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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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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).

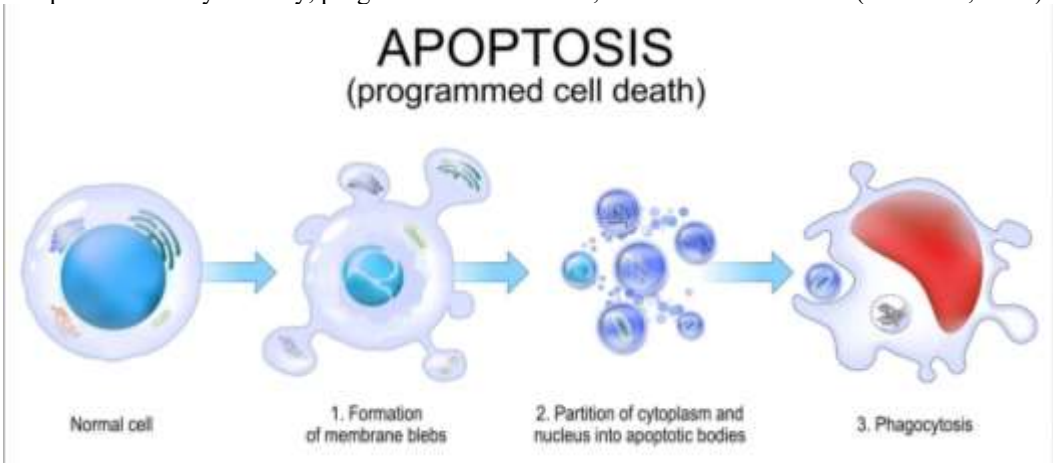

Figure (1) *Stages of Apoptosis*

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%)

Table 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

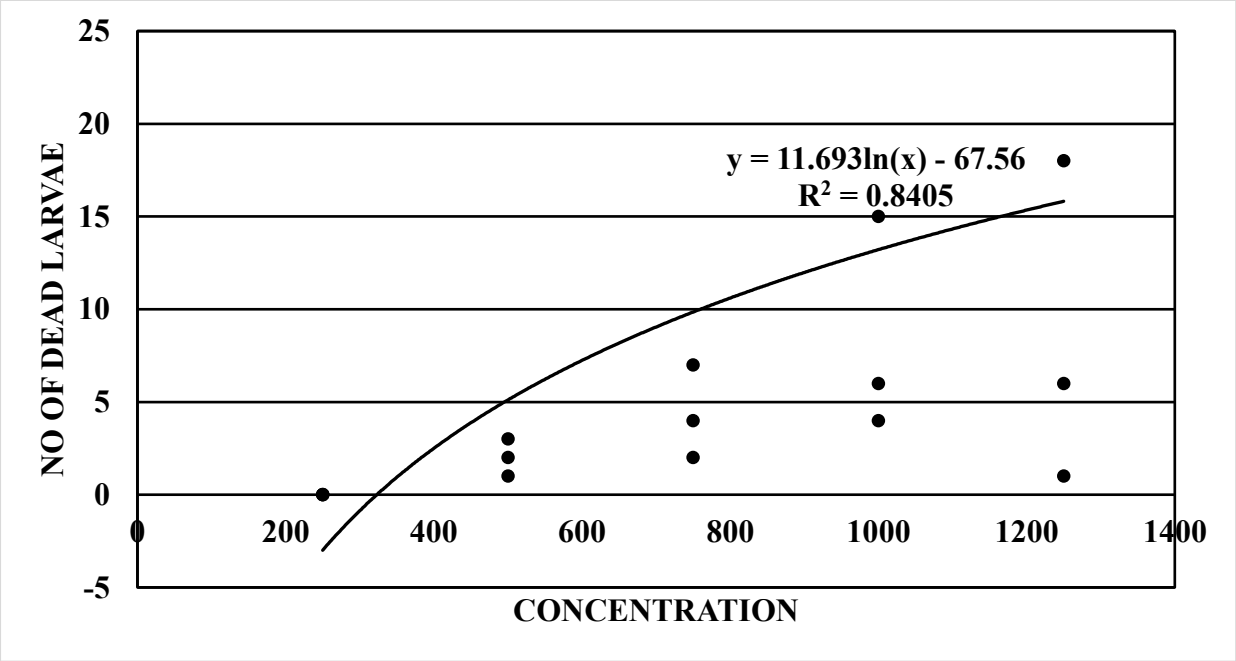

Figure 1: Mortality rates of larvae against the concentration (ppm)of aqueous extract of citrus aurantifolia leave and time period

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).

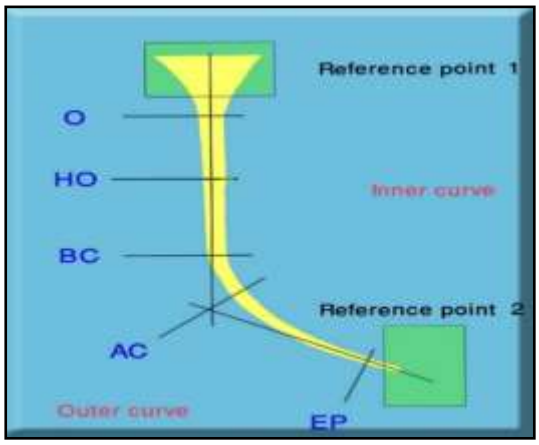

Fig. (1): *The five levels of measurement.*

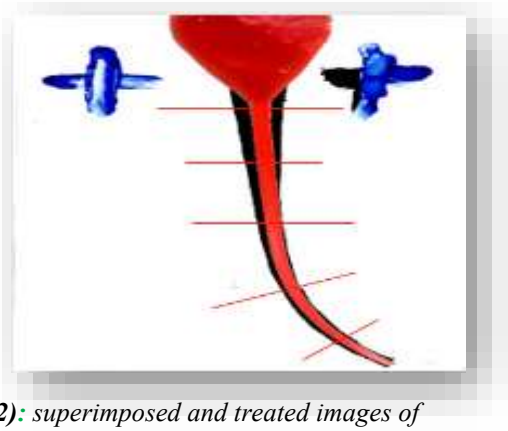

Fig. (2): *superimposed and treated images of unprepared and prepared canal with computerized program Adobe Photoshop (C).*

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.

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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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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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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	P. value = 0.001* Chi-square = 9.71
Oxacillin	89	80.91	21	19.09	
Gentamicin	57	51.82	53	48.18	
Tobramycin	56	50.91	54	49.09	
Levofloxacin	57	51.82	53	48.18	
Moxifloxacin	72	65.45	38	34.55	
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%)	< 0.0001
Wounds	38	(34.5%)	
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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