genetic variation among *blaZ* variants in clinical *S. aureus* isolates. Similarly, Lee et al. (2025) demonstrated that variation in the regulatory mechanisms of *blaZ* can influence β-lactamase expression. In contrast, a recent study of *blaZ*-positive *S. aureus* isolates under cefazolin exposure reported no consistent increase in *blaZ* expression under the tested conditions, suggesting that expression may also depend on the bacterial strain and experimental conditions. Therefore, the variation observed in the present study may reflect isolate-specific genetic and regulatory characteristics of the *blaZ* system.

The melting curve (Melt Curve) of the *blaZ* gene amplification product was analyzed to verify the specificity and quality of the Real-Time qPCR reaction. The curve showed a distinct main melting peak at approximately 81–82°C, supporting the presence of a specific *blaZ* amplification product and the absence of evident nonspecific amplification products, as shown in Figure (5).

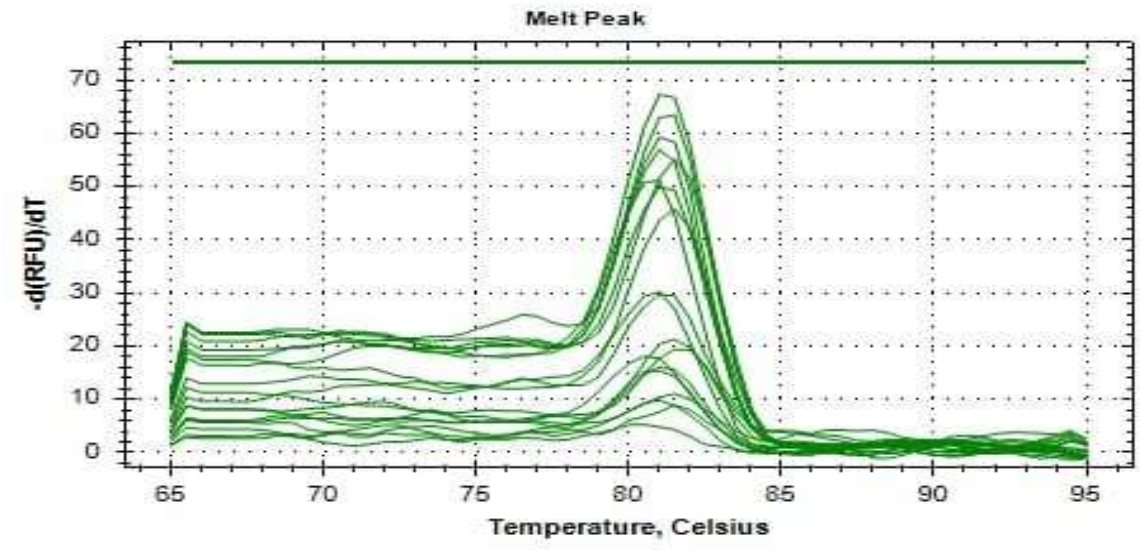


Figure 5: Melt curve analysis of the *blaZ* gene amplification produce using RT-qPCR.

Overall, the variation in *blaZ* expression observed among the clinical *S. aureus* isolates highlights the differences in *blaZ* expression between isolates and supports the importance of examining this gene in clinical isolates.

Conclusions

The present study demonstrated variable antimicrobial susceptibility patterns among clinical *Staphylococcus aureus* isolates, with complete resistance to ampicillin and high susceptibility to several of the tested antibiotics. Molecular analysis detected the *blaZ* gene in the selected β-lactamase-positive isolates, while real-time quantitative PCR revealed considerable variation in *blaZ* expression among the examined isolates. The observed differences in expression may reflect isolate-specific genetic and regulatory characteristics of the *blaZ* system. Overall, these findings characterize antimicrobial resistance patterns and *blaZ* expression among the clinical *S. aureus* isolates examined in this study.

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Nexus International University ISSN: 3136-6449; 12(2) 145-151

Pathogenicity of *Beauveria Bassiana* Against the Whitefly (*Bemisia Tabaci*) on Tomato Plants Under Laboratory Conditions

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Abstract. The pathogenic efficacy of Beauveria bassiana against Bemisia tabaci adults and second-instar nymphs on tomato plants was evaluated under laboratory conditions and the effect of exposure time and application rate of conidia on the adults and second-instar nymphs of B. tabaci was investigated. The three concentrations of conidia (1 × 106, 1 × 107 and 1 × 108 conidia/mL) and the control treatment were tested. Mortality was observed at day 3, 5, 7 and 10 following treatments. The higher the concentration of conidia, the higher the number of deaths in both developmental stages, and the longer the exposure time. In adults, the 1 × 108 conidia/mL treatment produced the highest mortality (86.6%) after 10 days, followed by 65.7% and 53.4% at 1 × 107 and 1 × 106 conidia/mL, respectively. The reported LT50 values decreased from 8.72 days at 1 × 106 conidia/mL to 6.76 and 4.93 days at 1 × 107 and 1 × 108 conidia/mL, respectively. There was no mortality in the control treatment. In nymphs, the highest mortality was 78.7% at 1 × 108 conidia/mL after 10 days, compared with 62.0% and 51.0% at 1 × 107 and 1 × 106 conidia/mL, respectively. The reported LT50 values were 9.34, 8.15, and 6.22 days at 1 × 106, 1 × 107, and 1 × 108 conidia/mL, respectively. The results showed concentration and time-dependent pathogenic activity of B. bassiana against adults and nymphs of B. tabaci, which confirmed its suitability for biological control in IPM of tomato whitefly.

Keywords: Beauveria bassiana; Bemisia tabaci; whitefly; biological control; entomopathogenic fungus.

1. Introduction

Tomato (Solanum lycopersicum) is an economically valuable crop and is highly grown for food production and export. The nutritional value of tomatoes is attributed to the presence of vitamins, minerals, dietary fiber, proteins, essential amino acids, monounsaturated fatty acids as well as carotenoids (Chaudhary et al., 2018; Vats et al., 2020). The production of tomatoes has further grown in the world indicating its significance in agricultural markets (Rudrapur & Hiremath, 2024). The tomato is also an important model crop for plant and agricultural research (Makhadmeh et al., 2022).

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae), whitefly is one of the most important of tomato plants in Iraq. It is very polyphagous and has been reported from over 600 plant species. Its persistence may be favored and spread under favorable environmental conditions by continuous crop production (Haj Hasan et al., 2015).

Whiteflies cause damage to the plants by inserting proboscis into the phloem and sucking plant sap, causing leaf yellowing, drying and early leaf drop. Honeydew excretion also favors sooty mold growth which decreases plant marketability and photosynthetic activity of affected plants (Horowitz et al., 2011).

Bemisia tabaci is also a vector of many plant viruses leading to losses. It has the ability to develop resistance against insecticides which makes chemical control more challenging, and requires alternative biological control measures to be found (Gerling, 1990; Kareem et al., 2020).

Beauveria bassiana, an entomopathogenic fungus, has been shown to be pathogenic against B. tabaci and other economically important insect pests. Some of its merits are that it can be used in a biological control program and it may be effective in insects upon contact with fungal conidia (Faria & Wraight, 2001; Sani et al., 2020). B. bassiana has been recently studied as a part of sustainable pest management, such as B. bassiana application to tomato pests (Matrood & Fotouhifar, 2023; Keerthi et al., 2023). Accordingly, the pathogenicity of B. bassiana was assessed against adult and nymphal B. tabaci in laboratory conditions in the present study.

2. Materials and Methods

2.1 Rearing of the Insect

A colony of B. tabaci was started from specimens that were collected from infested tomato leaves in Wasit Governorate, Iraq. The insects were reared under tomato plants (Solanum lycopersicum) in net cages 60 cm high and 60 cm diameter, at the temperature of 25 ± 2 °C, relative humidity 65 ± 5% and photoperiod of 16 h light and 8 h dark. The rearing protocol was as described by Tan et al. (2014).

2.2 Fungal Culture

The B. bassiana isolate was collected from Agricultural Research Center, Ministry of science and technology, Iraq. The isolate was grown on Potato Dextrose Agar (PDA) with chloramphenicol to keep bacteria to a minimum. Plates were incubated for 4-7 days at a temperature of 27 ± 2 °C and 65 ± 5% RH. The isolate was identified morphologically, following standard taxonomic procedures (Rehner et al., 2011), by characteristics of the colonies and the conidial morphology. Conidial suspension was prepared for each culture by adding 10 mL of sterile distilled water with 0.05% Tween 80 to the culture. Conidia were gently scraped, vortexed for 2 min and passed through sterile muslin cloth to remove mycelial fragments and debris. The conidial concentrations were adjusted to 1 × 106, 1 × 107, and 1 × 108 conidia/mL using a hemocytometer (Lacey, 2012).

2.3 Culture Medium Used for Fungal Cultivation

2.3.1 Potato Dextrose Agar (PDA)

PDA was used for cultivation of B. bassiana. Commercial PDA powder was prepared according to manufacturer's instructions at 39 g/L distilled water and then it was sterilized before pouring into sterile Petri dishes.

2.4 Laboratory Experiments

2.4.1 Activation of the Fungal Isolate

PDA was used for activation of the fungal isolate. Commercial PDA powder was dissolved in 1 L of distilled water and chloramphenicol was added at 250 mg/L to inhibit bacterial contamination, thus preparing the medium. The medium was poured into sterile Petri dishes and kept in a refrigerator at 4 °C until used. The B. bassiana isolate was reactivated by transferring a 0.5 cm diameter agar plug from the stored culture to PDA and growing it for one week at 25 °C for fungal growth and sporulation. The 0.5-cm diameter agar plugs were transferred to the sterile tube filled with PDA slant, cultured at 25 ± 2 °C for 1 week, and stored at refrigerated temperature (Wang et al., 2010).

2.4.2 Laboratory Bioassay of B. bassiana Against B. tabaci

2.4.2.1 Effect of B. bassiana on Adult Mortality of B. tabaci

The effect of B. bassiana on the mortality of adult B. tabaci was evaluated.

Adult B. tabaci (2–3 days old) were used in the study of the effect of B. bassiana on this species. Three conidial concentrations (1 × 106, 1 × 107 and 1 × 108 conidia/mL) and a distilled-water control were tested, each with three

replications. Adult whiteflies were placed in each replicate (10 per replicate). Appropriate suspension was applied to each replicate with one milliliter of the suspension per replicate on a hand sprayer in small plastic cages. The treated insects were then placed in 9-cm-diameter Petri dishes. Distilled water was given to the control insects. Mortality was noted daily and dead insects were examined for symptoms of fungal infection.
Corrected mortality (%) was calculated using Abbott's formula:
Corrected mortality (%) = [(T − C) / (100 − C)] × 100

Where T is the mortality percentage in the treated group and C is the mortality percentage in the control group (Abbott, 1925). Because control mortality was 0% at all assessment times in the present experiment, the Abbott-corrected mortality values were numerically identical to the observed treatment mortality values.

2.4.2.2 Effect of B. bassiana on Mortality of B. tabaci Nymphs

Using a soft brush, 2nd instar nymphs of B. tabaci were transferred to 30-day-old tomato plants. Using a hand sprayer, the nymphs were treated with 1 mL of a B. bassiana suspension at a concentration of 1 × 106, 1 × 107 or 1 × 108 conidia/mL. Distilled water was used for the control plants. Plastic cages 60 cm high and 60 cm in diameter covered with gauze were used for individual plant cultivation in tomato plants. Each treatment was replicated 3 times, and consisted of 10 nymphs per experimental unit. The daily mortality of the nymphs was recorded until 10 days after treatment (d.a.t.) (Flawerina et al., 2021).

2.5 Statistical Analysis

The experiments were designed as a Completely Randomized Design (CRD). Data analysis was performed using SPSS version 20. Treatment means were compared using Duncan's Multiple Range Test (DMRT), and differences were considered significant at $P \leq 0.05$. The letter groupings in Tables 1 and 2 represent the comparisons reported for each assessment time. The manuscript did not contain replicate-level mortality data; therefore, the reported LT50 estimates were retained as originally reported and were not independently recalculated during the present revision. No new LT50 values were introduced.

3. Results and Discussion

3.1 Efficacy of Beauveria bassiana Against Adult Bemisia tabaci

The mortality of B. tabaci adults increased with the concentration and time upon exposure to B. bassiana (Table 1). After 10 days, mortality reached 53.4%, 65.7%, and 86.6% at 1 × 106, 1 × 107, and 1 × 108 conidia/mL, respectively, whereas no mortality was recorded in the control. At 1 × 108 conidia/mL, mortality increased from 23.3% after 3 days to 46.7%, 73.3%, and 86.6% after 5, 7, and 10 days, respectively. The reported LT50 values were 8.72, 6.76, and 4.93 days for 1 × 106, 1 × 107, and 1 × 108 conidia/mL, respectively. Therefore, the highest conidial concentration resulted in the highest mortality and shortest reported LT50.

Table (1): Effect of different concentrations of B. bassiana on the mortality of B. tabaci adults

Spore concentrations (ml^{-1})	Percentage mortality % at different exposure periods				
	3days	5days	7days	10days	LT_{50}
1×10^6	6.7% (a)	24.3% (a)	43.4% (a)	53.4% (a)	8.72
1×10^7	16.7% (ab)	33.2% (a)	56.7% (b)	65.7% (b)	6.76
1×10^8	23.3% (b)	46.7% (b)	73.3% (c)	86.6% (c)	4.93
Control	0.00	0.00	0.00	0.00	-

Note: Values are means of three replicates. Different letters within a column indicate significant differences at $P \leq 0.05$ (DMRT).

The present findings demonstrated a clear concentration- and time-dependent response. Adult mortality at 10 days increased from 53.4% at 1 × 10^6 conidia/mL to 65.7% at 1 × 10^7 conidia/mL and 86.6% at 1 × 10^8 conidia/mL, while the corresponding reported LT50 values decreased from 8.72 to 6.76 and 4.93 days. The same directional pattern was

observed in second-instar nymphs, supporting the conclusion that greater conidial exposure was associated with higher mortality and a shorter time to reach 50% mortality.

The increase in mortality with increasing conidial concentration was consistent with the pathogenic activity reported for entomopathogenic fungi. B. bassiana can infect the host after the conidia attach to the surface, germinate and penetrate the cuticle, and then grow inside the host. Secondary metabolites that are generated during infection can also cause impairment and mortality in the host (Pedrini, 2022). Based on the findings of the present study, the tested B. bassiana isolate has activity dependence on the concentration against the adult B. tabaci. The same biological-control ability has been observed in the recent reviews and experiments (Keerthi et al., 2023; Kumar et al., 2024).

3.2 Efficacy of Beauveria bassiana Against Bemisia tabaci Nymphs

As seen in Table 2, B. bassiana also caused mortality in B. tabaci nymphs and exposure duration and conidial concentration had a significant influence on the nymph mortality. At 1 × 108 conidia/mL, mortality increased from 20.0% after 3 days to 40.4%, 61.3%, and 78.7% after 5, 7, and 10 days, respectively. At 10 days, mortality was 51.0% at 1 × 106 conidia/mL and 62.0% at 1 × 107 conidia/mL, with no mortality in the control. The reported LT50 values were 9.34, 8.15, and 6.22 days at 1 × 106, 1 × 107, and 1 × 108 conidia/mL, respectively.

This pattern could be explained by the concentration-dependent efficacy of entomopathogenic fungi (Figure 2) which may be attributable to the variety of mechanisms used by the fungi to infect and kill the insect hosts. The broad range of biologically active secondary metabolites with antimicrobial properties that entomopathogenic fungi are known for, as reported by Wadhwa et al. (2024), also appeals to their pathogenicity, overcoming host defense mechanisms, and supports their use in biological control programs.

The rising death rate in nymphs due to the duration of exposure was consistent with the infection process of entomopathogenic fungi. After the contact with the insect surface, conidia stick, germinate, penetrate the insect cuticle and multiply inside the insect. The time needed for these stages coincides with the progressive mortality obtained in the present experiment. The greatest mortality was associated with the highest conidial concentration, and the shortest reported LT50, suggesting a greater pathogenic activity with increased conidial density. Concentration- and time-dependent mortality of B. bassiana-treated specimens has also been reported in previous studies (Aş et al., 2024; Aly et al., 2025).

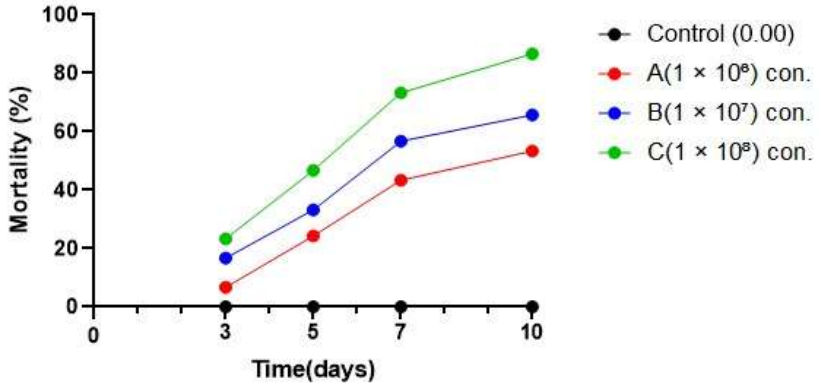


Figure 1: *Adult mortality of B. tabaci treated with different concentrations of B. bassiana at different exposure periods.*

The results of the current study are similar to others which have demonstrated higher mortality rates of insects as a result of increasing entomopathogenic fungal conidia. Aly et al. (2025) found that B. bassiana led to a greater number of dead larvae of S. frugiperda as the concentration of the fungus increased. Similarly, Aş et al. (2024) found that as the concentration and exposure duration of Zophobas morio larvae increased, so did the rate of mortality. The results

indicate that the conidial concentration and exposure period are important factors to consider in testing B. bassiana pathogenicity against insect hosts.

Table (2): Effect of different concentrations of B. bassiana on the mortality of B. tabaci nymphs

Concentrations ML^{-1}/(spores)	Percentage mortality % at different exposure periods				
	3days	5days	7days	10days	LT_{50}
1×10^{6}	3.3% (a)	26.7% (a)	36.7% (a)	51.0% (a)	9.34
1×10^{7}	13.3% (b)	30.0% (a)	46.7% (b)	62.0% (b)	8.15
1×10^{8}	20.0% (b)	40.4% (b)	61.3% (c)	78.7% (c)	6.22
Control	0.00	0.00	0.00	0.00	–

Note: Values are means of three replicates. Different letters within a column indicate significant differences at $P \leq 0.05$ (DMRT).

This indicates that increasing the spore concentration not only increased the final mortality rate, but also contributed to reaching of 50% mortality. The fungal concentration and the exposure time of the B. tabaci nymphs were shown to be influential in the results, suggesting that B. bassiana can be used as a biological control agent for B. tabaci nymphs. The increase of mortality of nymphs could be attributed to the sequential process of infection of entomopathogenic fungus (EPF) from the initial spore adhesion to the body surface to the subsequent growth phases of germination and penetration of the cuticle to the growth phase within the host body and the production of compounds that affect the host eventually leading to death. As a result, the mortality effect cannot be seen immediately following treatment, but can gradually increase with length of treatment as the fungal infection works its way through the host (Figure 2).

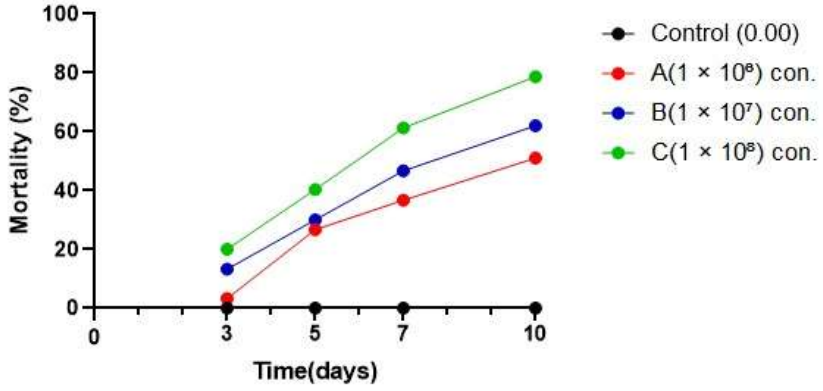


Figure 2: *Mortality of second-instar B. tabaci nymphs treated with different concentrations of B. bassiana at different exposure periods.*

The findings that fungal concentration is positively correlated with the mortality rate of treated nymphs were similar to those of several recent studies. As per Aly et al. (2025), as the numbers of spores per mL increased the probability of fungal infection increased, causing more spores to infect the third instar larvae of S. frugiperda, and thus increasing the pathogenic effect of the fungus.

The present results are similar to Aş et al. (2024) who showed that the effectiveness of B. bassiana against third instar Zophobas morio larvae significantly increased as exposure period increased and was continued. The mortality rate reached 50% at a concentration of 1×10^{5} conidia/mL, while the mortality increased to 100% at 1×10^{7} conidia/mL after six days of treatment. The results of the current study indicated the mortality rate of B. tabaci nymphs increased with the increasing number of spores, especially when the spore concentration was 1×10^{8} conidia/mL, which is consistent with the results of the above-mentioned study.

3. Conclusion

The high mortality rates seen in B. bassiana treated insects could be partly due to its multiple pathogenic mechanisms; B. bassiana can produce a number of secondary metabolites with toxic and immunosuppressive effects that are important to the augmentation of its virulence and reduction of insect host defenses, leading to insect host mortality (Pedrini, 2022). The increase in mortality was significant with increasing concentration, and the pathogenic mechanisms were similar to that observed in the present study, which showed that B. bassiana isolate is effective against adult B. tabaci.

Beauveria bassiana exhibited pathogenic activity on both adult and nymphal stage of Bemisia tabaci in the laboratory conditions tested. The higher the concentration of conidia and exposure time, the more deaths were observed. The highest mortality was obtained following the treatment with the 1 × 108 conidia/mL after 10 days with 86.6% and 78.7% mortality in adults and nymphs, respectively. A direct correlation between the reported LT50 values and concentration of conidia was also found. The results here indicate that B. bassiana is promising for use as a biological control agent against B. tabaci of tomato plants.

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Nexus International University ISSN: 3136-6449; 12(2) 153-178

Efflux-Mediated Synergy and Preserved in Vitro Selectivity of Zinc Oxide Nanoparticle–Ampicillin Combinations against Multidrug-Resistant *Klebsiella Pneumoniae* and *Pseudomonas Aeruginosa*

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Abstract
Background: Zinc oxide nanoparticles have the ability to rescue the β-lactam activity against multidrug resistant Gram-negative pathogens, but only concentrations that synergy that do not harm the host cells are therapeutically useful.
Objectives: To quantify the synergy between ZnO-NP and ampicillin in clinical isolates of MDR Klebsiella pneumoniae and Pseudomonas aeruginosa, as well as to parallelly determine if the synergistic concentrations are selective to the bacteria compared with the human lung fibroblasts and keratinocytes.
Methods: One hundred and forty-five non-duplicate clinical isolates (71 K. pneumoniae, 74 P. aeruginosa) recovered at Fallujah General Hospital, Anbar, Iraq, were studied. MICs and MBCs were obtained using broth microdilution and synergy was evaluated by checkerboard assays and by the fractional inhibitory concentration index, dose-reduction index, ZIP, Bliss, Loewe and highest-single-agent models. Two-way mixed-design ANOVA with Greenhouse–Geisser correction and Holm adjusted paired contrasts were used to compare normalised growth under six conditions. Dose–response curve with MTT was fitted to four-parameter logistic model and the selectivity index was calculated for WI-38 and HaCaT cells. Mechanistic endpoints included ethidium bromide retention, membrane permeability, qPCR of acrA, acrB, mexA and mexB and molecular docking. Biological inclusion levels of low dose hormesis (0.2%, 0.4%, and 0.8% ZnO MIC) were tested on the biofilm biomass, metabolic activity and extracellular polymeric substances (EPS) as well as the markers of cytotoxicity, LDH release, apoptosis and necrosis, and mitochondrial membrane potential.
Results: Baseline susceptibility was closely matched between species (median ampicillin MIC 256.00 μg/mL, IQR 128.00–512.00, P = 0.386; median ZnO MIC 62.50 μg/mL, IQR 31.25–125.00, P = 0.825). Synergy occurred in 133/145 isolates (91.7%): 66/71 K. pneumoniae (93.0%) and 67/74 P. aeruginosa (90.5%), with median FICI values of 0.391 and 0.359 and a median 8-fold ampicillin dose reduction in both species. Combination growth fell to 24.26 ± 11.33% of control versus 98.76 ± 0.70% in untreated controls (partial $\eta^2 = 0.974$ for treatment; no species effect, P = 0.931). On WI-38 and HaCaT cells, the combination IC_{50}s were 558.8 and 613.0 μg/mL (K. pneumoniae) and 542.8 and 598.2 μg/mL (P. aeruginosa), respectively, while ampicillin alone could not be determined in the 0–1000 μg/mL range. Median SI values were 38.57–43.27 and 41.52–44.19; SI > 1 occurred in 97.2% and 94.6% of combinations. EtBr retention (1.94-1.96-fold), membrane permeability (1.59-1.64-fold), pump-gene repression (0.66-0.69-fold) and docking scores (−6.3 to −6.4 kcal/mol) all pointed towards an efflux/permeability mechanism. Host-cell injury remained low (LDH 10.2–10.3%; apoptosis 6.9–7.4%; necrosis 3.3–3.4%; MMP 87.9–89.4% of control). But, 0.2-0.8% of the ZnO MIC enhanced the formation of biofilm, metabolic activity and EPS up to 109% of control.
Conclusions: The results demonstrate that the combinations of ZnO-NP/ampicillin are synergistic and selective for both species in vitro, with the combination showing a narrow sub-inhibitory exposure, so that there is a need for dose-controlled and mechanism-informed development rather than low dose application.

Keywords: Zinc oxide nanoparticles; ampicillin; efflux-mediated synergy; selectivity index.

1. Introduction

Antimicrobial resistance in Gram-negative pathogens has moved from a surveillance concern to a measurable mortality burden. A synthesis of epidemiological surveillance across 187 countries documented a 43% global increase in multidrug-resistant infections, with a 67% rise in healthcare-associated and 38% rise in community-acquired infections in settings with high antibiotic misuse, and annual healthcare costs exceeding USD 100 billion (Marıno *et al.,* 2025). *Klebsiella pneumoniae* alone accounted for approximately 800,000 deaths worldwide in 2019, 80% of them associated with resistance, with carbapenem and third-generation cephalosporin resistance responsible for more than half of the resistant mortality (Song *et al.,* 2025). *Pseudomonas aeruginosa* occupies the same ecological niche in hospital-acquired infection, and the two organisms frequently share carbapenemase and efflux-mediated resistance machinery. Throughout this paper, multidrug resistance is used in its consensus sense — non-susceptibility to at least one agent in three or more antimicrobial categories (Sendra *et al.,* 2024).

The Iraqi picture is consistent with this global trend but is documented unevenly. Multicentre analysis of 846 de-duplicated isolates from four governorates found an overall multidrug resistance prevalence of 57.3% (95% CI 53.9–60.6), with third-generation cephalosporin resistance in 44.4% of *K. pneumoniae* isolates and carbapenem resistance in 11.1% of *K. pneumoniae* and 29.4% of *P. aeruginosa* isolates (Omer *et al.,* 2026). A four-year national analysis reported *P. aeruginosa* resistance rising from 50% in 2021 to 54% in 2023, while MDR *K. pneumoniae* peaked at 65% in 2021 and declined to 58% (Alhilfi et al., 2026). A nationwide review of 11,592 clinical records identified ampicillin as an ineffective agent against isolates recovered from adults, and among the agents most resisted by paediatric isolates (Al-Fahad *et al.,* 2024). Ampicillin is therefore a molecule of genuine residual interest in this setting: inexpensive, universally available, mechanistically well characterised, and almost completely inactive against the local MDR population.

Zinc oxide nanoparticles offer a plausible route to reactivating such agents. Their antibacterial action proceeds through several non-exclusive mechanisms: generation of reactive oxygen species with consequent lipid peroxidation and membrane disruption, release of Zn^{2+} ions that bind thiol groups and inhibit glycolytic and respiratory enzymes, and interference with DNA replication and protein synthesis (Mendes *et al.,* 2022)(Gudkov *et al.,* 2021)(Gomaa, 2022), with cell-surface ROS generation scaling with available particle surface area (Hadi *et al.,* 2025). ZnO-NPs retain broad-spectrum activity against β-lactamase-producing Gram-negative pathogens, with reported MICs of 0.04–0.08 mg/mL and MBCs of 0.12–0.24 mg/mL, and they appear to interfere with β-lactamase activity in addition to damaging the envelope (Krishnamoorthy *et al.,* 2022). Susceptibility to ZnO-NPs is nevertheless species-dependent: MIC values of 80 μg/mL for *E. coli*, 60 μg/mL for *K. pneumoniae* and 30 μg/mL for *P. aeruginosa* have been reported (Sanchez *et al.,* 2021), which cautions against extrapolating a single effective concentration across genera.

Combining ZnO-NPs with β-lactams is attractive because the nanoparticle acts at multiple targets simultaneously, so that the probability of a single mutational event conferring resistance is low, and because dose sparing of the antibiotic reduces both cost and selective pressure (Ozdal & Gurkok, 2022). Experimental support is accumulating: ZnO-NPs with piperacillin–tazobactam showed enhanced activity against MDR *E. coli* and *P. aeruginosa* (Yu *et al.,* 2024); ZnO-NPs with ciprofloxacin and ceftazidime increased activity against resistant *Acinetobacter baumannii* through membrane damage and intracellular accumulation of the antibiotic (Abdelghani *et al.,* 2022); combinations with colistin, ciprofloxacin and meropenem produced synergistic fractional inhibitory concentration indices (Irede et al., 2024); and incorporation of meropenem into ZnO nanoflakes raised the inhibition zone against metallo-β-lactamase-producing clinical isolates to an average of 15.2 mm (Singh *et al.,* 2023). ZnO-NPs conjugated directly to clinically approved antibiotics, ampicillin included, enhanced antibacterial efficacy while showing minimal cytotoxicity against human cell lines (Akbar *et al.,* 2021). Computational work against carbapenem-resistant *K. pneumoniae* porins has added a further plausible target, with ZnO-NPs showing the highest predicted affinity (−9.1 kcal/mol) among tested ligands and an experimental MIC of 0.2 mg/mL, an MBC of 0.5 mg/mL and an inhibition zone of 24 mm (Elsayim *et al.,* 2023).

A second, less widely exploited mechanism is interference with drug efflux. The resistance-nodulation-division transporters AcrAB-TolC in Enterobacteriaceae and MexAB-OprM in *P. aeruginosa* are principal determinants of

intrinsic and acquired multidrug resistance, and their inhibition restores the activity of clinically relevant agents (Alenazy, 2022) (Ornik-Cha et al., 2021). Pharmacological efflux-pump inhibitors such as phenylalanine-arginine β-naphthylamide are used in vitro to raise intracellular antibiotic accumulation in Gram-negative bacteria, but adverse toxicology and pharmacokinetic profiles identified in early clinical trials preclude their therapeutic use, and they remain research tools (Langendonk *et al.,* 2021); PAβN also permeabilises the outer membrane at higher doses, which complicates the attribution of its effects to efflux inhibition alone (Al-Marzooq *et al.,* 2023). Nanoparticles can bypass efflux either by delivering the antibiotic intracellularly or by irreversibly blocking the pump (Modi *et al.,* 2023), and efflux inhibition additionally reduces biofilm adhesion and maturation, making it an attractive anti-biofilm target (Musioł, 2023). Direct evidence exists in *P. aeruginosa*: polyol-coated CuO, ZnO and CuZn nanoparticles acted synergistically with meropenem and ciprofloxacin, and at sub-inhibitory concentrations promoted intracellular accumulation of ethidium bromide, the classic efflux substrate (Eleftheriadou *et al.,* 2021).

The problem, and the reason the present study was designed as it was, is that the same properties that make ZnO-NPs effective antibacterial agents also make them potentially cytotoxic to host cells. Particle size strongly governs ZnO toxicity: in a comparative study on HaCaT keratinocytes and A549 lung epithelial cells, micrometric ZnO was the most cytotoxic, followed by 100 nm and then 50 nm particles, with membrane integrity more affected in keratinocytes (Mitjans *et al.,* 2023); ZnO-NPs reduced HaCaT viability with a half-lethal concentration of approximately 12.5 µg/mL under combined exposures (Wang *et al.,* 2024); and in human skin, keratinocyte cytotoxicity has been associated with exogenous zinc concentrations of 21–31 µg/mL (Khabir *et al.,* 2021). Against this, green-synthesised ZnO-NPs have been reported as considerably less toxic to normal fibroblasts, with an IC_{50} of 568.59 µg/mL against WI-38 cells compared with 409.70 µg/mL against A-431 cells (Naiel *et al.,* 2022). Because a combination can only be clinically meaningful if the concentration that kills bacteria is measurably lower than the concentration that injures host cells, the selectivity index — the ratio of off-target to target toxicity, conventionally IC_{50} divided by MIC (Ye *et al.,* 2023) — and the related therapeutic index (CC_{50}/IC_{50}), which should exceed unity for a usable agent (Al-Hakkani *et al.,* 2022), must be reported alongside any synergy claim.

A final complication is the low-dose, or hormetic, response. Sub-inhibitory exposure of bacteria to antimicrobials can stimulate rather than suppress growth, and low-dose nanoparticles are no exception: low concentrations of ZnO-NPs have been shown to trigger growth and biofilm formation in *P. aeruginosa* as a biphasic hormetic response (Al-Momani *et al.,* 2024); ZnO-NPs at sub-inhibitory concentrations induced biofilm formation in *K. pneumoniae* and *Staphylococcus aureus* (Sreekanth *et al.,* 2023); and the lowest dose of copper-containing nanoparticles enhanced biofilm formation of *S. aureus*, *P. aeruginosa* and *Candida albicans* by 11.3 ± 1.7%, 11.7 ± 1.5% and 14.2 ± 0.9% respectively (Almahdy *et al.,* 2024). Across engineered nanomaterial studies generally, motility and community functions including biofilm formation were affected in 50% of studies conducted at sub-inhibitory concentrations but in only 10–20% of studies using inhibitory concentrations (Mortimer *et al.,* 2021). Any dosing strategy for a nanoparticle–antibiotic combination therefore has a lower boundary as well as an upper one.

Taken together, the literature supports synergy and supports efflux involvement, but it does not resolve whether the two are accompanied by an adequate selectivity window in clinically relevant MDR isolates, nor how narrow that window is in relation to sub-inhibitory exposure. The present work addresses that gap directly. Its objectives were: (1) to establish the baseline susceptibility profile of *K. pneumoniae* and *P. aeruginosa* clinical isolates from a single Iraqi hospital to ampicillin and ZnO-NPs; (2) to quantify the interaction by FICI, DRI and four model-based synergy scores; (3) to determine the cytotoxicity of the combinations in two human cell lines and derive selectivity indices; (4) to test phenotypic, transcriptional and computational evidence for an efflux/permeability mechanism; (5) to measure low-dose hormesis in biofilm, metabolic activity and EPS; and (6) to integrate these domains statistically in order to judge whether an in vitro selectivity window exists that could justify further development.

2. Materials and methods

2.1 Study design, setting and bacterial isolates

The study was conducted on clinical bacterial isolates recovered from routine diagnostic specimens at Fallujah General Hospital, Anbar Province, Iraq, between ⟨collection period⟩. A single-centre, cross-sectional in vitro design was used.

A total of 145 non-duplicate clinical isolates were included: 71 *Klebsiella pneumoniae* and 74 *Pseudomonas aeruginosa*, obtained from ⟨specimen types⟩. Species-level identification was performed with ⟨identification platform⟩ according to the routine procedure of the hospital microbiology unit. Multidrug resistance was defined, in line with the consensus international definitions, as non-susceptibility to at least one agent in three or more antimicrobial categories (Sendra *et al.,* 2024). Isolates were stored in glycerol at −80 °C and subcultured twice on fresh medium before testing. The study was approved by ⟨ethics committee and approval reference⟩; informed consent was waived because only de-identified isolates obtained during routine diagnostic testing were used.

2.2 Antibacterial agents

ZnO-NPs were used as received, without further surface modification or purification ⟨supplier, lot number and nominal particle size as stated by the manufacturer⟩. Working suspensions were prepared by ultrasonication in assay medium immediately before each experiment, and fresh suspensions were prepared for every assay. The same stock, dispersion protocol, sonication time and medium were used throughout, so that the material delivered to each well remained constant across all experiments.

ZnO-NPs are conventionally characterised by X-ray diffraction, electron microscopy, dynamic light scattering and zeta potential, and these parameters govern both their antibacterial and their cytotoxic behaviour (Ahmad et al., 2023) (Ullah et al., 2024). In the present study the material was used as received and was not characterised in-house; the consequences of this for the interpretation of the absolute concentrations reported here are addressed explicitly in the Limitations (Section 4.6). Ampicillin was prepared as a stock solution in accordance with the manufacturer's instructions and diluted in assay medium on the day of use.

2.3 MIC and MBC determination

MICs were determined by broth microdilution using cation-adjusted Mueller–Hinton broth, a final inoculum of approximately 5×10^5 CFU/mL, and incubation at 35 ± 1 °C for 18 ± 2 h before reading (Tang et al., 2021) (Kadeřábková et al., 2024). Working suspensions were adjusted to the 0.5 McFarland standard, equivalent to approximately 1×10^8 CFU/mL, and then diluted 1:100 to give the final inoculum (Balogun et al., 2024). Because ZnO-NPs scatter light and may sediment in the assay medium, plates were read after gentle resuspension and endpoints were confirmed visually; the concentration range tested for each agent was identical for the two species. MBCs were determined by subculturing the contents of wells showing no visible growth, together with the two concentrations above the MIC, onto Mueller–Hinton agar; the lowest concentration preventing growth after 24 h at 37 °C was recorded as the MBC (Kathirvalu, 2023). Bactericidal activity was classified as an MBC/MIC ratio ≤ 4 and bacteriostatic activity as a ratio > 4 (Jarczak et al., 2021). All determinations were performed in triplicate. MIC and MBC values for ampicillin and ZnO-NPs were obtained both as single agents and within the combination.

2.4 Checkerboard synergy assays and interaction modelling

Two-dimensional checkerboard assays were performed in 96-well microplates with two-fold serial dilutions of each agent across the plate, read after 18 h by optical density (Bellio et al., 2021). FICI was calculated as FICI = FIC_A + FIC_B, where FIC_A and FIC_B are the MICs of each agent in combination divided by their MICs alone (Ratia et al., 2023). FICI ≤ 0.5 was interpreted as synergy, 0.5 < FICI ≤ 1 as additivity, 1 < FICI ≤ 4 as indifference and FICI > 4 as antagonism, following the conservative thresholds adopted to permit comparison across studies (Ibrahem & Alfadil, 2026). The DRI was derived to express the fold reduction in the effective concentration of each agent in the combination. In addition, the ZIP, Bliss, Loewe and HSA scores were computed as model-based synergy metrics, so that the interaction could be assessed independently of the specific concentration grid tested (Greger et al., 2025). Because exhaustive higher-dimensional testing of combinations is combinatorially prohibitive, model-based scores computed on a normalised concentration design provide a practical and internally consistent complement to FICI (Brennan et al., 2022). For display, mean normalised growth across the panel was assembled into a single checkerboard matrix after normalisation of ZnO and ampicillin concentrations to each isolate's own MIC, so that isolates with high absolute MICs would not compress the common concentration scale.

2.5 Growth inhibition under defined treatment conditions

Normalised growth of each isolate was measured under six conditions: untreated control, ZnO alone, ampicillin alone, ZnO + ampicillin combination, efflux-pump inhibitor control, and a reference strain control. Growth was expressed as a percentage of the untreated control. Concentrations of each agent in the combination were selected from the checkerboard-derived effective concentrations. The efflux-pump inhibitor control used ⟨inhibitor and final concentration⟩, a compound of the class routinely employed in vitro to increase intracellular antibiotic accumulation in Gram-negative bacteria (Langendonk *et al.,* 2021). The reference strain control used ⟨reference strain designation⟩.

2.6 Cytotoxicity in human cell lines and selectivity analysis

Cytotoxicity was evaluated in two human cell lines: WI-38 human lung fibroblasts and HaCaT human keratinocytes. Viability was measured by the MTT assay, which quantifies the reduction of tetrazolium dye to formazan by mitochondrial dehydrogenases of metabolically active cells (Lee et al., 2022), with absorbance read on a microplate reader. Cells were exposed to a two-fold concentration series of the combination, and to ampicillin alone across 0–1000 µg/mL, for ⟨exposure duration⟩. Concentration–response data were fitted by four-parameter logistic nonlinear regression, the standard approach for sigmoidal dose–response data in which the half-maximal inhibitory concentration and the slope of the curve are estimated simultaneously (Naiel *et al.,* 2022) (Ye *et al.,* 2023), and IC_{50} values were reported with 95% confidence intervals and Hill slopes. The selectivity index was calculated as the IC_{50} divided by the effective ZnO concentration of the combination, following the convention in which the SI expresses the ratio of off-target to target toxicity (Ye et al., 2023); the closely related therapeutic index (CC_{50}/IC_{50}) should exceed unity for an agent to be considered selective (Al-Hakkani et al., 2022).

2.7 Efflux, membrane permeability, gene expression and docking

Efflux activity was assessed phenotypically by EtBr retention, a fluorometric assay in which increased intracellular fluorescence indicates reduced efflux (Lee et al., 2024) (Ornik-Cha et al., 2021); the agar cartwheel method provides a complementary format for classifying efflux activity in clinical isolates (Ugwuanyi et al., 2021). Membrane permeability was quantified as a fold change relative to untreated controls. Transcriptional responses of the RND pump genes *acrA* and *acrB* (*K. pneumoniae*) and *mexA* and *mexB* (*P. aeruginosa*) were quantified by real-time PCR, with relative expression calculated by the comparative Ct method and inferential comparisons performed on ΔCt values (Ernst et al., 2026); ⟨housekeeping gene⟩ served as the internal reference. Molecular docking of the same four targets was performed with ⟨docking program and version⟩, following published protocols in which protein and ligand input files are prepared in PDBQT format and binding energies are reported in kcal/mol (Elsayim et al., 2023). Docking was treated as a hypothesis-generating step only, because binding energies obtained by rigid-receptor docking are approximations of affinity, and a realistic description of ligand–pump interaction requires flexible-receptor simulation of the kind used to characterise the promiscuous AcrB and AdeB binding sites (Ornik-Cha et al., 2021).

2.8 Cellular mechanisms of cytotoxicity

Cellular injury was characterised by four complementary endpoints: LDH release as a marker of membrane damage and necrotic death (Yadav et al., 2021) (Awashra & Młynarz, 2023), apoptosis and necrosis by flow cytometry, and mitochondrial membrane potential using a cationic fluorescent probe, a standard approach because mitochondrial dysfunction is an early and sensitive indicator of nanoparticle cytotoxicity (Stachyra et al., 2022) (Zhang et al., 2022). Together with MTT viability and LDH release, these endpoints provide a multi-parameter toxicity profile rather than relying on a single metabolic readout.

2.9 Low-dose hormesis

Biofilm biomass, metabolic activity and EPS production were measured at 0.2%, 0.4% and 0.8% of the ZnO MIC in a Species × Dose design. Biofilm was quantified by the crystal violet microtitre method, metabolic activity by reduction-based colorimetry, and EPS by a standard extraction and colorimetric procedure. These endpoints were

selected because the low-dose response of bacteria to antimicrobials is preferentially expressed in community-associated functions such as biofilm formation (Mortimer et al., 2021).

2.10 Statistical analysis

Analyses were performed in IBM SPSS Statistics version 32.0.0 and GraphPad Prism version 11.1.0. Distributional assumptions were checked by the Shapiro–Wilk test and visually. Non-normally distributed continuous variables were summarised as median with interquartile range and compared between species by the Mann–Whitney U test, with the rank-biserial correlation reported as an effect size. Normally distributed descriptive variables were summarised as mean ± standard deviation. Categorical interaction profiles were compared by the chi-square test, with Fisher's exact test used for sparse contingency structures. Growth responses across the six treatment conditions were analysed by two-way mixed-design ANOVA, with species as the between-isolate factor and treatment as the within-isolate factor, applying the Greenhouse–Geisser correction to the within-isolate factor and reporting partial eta squared; prespecified paired contrasts were performed with the Wilcoxon signed-rank test and Holm adjustment. The same model structure was used for the low-dose biofilm, metabolic and EPS analyses. MTT concentration–response data were fitted by four-parameter logistic regression. Cross-domain associations were assessed by Spearman rank correlation with Benjamini–Hochberg control of the false discovery rate across the prespecified correlation set (Huang & Peddada, 2023). Two-sided $P < 0.05$ was considered statistically significant. No a priori power calculation was performed; sample size was determined by the number of eligible non-duplicate isolates available during the collection period.

3. Results

3.1 Baseline susceptibility was closely matched between species

The baseline susceptibility profile of the two Gram-negative species was nearly identical. Median ampicillin MIC was 256.00 µg/mL (IQR 128.00–512.00 µg/mL) in both species ($P = 0.386$; rank-biserial r = −0.081), and median ZnO MIC was 62.50 µg/mL (IQR 31.25–125.00 µg/mL) in both ($P = 0.825$; r = 0.021). Median ampicillin MBC was 512.00 µg/mL (IQR 256.00–1024.00 µg/mL; $P = 0.319$; r = −0.094) and median ZnO MBC was 125.00 µg/mL (IQR 62.50–250.00 µg/mL; $P = 0.628$; r = 0.045). Within the combination, the median ampicillin MBC fell to 32.00 µg/mL (IQR 16.00–64.00) for *K. pneumoniae* and 48.00 µg/mL (IQR 32.00–112.00) for *P. aeruginosa* ($P = 0.499$; r = −0.064), while the median ZnO MBC in combination was 21.41 µg/mL (IQR 11.19–45.06) and 20.05 µg/mL (IQR 8.08–44.77) respectively ($P = 0.517$; r = 0.063). All rank-biserial effect sizes were small. This equivalence is analytically important: because the two species entered the interaction experiments with statistically indistinguishable MIC and MBC distributions, any subsequent difference in combination behaviour cannot be attributed to a pre-existing imbalance in susceptibility.

Table 1: Baseline Antimicrobial Susceptibility by Bacterial Species

Measure	K. pneumoniae (n=71), median (IQR)	P. aeruginosa (n=74), median (IQR)	P value	Rank-biserial r
Ampicillin MIC alone (µg/mL)	256.00 (128.00–512.00)	256.00 (128.00–512.00)	0.386	-0.081
ZnO MIC alone (µg/mL)	62.50 (31.25–125.00)	62.50 (31.25–125.00)	0.825	0.021
Ampicillin MBC alone (µg/mL)	512.00 (256.00–1024.00)	512.00 (256.00–1024.00)	0.319	-0.094
ZnO MBC alone (µg/mL)	125.00 (62.50–250.00)	125.00 (62.50–250.00)	0.628	0.045
Ampicillin MBC in combination (µg/mL)	32.00 (16.00–64.00)	48.00 (32.00–112.00)	0.499	-0.064
ZnO MBC in combination (µg/mL)	21.41 (11.19–45.06)	20.05 (8.08–44.77)	0.517	0.063

The susceptibility profile for the two Gram-negative species was very similar at baseline. For K. pneumoniae and P. aeruginosa, the median MIC for ampicillin was both 256 µg/mL with interquartile range 128–512 µg/mL (P = 0.386).

ZnO MIC alone was also identical at a median of 62.5 µg/mL (IQR 31.25–125 µg/mL; P = 0.825). There was no difference among the corresponding MBC distributions and no difference in the MBC values measured within the combination of agents for any species. No material effect was found in this dataset on baseline antimicrobial susceptibility as all rank-biserial effect sizes were small. This similarity is significant as subsequent differences in the combination behaviour can then be understood against widely similar baseline MIC/MBC distributions that do not involve a large pre-existing susceptibility imbalance.

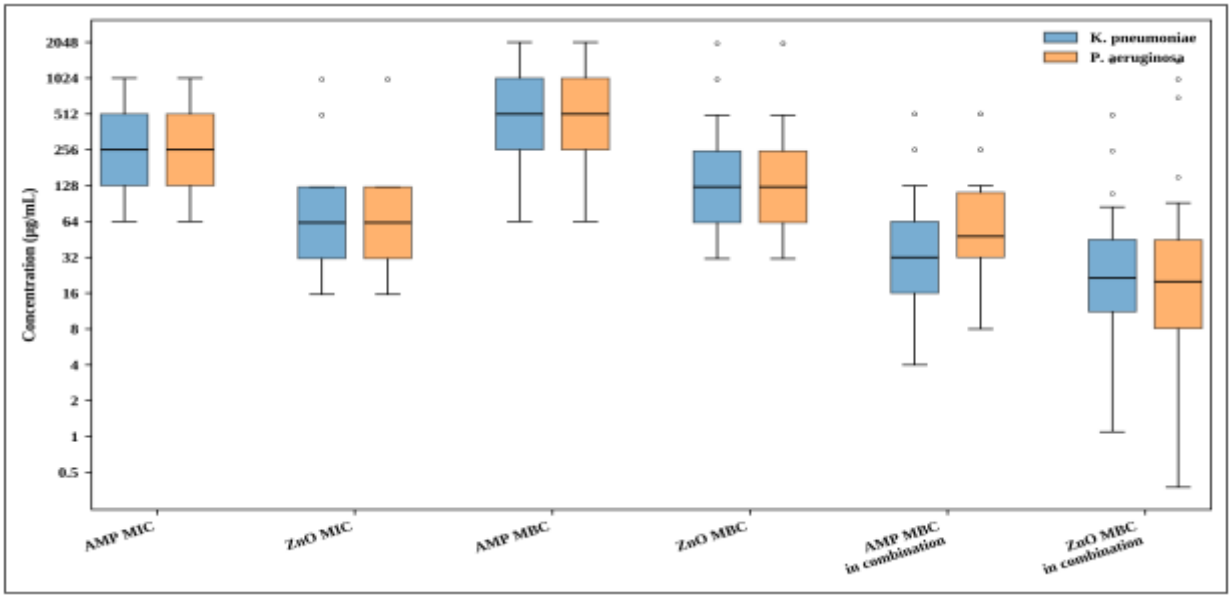


Figure 1: *MIC and MBC Distribution by Bacterial Species*

The initial susceptibility profile was very similar for the two Gram-negative species. The median of Ampicillin MIC was also the same 256 µg/mL for both K. pneumonia and P. aeruginosa, and the interquartile range was 128–512 µg/mL (P = 0.386). ZnO MIC alone was also identical at a median of 62.5 µg/mL (IQR 31.25–125 µg/mL; P = 0.825). The distribution of the corresponding MBCs was not significantly different among the two agents and no species difference was found in MBCs taken in the combination. All rank-biserial effect sizes were small indicating that in this dataset, baseline antimicrobial susceptibility was not materially influenced by species. This similarity is significant as the subsequent differences in combination behaviour may be interpreted in the context of broadly similar MIC/MBC distributions and not against a major pre-existing susceptibility imbalance.

3.2 The interaction was synergistic and reproducible in both species

Combination testing produced predominantly synergistic interactions. Synergy was recorded in 133 of 145 isolates (91.7%): 66/71 *K. pneumoniae* (93.0%) and 67/74 *P. aeruginosa* (90.5%). Median FICI was 0.391 in *K. pneumoniae* and 0.359 in *P. aeruginosa* (P = 0.184; FDR q = 0.369; r = 0.128), both well below the 0.5 synergy threshold. The median ampicillin DRI was 8-fold in both species (P = 0.549; q = 0.549; r = 0.055). Model-based scores were comparable between species: ZIP 13.38 (11.77–14.72) versus 14.01 (12.16–15.13); Bliss 12.56 (10.70–13.55) versus 13.05 (11.66–14.29); Loewe 14.14 (12.73–15.41) versus 14.63 (12.12–16.27); and HSA 11.48 (9.86–12.67) versus 12.24 (11.00–13.43). Only the unadjusted HSA comparison reached nominal significance (P = 0.043), and this disappeared after false-discovery-rate correction (q = 0.260), which illustrates why uncorrected P values should not be over-interpreted in multi-endpoint designs (Huang & Peddada, 2023). The overall distribution of synergy, additive and indifferent categories did not differ between species (χ^2, P = 0.267), and antagonism was recorded in 0/71 *K. pneumoniae* isolates (0.0%) and 3/74 *P. aeruginosa* isolates (4.1%). The single normalised checkerboard matrix for the whole panel showed a progressive increase in mean growth inhibition with increasing fractional concentration of

either component, with the strongest suppression at paired sub-MICs; normalisation to each isolate's own MIC permitted all isolates to be represented on one common concentration scale.

Table 2: Combination and Synergy Profile by Bacterial Species

Synergy metric	K. pneumoniae median (IQR)	P. aeruginosa median (IQR)	P value	FDR q	Rank-biserial r
FICI	0.39 (0.33–0.45)	0.36 (0.30–0.45)	0.184	0.369	0.128
Ampicillin DRI	8.00 (4.00–16.00)	8.00 (4.00–8.00)	0.549	0.549	0.055
ZIP score	13.38 (11.77–14.72)	14.01 (12.16–15.13)	0.260	0.391	-0.108
Bliss score	12.56 (10.70–13.55)	13.05 (11.66–14.29)	0.149	0.369	-0.139
Loewe score	14.14 (12.73–15.41)	14.63 (12.12–16.27)	0.420	0.504	-0.078
HSA score	11.48 (9.86–12.67)	12.24 (11.00–13.43)	0.043	0.260	-0.195
Interaction profile					
Synergy	66/71 (93.0%)	67/74 (90.5%)			
Additive	1/71 (1.4%)	2/74 (2.7%)			
Indifferent	4/71 (5.6%)	2/74 (2.7%)			
Antagonism	0/71 (0.0%)	3/74 (4.1%)			
Overall category distribution			0.267		

Combination testing showed a mainly synergistic effect for both species. Synergy was recorded in 133 of 145 isolates (91.7%), including 66/71 K. pneumoniae isolates (93.0%) and 67/74 P. aeruginosa isolates (90.5%). The median FICIs were 0.391 and 0.359 for the two species, respectively, with a median dose-reduction index of 8-fold for ampicillin in both cases. ZIP, Bliss, and Loewe scores also were comparable among species. The difference was not significant when using the false-discovery-rate method ($q = 0.260$), but was significant when the HSA was unadjusted ($P = 0.043$). There was no significant difference in the overall distribution of synergy values, additive and indifferent (χ^2 test, $P = 0.267$) or antagonistic values between species. Overall, the synergy was significant and repeatable for both organisms and there was no indication that one organism caused the synergy systematically.

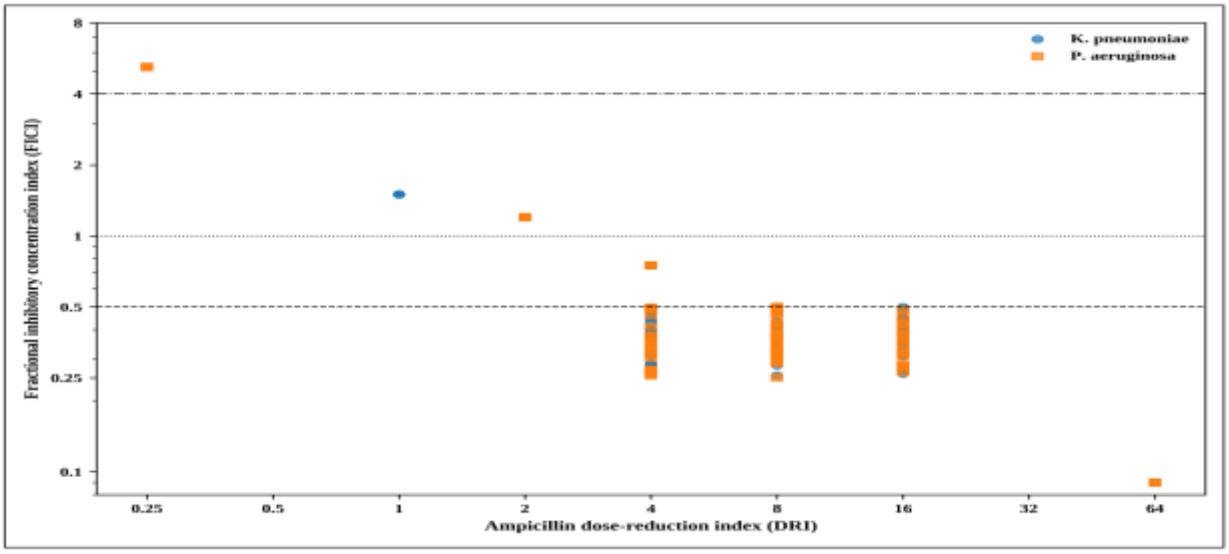


Figure 2: Synergy Profile and Dose-Reduction Index

Combination testing revealed mostly synergistic effects for both species. Synergy was recorded in 133 of 145 isolates (91.7%), including 66/71 K. pneumoniae isolates (93.0%) and 67/74 P. aeruginosa isolates (90.5%). The median value

of FICI was 0.391 and 0.359 in both species, respectively, and the median ampicillin dose-reduction index was 8-fold in both species. There was also no difference in the scores for ZIPS, Bliss and Loewe between species. The unadjusted HSA comparison resulted in P = 0.043, but this difference was not significant when false-discovery-rate corrected (q = 0.260). No significant difference was observed in the grand distribution of synergy, additive, indifferent and antagonistic categories between the species (χ^2 test, P = 0.267). The combination effect was significant overall and consistent across both organisms, with no apparent systematic evidence that one species consistently drove the synergistic effect.

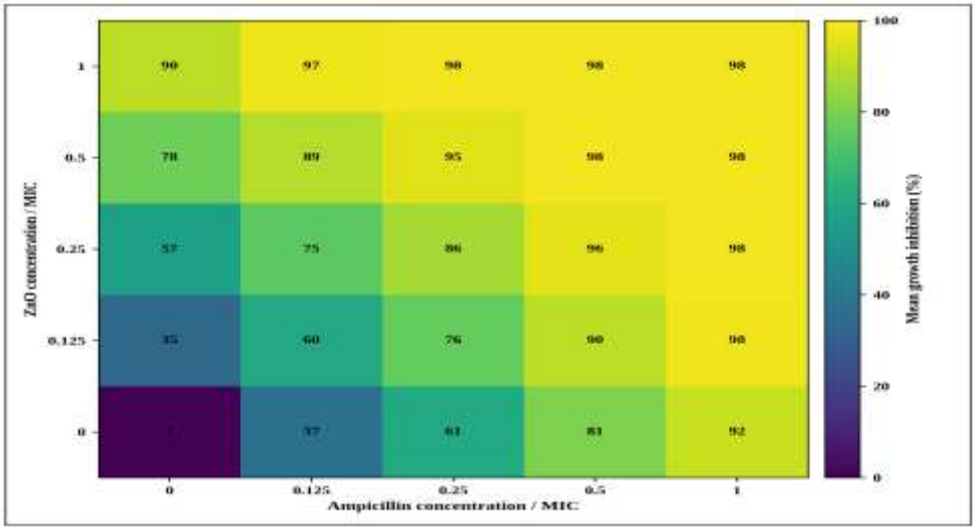


Figure 3: Mean Normalized Checkerboard Synergy Matrix

The single normalized checkerboard matrix shows the concentration-response pattern for the entire panel of isolates, with ZnO and ampicillin concentrations normalized to each isolate's MIC. There was a progressive increase in mean growth inhibition with increasing fractional concentration of either component and highest when both agents were used in higher fractional concentration. Importantly, there was a high amount of suppression observed at paired sub-MICs, and this is reflected visually in the predominance of FICI-defined synergy in the isolate-level analysis. The normalization to the individual MIC values makes it possible to show the checkerboard response in one common matrix while avoiding the possibility of suppressing the concentration scale by isolates with higher absolute MIC values.

3.3 Treatment condition, not species, governed growth inhibition

Growth differed markedly across treatment conditions but not between species. Untreated growth was 98.78 ± 0.67% for *K. pneumoniae* and 98.74 ± 0.74% for *P. aeruginosa*. ZnO alone reduced growth to 59.15 ± 6.07% and 60.37 ± 7.73% respectively; ampicillin alone to 64.78 ± 5.27% and 63.32 ± 7.27%; and the combination further reduced growth to 24.05 ± 8.88% and 24.46 ± 13.32% (overall mean 24.26 ± 11.33% versus 98.76 ± 0.70% untreated, 59.77 ± 6.97% for ZnO alone and 64.04 ± 6.39% for ampicillin alone). The pump-inhibitor condition produced the lowest growth (13.07 ± 9.34% and 13.36 ± 13.55%; overall 13.22 ± 11.64%), and the reference strain control gave 18.06 ± 9.24% and 18.28 ± 12.91% (overall 18.17 ± 11.23%). Two-way mixed-design ANOVA showed a very large treatment effect (Greenhouse–Geisser $\varepsilon = 0.341$; $F = 5396.507$; $P < 0.001$; partial $\eta^2 = 0.974$), no species main effect ($F = 0.008$; $P = 0.931$; partial $\eta^2 = 0.000$) and no Species × Treatment interaction ($F = 0.924$; $P = 0.385$; partial $\eta^2 = 0.006$). Holm-adjusted paired contrasts confirmed that combination growth was significantly lower than untreated, ZnO-only and ampicillin-only growth (all adjusted $P < 0.001$), and also lower than the pump-inhibitor condition (adjusted $P < 0.001$).

Table 3: Species × Treatment Factorial Analysis

Treatment/control condition	K. pneumoniae mean ± SD	P. aeruginosa mean ± SD	Overall mean ± SD
Untreated	98.78 ± 0.67	98.74 ± 0.74	98.76 ± 0.70
ZnO only	59.15 ± 6.07	60.37 ± 7.73	59.77 ± 6.97
Ampicillin only	64.78 ± 5.27	63.32 ± 7.27	64.04 ± 6.39
ZnO+Ampicillin	24.05 ± 8.88	24.46 ± 13.32	24.26 ± 11.33
Pump inhibitor	13.07 ± 9.34	13.36 ± 13.55	13.22 ± 11.64
Reference strain	18.06 ± 9.24	18.28 ± 12.91	18.17 ± 11.23
Factorial effect	F	P value	Partial η^2
Species	0.008	0.931	0.000
Treatment (GG ε=0.341)	5396.507	<0.001	0.974
Species × Treatment	0.924	0.385	0.006
Key paired contrasts (Holm-adjusted)			
Combo vs Untreated		<0.001	
Combo vs ZnO only		<0.001	
Combo vs Ampicillin only		<0.001	
Combo vs Pump inhibitor		<0.001	

Growth varied significantly under the 6 treatment/control conditions while the 2 species were similar under these conditions. Untreated growth was around 99% in both species, where the mean growth in ZnO alone and ampicillin alone was about 59–60% and 63–65%, respectively. The combination of ZnO and ampicillin resulted in further decrease of mean growth to ~24% in both species. The two-way mixed analysis showed a very large treatment effect (Greenhouse–Geisser-corrected $P < 0.001$; partial $\eta^2 = 0.974$), but no main effect of species ($P = 0.931$) and no Species × Treatment interaction ($P = 0.385$; partial $\eta^2 = 0.006$). Holm adjusted paired comparisons were performed and revealed that combination growth was significantly below the untreated, ZnO-only and ampicillin-only growth (all adjusted $P < 0.001$). Therefore, treatment condition was a major factor as opposed to the species of bacteria.

3.4 Cytotoxicity was concentration-dependent but far above the synergistic range

Both healthy cell lines showed concentration-dependent loss of viability, and the four-parameter logistic models described the pooled data well, with R^2 values at or above 0.9999 for the combination curves and narrow 95% confidence intervals. For *K. pneumoniae*-associated combinations, mean IC_{50} was 558.8 µg/mL (95% CI 540.4–577.2; Hill slope 1.415) in WI-38 cells and 613.0 µg/mL (95% CI 593.0–632.9; Hill slope 1.391) in HaCaT cells. For *P. aeruginosa*-associated combinations, the corresponding values were 542.8 µg/mL (95% CI 529.3–556.4; Hill slope 1.421) and 598.2 µg/mL (95% CI 564.3–632.1; Hill slope 1.338). Hill slopes clustered between 1.34 and 1.42 in all four species–cell-line combinations, indicating a consistent sigmoidal response. For ampicillin alone, 50% loss of viability was not reached within 0–1000 µg/mL; extrapolated values for this condition were not treated as experimentally observed endpoints.

Table 4: Cytotoxicity and Dose–Response Parameters

Species	Cell line	4PL IC50 (µg/mL)	95% CI	Hill slope	R^2
K. pneumoniae	WI-38	558.8	540.4–577.2	1.415	1.0000
K. pneumoniae	HaCaT	613.0	593.0–632.9	1.391	1.0000
P. aeruginosa	WI-38	542.8	529.3–556.4	1.421	1.0000
P. aeruginosa	HaCaT	598.2	564.3–632.1	1.338	1.0000
Ampicillin-only note	Both cell lines	IC50 not reached within 0–1000 µg/mL			

The MTT dose–response data indicated that both healthy cell lines suffered from concentration-dependent loss of viability, and the dose–response data for all cell lines were well described by the four-parameter logistic models with $R^2 \geq 0.9999$ for the combination curves. The fitted IC50 for the combination of K. pneumoniae was 558.8 µg/mL in WI-38 cells and 613.0 µg/mL in HaCaT cells. The values corresponding to P. aeruginosa associated combinations were 542.8 and 598.2 µg/mL, respectively. The 95% confidence intervals were fairly small, and the Hill slopes were around 1.34–1.42, suggesting a similar sigmoidal response among species and cell lines. In the ampicillin-only treatment, a concentration level of 0–1000 µg/mL was not sufficient to achieve a 50% loss of viability - extrapolated values for this treatment were not considered as experimentally observed points.

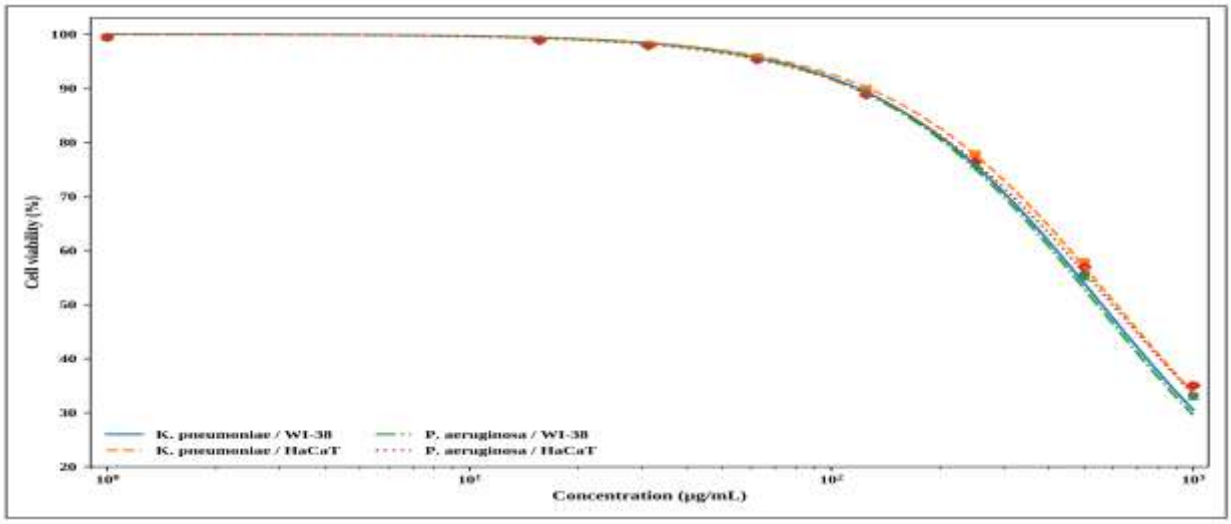


Figure 4: MTT Dose–Response Curves for WI-38 and HaCaT Cells

The concentration-dependent loss of viability in both cell lines from the MTT dose–response data, and the excellent fits of the four-parameter logistic models to the pooled raw observations ($R^2 \geq 0.9999$ for the combination curves), confirm that the combined data are sufficient for the construction of a single model.The concentration-dependent loss of viability observed in both cell lines in the MTT dose–response data, and the excellent fits of the four-parameter logistic models to the pooled raw observations ($R^2 \geq 0.9999$ for the combination curves) confirm that the combined data are sufficient for the construction of a single model. The fitted IC50 values for K. pneumoniae-associated combinations were 613.0 µg/mL for HaCaT cells and 558.8 µg/mL for WI-38 cells. The values were 542.8 and 598.2 µg/mL, respectively for P. aeruginosa associated combinations. The 95% confidence intervals were relatively narrow and the Hill slopes were reasonably close to 1.34–1.42, which would indicate a consistent sigmoidal response for all species and cell lines. The ampicillin-only IC50 values were not considered to be experimentally observed endpoints, as the 0-1000 µg/mL range did not result in 50% loss of viability.

3.5 A selectivity window was present in the great majority of combinations

The effective concentrations of the combination lay well below the cytotoxic midpoint of both cell lines. Median effective ZnO concentration was 15.12 µg/mL (IQR 7.78–27.69) for *K. pneumoniae* and 13.61 µg/mL (IQR 7.58–27.00) for *P. aeruginosa* ($P = 0.777$); median ampicillin concentration in the combination was 32.00 µg/mL (IQR 16.00–64.00) in both species ($P = 0.223$); and median ampicillin DRI was 8.00 in both ($P = 0.549$). Median selectivity index on WI-38 cells was 38.57 (IQR 21.37–68.78) for *K. pneumoniae* and 41.52 (IQR 19.60–75.62) for *P. aeruginosa* ($P = 0.851$); on HaCaT cells it was 43.27 (IQR 19.92–78.92) and 44.19 (IQR 19.33–87.31) respectively ($P = 0.814$). Isolates with SI > 1 numbered 69/71 (97.2%) for *K. pneumoniae* and 70/74 (94.6%) for *P. aeruginosa* on both cell lines. The minority of combinations with SI ≤ 1 are reported explicitly rather than averaged into the group summary, since they define the boundary of the window.

Table 5: Selectivity Index and Therapeutic Safety Window

Metric	K. pneumoniae	P. aeruginosa	P value
Effective ZnO concentration in combination (µg/mL)	15.12 (7.78–27.69)	13.61 (7.58–27.00)	0.777
Ampicillin concentration in combination (µg/mL)	32.00 (16.00–64.00)	32.00 (16.00–64.00)	0.223
Ampicillin DRI	8.00 (4.00–16.00)	8.00 (4.00–8.00)	0.549
SI on WI-38	38.57 (21.37–68.78)	41.52 (19.60–75.62)	0.851
SI on HaCaT	43.27 (19.92–78.92)	44.19 (19.33–87.31)	0.814
Isolates with SI > 1 on WI-38	69/71 (97.2%)	70/74 (94.6%)	—
Isolates with SI > 1 on HaCaT	69/71 (97.2%)	70/74 (94.6%)	—

The selectivity analysis showed that the concentrations of the bacterial combination were in general not very close to the cytotoxic midpoint, as determined in healthy cell lines. The median selectivity indices of K. pneumoniae combinations were 38.57 and 43.27 in WI-38 and HaCaT cells, respectively, while for P. aeruginosa combinations they were 41.52 and 44.19, respectively. No significant difference was found in either selectivity index, effective ZnO concentration, ampicillin concentration in the combination or dose-reduction index among species. For all healthy cell lines, 97.2% of the combinations of K. pneumoniae and 94.6% of the combinations of P. aeruginosa had an SI > 1. These data suggest the existence of an in-vitro selectivity window for most of the combinations tested, and the small number of combinations having SI ≤ 1 should be kept in the foreground, rather than being averaged together and covered over.

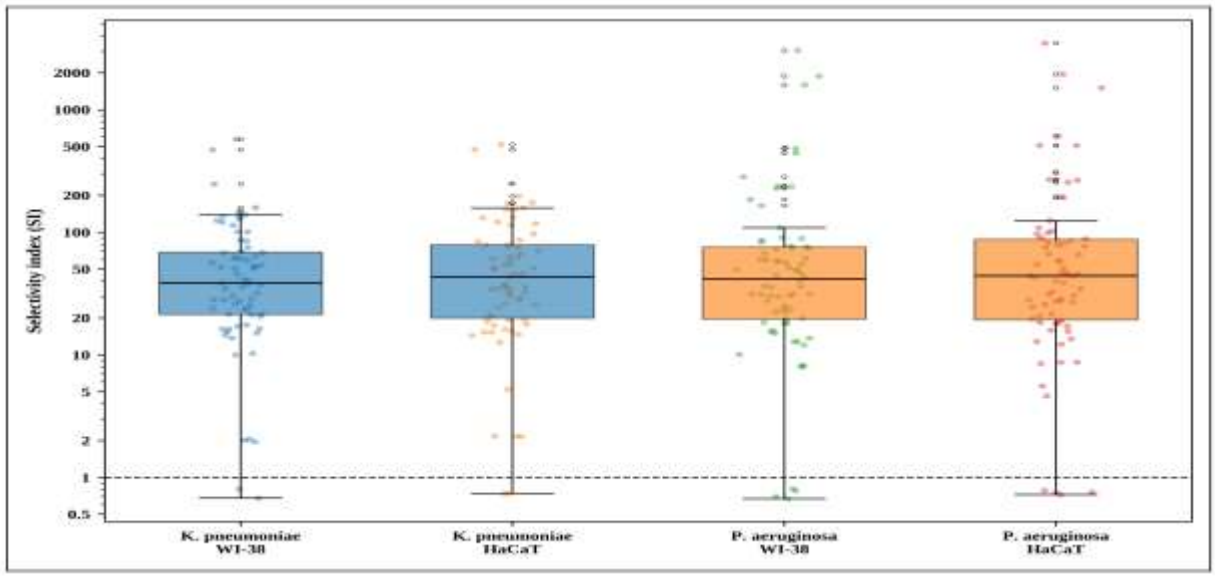


Figure 5: Selectivity Index and Safety Window

The selectivity analysis showed that in general, the bacterial combination concentrations were at levels much lower than the cytotoxic midpoint of the healthy cell lines. The median selectivity indices for K. pneumoniae combinations were 38.57 and 43.27 on WI-38 and HaCaT cells, respectively and 41.52 and 44.19 on WI-38 and HaCaT cells for P. aeruginosa combinations. No significant species difference was observed for either of the selectivity indices, effective ZnO concentration or ampicillin concentration in the combination and dose-reduction index. For each healthy cell line, 97.2% (94.6%) of K. pneumoniae (P. aeruginosa) combinations had an SI > 1. These data would confirm the existence of an in-vitro selectivity window for most combinations tested and would highlight that a few combinations (SI ≤ 1) should not be hidden from the view but rather be clearly visible.

3.6 Phenotypic, transcriptional and computational evidence converged on an efflux/permeability mechanism

Efflux-related phenotypes did not differ between species. Median EtBr retention was 1.94-fold (IQR 1.82–2.09) in *K. pneumoniae* and 1.96-fold (IQR 1.81–2.13) in *P. aeruginosa* ($P = 0.765$), and median membrane permeability was 1.64-fold (IQR 1.51–1.73) and 1.59-fold (IQR 1.50–1.73) respectively ($P = 0.700$). Pump-gene expression was below unity for all four targets: *acrA* 0.69 (0.60–0.75), *acrB* 0.66 (0.61–0.72), *mexA* 0.68 (0.60–0.74) and *mexB* 0.67 (0.60–0.74), with significant treatment-related down-regulation of all targets on the raw ΔCt scale ($P < 0.001$ for each gene). Docking scores clustered between −6.3 and −6.4 kcal/mol: *acrA* −6.44 (−6.78 to −6.17), *mexA* −6.43 (−6.71 to −6.15), *acrB* −6.30 (−6.56 to −6.04) and *mexB* −6.34 (−6.65 to −6.16). Increased EtBr retention, increased membrane permeability, reduced pump-gene transcription and favourable predicted binding energies therefore point in the same direction.

Cellular injury markers were correspondingly low and species-independent. Median LDH release was 10.20% (IQR 8.30–12.65) for *K. pneumoniae* and 10.30% (IQR 8.72–12.57) for *P. aeruginosa* ($P = 0.752$; $r = −0.031$); median apoptosis was 6.90% (IQR 5.90–8.20) and 7.40% (IQR 5.50–8.78) ($P = 0.972$; $r = 0.004$); median necrosis was 3.30% (IQR 2.70–3.95) and 3.40% (IQR 3.02–4.00) ($P = 0.347$; $r = −0.091$); and MMP was maintained at 89.40% (IQR 85.65–91.50) and 87.90% (IQR 85.53–91.57) of control ($P = 0.570$; $r = 0.055$). All effect sizes were small, and no species-dependent difference in host-cell toxicity was detected under combination conditions.

Table 6: Efflux Pump Activity, Membrane Permeability and Gene Expression

Domain	Endpoint	K. pneumoniae / acr	P. aeruginosa / mex	P value
Phenotypic	EtBr retention (fold)	1.94 (1.82–2.09)	1.96 (1.81–2.13)	0.765
Phenotypic	Membrane permeability (fold)	1.64 (1.51–1.73)	1.59 (1.50–1.73)	0.700
qPCR	acrA / mexA fold change	0.69 (0.60–0.75)	0.68 (0.60–0.74)	<0.001 within each gene
Docking	acrA / mexA score (kcal/mol)	-6.44 (-6.78–-6.17)	-6.43 (-6.71–-6.15)	—
qPCR	acrB / mexB fold change	0.66 (0.61–0.72)	0.67 (0.60–0.74)	<0.001 within each gene
Docking	acrB / mexB score (kcal/mol)	-6.30 (-6.56–-6.04)	-6.34 (-6.65–-6.16)	—

The efflux-related phenotypes and molecular measurements did not differ between the two species. The medians of the retention of EtBr and the permeability of the membrane were of the order of 1.94–1.96 and 1.59–1.64, respectively, and were not significantly different among the various species. The median pump-gene expression was <1 for the following four targets: acrA (0.69), acrB (0.66), mexA (0.68) and mexB (0.67) at the level of transcription. The raw qPCR ΔCt values showed significant treatment-related down-regulation of all targets (all $P < 0.001$). The docking scores for the four target molecules associated with the pump were clustered around the average score of −6.3 to −6.4 kcal/mol. The internal consistency of the phenotypic, molecular and computational evidence for an efflux/permeability component in the combination response is supported by the increased EtBr retention, increased membrane permeability, and reduced pump-gene expression along with the favorable docking scores.

Table 7: Cellular Mechanisms of Cytotoxicity

Endpoint	K. pneumoniae median (IQR)	P. aeruginosa median (IQR)	P value	Rank-biserial r
LDH release (%)	10.20 (8.30–12.65)	10.30 (8.72–12.57)	0.752	-0.031
Apoptosis (%)	6.90 (5.90–8.20)	7.40 (5.50–8.78)	0.972	0.004
Necrosis (%)	3.30 (2.70–3.95)	3.40 (3.02–4.00)	0.347	-0.091
MMP (% of control)	89.40 (85.65–91.50)	87.90 (85.53–91.57)	0.570	0.055

Both species-linked combination groups had similar cellular toxicity markers. Median LDH release was 10.2% and 10.3% for the combinations of K. pneumoniae and P. aeruginosa, respectively, while apoptosis was 6.9% and 7.4%, respectively, and necrosis 3.3% and 3.4%, and mitochondrial membrane potential was close to 88–89% of the control. There were no significant differences among the endpoints, and all the rank biserial effect sizes were small. In

consequence, the measured cellular injury profile did not reveal any species-dependent difference in host-cell toxicity as tested for the combination conditions.

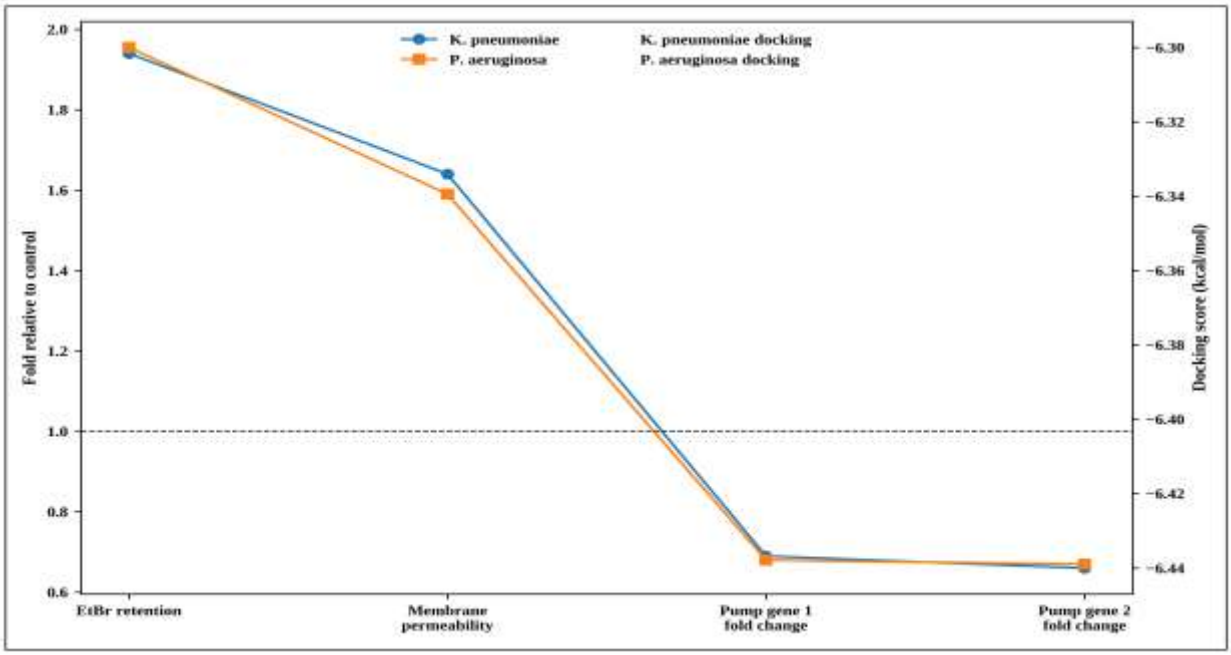


Figure 6: Efflux Pump and Molecular Response Profile

The efflux related phenotypes and molecular measurements were comparable between the two species. There were no significant difference between the species with regard to median EtBr retention, which was around 1.94–1.96 fold and membrane permeability, which was around 1.59–1.64 fold. Median pump-gene expression at the transcriptional level was found to be below unity for all four targets: acrA (0.69), acrB (0.66), mexA (0.68) and mexB (0.67). The raw qPCR ΔCt values were analysed and showed significant down-regulation in all treatments (all $P < 0.001$). The docking scores of the four targets associated with the pump were roughly similar in the center of the range from −6.3 to −6.4 kcal/mol. All the above three factors give internally consistent phenotypic, molecular and computational information that the combination response results from an efflux/permeability component.

3.7 Low-dose exposure stimulated bacterial community phenotypes

A clear biphasic dose–response was observed for all three low-dose endpoints, in both species. Biofilm biomass rose from 102.09 ± 2.06% and 102.76 ± 2.31% of control at 0.2% of the ZnO MIC, through 103.82 ± 3.82% and 104.71 ± 4.12% at 0.4%, to 107.06 ± 7.10% and 108.69 ± 7.83% at 0.8%. Metabolic activity rose from 101.53 ± 1.84% and 101.18 ± 1.79%, through 102.94 ± 3.18% and 102.22 ± 3.41%, to 105.64 ± 5.84% and 104.18 ± 5.95%. EPS production rose from 102.59 ± 2.49% and 102.63 ± 2.49%, through 105.19 ± 4.67% and 105.21 ± 4.43%, to 109.41 ± 8.73% and 109.49 ± 8.23%. The dose effect was significant for all three endpoints (Greenhouse–Geisser-corrected $P < 0.001$) with large partial η^2 values: 0.505 for biofilm, 0.410 for metabolic activity and 0.554 for EPS. The species main effect and the Species × Dose interaction were non-significant for every endpoint (species $P = 0.158$, 0.164 and 0.958; interaction $P = 0.281$, 0.119 and 0.955 for biofilm, metabolic activity and EPS respectively), indicating that the low-dose stimulation was shared rather than species-specific.

Table 8A: Low-Dose Hormesis Analysis

Outcome	Dose (% MIC)	K. pneumoniae mean ± SD	P. aeruginosa mean ± SD	Dose P	Species P	Interaction P	Partial η^2 (dose)
Biofilm	0.2	102.09 ± 2.06	102.76 ± 2.31	<0.001	0.158	0.281	0.505
Biofilm	0.4	103.82 ± 3.82	104.71 ± 4.12				
Biofilm	0.8	107.06 ± 7.10	108.69 ± 7.83				
Metabolic activity	0.2	101.53 ± 1.84	101.18 ± 1.79	<0.001	0.164	0.119	0.410
Metabolic activity	0.4	102.94 ± 3.18	102.22 ± 3.41				
Metabolic activity	0.8	105.64 ± 5.84	104.18 ± 5.95				
EPS	0.2	102.59 ± 2.49	102.63 ± 2.49	<0.001	0.958	0.955	0.554
EPS	0.4	105.19 ± 4.67	105.21 ± 4.43				
EPS	0.8	109.41 ± 8.73	109.49 ± 8.23				

A clear hormetic dose-response curve was observed for the low-dose series. Biofilm biomass, metabolic activity and EPS for 0.2%, 0.4% and 0.8% of the ZnO MIC were 102%, 101% and 103% of control, while increases were 107–109%, 104–106% and 109% of control, respectively. The dose effect was significant for all three endpoints (all Greenhouse–Geisser-corrected $P < 0.001$), with large partial η^2 values of 0.505 for biofilm, 0.410 for metabolic activity and 0.554 for EPS. This low-dose stimulation was observed in both species (no Species × Dose interaction) and was not significant. The integrated correlations also revealed a closer relationship between the efflux/permeability response and the lower FICI values with higher EtBr retention ($\rho = -0.627$, FDR $q < 0.001$), higher membrane permeability ($\rho = -0.528$, $q < 0.001$) and lower mean pump-gene expression ($\rho = 0.685$, $q < 0.001$). Additionally, with increasing dose reduction, pump expression was reduced ($\rho = -0.286$, $q < 0.001$). In contrast, the selectivity indexes did not significantly correlate with LDH release or apoptosis after FDR correction, distinguishing the bacterial synergy signal from the cells' injury markers, indicating healthy cells. Thereby in contrast, the selectivity indexes did not show significant correlation with LDH release or apoptosis after FDR correction, separating the bacterial synergy signal from the cells' injury markers and indicating healthy cells.

Table 8B: Integrated Cross-Domain Associations

Variable 1	Variable 2	Spearman ρ	P value	FDR q
FICI	DRI	-0.253	0.002	0.003
FICI	EtBr retention	-0.627	<0.001	<0.001
FICI	Membrane permeability	-0.528	<0.001	<0.001
FICI	Mean pump-gene fold change	0.685	<0.001	<0.001
DRI	Mean pump-gene fold change	-0.286	<0.001	<0.001
Mean docking score	Mean pump-gene fold change	0.586	<0.001	<0.001
SI WI-38	LDH release	0.043	0.611	0.667
SI HaCaT	Apoptosis	0.059	0.479	0.602
EtBr retention	Mean pump-gene fold change	-0.497	<0.001	<0.001
Membrane permeability	Mean pump-gene fold change	-0.479	<0.001	<0.001
FICI	Low-dose biofilm	-0.056	0.501	0.602
FICI	Low-dose EPS	0.021	0.800	0.800

There was a clear hormetic trend in the low dose series. For the 0.2%, 0.4% and 0.8% of the ZnO MIC, the biofilm biomass was approximately 102%–107–109% of control, metabolic activity was approximately 101%–104–106% of control and EPS was approximately 103%–109–106% of control. All three endpoints showed a dose effect (all Greenhouse–Geisser-corrected $P < 0.001$) and large partial η^2 values for biofilm (0.505), metabolic activity (0.410) and EPS (0.554). There was no significant species main effect and no significant Species × Dose interaction, suggesting that this stimulation at the low dose level was similar for both species. The integrated correlations further correlated efflux/permeability response with higher lower FICI values ($\rho = -0.627$, FDR $q < 0.001$), higher membrane permeability ($\rho = -0.528$, $q < 0.001$) and lower mean pump-gene expression ($\rho = 0.685$, $q < 0.001$). A reduction in the dose was also correlated with lower expression of the pump genes ($\rho = -0.286$, $q < 0.001$). In contrast, after FDR correction, selectivity indices were not significantly related with LDH release or apoptosis, allowing the separation of the bacterial synergy signal from the measured markers of injury of healthy cells.

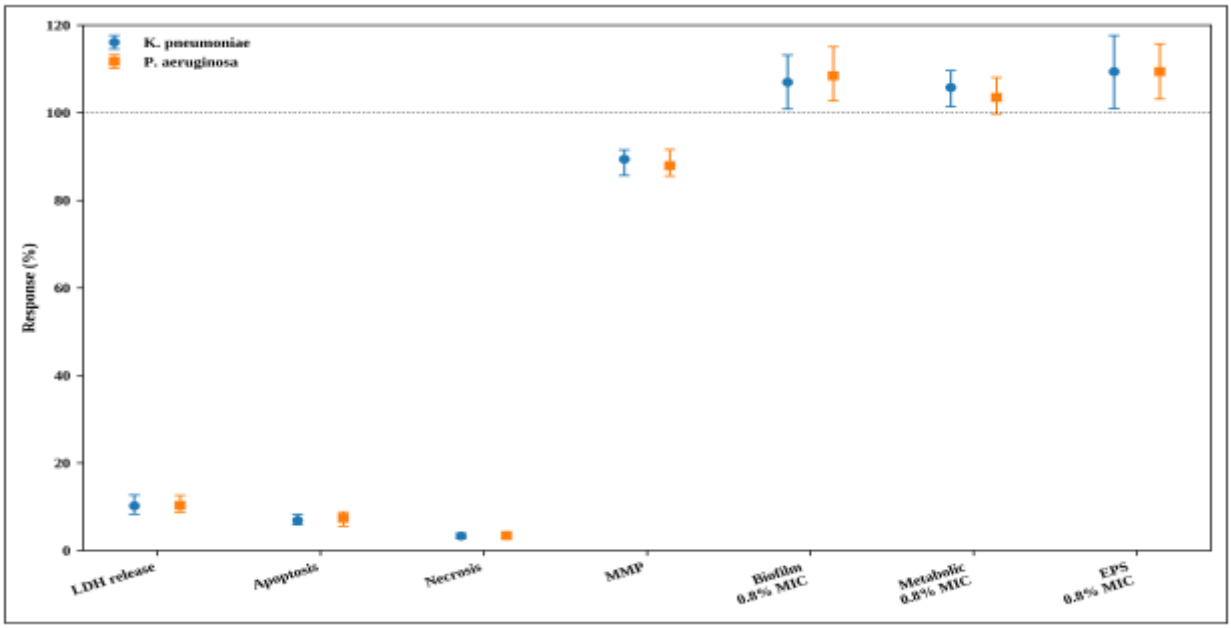


Figure 7: Cellular and Low-Dose Biological Response Profile

The single response-profile plot compares the injury indicators in the host cells with the major low-dosage bacterial responses in a common percentage format. In both species linked combination groups, LDH release, apoptosis and necrosis were not significantly elevated and the mitochondrial membrane potential was maintained at approximately 88–89% of control. At the low dose endpoint (0.8% of the ZnO MIC), bacterial endpoints differed from the control in opposite ways, with an increase in biofilm biomass and metabolic activity and EPS production for both organisms. The similarity of the two species for all seven endpoints is consistent with the formal analyses that did not find a meaningful species effect for the cellular toxicity endpoints and a lack of Species × Dose interaction for the low-dose responses. The plot thus emphasizes the difference between the limited (tested) combination conditions and the measurable stimulation of the bacteria in the very-low-dose range.

3.8 Cross-domain correlations separated the antibacterial signal from the cytotoxicity signal

Integration of the prespecified correlation set clarified the structure of the dataset. Lower FICI values, that is stronger synergy, were associated with higher EtBr retention ($\rho = -0.627$; FDR $q < 0.001$), higher membrane permeability ($\rho = -0.528$; $q < 0.001$) and lower mean pump-gene expression ($\rho = 0.685$; $q < 0.001$). Greater ampicillin dose reduction correlated with lower pump-gene expression ($\rho = -0.286$; $q < 0.001$), and the mean docking score correlated with mean pump-gene expression ($\rho = 0.586$; $q < 0.001$). FICI and DRI were themselves inversely related ($\rho = -0.253$; $P = 0.002$; $q = 0.003$). By contrast, neither selectivity index correlated significantly with LDH release ($\rho = 0.043$; $P =$

0.611; q = 0.667) or with apoptosis (ρ = 0.059; *P* = 0.479; q = 0.602), and FICI did not correlate with either low-dose biofilm (ρ = −0.056; *P* = 0.501; q = 0.602) or low-dose EPS (ρ = 0.021; *P* = 0.800; q = 0.800). The statistical separation of the synergy axis from the host-cell injury axis, and its disconnection from the hormetic axis, is the central structural finding of this analysis.

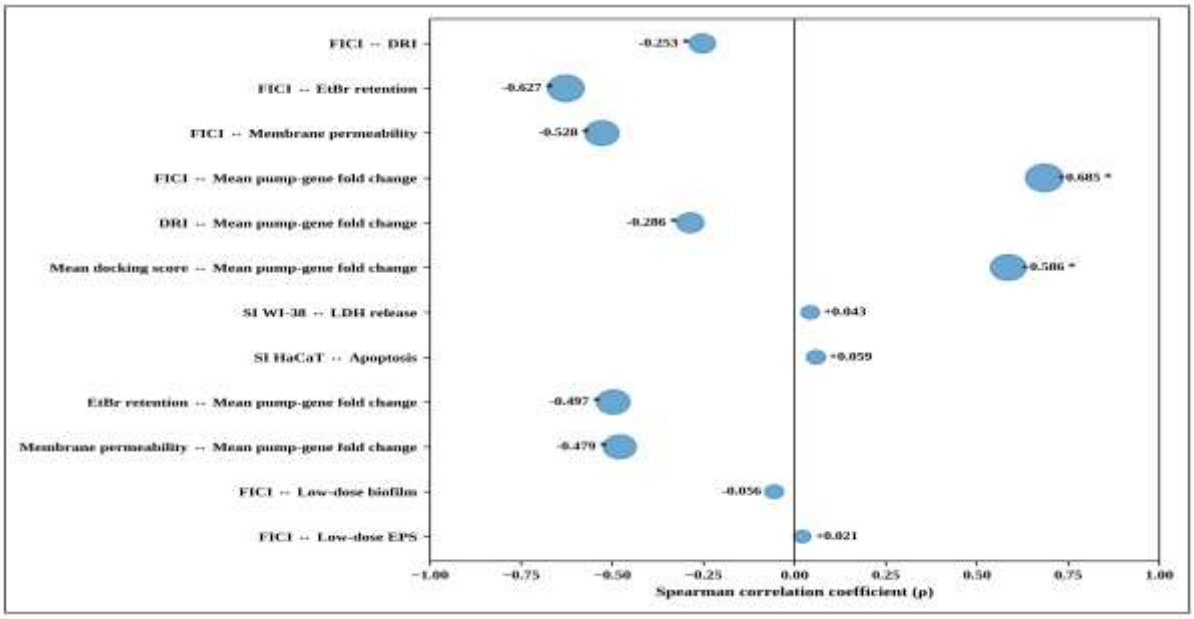


Figure 8: Integrated Cross-Domain Correlation Bubble Plot

The correlation bubble plot is a summary of the direction and strength of the pre-specified cross-domain relationships, but without the heatmap. Lower FICI values were associated with higher EtBr retention (ρ = −0.627, FDR q < 0.001), higher membrane permeability (ρ = −0.528, q < 0.001) and lower mean pump-gene expression (ρ = 0.685, q < 0.001). There were also associations between greater dose reduction of ampicillin and lower pump-gene expression (ρ = −0.286, q < 0.001); and between mean docking score and mean pump-gene expression (ρ = 0.586, q < 0.001). On the other hand, the selectivity indices were not significantly related to LDH release or apoptosis at FDR p<0.05 and the FICI index was not significantly correlated with the low-dose biofilm nor the EPS measures. The size of the bubbles indicates the strength of the correlation, and the x-axis indicates the sign and strength of the Spearman correlation.

4. Discussion

4.1 Principal findings

Three results define this study. First, the combination of ZnO-NPs with ampicillin was synergistic against 133 of 145 MDR clinical isolates (91.7%), with median FICI values of 0.391 and 0.359 and a median eight-fold reduction in the effective ampicillin concentration, and with no evidence that either species systematically drove the effect. Second, the synergistic concentrations sat far below the cytotoxic midpoint of both healthy cell lines, giving median selectivity indices of 38.57–43.27 for *K. pneumoniae* and 41.52–44.19 for *P. aeruginosa*, with SI > 1 in 97.2% and 94.6% of combinations. Third, and tempering the first two, exposure at 0.2–0.8% of the ZnO MIC increased biofilm biomass, metabolic activity and EPS by up to approximately 109% of control in both species. A narrow but real therapeutic corridor therefore exists, bounded above by cytotoxicity and below by hormesis.

4.2 The synergy is mechanistically coherent

The convergence of phenotypic, transcriptional and computational endpoints is the strongest argument that the observed synergy is not an artefact of the checkerboard format. EtBr retention rose approximately two-fold while membrane permeability rose approximately 1.6-fold, indicating both impaired extrusion and a more permeable

envelope. Pump-gene transcription fell to 0.66–0.69 of control for *acrA*, *acrB*, *mexA* and *mexB*, and docking scores for the same four proteins clustered tightly between −6.3 and −6.4 kcal/mol. Crucially, the correlation structure is internally consistent: stronger synergy (lower FICI) accompanied higher EtBr retention, higher permeability and lower pump-gene expression, and greater ampicillin dose reduction accompanied lower pump-gene expression. This is the pattern predicted when a nanoparticle acts as an efflux-pump inhibitor and envelope disruptor, allowing an otherwise expelled β-lactam to accumulate at its target. It also matches independent evidence that polyol-coated metal oxide nanoparticles at sub-inhibitory concentrations promote EtBr accumulation in MDR *P. aeruginosa* and act synergistically with meropenem and ciprofloxacin (Eleftheriadou *et al.,* 2021), and that nanoparticles can circumvent efflux either by intracellular delivery or by pump blockade (Modi *et al.,* 2023). Because the RND transporters AcrAB-TolC and MexAB-OprM are principal determinants of multidrug resistance in Enterobacteriaceae and *P. aeruginosa* (Alenazy, 2022), and because efflux inhibition also attenuates biofilm adhesion and maturation (Musioł, 2023), the mechanism identified here is of general rather than species-specific significance.

Two cautions belong with this interpretation. First, the docking energies reported here are modest in absolute terms: binding modes of RND pump substrates and inhibitors obtained with a flexible binding site differ substantially from rigid-docking estimates, and the AcrB and AdeB pockets accommodate chemically diverse ligands through different interaction patterns (Ornik-Cha *et al.,* 2021). Scores between −6.3 and −6.4 kcal/mol are therefore best read as evidence of plausible interaction, not as proof of inhibition. Second, because PAβN itself permeabilises the outer membrane at higher doses while retaining efflux-inhibitory activity at lower doses (Al-Marzooq *et al.,* 2023), the efflux and permeability components of the mechanism cannot be fully separated by phenotype alone; the convergence of four independent readouts in the same direction is what gives the interpretation weight.

The envelope-directed component is equally consistent with the ZnO literature. ZnO-NPs act through ROS generation with lipid peroxidation and membrane rupture, and through Zn^{2+} release with thiol-group oxidation and inhibition of glycolytic enzymes (Gomaa, 2022), with surface-area-dependent ROS generation (Hadi *et al.,* 2025). Independent work has shown that ZnO-NPs are active against β-lactamase-producing Gram-negative pathogens, with MICs of 0.04–0.08 mg/mL and MBCs of 0.12–0.24 mg/mL, and that this activity involves membrane leakage, DNA damage and apparent β-lactamase inhibition (Krishnamoorthy *et al.,* 2022). A membrane-permeabilising and efflux-inhibiting nanoparticle combined with a cell-wall-directed β-lactam attacks two independent barriers at once, which is a plausible explanation for FICI values as low as 0.36–0.39 rather than the additivity that simple co-administration would predict.

4.3 The selectivity window is real but should be read quantitatively

The cytotoxicity data are unambiguous in direction and large in magnitude. Combination IC_{50} values of 542.8–613.0 µg/mL were two orders of magnitude above the median effective ZnO concentration of 13.61–15.12 µg/mL in the combination. Ampicillin alone did not reach IC_{50} within 0–1000 µg/mL, so the cytotoxic signal is attributable to the nanoparticle component rather than to the antibiotic. Hill slopes of 1.34–1.42 and R^2 at or above 0.9999 indicate that the fitted curves were not model artefacts, and the low and species-independent injury markers — LDH release of 10.2–10.3%, apoptosis of 6.9–7.4%, necrosis of 3.3–3.4% and MMP maintained at 87.9–89.4% of control — indicate that at the effective combination concentrations the host-cell response remained sub-lethal across several independent mechanisms of injury.

This picture should nevertheless be read in the context of the nanoparticle literature, which reports wide variation in ZnO cytotoxicity according to particle size, formulation and cell type. Micrometric ZnO has been shown to be more cytotoxic than 100 nm and 50 nm particles, with keratinocytes more affected than lung epithelial cells (Mitjans *et al.,* 2023); ZnO-NPs have been reported to cause approximately 50% HaCaT death at combination exposures involving 10–12.5 µg/mL (Wang *et al.,* 2024); and exogenous zinc concentrations of 21–31 µg/mL have been associated with keratinocyte cytotoxicity (Khabir *et al.,* 2021). Conversely, green-synthesised ZnO-NPs have been reported with an IC_{50} of 568.59 µg/mL against WI-38 fibroblasts (Naiel *et al.,* 2022), a value very close to the 558.8 µg/mL obtained here for *K. pneumoniae*-associated combinations on the same cell line. The most economical interpretation is that particle physicochemical characteristics, which can differ substantially between synthesis routes, dominate the cytotoxicity outcome, and that this is why selectivity must be measured rather than assumed for each preparation (Chumachenko *et al.,* 2023). Reporting the selectivity index alongside MIC or FICI is therefore not optional: the index

formalises the requirement that the pathogen be killed before the host cell is damaged (Ye *et al.,* 2023), and the related therapeutic index should exceed unity for an agent to be considered usable (Al-Hakkani *et al.,* 2022).

4.4 Sub-inhibitory exposure is the practical constraint

The hormesis data are the most operationally important negative finding. At 0.2% of the ZnO MIC, biofilm biomass, metabolic activity and EPS were already at 101–103% of control; by 0.8% of the MIC, biofilm had risen to 107.06% (*K. pneumoniae*) and 108.69% (*P. aeruginosa*), metabolic activity to 105.64% and 104.18%, and EPS to 109.41% and 109.49%, with large dose effects (partial η^2 0.410–0.554) and no Species × Dose interaction. This reproduces, in *K. pneumoniae* as well as *P. aeruginosa*, the biphasic response previously described for low-dose ZnO-NPs in *P. aeruginosa*, where growth and biofilm formation were stimulated at low concentrations (Al-Momani *et al.,* 2024), and for ZnO-NPs inducing biofilm formation in *K. pneumoniae* and *S. aureus* at sub-inhibitory concentrations (Sreekanth *et al.,* 2023). Comparable stimulation has been reported for the lowest dose of copper-containing nanoparticles, which enhanced biofilm formation of *S. aureus*, *P. aeruginosa* and *C. albicans* by 11.3 ± 1.7%, 11.7 ± 1.5% and 14.2 ± 0.9% (Almahdy *et al.,* 2024). More broadly, community-associated functions such as biofilm formation and quorum sensing are affected in 50% of nanomaterial studies conducted at sub-inhibitory concentrations, compared with only 10–20% of studies at inhibitory concentrations (Mortimer *et al.,* 2021).

The therapeutic implication is that for this combination the relevant parameter is not the minimum effective concentration but the minimum concentration that remains inhibitory in the target compartment. Because topical and wound applications inevitably generate concentration gradients, sub-MIC regions at the periphery of an applied dose are a realistic clinical scenario; the fact that FICI did not correlate with low-dose biofilm ($\rho = -0.056$; $P = 0.501$) or EPS ($\rho = 0.021$; $P = 0.800$) indicates that synergy and hormesis behave as independent axes, so a strongly synergistic pair is not automatically protected against low-dose stimulation.

4.5 Comparison with the wider ZnO–antibiotic literature

The direction of the present result agrees with earlier work combining ZnO-NPs with β-lactams and other antibiotics. ZnO-NPs with piperacillin–tazobactam enhanced activity against MDR *E. coli* and *P. aeruginosa* (Yu *et al.,* 2024); ZnO-NPs with ciprofloxacin or ceftazidime increased activity against resistant *A. baumannii* through membrane damage and intracellular accumulation (Abdelghani *et al.,* 2022); FICI-documented synergy has been reported with colistin, ciprofloxacin and meropenem (Irede *et al.,* 2024); and meropenem-loaded ZnO nanoflakes achieved an average inhibition zone of 15.2 mm against metallo-β-lactamase-producing clinical isolates (Singh *et al.,* 2023). Direct conjugation of ZnO-NPs to clinically approved antibiotics, ampicillin included, has also enhanced antibacterial efficacy while producing minimal cytotoxicity against human cell lines (Akbar *et al.,* 2021). What the present study adds is not the existence of synergy — that is now well documented — but its quantification against a controlled, species-matched, multidrug-resistant baseline in parallel with a formal selectivity analysis and a mechanistic correlation structure, applied to 145 clinical isolates rather than to reference strains. The species-matched baseline is a methodological point of some weight: because the two organisms entered the interaction experiments with statistically indistinguishable MIC and MBC distributions, the similarity of their synergy responses is a genuine finding rather than a consequence of unequal starting susceptibility.

4.6 Limitations

Design and scope. The work is an in vitro study, and neither intracellular bacterial survival nor biofilm-embedded killing was evaluated, so the observed growth inhibition of planktonic cells cannot be extrapolated directly to biofilm-associated infection. The 145 isolates came from a single hospital in Anbar Province, which limits generalisability to other Iraqi or non-Iraqi settings even though it strengthens relevance to the local resistance epidemiology (Al-Fahad *et al.,* 2024). The combination was characterised with a fixed 1:1 design in the growth experiments, so the ratio dependence of synergy was not explored, and only two human cell lines were used, both immortalised; primary respiratory epithelial or macrophage models would strengthen the cytotoxicity assessment. The ampicillin-only IC_{50} was not reached within the tested range, so no direct cytotoxic reference point for the antibiotic component is available.

Material characterisation. The ZnO-NP preparation was not characterised in-house by X-ray diffraction, electron microscopy, dynamic light scattering or zeta potential, so particle size, morphology, agglomeration state in the assay medium and surface charge are not documented. Because particle size strongly influences ZnO cytotoxicity (Mitjans *et al.,* 2023), the absolute IC_{50} values reported here should be regarded as specific to the material lot used, and batch-to-batch reproducibility cannot be verified from these data. This limitation applies to the absolute concentrations rather than to the internal comparisons: because the same stock, dispersion protocol and medium were used throughout, the relative comparisons between species, between treatment conditions and between concentrations remain internally valid. Nevertheless, any attempt to reproduce these values with a different ZnO preparation should expect the antibacterial and cytotoxic thresholds to shift, and physicochemical characterisation of the material is the first requirement for any subsequent formulation work.

Mechanistic evidence. The mechanistic evidence is in part associative. Docking scores of −6.3 to −6.4 kcal/mol indicate plausible binding rather than confirmed inhibition, and were not validated by efflux-pump knockout or by a protonophore control; rigid-receptor docking is known to overstate the stability of inhibitor binding relative to flexible-receptor simulation (Ornik-Cha *et al.,* 2021). Gene expression was measured at a single time point, and the efflux and outer-membrane permeabilisation components of the mechanism cannot be fully separated by phenotype, since efflux inhibitors of the PAβN class themselves permeabilise the outer membrane at higher doses (Al-Marzooq *et al.,* 2023).

Safety endpoints. The cytotoxicity endpoints measured at the synergistic concentrations were sub-lethal, but longer exposures, repeated dosing and evaluation of zinc accumulation and clearance were not examined, and safety in vivo is outside the scope of this work. No time-kill kinetics were performed, so the combination is characterised as inhibitory rather than as bactericidal on a kinetic basis; time-kill data would be required to establish whether the eight-fold dose reduction translates into sustained killing, and comparative in vitro work on nanomaterial–antibiotic combinations identifies time-kill as one of the more informative endpoints for this purpose.

4.7 Implications for development

If this combination is to be taken forward, the data argue for three priorities. First, formulation: because cytotoxicity in ZnO systems is strongly dependent on particle size (Mitjans *et al.,* 2023), the immediate engineering objective is to widen the selectivity window by shifting the cytotoxic threshold upward rather than by increasing antibacterial potency. Second, dosing: the hormesis threshold must be avoided, which favours formulations that release ZnO or Zn^{2+} in a controlled manner above 0.8% of the MIC at the site of infection, and makes sustained-release or surface-immobilised systems more attractive than free nanoparticle suspensions. Third, translation: nanoparticle–antibiotic combinations face unresolved obstacles in reproducibility, scalability, characterisation and regulatory classification, and the absence of dedicated regulatory pathways for such materials remains a recognised barrier alongside cost of production and excretion concerns (Doolan *et al.,* 2022) (Huang *et al.,* 2024) (Mamun *et al.,* 2021). Early engagement with these questions — full physicochemical characterisation, standardised batch-to-batch reproducibility, and explicit safety documentation — materially accelerates the path to clinical evaluation (Muir *et al.,* 2025) (Blackman *et al.,* 2022).

5. Conclusions

ZnO nanoparticle–ampicillin combinations were synergistic against 133 of 145 multidrug-resistant clinical isolates (91.7%), comprising 93.0% of *K. pneumoniae* and 90.5% of *P. aeruginosa* isolates, with median FICI values of 0.391 and 0.359 and a median eight-fold reduction in the effective ampicillin concentration. The synergy was accompanied by a favourable in vitro selectivity window: IC_{50} values of 542.8–613.0 µg/mL against WI-38 and HaCaT cells, median selectivity indices of 38.57–44.19, and SI > 1 in 97.2% and 94.6% of combinations. Phenotypic, transcriptional and computational evidence converged on an efflux/permeability mechanism, with lower FICI values associated with higher EtBr retention ($\rho = -0.627$), higher membrane permeability ($\rho = -0.528$) and lower pump-gene expression ($\rho = 0.685$). Host-cell injury markers remained low, with LDH release of 10.2–10.3%, apoptosis of 6.9–7.4%, necrosis of 3.3–3.4% and mitochondrial membrane potential at 87.9–89.4% of control. However, exposure at 0.2–0.8% of the ZnO MIC stimulated biofilm biomass, metabolic activity and EPS by up to approximately 109% of control in both species, establishing a lower boundary to the useful dose range that is statistically independent of the synergy axis.

The combination therefore merits further development as a dose-controlled, mechanism-informed strategy rather than as a low-dose or uncontrolled-release preparation.

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Nexus International University ISSN: 3136-6449; 12(2) 179-188

Zingiber officinale: Hot Aqueous Extract against the Growth and Production of MR Staphylococcus aurous Biofilms

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Abstract

Aims: to determine the efficacy of Zingiber officinale (Ginger) as a medicinal plant containing active compounds against the growth and biofilm production of methicillin-resistant Staphylococcus aureus (MRSA).
Study design: the Case control study.
Place and Duration of Study: Department of Biology Faculty of Education /Kufa University and hospitals of Al-Najaf Al-AShraf /Iraq for the period (September 2 to December 25 /2025)
Methodology: Twenty clinical isolates of MR Staphylococcus aureus and were diagnosed by using VITEK 2 compact system. Its efficiency in production of biofilm was performed by Congo Red Agar and Tissue culture plate methods. Four concentrations (2.5%, 5%, 10%, and 20%) of the hot aqueous ginger extract were prepared, and their efficacy against MR Staphylococcus aureus isolates was tested using both the well-diffusion and drop methods. Additionally, the effect of these concentrations on biofilm formation was evaluated using the tissue culture plate method.
Results: The aqueous ginger extract demonstrated the ability to inhibit the growth of multidrug-resistant Staphylococcus aureus specifically methicillin-resistant isolates achieving a maximum inhibition zone diameter of 35 mm via the agar well diffusion method at a 20% extract concentration. Although the isolates were capable of producing biofilms as evidenced by Congo Red agar and tissue culture plate assays (conducted both with and without glucose) various concentrations of the aqueous ginger extract reduced biofilm production levels, while higher concentrations completely inhibited its formation. Conclusion: Aqueous ginger extract plays active role in inhibiting the growth of MR Staphylococcus aureus and preventing its biofilm formation. Consequently, aqueous ginger extract can be recommended as a safe, natural alternative to address the issue of antibiotic resistance in S. aureus specifically the adverse effects associated with biofilm formation, which pose a major obstacle to treating the infectious diseases caused by this bacterium.

Keywords: Biofilm, MR Staphylococcus aureus, Zingiber officinale, Congo Red, Ginger.

1. Introduction

Staphylococcus aureus are among the leading infectious agents responsible for high mortality rates in 135 countries, according to a study published in The Lancet. The highest mortality rates associated with this bacterium have been observed in individuals over 15 years of age (Liu et al., 2024).

Virulence factors and biofilm formation are key attributes that protect Staphylococcus aureus from antimicrobial agents and the host immune system, while also facilitating bacterial adhesion to host cell surfaces. Implanted medical devices serve as a substrate for Staphylococcus aureus biofilm formation, leading to the development of infections

that are highly resistant to antimicrobial agents. According to the literature, biofilm-forming bacteria are 10 to 1000 times more resistant to antibiotics dispersed (Douglas et al., 2023).

The ability of bacteria to form antibiotic-resistant biofilms composed of extracellular polymeric substances (EPS), a complex mixture of extracellular DNA (eDNA), proteins, and polysaccharides makes eradicating pathogenic bacteria difficult (Idrees et al., 2021). The efficacy of penicillin-binding protein 2 (PBP2) is modulated by the fem gene family via the regulation of peptidoglycan precursor metabolism (Ambade et al .2023; Hou et al. 2023) while the agr quorum-sensing system indirectly influences resistance characteristics (including biofilm formation and antibiotic resistance) by dynamically (Rimi et al. 2024). It has recently been observed that inappropriate antibiotic treatment can have counterproductive results in treating Staphylococcus aureus biofilm-associated infections, as it promotes the manipulation of cellular systems and enhances biofilm growth (He et al., 2023). Other researchers have also noted that biofilm growth in the presence of antibiotics occurs in a dose-dependent manner, resulting in a more robust and adhesive biofilm (Rabin et al., 2015).

Zingiber officinale - Ginger- has long been used as a spice and an herbal medicine. Numerous bioactive compounds have been identified in ginger, such as phenolic and terpenoid compounds. The phenolic compounds in ginger consist primarily of gingerols, shogaols, and paradols, which are responsible for various biological activities (Kieliszek et al., 2020). Many studies have demonstrated that ginger possesses biological properties, including antioxidant, anti-inflammatory (Nile and park (2015) and antimicrobial activities (Akbari et al.2023).

Given the rising antibiotic resistance in Staphylococcus aureus compounded by its virulence factors and biofilm forming capabilities there is an urgent need to develop effective strategies to combat the global challenge posed by the infectious diseases it causes. One significant approach involves utilizing bioactive compounds derived from medicinal plants and herbs, particularly those with a history of safe human consumption in daily diets; Ginger is a key plant highlighted in this study, as it contains bioactive substances believed to play a role in inhibiting or preventing biofilm formation by Staphylococcus aureus.

2. Material and Methods

2.1 Bacterial Isolates

Twenty Clinical isolates of MR Staphylococcus aurous were obtained from the hospitals of Al-Najaf Al-AShraf /Iraq for the period (September 2 to December 25 /2025)

2.2 Identification of Staphylococcus aurous

For the purpose of diagnosing bacterial isolates, samples were initially cultured on manitol salt agar and blood agar media, then incubated at 37oC for 18 hours, and diagnosed according to the cultural characteristics both types of media (De la Maza et al.2020). the diagnosis of bacteria was confirmed by using VITEK compact system according to Manufacturers Instructions bioMe' rieux) ; ID for Gram positive Cocci Cards.

2.3 Collecting and preparing the aqueous extract of Zingiber officinale

The Ginger (Zingiber officinale) rhizomes were purchased from local markets. It was peeled, dried at room temperature and ground with electric grinder to obtain dry powder, the powder was packed I glass bottle until extraction and then prepared hot aqueous different concentrations (2.5, 5, 10, 20) %

2.4 Detection of crud active substances in Ginger Extract

The active crud materials were detected using each of: Meyer’s reagent for flavonoids, Molisch’s reagent Carbohydrates, Ferric chloride for phenols and (Lieberman- Burchard) reagent for identifies terpenoids, For the detection of volatile oils, a sodium hydroxide solution with drops of hydrochloric acid was used. (Harborne, 1998; Anyasor et al., 2010)

2.5 Testing MR Staphylococcus aurous against Antibiotic

The method of Bauer et al. (1996) was used to detect of MR Staphylococcus aurous resistance and sensitivity against different antibiotics resistance and sensitivity (Oxacillin, Amoxyclave, Rifampin, Ciprofloxacin, Ceftriaxone, Erythromycin, Gentamycin, Tigecycline, Fusidic acid). the bacterial isolates were considered sensitive or resistant to these antibiotics used based on international specifications contained in CLSI (2025). then it was confirmed that the isolation were diagnosed using the Vitek2 compact system ID and AST Biomerieux (France) specific card for gram positive bacteria.

2.6 Effectiveness of Zingiber officinale aqueous extract against MR Staphylococcus

The well diffusion method (Piddock, 1990) was used, MRS Staphylococcus aurous isolates (1.5 * 108 cell/ml) were spreading on Muller- Hinton Agar, the wells 8 mm were made by cork poorer, plant extract concentration (2.5, 5, 10, 20) % were added, and the negative control represented by normal saline. incubated at 37oC for 24 hours, The diameters of the inhibition zones were measured using a ruler, and then the well diameter was subtracted from the averages of the inhibition zones, each separately.

The spotting method also used by adding 5 microliter of each concentration on the bacteria grown on Muller- Hinton Agar, then the inhibition zone were measured to be compared with well diffusion.

2.7 Detection of MRS Staphylococcus aurous biofilm formation and impact of Ginger extract.

Six isolates of the most antibiotic-resistant Staphylococcus aurous were selected to test their efficiency in producing biofilms. There are two method were used to determine production of bacterial biofilm, the first was tissue culture plate method described by (Mathur et al., 2006) and the Second was Congo Red agar according to Freeman et al., (1989)

Tubes containing brain heart infusion broth were inoculated with Staphylococcus aurous isolates. A bacterial suspension was then prepared for each isolate at McFarland standard turbidity (1.5 *108) cell/ml. Twenty microliters of the bacterial suspension were taken and placed in individual wells of microtiter plate and incubated at 37oC for 24 hours. after that the plate was washing by phosphate buffer saline (pH=7.2) to remove non-adherent bacteria, then sodium acetate (2%) was added to fix the adhering bacterial cells. It was then stained with (0.1 w/v) crystal violet. washed with distilled water. The absorbance was then measured by ELISA auto reader at a wavelength of 570 nm. The same steps as above were followed when measuring the effect of the Ginger extract on biofilm production by adding equal volumes of different concentrations (2.5, 5, 10, 20) % of the extract to the bacterial isolates individually.

The ability of bacteria to form a biofilm was quantitatively estimated by measuring the optical density of the adhering dye, and the results were recorded according to the following table:

Biofilm formation	**Strong**	**Weak**	**Non produce**
Optical density	≥ 0.240	0.240 - 0.125	< 0.125

2.8 Statistical Analysis

The results were statistically analyzed using (SPSS) software by performing a one-way ANOVA and using the least significant difference (LSD) method with p value ≤ 0.05.

3. Results and Discussion

The results on the active raw compounds in the plant extract of ginger revealed that it contains flavonoids, as evidenced by the extract turning yellow after the addition of Meyer,s reagent. A purple ring also formed in the extract tube after the addition of Molisch,s reagent indicating the presence of sugars in the aqueous extract of the plant. A bluish-green

color is produced when using Lieberman- Burchard reagent to confirm the presence of terpenoids. When the extract was examined for volatile oils, a light oily ring was formed upon the addition of NaOH along with drops of HCl.

Studies have shown that ginger is rich in active compounds, as it contains phenols in various forms (Gingerols, Shogaols, and Paradols) that transform into other forms with changes in temperature and storage. Ginger also contains a number of terpenes, including α-curcumene, zingiberene, α-farnesene, β-bisabolene and β-sesquiphellandrene which are the main components of its volatile oils. (Yeh et al. ,2014). In addition, it contains polysaccharides, organic acids, lipids and crud plant fiber. (Schadich et al., 2016)

The results regarding resistance and sensitivity of the Staphylococcus aureus isolates, shown in Figure (3-1), indicate that the bacteria were 100% resistant to both Oxacillin and Penicillin, The second most common antibiotic was Erythromycin, with a percentage of 80%, Meanwhile, the bacterial isolates exhibited varying degrees of resistance to the remaining antibiotics, namely Ceftriaxone, Tig, clindamycin, fusidic acid, Ciprofloxacin and rifampin, respectively. While the isolates were sensitive to the antibiotic Vancomycin at a rate of 84%.

The intrinsic resistance mechanisms of Methicillin-resistant Staphylococcus aureus (MRSA) are due to the presence of the mecA gene, which is the main molecular basis of resistance. This gene is encoded by penicillin-binding protein 2a (PBP2a), the latter provides a strong defensive barrier against beta-lactam antibiotics by remodeling the cell wall biosynthesis pathway (Abebe and Birhanu 2023) . Several pathogenic bacterial isolates exhibit heterogeneous resistance to antibiotics, including a Subpopulation with a minimum inhibitory concentration (MIC) higher than the minimum inhibitory concentration (CBP), and are expected to better resist the drug concentrations achieved in the treated patient (Heidarian et al.,2024)

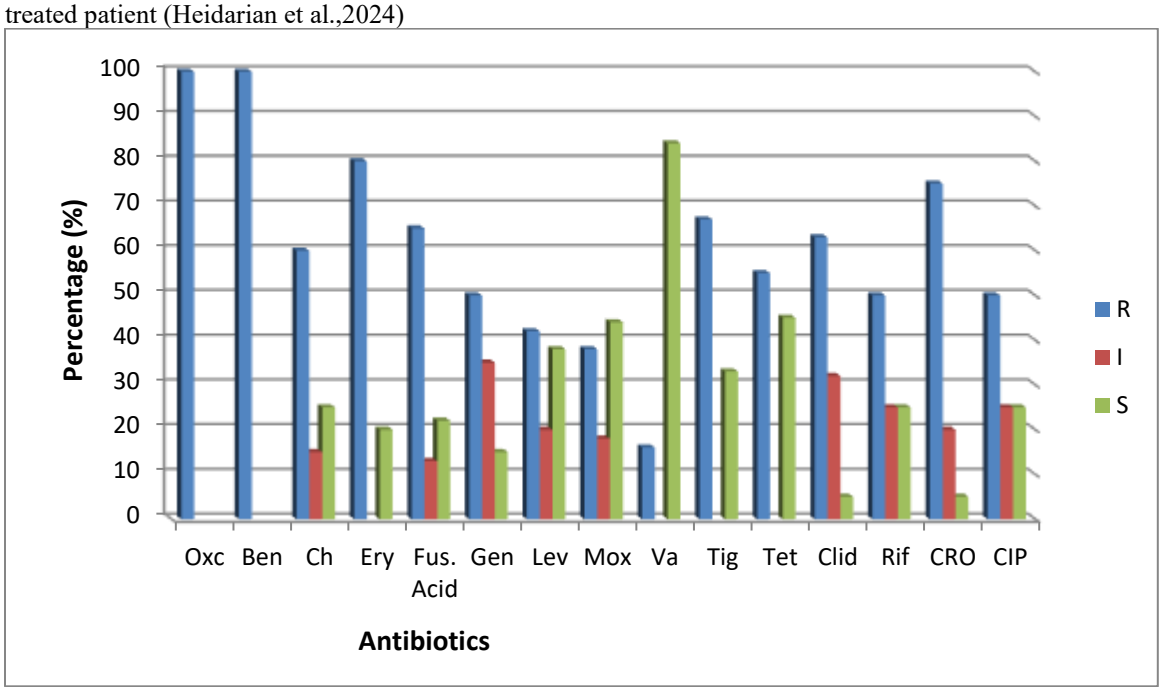


Figure (1): *Resistance and susceptibility of Staphylococcus aureus to the various antibiotics*

Table (1) Shows a comparison of the effect of Zingiber officinale extract on inhibiting the Staphylococcus aureus isolates under investigation using the well diffusion method (Figure 1) and the Spotting method. This finding shown in the table indicate that the average diameters of the inhibition zones increased significantly ($p \leq 0.05$) with increasing concentrations (5, 10,15,20) % of the Zingiber officinale extract, respectively, for both methods. A concentration of 20% gave the highest inhibition rate of bacteria at a diameter of 35 mm using the diffusion method as Figure (2). It is also noted that concentration 2.5% failed to produce any bacterial inhibition.

Molecular weight plays a crucial role in agar diffusion (Flanagan and Steck, 2017). These observations indicate that as the diffusion rate increases with increasing plant extract concentration, the inhibition zone increases proportionally with the depth of the agar layer. The accuracy of this test is affected by the uniformity of the agar depth, which is ideally 4 mm (CLSI, 2013).

Ginger contains gingerol, which is classified according to the length of its unbranched alkaloid chain, 6-gingerol is a bioactive compound with a mild pungent flavor in ginger and has anti-inflammatory and antibacterial properties (Sang et al. 2020). Some pathogenic bacteria, particularly Staphylococcus aureus, encode a unique enzyme, 6-hydroxymethyl-7,8-dihydropyrin pyrophosphate kinase (SaHPPK) (Dennis et al. 2016), against which no antibiotics have been found. Therefore, the active ingredients in the plant extract of ginger are likely to be anti- Staphylococcus aureus agents, especially those that are methicillin-resistant. Rampogu et al. (2018) proposed nominating both shogaol, and gingerenone-A as antibacterial drugs because molecular dynamics simulation results showed their directional stability and strong interactions with catalytic active residues in the catalytic pocket of the SaHPPK enzyme.

Table (1): Antibacterial effectiveness of different concentrations of Zingiber officinale extract on Staphylococcus aureus isolates.

Plant extract conc. (%)	Average of inhibition zone (mm) (main ± Sd)	
	Well diffusion method	Spotting method
2.5	0	0
10	5 ± 0.54	5 ± 0.86
15	15 ± 1.52 *	10 ± 1.45 *
20	35 ± 2.9 **	18 ± 2.08 **

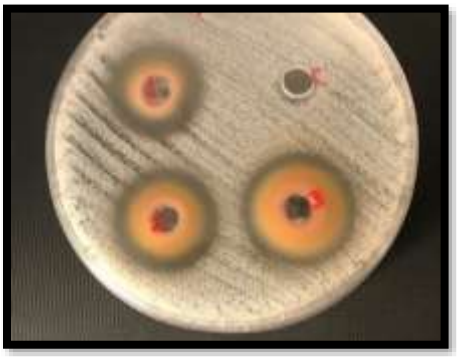


Figure (2): *Inhibition of Staphylococcus aureus using different concentrations of the extract by diffusion method. (the well at the top left is 5% conc., below left is 10 %, but the below right is 20%, while the top right is control)*

After conducting sensitivity and resistance tests on Staphylococcus aureus isolates, the six most efficient isolates were selected based on their multidrug resistance to the antibiotics used in the study. These isolates were then tested for biofilm production using both Congo Red Agar medium and tissue culture plate methods

The results obtained the ability of all six isolates to produce biofilm through their growth on Congo Red medium, as shown in Figure (3), the colonies appeared black after growing on the culture medium overnight, this is due to the presence of glucose, which reacts with Congo red dye through bacterial action, resulting in the production of a black color (Mathur et al., 2006). The Congo red agar method is simple, specific, and 100% sensitive, with an accuracy of 43% compared to other methods used to detect biofilm production (Banerjee et al., 2018)

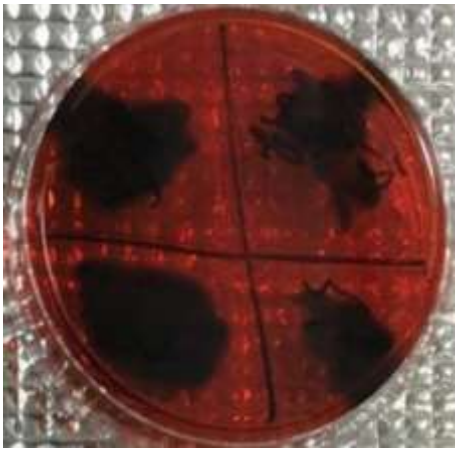


Figure (3): *Detection of Staphylococcus aureus biofilm formation by Congo Red Agar medium (Bacterial colonies appear black after growing on the culture medium for 24 hours)*

Regarding the production of biofilm using tissue culture, it can be observed from Figure (4) that the biofilm production of the isolates varied; some were highly productive, others moderately productive, with the exception of isolate number one, which was weak. Furthermore, there was an increase in production in the presence of glucose compared to its absence in the culture medium. This reinforces the findings of Wieland et al. (2025) who, during their study of two Staphylococcus aureus strains, Newman and Newman D2C, found that they differed significantly in their ability to adhere to non-living surfaces and form biofilms on them in various laboratory tests. However, when tested in a mouse model of foreign body-associated infection, both strains produced similar vegetative growth on the implanted catheter tube.

The culture medium used in bacterial cultivation is of paramount importance in increasing biofilm formation. Furthermore, the increased production of biofilms in the presence of glucose may be attributed to its phosphorylation. Vasu et al. (2017) found that phosphorylation by glucokinase (glkA) increased its activity fourfold, highlighting the important role this compound plays in biofilm formation.

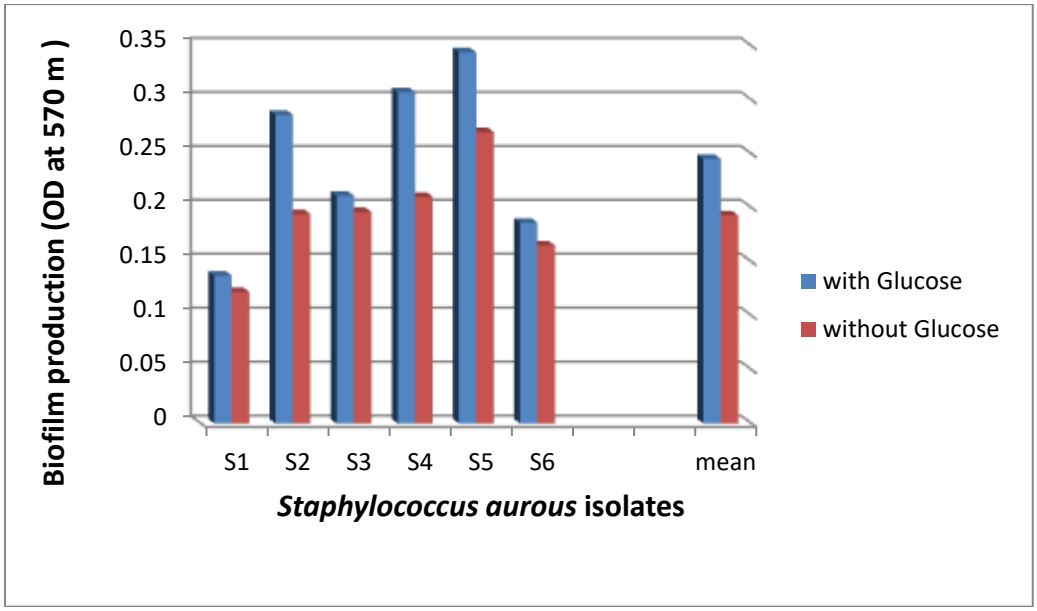


Figure (4): *Staphylococcus aureus biofilm production in the presence and absence of glucose.*

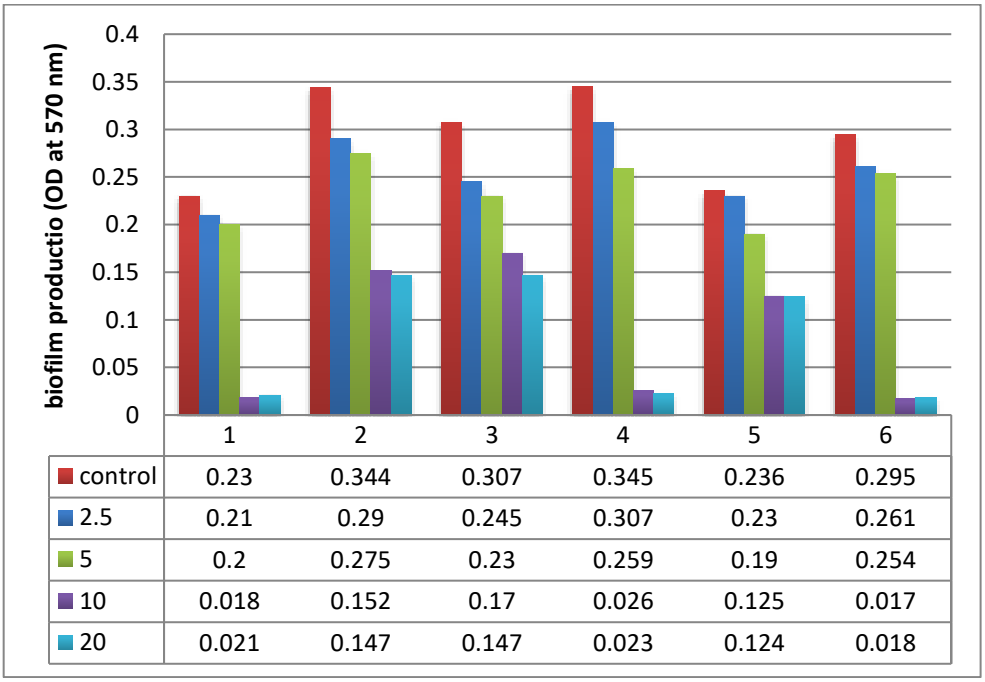
When investigating the effect of Zingiber officinale extract on the biofilm production of Staphylococcus aureus, the data in Figure (5), (6) showed that all isolates exhibited a significant decrease in biofilm production, transforming into non-biofilm-producing bacteria due to the extract, particularly at concentrations of 10% and 20%. This can be explained by Bioactive chemicals rise in tandem with extract concentration, which reduces the production of biofilms.

There are several bioactive substances with antibacterial properties can be found in ginger extracts. Some of these constituents have been identified, including zingerone, 6-gingerol, and 6-shogaol. According to certain research, zingerone is efficient in preventing Pseudomonas aeruginosa from forming biofilms (Kumar et al .2013), The current results also confirm what the researchers (Akbari et al.2023) have concluded: that ginger extract has a greater effect on Gram-positive bacteria, specifically B. subtilis, than on Gram-negative bacteria P. aeruginosa. Furthermore, the crude ginger extract and its methanolic fraction inhibited the adhesion and biofilm formation of Streptococcus mutans and its glucan synthesis by downregulating virulence genes. Consistent with the in vitro study, a reduction in the development of caries induced by this bacterium was observed in a group of treated rats (Hasan et al. 2015).

Initial surface adhesion is the first stage of biofilm formation, which is followed by biofilm growth and maturation and dissemination. It is an intricate and evolving process that encompasses alterations in physiological characteristics, the control of multiple genes, metabolic variability, and the capacity to react to environmental and cellular stimuli (Chai et al., 2024). Therefore, it is expected that the 20% concentration inhibited the adhesion process, which is the initial step in bacterial biofilm formation.

Plant extracts affect bacterial cell membranes through both direct and indirect mechanisms. The direct mechanism involves biologically active components disrupting bacterial cellular structures, destabilizing the biofilm matrix, or inhibiting vital enzymatic activities, thereby reducing bacterial adhesion, aggregation, and viability [Bandyopadhyay et al. (2024); Huang et al. (2025). For instance, ethanolic extracts of Torilis japonica have been found to inhibit the expression of adhesion genes—specifically sarA, icaA, and hla—in Staphylococcus aureus (strain KCTC 1927), thereby suppressing biofilm formation (Kim et al., 2022)

Based on the current results, concentrations of 20% and 15% inhibited biofilm formation in the Staphylococcus aureus isolates under study. This potential effect may be attributed to the higher concentration of active compounds in the ginger extract at these levels, as well as to direct and indirect effects on the bacteria that prevent adhesion and biofilm formation.



	1	2	3	4	5	6
control	0.23	0.344	0.307	0.345	0.236	0.295
2.5	0.21	0.29	0.245	0.307	0.23	0.261
5	0.2	0.275	0.23	0.259	0.19	0.254
10	0.018	0.152	0.17	0.026	0.125	0.017
20	0.021	0.147	0.147	0.023	0.124	0.018

Figure (5): *Impact of varying aqueous ginger extract concentrations on Staphylococcus aureus biofilm formation.*

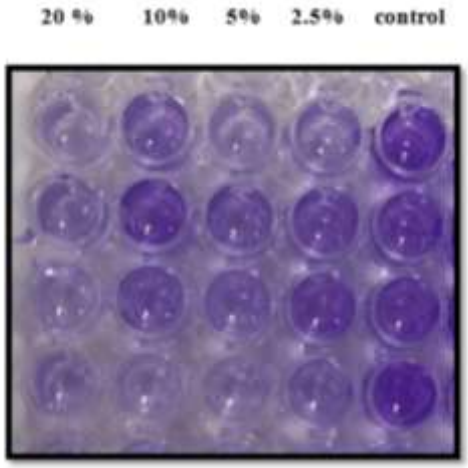


Figure (6): *Reduction of Biofilm Production Levels in Staphylococcus aureus and Inhibition of Its Formation by Various Concentrations of Aqueous Ginger Extract.*

4. Conclusion

The results of the current study indicate that the aqueous extract of ginger, at various concentrations, demonstrates high efficacy in inhibiting the growth of Methicillin resistant Staphylococcus aureus isolates and reducing their biofilm production levels, as well as preventing biofilm formation at higher concentrations. Given its content of active natural compounds, the aqueous ginger extract is considered safe for use and a suitable alternative to antibiotics for combating Methicillin resistant Staphylococcus aureus a bacterium responsible for serious diseases that are sometimes difficult to treat.

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Nexus International University ISSN: 3136-6449; 12(2) 189-195

Hospital-Based Epidemiological Distribution of Lung and Liver Cancer Among Patients Attending the National Oncology and Hematology Center in Najaf, Iraq (2021–August 2026)

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Abstract. This study aims to describe the hospital–based epidemiological distribution of registered lung and liver cancer cases at the National Oncology and Hematology Center in Najaf, Iraq, from 2021 to August 2026. A retrospective hospital-based descriptive study was conducted using records of 1,470 patients aged 10 years and above who were diagnosed with lung or liver cancer. Data were summarized using frequencies and percentages, and Pearson's chi-square test was used to assess associations among cancer type, sex, and residential area. Lung cancer accounted for 1,232 cases (83.81%), whereas liver cancer accounted for 238 cases (16.19%). Males represented 995 cases (67.7%), and females 475 cases (32.3%). Patients aged 50 years and above accounted for 1,290 cases (87.8%), with the largest proportion in the 60–69-year group (29.32%). The highest number of registered cases was recorded in 2025 (311 cases); however, 2026 data covered only January through August and were therefore not directly comparable with complete calendar years. A statistically significant association was observed between cancer type and sex (χ^2=5.938, df=1, p=0.0148), with liver cancer constiuting a higher proprotion of cases among females (19.6%) than among males (14.6%), and between sex and residential area (χ^2=8.833, df=1, p=0.0030), whereas the association between cancer type and residential area was not statistically significant (χ^2=1.645, df=1, p=0.200). These findings describe the distribution of registered hospital cases and should not be interpreted as population-based disease rates. Population-based studies incorporating relevant risk factors and population denominators are needed to further investigate the epidemiology of lung and liver cancer in Najaf.
In conclusion, lung cancer constituted the largest proportion of registered cases, males accounted for a higher proportion than females, and most cases occurred among patients aged 50 years and above. The observed associations with sex and residential area should be interpreted within the hospital -based nature of the dataset. Further population -based research is needed to estimate disease rates and examine the contribution of smoking, viral hepatitis, environmental exposures, and access to healthcare.

Keywords: Lung cancer, liver cancer, sex and age differences, Najaf, Iraq.

1. Introduction

Unlike benign tumors, which generally do not invade surrounding tissues or spread to distant sites, cancer comprises a heterogeneous set of disorders in which abnormal cells multiply and expand without normal regulatory control. This disease remains a major global public health burden. Based on the most recent GLOBOCAN 2024 figures, nearly 20.6 million newly diagnosed cases and 9.8 million deaths attributable to cancer occurred across the globe during 2024 [1]. One of the most prevalent malignancies in developing nations is liver cancer, especially hepatocellular carcinoma (HCC). It is intimately linked to liver cirrhosis brought on by alcohol use or other long-term liver conditions, as well as long-standing infection with hepatitis B and C viruses (HBV/HCV). According to recent research conducted in the Middle East and North Africa (MENA), the burden of liver cancer is rising due to several variables, such as obesity, type 2 diabetes, and viral infection [2][3]. Globally, lung cancer ranks first in both the number of new diagnoses and

the number of cancer deaths [4]. Tobacco use is a primary causal determinant and causes about 80-90% of cases of lung cancer; there are also carcinogenic agents, such as radon and environmental pollutants, that contribute to the development [5][7][8]. Lung cancer is distinguished by its rapid spread to other organs, such as the brain and bones [6]. According to the latest GLOBOCAN 2024 estimates, Iraq recorded 34279 newly diagnosed cases and 19963 deaths due to the disease during that year. Lung cancer accounted for 2758 new cases (8.0%) and 2621 deaths (13.1%), ranking second among cancers in both incidence and mortality [11]. Nationally, it represents one of the commonest malignancies and a leading contributor to cancer mortality [9]. Given the high rate of smoking and the pervasiveness of chronic liver disorders, concentrating on liver and lung cancer in Iraq emphasizes the necessity of bolstering prevention and early detection initiatives. Research on these cancers also helps to develop effective governmental programs to reduce the burden of these diseases [10].

2. Research Methodology

A retrospective, hospital-based descriptive study was conducted to describe registered cases of lung and liver cancer from 2021 to August 2026. Information extracted from the records included cancer type, sex, age group, residential area, and year of diagnosis. Altogether,1470 registered records were included in the analysis. Frequencies along with percentages were used for summarizing the data. At the same time, Pearson's chi-square test was applied to examine the relationships of cancer type with residential area, cancer type and sex, and sex and residential area. Because the dataset consisted of hospital-registered cases and didn't include population denominators, the results were interpreted as distributions of registered cases rather than population-based disease rates.

2.1 Research Site and Data Source

The research took place at the National Oncology and Hematology Center in Al-Najaf Al-Ashraf Governorate, Iraq. Records from January 2021 through August 2026 were reviewed. Eligible cases comprised both male and female patients whose age was 10 years or more and who had been diagnosed with lung or liver cancer. Registered cases were from urban and rural areas and included patients residing inside and outside the governorate.

2.2 Data Analysis

Statistical processing was performed with SPSS software (version 26), and counts with their proportions were calculated for all categorical measures. Pearson's chi-square procedure was used to examine relationships among categorical factors, with statistical significance set at $p > 0.05$.

3. Results

Our results indicated (Table 1) that 1470 registered cases were recorded from 2021 to August 2026. Of these, 83.81% were lung cancer and 16.19% were liver cancer. Urban residents accounted for 852 cases (58.0%), whereas rural residents accounted for 618 cases (42.0%). No significant statistical relationship was detected linking cancer type with residential area ($\chi^2=1.645$, df=1, p=0.200).

Cancer type	Urban	Rural	Total	%
Lung	723	509	1232	83.81
Liver	129	109	238	16.19
Total	852	618	1470	100.0

Table 1: Cancer type by residential area

Table (2) shows that the highest number of registered cases 311, was observed in 2025. The number of 2026 refers to the first eight months of the year and thus is not directly comparable with complete calendar years.

Year	Urban	Rural	Total
2021	128	80	208
2022	155	100	255
2023	121	100	221
2024	145	119	264
2025	196	115	311
2026 (Jan–Aug)	107	104	211
Total	852	618	1470

Table 2: Recorded cases by year and residential area.

Table (3) shows the distribution of registered cases by sex. Males accounted for 995 cases (67.7%), whereas females accounted for 475 cases (32.3%).

Sex	Number of Cases	Percentage %
Male	995	67.7%
Female	475	32.3 %
Total	1470	100 %

Table 3: Distribution of cancer cases according to sex.

Table (4) indicates that lung cancer represented the largest proportion of registered cases, with 1,232 cases (83.81%) of the total cancer cases recorded, while liver cancer had 238 cases (16.19%). Therefore, the bulk of the cases recorded over the study period were for lung cancer.

Cancer type	Male	Female	Total	%
Lung	850	382	1232	83.81
Liver	145	93	238	16.19
Total	995	475	1470	100.0

Table 4: Distribution OF cancer type by sex.

Table (5) shows that registered cases were concentrated in the older age groups. Among patients aged 50 years and above, 1,290 cases (87.8%) were recorded. The largest single age group was 60–69 years (29.32%), followed by 70–79 years (27.21%) and 50–59 years (22.31%).

Age group	Liver	Lung	Total	%
<20	4	3	7	0.48
20–29	6	5	11	0.75
30–39	7	19	26	1.77
40–49	33	103	136	9.25
50–59	58	270	328	22.31
60–69	56	375	431	29.32
70–79	56	344	400	27.21
≥80	18	113	131	8.91
Total	238	1232	1470	100.00

Table 5: Age distribution by cancer type.

The distribution of lung and liver cancer cases according to age group and sex is shown in Table 6. There were 1,470 registered cases, including 995 males and 475 females. Lung cancer accounted for 1,232 cases (850 males and 382 females), whereas liver cancer accounted for 238 cases (145 males and 93 females). The largest share of all registered cases occurred in the 60–69 years (29.32%), with the 70–79 years (27.21%) and the 50–59 years (22.31%) ranking next.

Age group	Liver Female	Liver Male	Lung Female	Lung Male	Total
<20	2	2	1	2	7
20–29	4	2	2	3	11
30–39	5	2	8	11	26
40–49	16	17	39	64	136
50–59	24	34	77	193	328
60–69	17	39	108	267	431
70–79	17	39	103	241	400
≥80	8	10	44	69	131
Total	93	145	382	850	1470

Table 6: Distribution of cases by age group, cancer type, and sex.

The distribution of registered cancer cases according to cancer type, sex, and residential area is presented in Table 7. Pearson's chi-square test was applied to evaluate the relationship between cancer type with residential area, cancer type and sex, and sex and residential area.

Variables	Urban	Rural	Total	χ^2	df	P value	Interpretation
Male	603	392	995				
Female	249	226	475				
Total	852	618	1470				
Cancer type × residential area				1.645	1	0.200	Not significant
Cancer type × sex				5.938	1	0.0148	Significant
Sex × residential area				8.833	1	0.0030	Significant

Table 7: Distribution of cancer cases and Pearson chi-square tests of association between cancer type, sex, and residential area. **Note:** $\alpha = 0.05$. Pearson chi-square test, without continuity correction. The descriptive sex × residential-area counts are shown above to support the corresponding chi-square test.

4. Discussion

The present study described the hospital-based distribution of registered lung and liver cancer cases at the National Oncology Center in Najaf from 2021 through August 2026. A total of 1,470 registered cases were included. Lung cancer accounted for 1,232 cases (83.81%), while liver cancer accounted for 238 cases (16.19%). Males represented 995 cases (67.7%), and females represented 475 cases (32.3%). Because the study was based on hospital records and did not include population denominators, these proportions describe the composition of registered cases and should not be interpreted as population-based rates or as evidence that one kind of cancer is more common in the Najaf population overall. The high proportion of registered cases attributed to lung cancer is consistent with the large global burden of this malignancy documented in international cancer statistics [1][11]. Smoking is well established as a major contributor to lung cancer risk, as are other exposures such as radon and occupational or environmental carcinogens [5][13]. Smoking status and occupational and environmental exposures were not available in the present dataset. Liver cancer accounted for 16.19% of the registered cases. Chronic hepatitis B and C infections and chronic liver disease are established risk factors for hepatocellular carcinoma [12][14]. However, information on viral hepatitis status and additional clinical risk factors was not available in the present study. There was a significant relationship between patients' sex and the cancer type diagnosed (χ^2=5.938, df=1, p=0.0148). Although males represented the majority of both lung and liver cancer cases, liver cancer constituted a higher proportion of cases among females (93/475;19.6%) than among males (145/995;14.6%). Differences between males and females in cancer patterns have been described internationally and may be related to differences in exposure to risk factors, occupational environments, behaviors, and biological factors [19][20]. However, the current study did not obtain sufficient exposure or clinical information to determine which factors explain the observed association. The association between sex and residential area was also statistically significant (χ^2=8.833, df=1, p=0.0030). This finding describes the distribution of sex across residential categories among the registered cases and should not be interpreted as evidence that place of residence or sex has a causal effect on the occurrence of cancer.

Regarding residential area, 852 cases (58.0%) were from urban areas, whereas 618 cases (42.0%) were from rural areas. A previous study conducted in Al-Najaf also included urban and rural residence among the demographic features recorded for registered patients with lung cancer at the Middle Euphrates Oncology Center [15][17]. Based on Pearson's chi-square test, cancer type was not significantly related to residential area (χ^2=1.645, df=1, p=0.200). Thus, among the registered hospital cases, the distribution of lung and liver cancer by residential area did not differ significantly. This finding should be interpreted as a description of the registered cases rather than as evidence that residential area is unrelated to cancer risk. Studies of urban–rural cancer differences indicate that such patterns can vary according to cancer site and may be influenced by socioeconomic and healthcare-related factors [16].

Furthermore, the present study didn't provide population denominators or information on environmental exposures, healthcare access, or other potential confounders. Therefore, the observed urban–rural distribution cannot be

interpreted as a population-based difference in cancer incidence. Population -based cancer incidence rates require the number of newly diagnosed cases to be related to the population at risk, emphasizing the importance of population denominators when interpreting cancer incidence [18]. Patients aged 50 years and above comprised 1,290 cases (87.8%). Most cases were concentrated in the 60–69 year age group (431 cases; 29.32%), with the next highest numbers in the 70–79 year group (400 cases; 27.21%) and 50–59 years (328 cases; 22.31%). In lung cancer, registered cases peaked in the 60–69-year age group (375 cases), whereas liver cancer was most frequently registered among patients aged 50–59-years (58 cases). The concentration of cases in older age groups reflects the widely recognized association of advancing age with a greater cancer burden [21][22]. However, since the study was not population-based, the age distribution should be interpreted as the age composition of the registered cases rather than as an estimate of age-specific cancer risk.

The number of registered cases varied across calendar years. In 2025, 311 cases were registered. The total for 2026 was 211cases; however, these data included only January through August and therefore can't be directly compared with complete calendar years from 2021 through 2025. The increase observed in 2025 may be related to changes in case detection, referral patterns, healthcare utilization, or cancer registration, none of which were directly measured in the current study. Therefore, the observed year-to-year disease occurrence at the population level [23].

In summary, the results provide a useful description of the demographic and residential characteristics of lung and liver cancer registered at the study center. The main limitations are the hospital -based design, lack of population determinants, including tobacco use, viral hepatitis status, work-related and environmental exposures, and access to healthcare. Future population-based studies with standardized case ascertainment, appropriate population denominators, and detailed clinical and exposure information would provide stronger evidence regarding the determinants of lung and liver cancer in Najaf [24][25].

5. Ethical Considerations

All information on patients was collected from hospital records for research. Patient identifiers were removed to ensure patient privacy and confidentiality. This investigation was conducted following medical research ethics.

6. Conclusion

This hospital -based retrospective study described the distribution of registered lung and liver cancer cases at the National Oncology Center in Najaf between 2021 and August 2026. Lung cancer represented the largest proportion of recorded cases, and most cases occurred among older adults. Male patients made up a larger share of the registered patient population than females. A significant relationship was detected between the type of cancer and sex, whereas cancer type showed no meaningful statistical link with the residential area. These findings should be interpreted as a description of registered hospital cases rather than population- based disease rates. Further population-based studies incorporating smoking history, viral hepatitis status, environmental exposures, and healthcare access are needed to investigate the determinants of lung and liver cancer in Najaf.

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Nexus International University ISSN: 3136-6449; 12(2) 197-203

Serum Albumin Levels and Risk of Pressure Injuries in Intensive Care Unit Patients: A Single-Center Prospective Cohort Study

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Abstract.

Background: Pressure ulcers (PUs) / pressure injuries are a significant complication that can occur and be prevented in intensive care units (ICUs). Given the common association between hypo-albuminemia and PU development, unadjusted associations are found to exaggerate its independent predictive potential by confounding with severity of illness and systemic inflammation. We prospectively studied the association of baseline/2-week serum albumin levels with PU incidence in a cohort of critically ill patients.
Methods: This is a STROBE-compliant, prospective observational study carried out in the Al-Hussain Teaching Hospital (Samawa, Iraq) from October 2023 until October 2025. Seventy patients aged 40–80 years with a ICU length of stay ≥ 30 days were included and. The serum albumin was assessed at admission and 2 weeks post-discharge using a standard BCG spectrophotometric assay (hypoalbuminemia < 3.5 g/dL). The primary outcome was development of stage 2+ PU during follow-up. Adjusted odds ratios (aOR) were determined using univariate tests and multivariable logistic regression.
Results: PUs occurred in 40/70 patients (57.1%), all <55–80 years of age. Highest incidence was observed in stroke/CVA (72.7%) and postoperative (60.0%) patients, while the lowest incidence was detected in pancreatitis (0.0%). Admission hypoalbuminemia (< 3.5 g/dL) was seen in 20 patients (28·6%) based on cut-off levels, whereas a mean of only 60 days after admission had hypoalbuminemia at two weeks to reflect an acute depletion trajectory among these patients with support for 1500 human risks [85.7%]. Hypoalbuminemia was present in 35/40 (87.5%) PU patients versus 5/30 (16.7%) non-PU patients at PU onset / 2-week follow-up (unadjusted OR 35·0,95% CI 9·2–133·9; p < 0·001). In multivariable logistic regression, controlling for age and admission category, low serum albumin remained a powerful independent predictor of PU development in the orthopedic group (aOR 18.4, 95% CI 3.8–89.2; p < 0.001). For PU, there was a complete separation between people who required non-oral tube feeding (100%) compared to those that did not (0%), indicating immobility and illness severity.
Conclusion: Hypoalbuminemia, an independent and powerful predictor of pressure ulcer risk in long-stay ICU patients. Incorporating standardized serum albumin tracking with clinical risk scales and early mobility protocols can advance PU risk stratification in critical care.

Keywords: Pressure ulcer, Pressure injury, Hypoalbuminemia, Serum albumin, Critical care, Intensive care unit, Risk stratification.

1. Introduction

Background: Pressure ulcers (PUs), otherwise known as pressure injuries or bed sores, are defined as localized damage of the skin and underlying subcutaneous tissues arising from sustained pressure, shear forces and friction in

tissue over bony prominences. PU in intensive care units (ICUs) are one of the most common and severe preventable adverse events, with incidence ranging from 1% to >50% depending on population case mix, severity of illness, and diagnostic vigilance [1, 6]. PUs does cause physical harm, but they also prolong length of ICU stay, increase risk of secondary bacterial sepsis, hospital mortality and healthcare costs [1, 7].

The pathogenesis by which pressure injuries develop is a dynamic interplay of extrinsic (interface pressure, shear, microclimate) and intrinsic patient factors that decrease tissue tolerance [2]. Critically-ill patients, marked by extensive immobility, mechanical ventilation, deep sedation and peripheral vasoconstriction induced by high doses of vasopressors are especially prone to acute tissue hypoperfusion which can occur in the absence of load mitigation within hours [12].

Of metabolic and nutritional biomarkers, serum albumin has been studied extensively. Albumin represents the major factor of plasma colloid oncotic pressure. Profound decrease in serum albumin causes interstitial fluid redistribution and dermal edema, which increases diffusion distance for oxygen and metabolic substrates at the skin microvascular level [15]. In addition, hypoalbuminemia is important as a negative acute-phase reactant in time of systemic inflammation, it is both an index of metabolism and a strong surrogate for severity of critical illness [15, 16]. Although low serum albumin has consistently been associated with both PU development and impaired wound healing in clinical studies [13, 14], unadjusted observational studies often report extremely high effect sizes that likely represent confounding due to overall severity of illness and patient immobility.

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2. Methods

2.1 Study Design and Setting

Design: A two-year, prospective observational cohort study set in the Intensive Care Unit in Al-Hussain Teaching Hospital (Samawa, Al-Muthanna Governorate, Iraq) between October 2023 and October 2025. The protocol of this study has been approved by the Institutional Review Board of Al-Hussain Teaching Hospital (IRB Ref No: ATH-2023-0892). Patients, or their legally authorized representatives, provided written informed consent prior to enrollment. This manuscript was prepared according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

2.2 Study Population and Eligibility Criteria

Data were collected from seventy patients over 18 years old with an ICU admission between November 2016 and September 2020. Inclusion criteria were: (1) adult patients aged 40–80 years; (2) admission to the ICU because of any medical or surgical reason; and (3) ICU length of stay more than 30 days. Exclusions included patients with ICU stay≤ 30 days, transfer to another facility or death before 30-day of follow-up ICIU and the presence of existing pressure

injuries at admission. The final cohort included 40 postoperative surgical admissions and 30 medical admissions (22 stroke/cerebrovascular accident [CVA] and 8 acute pancreatitis patients).

2.3 Data collection and clinical variables

Inclusion criteria were prospective recording of demographic and clinical data at the time of admission such as age, sex, main diagnostic category on admission, height (cm), weight (kg), whole-body fat percentage (%) using Jackson-Pollock body composition equations and enteral/parenteral feeding route (non-oral packing tube vs oral feeding status). Non-oral feeding included total enteral nutrition through nasogastric/jejunal tubes complemented with total or partial parenteral nutrition. Certified critical care nurses performed daily physical skin examinations, which were verified by study investigators. Identification, location and categorization of PUs by the National Pressure injury advisory panel (NPIAP) clinical criteria [9] The main outcome measure was the development of a new stage 2 or higher pressure ulcer during follow-up.

2.4 Serum Albumin Assessment in the Laboratory

Venous blood was collected at two predetermined prospective time points: (1) Baseline admission (within 24 hours of ICU admission) and; (2) Follow-up at 14 days post-admission (or, at the exact time of PU diagnosis if prior to day 14). Serum albumin was measured with a standardized automated Bromocresol Green (BCG) dye-binding spectrophotometric colorimetric assay (Roche Cobas c501 analyzer, Roche Diagnostics, Mannheim, Germany). Hypoalbuminemia was defined quantitatively as a serum albumin concentration of < 3.5 g/dL (< 35 g/L), according to international laboratory standards. Serum albumin, both continuous-like and as a binary diagnostic classification (Normal ≥ 3.5 g/dL versus Reduced < 3.5 g/dL) were assessed in each study event on a random effect basis.

2.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Categorical data were shown as absolute frequencies and percentages (n (%)). The Pearson Chi-Square (χ^2) test or Fisher's Exact test (depending on expected cell counts ≤ 5) were used for univariate comparisons between patients who developed PUs and those who did not. Primary exposure variables were analyzed by unadjusted Odds Ratios (OR), Relative Risks (RR) with 95% Confidence Intervals (95% CI).

A multivariable binary logistic regression model was created to isolate the independent predictive value of serum albumin and statistically control for critical demographic/clinical confounders. Analyses were adjusted for age (continuous) and admission diagnosis category (Postoperative vs Stroke vs Pancreatitis) and included serum albumin status (at follow-up/PU onset). Selection of variables was driven by biological plausibility and univariate significance. The feeding route variable (100% PU incidences in the nonoral fed group versus 0% in the oral fed group) could not be tested by parametric regression due to complete separation and it was assessed as a master clinical surrogate for immobility and illness severity. P values were considered statistically significant if <0.05 (two-sided).

3. Results

3.1 Baseline Characteristics

There were 70 ICU patients evaluated (45 males, 64.3% and 25 females, 35.7%) aged between 40–80 years old. Forty of those patients (57.1%) were postoperative surgical cases while the others included a total of 30 medical cases (42.9%), consisting of 22 stroke/CVA patients (31.4%) and eight acute pancreatitis patients (11.4%). In total, 30 patients (42.9%) were able to take nutrition orally and 40 patients (57.1%) required non-oral tube/parenteral feeding. Table 1 presents baseline characteristics.

Table 1: Baseline demographic and clinical characteristics of the study cohort (N = 70).

Baseline Characteristic	Sample Count (n)	Percentage (%)
Sex – Male	45	64.3%
Sex – Female	25	35.7%
Admission Category – Postoperative	40	57.1%
Admission Category – Cerebrovascular Accident (CVA)	22	31.4%
Admission Category – Acute Pancreatitis	8	11.4%
Feeding Route – Non-oral (Tube/Parenteral)	40	57.1%

3.2 The Overall Incidence and Subgroup Stratification

Forty of 70 (57.1%) patients developed pressure ulcers. Pus developed in the first 14 days after ICU admission exclusively among case patients aged 55 to 80 years. The incidence of PU between admission diagnoses was significantly different ($\chi^2 = 12.98$, df = 2, p= 0.002). The patients with highest incidence, as seen in Figure 1A along with the specific type of PU that developed are stroke/CVA (16/22, 72.7%), postoperative surgical patients (24/40:60.0%) while those admitted with acute pancreatitis had zero PU development (0/8, 0.0%) (Table 2 and Figure 1A).

Table 2: Pressure injuries incidence based on admission diagnosis category ($\chi^2 = 12.98$, p = 0.002).

Admission Diagnosis	Total Patients (n)	PUs Developed (n)	Incidence (%)
Cerebrovascular Accident (Stroke)	22	16	72.7%
Postoperative Surgical	40	24	60.0%
Acute Pancreatitis	8	0	0.0%
Total Cohort	70	40	57.1%

3.3 Incidence by Sex and Age

Example score of pressure ulcers in male patients 25/45 (55.6%) and female patients 15/25 (60.0%). There was no statistically significant difference regarding this (unadjusted OR 0.83, 95% CI 0.31–2.25; p = 0.72) (Table3). Out of the 40 patients involved, all were aged between 55 and 80 years confirming older age is a risk factor for cluster disease.

Table 3: Pressure injury incidence by patient gender (p = 0.72).

Sex Category	Total Patients (n)	PUs Developed (n)	Incidence (%)
Male	45	25	55.6%
Female	25	15	60.0%
Total Cohort	70	40	57.1%

3.4 Prediction of the Future Trends of Serum Albumin

At baseline admission, 50 (71·4%) patients had normal serum albumin (≥ 3·5 g/dL) and 20 (28·6%) presented with hypoalbuminemia (< 3·5 g/dL). All patients were stable at week 1:91 patients (88.6%) had a normal serum albumin (<3.5 g/dL), followed by a significant metabolic shift in week 2 of ICU stay, with reduced serum albumin observed in 60 patients (85.7%) vs10 patients (14.3%) who remained within the normal range (Table4). The acute depletion of circulating albumin that may occur during prolonged critical illness is illustrated in the longitudinal perspective shown in Figure 1B.

Table 4: Potential progression of serum albumin status between ICU admission and 2-week follow-up (N = 70).

Serum Albumin Category (< 3.5 g/dL)	Baseline Admission n (%)	2-Week Follow-up n (%)
Normal (≥ 3.5 g/dL)	50 (71.4%)	10 (14.3%)
Reduced / Hypoalbuminemia (< 3.5 g/dL)	20 (28.6%)	60 (85.7%)

Serum albumin and pressure injury (1)

Post-hoc analysis: Cross-tabulation of follow-up / PU evaluation serum albumin status with pressure injury onset Figure 1B shows that out of the 40 patients who developed PUs, 35 (87.5%) were found to have hypoalbuminemia (3.5 g/dL). In contrast, of the 30 PU-free patients, 25 (83.3%) had normal albumin levels and 5 (16.7%)

hypoalbuminemia ($\chi^2 = 35{\cdot}1$, $p < 0{\cdot}001$; Fisher's exact $p < 0{\cdot}001$) (Table √ in full-text PDF version for subscription link; Figure √ in full-text PDF). One in unadjusted univariate analysis gave an OR, 35.0 (95% CI 9.2–133.9; RR = 5.3).

The multivariable logistic regression model, adjusting for age and admission diagnosis, showed that hypoalbuminemia was an independent risk factor associated with PU development (aOR: 18.4 [95% CI 3.8–89.2; $p < 0.001$]).

Table 5: Cross-tabulation of follow-up serum albumin status and incident pressure injury ($\chi^2 = 35.1$, $p < 0.001$; Unadjusted OR = 35 & Multivariable aOR = 18.4).

Serum Albumin Status	PU Developed (n = 40)	No PU (n = 30)	Total Cohort (N = 70)
Reduced / Hypoalbuminemia (< 3.5 g/dL)	35 (87.5%)	5 (16.7%)	40 (57.1%)
Normal Albumin (≥ 3.5 g/dL)	5 (12.5%)	25 (83.3%)	30 (42.9%)
Total	40 (100.0%)	30 (100.0%)	70 (100.0%)

3.6 Feeding Path and Fractionation Function

Statistical separation was absolute as to feeding route: absolutely none of the 30 orally-fed patients developed a PU (0%); while all 40 patients requiring non-oral tube/parenteral nutrition did develop a PU (100%) (Fisher's exact p 3.5 g/dL [blue]) and hypoalbuminemia (<3.5 g/dL [red]) The line indicates the gender-based cut-off (denoted by dashed and dotted lines) Y-axis corresponds to Serum Albumin Distribution (%) The left column depicts baseline pathway to admission (− 28.6% compared with ED only, while the middle and right columns depict cross-tabulation of follow-up vs PU evaluation. The prevalence of hypoalbuminemia was significantly higher in PU patients compared to non-PU patients (87.5% vs 16.7%; OR unadjusted = 35.0, 95% CI 9.2–133.9; multivariable aOR = 18.4, 95% CI 3.8–89.2; p <.001).

4. Discussion

This is a prospective single-center study of the incidence of pressure injuries in critically ill ICU patients with a length of stay longer than 30 days; 57.1% of patients developed PIs. This residency is at the upper end of reported international benchmarks found in critical care literature (1%–56%) [1, 6, 18] and reflects a high-risk cohort of long-stay patients with a considerable burden of baseline organ impairment.

The strongest result of this exploration is the independent association between hypoalbuminemia (< 3.5 g/dL) and PU development. Unadjusted OR by univariate analysis was very high (unadj OR 35.0), but this remained highly significant in multivariable regression adjusted for age and primary diagnostic category (aOR 18.4, 95% CI 3.8–89.2; $p < 0.001$). This confirms that hypoalbuminemia is not simply a coincidental consequence of age or diagnostic group but rather an independent correlate of tissue failure.

PU susceptibility was highly influenced by the diagnostic category (Figure 1A); tissue degradation occurred at a markedly high rate in stroke/CVA patients (72.7%) but also in the major postoperative admissions group (60.0%). In addition, a visual summary of the decline over time in serum albumin level and its historical separation between patients with PU (87.5% hypoalbuminemia) and those without (16.7% hypoalbuminemia) pre-to-follow-up is encapsulated in figure 1B.

Pathophysiological Process underlying this relationship are explained by several interlocking mechanisms. Eighty percent of plasma oncotic force is made by albumin. Interstitial fluid leaks into tissue spaces and cause localized edema due to severe hypoalbuminemia. The swelling of the skin is likely to increase the distance diffusion processes need to travel via capillaries through tissue into dermal cells for oxygen and essential nutrients, thus reducing microvascular ischemia thresholds during mechanical loading [15]. Second, serum albumin synthesis decreases markedly during systemic inflammation (mediated primarily by pro-inflammatory cytokines IL-6 and TNF-α), which have been known to simultaneously promote protein catabolism and increase vascular permeability [15, 16]. Thus, low serum albumin represents an amalgam of malnutrition, chronic metabolic input and prominent systemic inflammation [13, 14, 22].

The statistically significant results for feeding route, where 100% of patients fed with tubes developed pressure ulcers (PUs) against none who were fed orally, should be interpreted cautiously in clinical practice. In this cohort the dependency on enteral feeding tube was taken as a surrogate for severe neurological impairment (i.e. longstanding ischemic stroke), mechanical ventilation, and complete immobility in bed state. Again, tube feeding identifies an ultra-high risk population requiring very thorough preventive care [22, 24, 25] rather than enteral nutrition being the cause of PUs.

4.1 Study Limitations

Several limitations should be acknowledged. First, sample size was small (N = 70) and confined to a single tertiary care site which limits generalizability. Second, although our data were rich in clinical content, formal clinical illness severity scores (eg APACHE II or SOFA) could not be systematically collected at all baseline timepoints for our cohort hence the exclusion of such variables from the regression model. Third, the tube-feeding variable was treated using a complete separation method that blocked its parametric entry into multivariable modeling. These findings should be validated in a multicenter prospective study using high frequency daily monitoring of albumin and detailed severity scoring.

5. Conclusion

Serum albumin < 3.5 g/dL is a strong independent risk factor for pressure injury in long-stay intensive care patients. Against this background we demonstrated how routine serum albumin trajectories have strong prognostic potential for distinguishing at-risk phenotypes — in this case older adults pre- and post-stroke, and peri-operative tube-fed surgery patients. Implementing dynamic monitoring of albumin together with a universal assessment strategy of risk may substantially reinforce prevention protocols in intensive units for pressure sores.

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Nexus International University ISSN: 3136-6449; 12(2) 205-216

A Study of Some Physicochemical and Bacteriological Parameters of Water Treatment Plants in Al-Muthanna Governorate with the Application of Statistical Factors (Independent Samples t-test)

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Abstract. This study was conducted to evaluate the efficiency of water treatment plants in Al-Muthanna Governorate by examining the physical and chemical properties of raw and treated water and comparing them with Iraqi standard specifications. The study was conducted on four plants (Al-Samawah, Al-Rumaytha, Al-Warka, and Al-Khidr) for eleven variables: temperature, pH, electrical conductivity, total dissolved solids, turbidity, total hardness, sodium, calcium, magnesium, sulfates, and chlorides. The study was conducted in two phases. The first phase, the wet period, extended from November 2023 to March 2024. The second phase, the dry period, extended from May 2024 to September 2024. The results showed that the physical and chemical parameters were outside the permissible limits according to Iraqi specifications, and that the water was unfit for consumption.

1. Introduction

Water is essential for life and its continuation, without neglecting other elements, most importantly air. However, its direct importance lies in its comprehensive role in sustaining the lives of various living organisms, regardless of their specific needs. Humans rely on water directly and benefit from it in numerous ways. We must not overlook the fact that they use clean water daily to maintain their health (Al-Zahrani, 2002, p. 315), which is provided through water purification plants. Therefore, it is essential that drinking water be free from pollutants that could cause harm to the drinker, such as illness or pain (Al-Saadi et al., 1989, p. 510). Statistics and studies in the United States indicate that 59% of diseases are caused by drinking water pollution (Al-Safawi et al., 2013, p. 86). Reports from the United Nations Programme of Education (UNEP) in 1999 indicate that more than 80% of diseases and more than 33% of deaths in developing countries are caused by the pollution of drinking water sources (Raml, 2010, p. 20). Since there is no 100% pure water in nature (Cabelli, 1979), humans have long tried to control the problems of polluted water and have used several methods to purify it. One of these methods is boiling water, which is the first method humans used to obtain healthy water. In addition to its ease, it can be done by anyone and is suitable in emergency situations. However, the disadvantages of this method are that it produces undesirable water and is used for small quantities of water (Laubusch, 1971).

1.1 Area Description

Four drinking water treatment plants were selected in Al-Muthanna Governorate, drawing their water from the Euphrates River or its tributaries. Table (1) lists the names of the treatment plants and their associated complexes included in the study.

Table (1): Names of Treatment Plants in Al-Muthanna Governorate

No.	Location	Complex or Station	Source
1	Samawah	Barbuti Complexes	Samawah Canal / Euphrates River
2	Rumaytha	First Teachers' Neighborhood Complex	Rumaytha Canal / Euphrates River
3	Khidr	Al-Soub Al-Saghir Water Complex	Euphrates River
4	Warka	Al-Warka Water Project	Euphrates River

Source: Ministry of Construction, Housing, and Municipalities, General Directorate of Water, Department of Environment and Quality Control, Al-Muthanna Governorate, Unpublished Data, 2023.

1.2 Sampling Method

Samples were collected from water treatment plants between November 10, 2023, and September 15, 2024, using plastic containers that were washed twice before filling. Samples were taken from two locations at each plant: one for raw water and the other for treated water. The required tests were conducted at the Ministry of Environment/Al-Muthanna Governorate Environment Directorate/Al-Muthanna University/College of Agriculture.

1.3 Approved Specifications

The samples taken from the study area were compared with the updated Iraqi local standard specifications of 2009 (Table 2).

Table 2: Iraqi Environmental Specifications for Water Suitability for Human Use

No.	Element	Allowable limit	Unit of measurement
1	Temperature	-	C
2	PH	6.5-8.5	----------
3	Electrical Conductivity	2000	MS/CM
4	Total Dissolved Solids	1000	Mg/l
5	Turbidity	5	NTU
6	Hardness	500	Mg/l
7	Sodium	200	Mg/l
8	Potassium	10-12	Mg/l
9	Magnesium	100	Mg/l
10	Calcium	150	Mg/l
11	Sulfate	400	Mg/l
12	Chloride	350	Mg/l
13	Residual Chlorine	1.45 - 3	
14	Coliforms	0	Cell / 100
15	Fecal Bacteria	0	Cell / 100
16	Coliform Count	50	Cell / 100

Source: Ministry of Environment, Monitoring and Planning Department, Iraqi Drinking Water Specifications No. (417) of 2009, Second Update.

2. Results and Discussion

First: Physical and Chemical Tests of Raw and Treated Water from Water Treatment Plants in the Study Area

1: Temperature

Living organisms are affected by temperature changes, which in turn affect the physical and chemical properties of their environment (Al-Hadithi, 1986). Evison (1988) also indicated that temperature is one of the main environmental factors affecting the survival of microorganisms in water. Furthermore, the rate of corrosion in the water transmission pipe network is related to high temperature (Besner et al., 2002). Therefore, water temperature is used to determine the corrosion of water transmission pipes in the distribution network. It has been shown that the nitrification process, which leads to corrosion, often occurs at high temperatures exceeding 15°C (Kimeyer et al., 2002).

Table (3) shows that the temperature measurements during the wet and dry periods of raw water varied. The lowest temperature was recorded at the Rumaytha station, at approximately 10°C, while the highest was recorded at the Warka station, reaching approximately 17°C. For treated water, the lowest temperature recorded was approximately 26°C at

the Warka station, while the highest was recorded at the Samawah station, reaching approximately 32°C. This variation in temperature values between the wet and dry periods is a normal occurrence related to climate.

Table (3): Temperature Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
16	16	17	17	10	10	13	13	Wet period
28	28	26	26	27	27	32	32	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Second: pH

PH reflects the activity and effectiveness of hydrogen ions. Its rise and fall result from the presence of pollutants in the water (Abawi & Hassan, 1990). The pH values did not differ significantly across all study stations and water types (raw and treated), and they remained within the permissible limits according to Iraqi specifications, as shown in Table (4). The highest pH value during the wet period was recorded for raw water (7.9) at the Al-Khidr station, and the lowest value (7.6) at the Al-Warka station. For treated water, the highest value was recorded at the Al-Khidr station (7.6), while the lowest value was (7.3) at the Al-Warka station. Regarding the dry period, the Al-Khader station recorded the highest pH value for raw water at (8.1) and the lowest at (7.8). For treated water, the values ranged between (7.4-7.6) for all stations.

Table No. (4) pH Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
7.6	7.9	7.3	7.6	7.5	7.7	7.4	7.8	Wet period
7.6	8.1	7.4	7.8	7.6	7.9	7.6	7.9	Dry period

Source: Research by the researcher based on analyses conducted in the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from (10/11/2023) to (15/9/2024).

Electrical Conductivity

By observing Table No. (5), which shows the electrical conductivity values for raw and treated water for all stations in the study area and for the wet and dry periods, it is evident that the values vary between the stations. During the wet period, the Al-Khader station recorded the highest values for both raw and treated water, ranging from (2564-2554 µmH/cm), which exceeds the permissible limits according to Iraqi specifications. The remaining stations were below the permissible limits according to Iraqi standards for the same period. During the dry period, all studied stations recorded high values, ranging from (2979-2927 µmH/cm) for raw water and from (2965-2914 µmH/cm) for treated water. The water concentration (µS/cm) in the treated water exceeded the limits permitted by Iraqi specifications, which are set at 2000 µS/cm. This is due to insufficient water supply to these stations because of low river levels and the lack of rainfall during the winter season in quantities sufficient to raise the water level.

Table No. (5) Electrical Conductivity Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
2554	2564	1757	1776	1618	1632	1915	1924	Wet period
2943	2955	2453	2547	2965	2979	2914	2927	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Total Dissolved Solids (TDS): Table 6 shows the TDS values during the study period. The test results indicated that all values at all stations were outside the permissible limits according to Iraqi standards, which specify a maximum concentration of 1000 mg/L.

For raw water and wet-dry water, TDS values ranged between 1040 and 1526 mg/L. While the total dissolved solids (TDS) levels ranged between 1314 and 1712 mg/L during the dry period, the levels in treated water ranged between 1032 and 1512 mg/L during the wet period, and between 1302 and 1698 mg/L during the dry period. Generally, the high TDS levels, in addition to the limited river water supply and the reduced water supply to the treatment plants, are attributed to the climatic conditions, specifically the extremely high temperatures during the dry period. Furthermore, the high salinity is due to the higher salinity of the soils through which the Euphrates River flows compared to the Tigris River (Al-Tufaili, 1999).

Table No. (6) Total Dissolved Solids Values for the Study Area Stations

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
1512	1526	1114	1128	1032	1040	1321	1376	Wet period
1698	1712	1302	1352	1302	1314	1388	1412	Dry period

Source: Research by the researcher based on analyses conducted in the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from (10/11/2023) to (15/9/2024).

Turbidity

Table No. (7) indicates the turbidity concentration values for all study stations during the wet and dry periods. We find that the raw water had very high turbidity concentrations for all stations, especially during the dry period, exceeding the permissible limits set by Iraqi specifications, which are (5). The Samawah and Al-Khader stations recorded the highest concentrations at (57-58). As for the treated water, the results showed that it did not conform to Iraqi specifications, as the turbidity concentration values were outside the permissible limits, less than (5) for most stations, with the exception of the Rumaytha station, which recorded a value of (2-3) during the wet and dry periods. The high turbidity levels observed in raw water at the studied stations and others indicate that the Euphrates River and its tributaries within Al-Muthanna Governorate contain significant amounts of sewage and industrial waste, in addition to numerous drainage canals and other contributing factors. The high turbidity levels in treated water at the stations are attributed to either the insufficient use of alum to effectively treat the turbidity or its complete absence during the filtration process. Furthermore, the lack of regular station maintenance and the fact that water consumption by residents exceeds the station's production capacity, preventing the water from settling in the sedimentation basins, exacerbate the problem.

Table (7) Turbidity Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
19	36	6	16	2	23	19	21	Wet period
28	57	14	31	3	55	36	58	Dry period

Source: Based on the researcher's work and analyses conducted at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Total Hardness: As indicated by the outcomes of the hardness test presented in Table 8, the results for both untreated and treated water exceeded the limit of 500 mg/L set by Iraqi standards, except for the Al-Rumaytha station, where the results were in the allowable range of 465-473 mg/L.

For raw water, the Al-Khader station recorded the highest concentration for both wet and dry periods, ranging from 648 to 739 mg/L. Similarly, for treated water, the same station recorded the highest concentrations for both wet and dry periods, ranging from 642 to 732 mg/L. The high hardness is attributed to the high salt content in the sedimentation basins and the lack of maintenance or regular tank cleaning.

Table (8) Total Hardness Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
642	648	507	513	465	473	589	596	Wet period
732	739	568	590	568	575	593	603	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Sodium (Sodium): Table (9) shows the sodium concentrations at the studied stations. Comparing these concentrations with Iraqi standards, they were within the permissible limits of 200 mg/L. For raw water during the wet period, the Al-Rumaytha station recorded the lowest value at 140 mg/L, while the treated water at the same station recorded 135 mg/L. However, the Al-Khader station exceeded the permissible limits for both the wet and dry periods. The sodium concentration in the raw water reached 229-280 mg/L, while the treated water concentration for both periods ranged from 220-275 mg/L.

Table No. (9) Sodium values for stations in the study area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
220	229	150	150	135	140	178	180	Wet period
275	280	185	200	180	190	188	197	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Calcium: The amount of calcium may decrease, increase, or remain unaffected during filtration. This is attributed to the cleanliness of the sedimentation basins, filters, and water tanks, as well as the project's production capacity and local water consumption (Hamza, 1999). Although the results of the raw and treated water analyses were close to the permissible limit, they did not exceed it. This is evident from Table (10), which shows the calcium concentrations for all stations during both the wet and dry periods. These concentrations were within the limits permitted by Iraqi specifications, which set the limit at 150 mg/L, except for the Al-Khader station, which recorded values outside the permissible limits during the dry period for both raw and treated water, at 153-154 mg/L.

Table No. (10) Calcium values for stations in the study area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
133	134	109	110	101	102	120	125	Wet period
153	154	119	123	119	120	123	129	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Magnesium

Table 11 shows that the magnesium concentration values were within the permissible limits according to Iraqi specifications, which are set at 100 mg/L.

For raw and treated water during the wet period, the Al-Rumaytha station recorded the lowest values (54 mg/L) and (53 mg/L) respectively, and the highest concentration at the Al-Khader station (76 mg/L) and (75 mg/L). During the dry period, the same stations recorded the lowest concentrations for raw and treated water (67 mg/L) and (68 mg/L) at the Al-Rumaytha station, and the highest concentrations (86 mg/L) and (84 mg/L).

Table No. (11) Magnesium values for stations in the study area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
75	76	57	58	53	54	62	69	Wet period
84	86	66	69	68	67	70	71	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Directorate, from November 10, 2023, to September 15, 2024.

Sulfate

Table 12 indicates that sulfate concentrations varied between stations during the dry and wet periods. Regarding raw water during the wet period, the Al-Rumaytha and Al-Warka stations recorded the lowest values, ranging between 347 and 399 mg/L respectively, thus falling within the permissible limits of 400 mg/L according to Iraqi standards. Similarly, the treated water from the same stations recorded values of 344 and 394 mg/L respectively, also within the permissible limits. However, the Al-Samawah and Al-Khidr stations recorded high concentrations exceeding the permissible limits according to Iraqi standards.

During the dry period, sulfate concentrations at all stations were outside the permissible limits for both raw and treated water. The Al-Rumaytha station recorded the highest concentrations for both raw and treated water, at values of 608 mg/L and 602 mg/L, respectively.

Table No. (12) Sulfate Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
504	510	394	399	344	347	470	474	Wet period
602	608	448	466	450	455	476	482	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Chloride

Table 13 shows that the chloride concentration values at the treatment plants were within the permissible limits according to Iraqi specifications, which is 350 mg/L. The chloride concentration values for raw water ranged between 208 and 329 mg/L during the wet period, while the concentration values for treated water ranged between 206 and 326 mg/L during the same period. While the lowest values for raw water were recorded at the Rumaytha station (270 mg/L) and the lowest values for treated water at the Warka station (266 mg/L), the exception was the Khidr station, which exceeded the permissible limits for both raw and treated water during the dry period, recording values ranging from 418 to 423 mg/L.

Table No. (13) Chloride Values for Stations in the Study Area

Al-Khader Station,		Al-Warka Station,		Al-Rumaytha Station		Al-Samawah Station		Station
processor	raw	processor	raw	processor	raw	processor	raw	
326	329	206	208	252	257	269	275	Wet period
418	423	266	287	259	270	300	307	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

(3-2) Bacteriological and Residual Chlorine Tests of Water Exhausted from Water Treatment Plants in the Study Area

Residual Chlorine

Chlorine is used to disinfect water intended for human consumption because it is an oxidizing agent that destroys microorganisms that were not removed by sedimentation and filtration processes (APHA, 1985). Therefore, the amount of chlorine added to the plants must be sufficient to ensure disinfection while maintaining a residue of (0.2–0.5) mg/L of free chlorine in the distribution networks (WHO, 1993).

The study results showed a similarity in residual chlorine values, as shown in Table (14), ranging between (1.5-3) mg/L during the wet period. This indicates that the values did not exceed the permissible limits according to Iraqi standards. However, during the dry period, the Samawah and Warka water treatment plants exceeded the permissible limit, recording values of (4) mg/L. This increase is attributed to the inverse relationship between residual chlorine and temperature, as chlorine decomposition increases with rising temperatures (Al-Qaisi, 2005). Therefore, it is essential to increase chlorine dosages in water treatment plant projects during the hot months of the year to ensure that sufficient chlorine levels reach the drinking water to eliminate any microorganisms present.

Table (14) Free Chlorine Values for Water Treatment Plants in the Study Area

Al-Khader Station, processor	Al-Warka Station, processor	Al-Rumaytha Station processor	Al-Samawah Station processor	Station
1.5	3	2	3	Wet period
3	4	3	4	Dry period

Source: Research conducted by the researcher based on analyses performed at the Soil and Water Laboratory, College of Agriculture, Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from November 10, 2023, to September 15, 2024.

Second: Bacteriological Tests

Among microorganisms, bacterial species constitute one of the main group present in water. Apart from indigenous bacterium associated with the aquatic environment and possibly capable of proliferating in it without being influenced by external agents, they could indicate the presence of another group of the bacteria which could flow into the water and be responsible for diseases creation. These invasive species may come from some aquatic sources including floods, melted snow, etc. The results of laboratory analysis conducted on the water from all sampling points is presented in Table 15. It is unequivocal that all treatment plants, regardless of weather conditions are free from coliforms and fecal bactefia. There is a decline of the amount of the bacteriological colonies at different sampling points over the period. The Samawah plant has demonstrated the highest content of bacteria colonies with an index of (66 cells/100) in the wet period. This number significantly exceeds the maximum permissible concentration of 50 cells/100. The incapacity of the plant to process water is owing to the fact that it is located in the city closer to the more active anthropogenic impact. It must also be noted that abundance of trees, fallen leaves and provide sufficient shade contributes to the higher amount of organic matter which together with low temperatures and less sunlight in winter create adverse conditions for microorganisms and thus prevents from effective treatment (Yehia and Sabea, 2011). The technological regime at the sites may not ensure the production of drinking water. In order to deal with the biological aspect of the treatment process, the procedures for the addition of chlorine must be implemented (Obaid, 2011).

In the remaining stations, the concentration of bacterial colonies ranged between (0-15 cells/mm^3) and did not exceed Iraqi environmental limits. This is attributed to the low level of human activity and the low percentage of organic matter in the surrounding areas. Additionally, the removal rate of these colonies was good, keeping the levels within acceptable limits.

Table No. (15) shows the numbers of coliform bacteria, fecal bacteria, and bacterial colonies at the stations in the study area.

Plate Count 37c/1ml	MPN of total E-coli 44c/100ml	MPN of total coliform 37c/100ml	Station	
66	0	0	Wet period	Al-Samawah Station
45	0	0	Dry period	
15	0	0	Wet period	Al-Rumaytha Station
10	0	0	Dry period	
13	0	0	Wet period	Al-Warka Station
0	0	0	Dry period	
14	0	0	Wet period	Al-Khader Station
0	0	0	Dry period	

Source: From the researcher's work based on analyses conducted in the Soil and Water Laboratory, College of Agriculture / Al-Muthanna University, and the Environmental Analysis Laboratory, Al-Muthanna Environment Department, from (10/11/2023) to (15/9/2024).

Third: Paired Samples t-test for comparing raw and treated water values.

The Independent Samples t-test was utilized to find whether there were any significant differences between the means of the two groups concerning some variable of interest that includes certain physical and chemical properties of water under observation (Ghadhban & Rasheed, 2021; Saeed & Mohammed, 2023). This statistical method is a technique that is used when there is a need for comparing the mean of a continuous variable, and this helps the researcher in determining whether the difference found between the means of groups is a true difference or occurred owing to random variation in the data (Ghufran, 2021). A significance level of 0.05 ($\alpha = 0.05$) was set for advocating the significance of the differences. The T-value is the test statistic that shows the degree of difference in the means of the two groups compared to the variation in the data, but in the same way, the "Sig." value is the probability value (p-value) to support statistically significant conclusions derived from the T-tests. When the Sig. value is smaller than 0.05, the difference between the groups is regarded as a statistically significant one; in case the Sig. value turns out to be greater than 0.05, the difference can be labelled as statistically insignificant.

Table (16): t-test comparing raw and treated water at the study plants.

Parameter	T	Sig.	Result
pH	7.348	0.005	Significant difference
Electrical Conductivity	5.719	0.011	Significant difference
Total Dissolved Solids (TDS)	2.098	0.127	Not significant
Turbidity	4.173	0.058	Not significant
Total Hardness	14.100	0.001	Highly significant difference
Sodium	1.958	0.134	Not significant
Calcium	2.000	0.139	Not significant
Magnesium	1.667	0.194	Not significant
Sulfate	6.971	0.006	Significant difference
Chloride	4.382	0.022	Significant difference

• If Sig. < 0.05 ⇒ There is a significant difference (statistically significant).
• If Sig. > 0.05 ⇒ There is no significant difference.

As shown in Table (16), the treatment processes resulted in different responses in terms of physical and chemical characteristics. While some variables showed statistically significant differences, others did not meet the level of significance. This variation is due to the differences in the characteristics of the water components and their features of response to the treatment processes.

pH

Findings from the Paired Samples t-Test indicated a statistically significant difference between raw and treated water in terms of pH level (T = 7.348; Sig. = 0.005, sig < 0.05). This shows that the treatment has successfully resulted in a

statistically significant change in pH, which can be explained by how different water treatment processes change the chemical balance of the constituents in the water solution; additionally, removing ions and dissolved substances is capable of changing acid-base characteristics of the water. pH is an important property of water quality as it is connected to solubility of different substances in a solution and their chemical properties.

Electrical Conductivity (EC)

Electrical conductivity tests displayed a notable contrast between untreated water and treated water (T = 5.719; Sig. = 0.011, less than 0.05). This displays that there is a significant statistical alteration in the ion composition and ionic material concentration of the water because of the treatment process. The ratio of electrical conductivity is significant since it shows the chemical content of water in terms of ionic products, and it's a recognised fact that electrical conductivity is directly proportional to the ion presence in water. Hence, the change of EC after the treatment will denote the presence of the lesser proportion of dissolved ionic materials, especially in the cases when membrane technology has been used (Al-Radaideh, 2010).

Total Dissolved Solids (TDS)

The T-value and Significance (Sig) for Total Dissolved Solids were 2.098 and 0.127 respectively. Since the latter exceeds the 0.05 limit, then it means that the difference between the raw and treated water is not statistically significant. However, the fact that there is no statistical significance does not mean that there was no actual reduction in the TDS; it rather means that the change was not enough to achieve statistical significance. The TDS relates to the total amount of substances, salts and dissolved solids in the water, thus the changes in the TDS happen depending upon how much salt comes into the treatment unit and how efficient the elimination devices are.

Turbidity

The T-value for turbidity was 4.173 with a Sig. value of 0.058. Though this value is slightly above the cut-off for significance level (0.05), turbidity was classified as statistically insignificant. However, because the Sig. value is approaching the significance threshold, this indicates that there may be some change occurring close to statistical significance. Turbidity measurements are actually of great importance in practical terms since they determine the efficiency of filtration and the removal of particles and suspended solids. Turbidity can be viewed as an important physical characteristic in assessing the quality of water because it provides information about the concentration of fine particles and suspended solids that provide the water with its appearance and affect efficiency at various stages of treatment process.

Total Hardness

With the T-value of 14.100 and Sig. value (significance value) of 0.001, total hardness, among all studied factors, had the highest coefficient of difference. The statistic shows a significant difference between raw and treated water. Total hardness is largely determined by the presence of calcium and magnesium ions in water, thus, changes in the level of hardness after water treatment will show how successful the processes of removal of those ions were. Additionally, reducing hardness is important from practical perspectives in order to lower the risk of scaling in pipes, equipment and facilities.

Sodium (Na)

When it comes to sodium, the T-value recorded was 1.958 with the Sig. value being equal to 0.134. Because the Sig. value is greater than 0.05, it can be concluded that there is not a meaningful difference between raw water and treated water in terms of sodium concentration as the changes observed were neither consistent nor substantial enough; thus, there could be other factors influencing the variations in sodium concentrations among stations and ion removal efficiencies during the water treatment process.

Calcium (Ca)

For calcium, the T-value recorded was 2.000, and the Sig. value was 0.139. Since it is more than 0.05, there was no significance in difference between the observed water data. While calcium is a major element with relation to hardness, the absence of significant difference between calcium levels focused on its distribution may indicate sample weight and magnitude of value differences of the attained readings. Thus, statistical significance evaluation is not connected only with the amount of reduction but also with the variability of obtained results.

Magnesium (Mg)

For magnesium, the T-value was 1.667 and the Sig. value was 0.194. As this exceeds 0.05, there is no statistically significant difference between the raw and treated water. While magnesium is associated with total water hardness, the lack of a significant difference in its concentration may be due to limited variation between measurements or differences in concentration levels across stations.

Sulfate (SO_4^{2-})

Sulfate levels showed a clear, significant difference, with a T-value of 6.971 and a Sig. value... = 0.006, which is less than 0.05. This result indicates a statistically significant change in sulfate concentration between the raw and treated water. Sulfates are important dissolved ions in water; therefore, their reduction following treatment suggests the removal of a portion of dissolved ions resulting from the separation and treatment processes.

Chloride (Cl^-)

Test results revealed a significant difference in chloride concentration, with a T-value of 4.382 and a significance (Sig.) value of 0.022—both below the 0.05 threshold. This result indicates a clear statistical change in chloride concentration after treatment. Chloride is a major dissolved ion in water and is linked to water salinity and chemical composition; thus, its reduction after treatment may reflect the removal of some salts and dissolved ions.

3. Results

Based on data analysis, it has been determined that the quality parameters of water collected from both sites of the facility are identical. Analysis of the quality parameters of both untreated and treated water shows high concentrations exceeding both Iraqi standards and making the water unsuitable for application purposes. Results of the study show that the technological devices of the facility do not function normally, and only serve to transport water. Large amount of physical and chemical constituents in raw water suggest that the Euphrates River is highly polluted. Results of the independent samples t-test demonstrate high statistical significance of the difference between the two groups for five parameters: pH, electrical conductivity, total hardness, sulfates, and chloride, with the biggest difference found for total hardness (Sig=0.001).

In turn, no statistically significant differences for Total Dissolved Solids, turbidity, sodium, calcium, and magnesium were noted, since Sig values for these variables were above 0.05.

4. Recommendations

1- Innovations at filtration facilities will update and upgrade treatment technologies to reduce the level of salt and total hardness:
Refurbish and upgrade filtration facilities with advanced treatment techniques. For example, one can consider the incorporation of technology for ion exchange or reverse osmosis. By doing so it is possible to eliminate the problem of rising levels of total hardness, TDS, sulfate and chloride ions, which conventional technologies fail to resolve.
2- Introduction of automation in the process of dose application for coagulants and disinfectants:
Integrate advanced methods of dosing chemicals into the process of adding treatment agents such as alum for turbidity reduction and chlorine for disinfection with flexibility to the seasonal fluctuations in the dose applied. This is done to

avoid discharging treated water with prolonged turbidity higher than national standards at Al Samawah, Al-Khidr and Al Warka plants.
3- Designing a maintenance plan for sedimentation and filtration basins:
Putting in place a comprehensive program for doing preventive maintenance, which will ensure regular cleaning and backwashing of sedimentation and filtration basins with the aim of preventing salt build up and sludge formation. Results of the experiment show that improper maintenance of the plants results in loss of efficiency of filtration process.
4- Monitoring pollution sources at the intakes, and eliminating pollution of the Euphrates and its tributaries:
Introducing strict environmental monitoring programs at the Euphrates River and its tributaries to ensure that no effluents from sewage, agricultural and industrial plants are discharged close to the plants' intakes. This is necessary in order to minimize the discharge of biological load and turbidity caused by the pollution of the water bodies.
5- Capacity building of the personnel of the Muthanna Water Directorate:
Organizing capacity-building initiatives aimed at development of the expertise of monitoring personnel. This is done in cooperation with educational institutions.
6- Implementation of statistical monitoring and quality control programs:
Implementation of the statistical methods of monitoring (t-tests and multivariate analyses) for the provision of quality reports that would help assess the effectiveness of each stage of the process used in the Muthanna environmental monitoring system.

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