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Enhancement of Antibiotics Activity Against Resistant Bacteria Using Specific Nanoparticles *in Vitro*

A Thesis

Submitted to the Council of Veterinary Medicine College/University of Shatrah, as Partial Fulfillment of the Requirements for a **Master's Degree (M.Sc.)** in Veterinary Sciences (**Microbiology**).

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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لِي مِنْ أَمْرِي مَخْرَجًا وَسَلَامًا بِصِيْرًا))

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"Certification"

We certify that this thesis (**Enhancement of Antibiotics Activity against Resistant Bacteria Using Specific Nanoparticles in *Vitro***), was prepared under our supervision at the Department of Microbiology, Faculty of Veterinary Medicine, Shatrah University, as a partial requirement for Master degree in Veterinary Medicine / **Microbiology**.

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Declaration

I declare that this thesis is my original work, except for citations. I also confirm that it has not been previously submitted to any other degree at the University of Shatrah or other universities and institutions.

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DEDICATION

To ALLAH, for all of His blessing and for giving me the strength to see this path through to the end.

To the Prophet of mercy and the light of the world's Our master Muhammad, may God bless him and his family and grant them peace....

To whom gave me his name ... My dear father .. To the one who mighty God made heaven under her feet & blessed her in his books...My Mother.

To my dear sisters, brothers & beloved...

To my dear husband... For his Patience understanding... and help To my children... For all my family for their support and their assistance.

I give them my humble work.

Huda

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Summary

The emergence of antimicrobial resistance (AMR) among clinically important bacteria represents a serious challenge to modern healthcare systems worldwide. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most common pathogens isolated from clinical samples, and they are frequently associated with severe infections. Their ability to develop multiple resistance mechanisms limits the effectiveness of conventional antibiotics and necessitates the exploration of alternative therapeutic strategies. The application of natural polymer-based nanoparticles, has gained increasing attention as a promising approach to enhance antimicrobial activity and overcome bacterial resistance. Chitosan, a biocompatible and biodegradable polysaccharide derived from chitin, possesses inherent antimicrobial properties and, in its nanoform, exhibits improved surface activity, stability, and interaction with microbial cells. Combining chitosan nanoparticles with conventional antibiotics may therefore provide synergistic effects and lead to more efficient therapeutic options. This study was designed to investigate the antibacterial activity of chitosan nanoparticles, both alone and in combination with three selected antibiotics, against clinical isolates of *S. aureus* and *P. aeruginosa*. One hundred samples were collected from patients admitted to hospitals in Thi-Qar province during the period from October 2024 to February 2025. The collected specimens were cultured, and further studies were performed in the Microbiology Laboratory at the College of Veterinary Medicine, Shatrah University. VITEK systems I and II were carried out in the microbiological laboratory. Using the disk diffusion method, the antibiotic sensitivity test was investigated. The presence of Betalactamase-resistance genes was molecularly studied in both species by investigating the presence of antibiotic resistance genes (*mecA* and *blaZ*) in *S. aureus* and (*AmpC* and *MexA*) in *P. aeruginosa*.

Chitosan nanoparticles were VI prepared and characterized for particle size, morphology, and stability. Erythromycin, Ampicillin, and Ceftazidime were selected and mixed with the nanoparticles in powder form under controlled laboratory conditions. Comparative analyses were performed to determine the enhancement of antibiotic activity in the presence of chitosan nanoparticles. Various concentrations of each antibiotic solution were prepared and mixed with CNPs, which also melted by using more than one solvent. The absorbency, purity and non-degradability of chitosan and chitosan-antibiotics combinations separately were investigated by using specific UV-Vis; the presence functional groups in chitosan-antibiotics combination were investigated by applying Fourier Transform Infrared Spectroscopy (FT-IR); Field emission scanning electron microscopy (FE-SEM) analysis was used as well to investigate the CNPs-antibiotic association. Out of 100 samples, 34 isolates were identified as *P. aeruginosa* and 23 isolates were identified as *S. aureus*. The results discovered that the antibiotic resistance genes *mecA* and *blaZ* were detected in 12 tested *S. aureus* isolates. In contrast, the *mexA* and *ampC* genes were detected in 8 tested *P. aeruginosa* isolates. This study reported that all *P. aeruginosa* and *S. aureus* isolates were antibiotic-resistant and showed that the prevalence of antibiotic resistance in patients with burns and wounds in Thi-Qar/ Iraq is alarmingly high. The results found that chitosan and antibiotic aqueous solutions separately had weak antibacterial activity; however, when both were mixed to form the binary combination antibiotic-Chitosan a synergistic effect was observed. The results demonstrated that while ceftazidime-CNPs had the most antibacterial activity against *P. aeruginosa*, displaying the biggest inhibition zones; Erythromycin-loaded chitosan nanoparticles were more efficient against *S. aureus*. The successful formulation of chitosan-based nanoparticles loaded with the

antibiotics ceftazidime and erythromycin using the ionic gelation method. Chitosan incorporation enhances the UV absorbance of Erythromycin and Ceftazidime, with VII a much more pronounced effect in the case of ceftazidime. The spectrophotometric data do not reveal any significant interaction between erythromycin and chitosan within the 300–800 nm range. The FTIR spectrum confirms the presence of hydroxyl, carbonyl, and amide groups, indicating successful conjugation or interaction of the antibiotic with the nanoparticle surface. Shifts in characteristic antibiotic peaks (C=O, N–H, C–N) compared to pure antibiotic spectrum would confirm binding; comparing with reference spectra is essential. The findings of this study highlight the promising potential of chitosan nanoparticles as an adjunct strategy to enhance the antibacterial efficacy of conventional antibiotics. This approach may contribute to reducing antibiotic dosages, minimizing side effects, and extending the lifespan of existing antimicrobial agents. Further studies are recommended to evaluate cytotoxicity *in vivo* activity.

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List of Abbreviations

<i>abbreviations</i>	<i>meaning</i>
AST	Antibiotic Sensitivity Test
CLIS	Clinical and laboratory standard institute
CNPS	Chitosan nanoparticules
FE-SEM	Field emission scanning electron microscopy
FT-IR	Fourier Transform Infrared Spectroscopy
HCAIs	Healthcare-associated infections
MDR	Multi drug resistant
MIC	Minimum inhibition concentration
MRSA	Methicillin resistant s. aureus
PAR	Pan drug-resistant
PBP	Pencillin binding protein
QS	Quorum sensing
UV-Vis	Ultra-Violet Visible Light Spectroscopy
VRSA	Vancomycin resistant s. aureus
WHO	World Health Organization
XDR	Extensively drug-resistant

Chapter One

Introduction

1.1 Introduction

Various kinds of microorganisms acquired the ability to resist the antibiotics and this phenomenon become more dangerous and threat the life of human and animal alike (Urban-Chmiel, *et al.*, 2022). Antibody-resistant bacteria were actually recognized more than 50 years ago by the late 1950 s the majority of isolates of *S. aureus* developed a resistance to penicillin, which was once the standard treatment for them (Kim and Cha, 2021). Antibiotic resistance has spread as a result of the several strategies that bacteria have developed to withstand the effects of these medications (Chinemerem *et al.*, 2022). Antimicrobial resistance is a natural phenomenon, according to the World Health Organization (WHO) that arises when bacteria become less responsive to medicines to which they were previously sensitive and that were once effective in treating diseases brought on by these microbes (Salam *et al.*, 2022).

The WHO first released a list of the 12 bacterial families that are the biggest threats to human health in 2017 (Mulani *et al.*, 2019). The WHO list divides bacteria into three priority groups: critical, high, and medium. This is based on how urgent it is to create new antibiotics to fight these infections (De Olivera *et al.*, 2020). The WHO released a list of pathogens that includes those that were assigned the highest (priority status) because they pose a serious threat to humans and are known by the acronym ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *pseudomonas aeruginosa*, and *Enterobacter* species((Breijyeh *et al.*, 2020). Unfortunately, the overuse and misuse of antibiotics as well as social or economic factors have accelerated the spread of antibiotic-resistant bacteria and making drug treatment ineffective (Chis, 2022). Hence, researchers have paid attention to come with new methods that may help

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and overcome like this phenomenon. There are many approaches that have been applied to connect antibiotics and nanoparticles. Nanomaterials are potential candidates for antibacterial activity. The characteristics of nanoparticles for biological applications have been extensively studied by researchers over the past years. Presently using nanotechnological solutions, which include nanoparticles, represents one of the possible ways to overcome the resistance to antibiotics in bacteria (Yougbaré, *et al.*, 2021).

Indeed, because of their size, nanoparticles can enter cells through different methods than tiny molecules. And that can vary depending on the nanoparticles' physicochemical characteristics, their capacity to recognize certain targets, their surface functionalization, and their employment as drug delivery vehicles (Rezić, 2022). In early studies, chitosan nanoparticles are a common contender. According to several reports, chitosan is GRAS (Generally Recognized As Safe) and bio absorbable (Thambiliyagodage, *et al.*, 2023). Because of its structural resemblance to natural glycoaminoglycans, it has low toxicity and biodegrades into harmless amino sugars that the body may absorb completely (Mikusova & Mikuš, 2021). New techniques to compact bacterial resistance and persistence are becoming more necessary due to the limited efficacy and effectiveness of existing antibiotics and the increased tolerance and resistance to multidrug. A deeper understanding of the factors that influence the development of resistant antibiotic bacteria is critical (Christaki *et al.*, 2020). For example, *S. aureus*, a Gram-positive bacterium, is one of the most notorious human pathogens, causing illnesses ranging from mild skin and wound infections to fatal sepsis or multi-organ failure and it has been known *S. aureus* bacteria resistance to most antibiotics. *P. aeruginosa* is a frequent cause of Healthcare-associated infections (HCAI) and has been identified as a

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source of infection in hospitals (Fazeli *et al.*, 2012; Mekonnen *et al.*, 2021). *P. aeruginosa* is G-ve, a motile, rod shaped bacterium around 0.5–1.0 µm broad and 1–5 µm length (Diggle and Whiteley 2020). It is a facultative aerobe noncapsulated but it has pseudo capsule, slime layers which they play as a barrier against antibiotics, phagocytes and, lymphocytes (Todar, 2011). Non-spore-forming, and can produce many types of pigments such as, pyoverdine and pyocyanin provide solid media its characteristic blue green color. *P. aeruginosa* has huge virulence factors which cause both extensive tissue damage and interfere with the immune defense weapons (Urgancı *et al.*, 2022).

1.2.Aim of the Study

The current study aims to improve the antibiotic activity against Multi-Drug Resistant bacteria (MDR) using nanoparticles by:

- 1-Isolate and identify two types of MDR bacteria including *p. aeruginosa* and *S. aureus* from clinical specimens.
- 2- Study the antibiotic sensitivity of *S. aureus* and *P. aeruginosa* against 11 antibiotic disks.
- 3- Study of the presence of antibiotic resistance genes (*mecA* and *blaZ*) in *S. aureus* and (*AmpC* and *MexA*) in *P. aeruginosa*.
- 4- Antibiotic Encapsulation with chitosan nanoparticles and applying drug delivery methods to examine the effectiveness of antibiotic encapsulated with Nanoparticles against MDR isolates *In Vitro*.
- 5- Study the synergistic effect of Nano-Chitosan combined with powdered antibiotics on the growth of antibiotic resistant bacteria.

Chapter Two

Literature Review

2.Literature Review

2.1. Bacterial Resistant to Antibiotic

Antibiotic resistance is the ability of bacteria to resist exposure to antibiotics designed to kill them or inhibit their growth. Although antibiotic resistance is a natural process due to genetic changes in the bacteria following antibiotics exposure, however, this phenomenon is being accelerated through the abuse of antibiotics (Mancuso *et al* 2021). Resistant bacteria are now on the rise around the world, complicating the treatment of Healthcare-associated infections (HAIs), and are becoming increasingly dangerous in developing nations (Elbehiry *et al.*, 2022). Most pathogenic bacteria, it appears, are capable of acquiring antimicrobial resistance to at least some antimicrobial agents. Limiting a drug's uptake, modifying a drug's target, inactivating a drug, and active effluxing of the drug are the basic mechanisms of resistance. These processes may be found in the bacteria themselves or have been acquired from other microbes (Flemming *et al.*, 2023).

Multi-Drug Resistant (MDR) pathogens can develop resistance to different antimicrobials via horizontal gene transfer and via gene mutations as a consequence of exposure to the drugs. Although the acquisition of resistance is a natural process, it is exacerbated by the misuse of antibiotics, inadequate surveillance, and the poorly controlled regulation of antibiotics in clinical medicine and in the livestock industry, which leads to the appearance and spread of MDR bacteria all over the world, that increase the need to develop new bactericidal materials. Consequently, there is a strong demand for developing novel strategies and new materials that can cope with these problems (Muteeb *et al.*, 2023) . The most serious concern with antibiotic resistance is that some bacteria have become

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resistant to almost all of the easily available antibiotics. These bacteria are able to cause serious disease and this is a major public health problem. Therefore, there has been a serious demand for the discovery of alternatives for the treatment of drug-resistant microbial infections apart from antibiotics (Elshobary *et al* 2025). Resistance was not only limited to multiple antibiotic resistance but also included the emergence of different degrees of MDR in bacteria, the terms extensively drug-resistant (XDR) and pan drug-resistant (PDR) have been introduced. Extensively drug-resistant (XDR) is the non-susceptibility of one bacteria species to all antimicrobial agents except in two or fewer antimicrobial categories. Within XDR, pan drug-resistant (PDR) is the non-susceptibility of bacteria to all antimicrobial agents in all antimicrobial categories (Naik *et al.*, 2021). As illustrated in the figure (2-1).

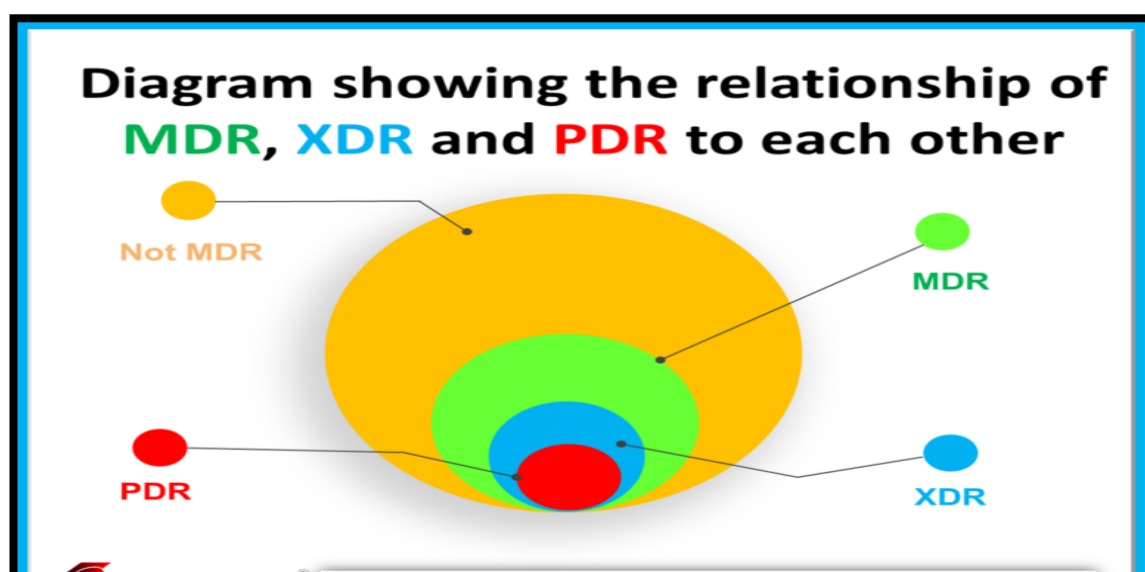


Figure (2-1) (MDR) has been divided into three categories depending on their resistance profile: 1. MDR—non-susceptible to at least 1 agent in 3 antimicrobial categories; 2. extensively drug-resistant (XDR)—non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories and 3. pan-drug-resistant (PDR) non-susceptible to all agents in all antimicrobial categories (Naik *et al.*, 2021).

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2.2. Biofilms

Biofilms are structured aggregates of bacteria capable of surviving hostile environmental conditions and exhibit resistance to the host's immunity and different chemotherapeutic agents (Shree *et al.*, 2023). Biofilms are complex of single or multiple species of bacteria enclosed in an extracellular polymeric substance (EPS) that is mainly composed of polysaccharides, nucleic acids, and proteins (Flemming *et al.*, 2023). Biofilm formation consists of five distinct stages: 1. Attachment: microbial cells adhere to the surface reversibly. 2. Colonization: microbial cells attach to the surface irreversibly via flagella, pili, exopolysaccharides, and etc. 3. Development: multilayered cells accumulate and produce extracellular polymeric substances (EPS) .4. Mature: stable formation of a three-dimensional community. 5. Active dispersal: microorganisms are disseminated from the aggregate biofilm and return to a planktonic state (Yin *et al.*, 2019). Bacterial biofilms are a serious global health concern due to their ability to tolerate antibiotics, host defense systems, and other external stresses, therefore it contributes to persistent chronic infections (Nelson *et al.*, 2020). Comprehensive studies of antimicrobial resistance have revealed that bacterial infections that resist antibiotics are not due to free bacteria but rather to bacteria existing within a biofilm. Biofilm-forming bacteria are resistant to conventional antimicrobials due to:

1. The inability of the antimicrobial to penetrate the biofilm.
2. Evolution of complex drug resistance properties.
3. Biofilm mediated inactivation or modification of antimicrobial enzymes. (Mirghani *et al* 2022).

As shown in the figure (2-2) biofilms provide protection to the microorganism not only from altered pH, Osmolarity, and nutrient scarcity,

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mechanical and shear forces but also block the access of bacterial biofilm communities from antibiotics and the host's immune cells. Therefore, the biofilm matrix gives additional resistance power to bacteria which makes them not only tolerate harsh conditions but also resistant to antibiotics which lead to the emergence of bad bacterial infections like MDR, XRD, and totally drug-resistant bacteria (Sharma *et al.*, 2019).

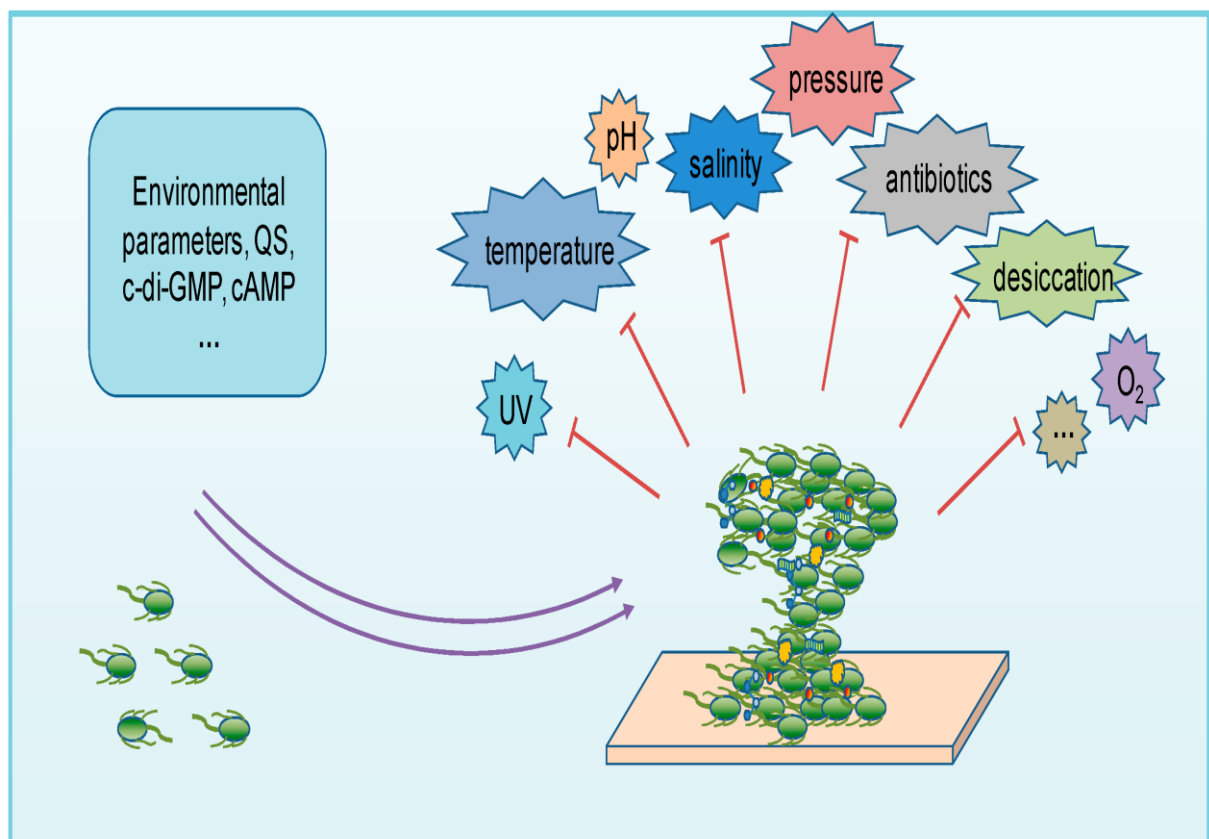


Figure (2-2) : A schematic representation of how biofilms work. The formation of biofilms can strengthen bacteria' resilience to a range of harsh conditions (Yin *et al.*, 2019).

2.3. Studied Isolates of Bacterial

2.3.1. *Staphylococcus aureus*

2.3.1.1.Characteristics and Taxonomy of *S. aureus* :

Staphylococci are Gram-positive cocci-shaped cells arranged in irregular clusters similar to grape clusters (Silva *et al*, . 2023) . Although *S. aureus* is the major bacterium of its genus, more than 50 Staphylococcus species are registered. These bacteria are facultative anaerobes, Staphylococci were first identified in 1880 and isolated from the pus of surgical abscesses by the Scottish surgeon Sir Alexander Ogston (Chieffi *et al*,. 2024). These frequently forms clusters that are referred to be "grape-like." The German physician Friedrich Rosenbach isolated and cultured staphylococci from humans in 1884 (Rasheed *et al*., 2021). He studied their characteristics and categorized them according to the production of golden or yellowish colonies, naming the species —aureus from the Latin word meaning golden. Its optimum growth occurs at temperatures between 18 °C and 40 °C (Fayisa and Tuli. 2023). Colonies are characterized by their large, smooth, and elevated appearance with a yellow color . Classification of *S. aureus* following (Xia and Wolz 2014)

Domain; Bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacilillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *aureus*

2.3.1.2. Epidemiology of *S. aureus*

Staphylococcus aureus is a dangerous human pathogen that is responsible for thousands of deaths annually in the U. S. alone. Virulence of *S. aureus* is due to a large repertoire of virulence factors. *S. aureus* infections become particularly dangerous when they are caused by strains that are resistant to commonly used antibiotics. (Cheung , 2021). *S. aureus* is a major human nosocomial and community-acquired pathogen that causes infections of the skin and soft tissues, and life-threatening systemic diseases (Cong *et al.*, 2019). Transmission of *S. aureus* can take place from one person to another by close or direct contact, sharing personal items (Rasheed *et al* 2021). *S. aureus* colonization in different parts of the body increases the risk of infection at the surgical site, as well as the infections of the lower respiratory and blood stream in hospitals (Linz *et al*, . 2023). It remains a challenging, global public health crisis due to the emergence and spread of (MRSA) and Vancomycin-resistant *S. aureus* (VRSA). Currently, MRSA and VRSA are categorized as agents of high significance with potential to cause considerably devastating worldwide mortality in the absence of effective containment and treatment options (Shoaib, *et al*, . 2023) .

2.3.1.3.Pathogenicity and Virulence Factors of *S. aureus*

Staphylococcus aureus is one of the most notorious human pathogens which causes illnesses from mild skin and wound infections to fatal sepsis or multi failure. This pathogen can cause a wide variety of diseases (Fayisa & Tuli, 2023). It is a leading causative agent in pneumonia and other respiratory tract infections, surgical sites, prosthetic joints, and cardiovascular infections, as well as nosocomial bacteremia (Fayisa Tuli , 2023). Usually not life threatening but may be accompanied by significant

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morbidity and pain. Due to their frequency (several million annually in the U.S.), they represent a considerable public health burden (Cheung *et al.*, 2021). These include a plethora of toxins and immune evasion factors, and a vast array of protein and non-protein factors that enable host colonization during infection (Cheung *et al.*, 2021). As in figure (2-3) virulence factors of *S. aureus* includes (Jiang *et al.*, 2023).

2.3.1.3.1.Cell wall components

The cell wall of *S. aureus* shows the typical features of gram-positive bacterial cell walls under the electron microscope it appears as a relatively thick (about 20 to 40 nm) homogeneous structure. The peptidoglycan, half of the cell wall by weight is peptidoglycan, has been known for a long time and serves as a protective barrier, The peptidoglycan consists of layers of glycan chains built with 10 to 12 alternating subunits of N-acetylmuramic acid and N-acetyl glucosamine. Oligopeptide side chains are attached to the N-acetylmuramic acid subunits and are then cross-linked with peptide bridges(Soe *et al.*, 2021). The peptidoglycan layer in Gram-positive organisms consists of many cross-linked layers, which make the cell wall more rigid (Nikolic *et al.*, 2023).

2.3.1.3.2.Enzymes

Many secreted *S. aureus* enzymes degrade host molecules or interfere with host metabolic or signaling cascades. Several of those are proteases, lipase, hyaluronidase and nucleases that help to evasion from immune system and many exotoxins such as four hemolysis (alpha, beta, gamma and delta, which promote the manipulation of the host's immune responses while ensuring bacterial survival. In keeping a staphylococcal infection localized such as complement factors, antimicrobial peptides and leading to tissue destruction (Tam and Torres, 2019).

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2.3.1.3.3.Toxins

Staphylococcus aureus produces toxins that may cause such diverse manifestations as septic shock, gastroenteritis, toxic shock syndrome and scalded skin syndrome. The virulence of *S. aureus* is defined by a large repertoire of virulence factors, among which secreted toxins, many *S. aureus* toxins damage the biological membranes, leading to cell death. (Divyakolu *et al.*, 2019). These toxins were grouped in two categories: first Exotoxin, cytoplasmic membrane is the target of bacterial toxins, that are produced by *S. aureus* and secretes numerous exotoxins, *S. aureus* is widely acknowledged as a significant food-borne pathogen (Tam K.Torres 2019). Second, Enterotoxin released toxins disrupt intestinal activity and induce staphylococcal food poisoning (SFP), which is characterized by nausea, vomiting, abdominal pain, and diarrhea without indications of toxic effects, such as fever or hypotension (Ahmad-Mansour *et al.*, 2021). They may not be completely denatured by mild cooking of contaminated food (Cieza *et al.*, 2024).

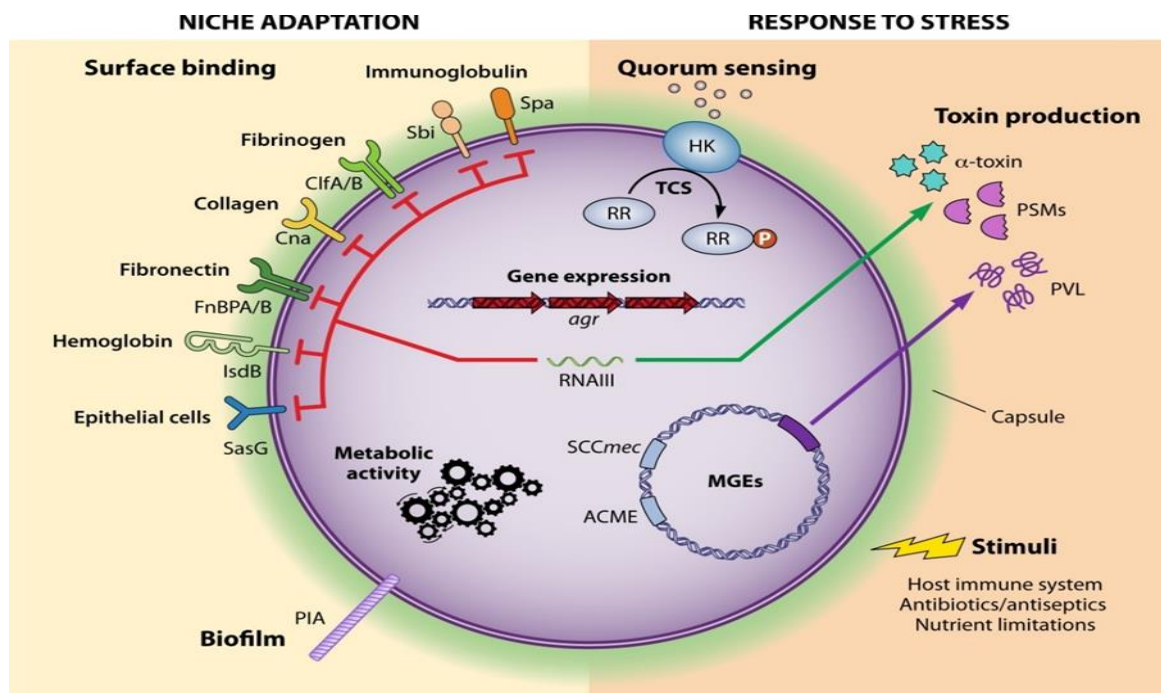


Figure (2-3): show the virulence Factors of *S. aureus* (Jiang Jet *al.*, 2023).

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2.3.1.4.Genomic of *S. aureus*

The genome of *S. aureus* consists of a single circular chromosome (2.7-2.8) Mbp plus an assortment of extra chromosomal accessory genetic elements: conjugative and non-conjugative plasmids, mobile elements (IS, Tn, Hi (plata *et al.*, 2009). The *mecA* gene is found on the staphylococcal cassette chromosome *mecA*, a movable genetic element that has been described in at least 13 distinct kinds (Miragaia 2018).). New *mecA* homologues, *mecB*, C, and D, have recently been discovered to confer β -lactam antibiotic resistance (Naji Hasan and Abdal karim jassem 2021). Utsui and Yokota in 1985 confirmed the role of an altered PBP in conferring methicillin and cephem resistance in *S. aureus*, the gene responsible for conferring the methicillin resistance phenotype to *S. aureus* was found to be located on the chromosome, unlike the β -lactamase gene which was found on a plasmid. This region on the chromosome was traced as a “foreign DNA” present only in resistant strains and absent in susceptible ones. later showed that it encodes a PBP(Pencillin Binding Protein), and the gene was later named *mecA*, with confirmation of its role in methicillin resistance(Bilyk *et al* 2022) .

Two mechanisms of resistance to β -lactams are described in *S. aureus*. The most important is the production of a β -lactamase enzyme, which is encoded by the *blaZ* gene, and hydrolyzes the β -lactam ring of the antibiotic and the second an anti-repressor (Mlynarczyk-Bonikowska, *et al*, .2022).

The second mechanism is more common in human isolates, being mediated by the *mecA* gene which is responsible for encoding PBP-2a MRSA (*S. aureus* resistant to methicillin), a synthetic wall PBP) of the bacterial cell with low affinity binding to β -lactam antibiotics (Ambade *et al.*, 2023).

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2.3.1.5. Antimicrobial Resistance of *S. aureus*:

A global problem of multi-drug resistance (MDR) among bacteria is the cause of hundreds of thousands of deaths every year. The drug resistance of *S. aureus* has gradually increased in the recent decades due to the misuse of antibiotics (Gharee *et al.*, 2023) . Most of the antibiotics available in the market become less active against resistant bacteria ; hence, new strategies are needed to treat MRSA infections .(Renata *et al.*, 2022), (Guo *et al.*, 2020) . MRSA has acquired resistance to all β -lactam antibiotics through two distinct mechanisms: (I) the production of penicillin-binding protein 2a (PBP2a), a trans peptidase that is highly resistant to inhibition by β -lactam antibiotics and can maintain peptidoglycan crosslinking, thus allowing the strain to survive in the presence β lactam, and (II) the production of β -lactamases, which hydrolyze the amide bond of the four-membered β -lactam ring, rendering the antibiotic inactive before it gets to the PBP target. (Lade and Kim, 2021).

The *mecA* gene is found on the staphylococcal cassette chromosome. The expression of *blaZ* is controlled by two adjacent genes, *blaI* and *blaR1*, the first being a *blaZ* transcription repressor, and the second an anti-repressor . (Naji Hasan *et al.*, 2021).

2.3.2. *pseudomonas aeruginosa* :

pseudomonas aeruginosa was isolated as a pure culture in 1882, by Gessard, it is a bacterial genus that belongs to the Pseudomonadaceae family. The term Pseudomonas is taken from the Greek word Pseudo, which means false, and the Latin word monas, which refers to a single unit (Urgancı *et al.*, 2022). The Pseudomonas genus is contained within the Gamma proteobacteria class, that considered one of the major bacteria

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groups. and aeruginosa means verdigris. *P. aeruginosa* classified as follows: (Diggle and Whiteley, 2020).

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: *Pseudomonas*

species: *aeruginosa*

2.3.2.1.Characterization of *P. aeruginosa*

In common these bacteria are Gram-negative, aerobic facultative anaerobic, aerobic respiratory metabolism by oxygen, but in some cases, the use nitrate oxidically. It is rod-shaped measuring 0.5 to 1.0 μm positive and grows at 37°C to 42°C (Sharma *et al*, . 2021). based on colony morphology, coloration, and oxidase positivity (Araos and D'Agata, 2019). They bacteria do not ferment carbohydrates but produces acid from sugars such as glucose, fructose, and xylose, growth at 42°C allows differentiation from other *Pseudomonas* species. The identification of *P. aeruginosa* is motile with a single polar flagellum (Shah, 1995). they grow on many types of culture media, as a single bacterium, or as short chains, forming smooth round colonies with a characteristic grapelike or (corn-taco) odor. This is due to its production of Amenoacetophon (Ezeador *et al.*, 2020). The green-blue color is due to the production of pyocyanin (blue) and pyoverdin (green) and this distinct color explains the species name (aeruginosa)(Abdelaziz *et al.*, 2023). *P. aeruginosa* has huge virulence factors that are classified into cell associated factors (Edward *et al.*, 2023).

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2.3.2.2. Epidemiology of *P. aeruginosa*

Pseudomonas aeruginosa can cause nosocomial infection and many severe as toxigenic infectent. *P. aeruginosa* has the ability to grow in many environments. It is called opportunistic pathogens. This bacterium was found in hospitals and even now it is one of the main causes of infection in hospitals. According to the Center for Disease Control and concentration, *P. aeruginosa* accounts for 10 % of all hospital-acquired infections and leads to death nearly 50 % of patients with cancer, cystic fibrosis, and burns (Qin *et al.*, 2022). The World Health Organization (WHO) has designated *P. aeruginosa* as (a top priority) with a critical urgency for finding novel antimicrobial therapies. Because this species is frequently resistant to a variety of antibiotics (Mashruwala and Bassler, 2020) .

2.3.2.2. Pathogenicity and Virulence Factors of *P. aeruginosa*

The current greatest global public health problem is the pathogenesis associated with the emergence of generalized infectious bacteria (Frieden and Buissonnière, 2021). An opportunistic pathogen, most infections occur in individuals with altered host defense mechanisms (Langendonk *et al.*, 2021). The bacteria have a wide set of virulence factors, allowing them to harm host cells and modulate human adaptive immune mechanisms(Grandy *et al.*, 2023). Its infection has been observed in COVID 19-infected intensive-care patients (Lansbury *et al.*, 2020). (LeBlanc *et al.*, 2021). Pathogenesis of this bacteria is not exclusively focused on the emergence of new infectious diseases, but also on biofilm formation and on the appearance of new antibiotic-resistant strains, or on the increase in prevalence of already known strains (Chadha *et al.*, 2021). The virulence factors of *p. aerugiosa* includes

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A-Outer Membrane Components ;

Lipopolysaccharide (LPS) elaborated by this organism is a key factor in virulence as well as both innate and acquired host responses to infection. It is a major component of the outer membrane in Gram-negative bacteria, (Paracini *et al.*, 2022). Composed of lipid A moiety, inner and outer core oligosaccharides, as well as the O-antigen, LPS may be the most extensively studied bacterial molecule due to its high immunogenicity and surface accessibility (Fux *et al.*, 2023). It also develops alginate to protect bacteria against toxins, phagocytosis, and environmental conditions and is involved in immune resistance. LPS plays a major role in pathogenesis and disrupts the immune response of the host and leads to high mortality rates, helps bacteria bind to host cell surfaces and contributes to nosocomial disease, chronic and acute infection (Huszczynski *et al.*, 2020). Paradoxically, LPS can stimulate neutrophils to release Neutrophil Extracellular Traps (NETs to capture invading pathogens, but they protect bacteria from phagocytosis) (Hidalgo *et al.*, 2023).

A. Biofilm Formation

These bacteria are characterized by their high ability to form a biofilm, especially in chronic infections. There are three stages for the formation of a biofilm, the first of which is the attachment of bacteria to the surface and adhesion, then growth and reproduction, through which micro-colonies are formed after which the mother cells mature and separate to form new colonies from the biofilms (Sauer *et al.*, 2022) . The biofilm has many important functions, including antibiotic resistance, as the bacteria inside the biofilm layer can resist a thousand times more than those that live freely or floating (Sharma *et al.*, 2019). In addition resistance to the process of phagocytosis and the expulsion of free radicals released by

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macrophages and neutrophil cells, escape from defense mechanisms and resistance to substances that inhibit the biofilm, also responsible for retaining water and nutrients in harsh environmental conditions (Tuon *et al.*, 2022).

B. Quorum Sensing (QS) :

A production of several virulence factors by *P. aeruginosa* is controlled according to cell density through two quorum-sensing systems lasL and lasR (Ghanem *et al.*, 2023). Quorum sensing is a bacterial cell-to-cell communication system, as show in figure (2-4), (Ghosh *et al.*, 2022).

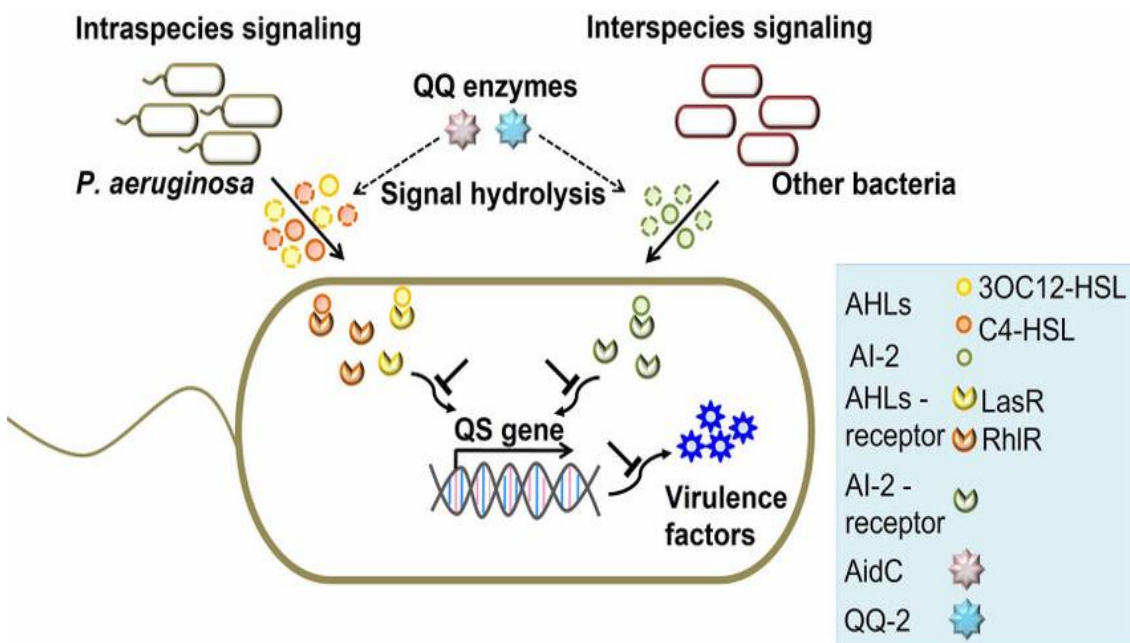
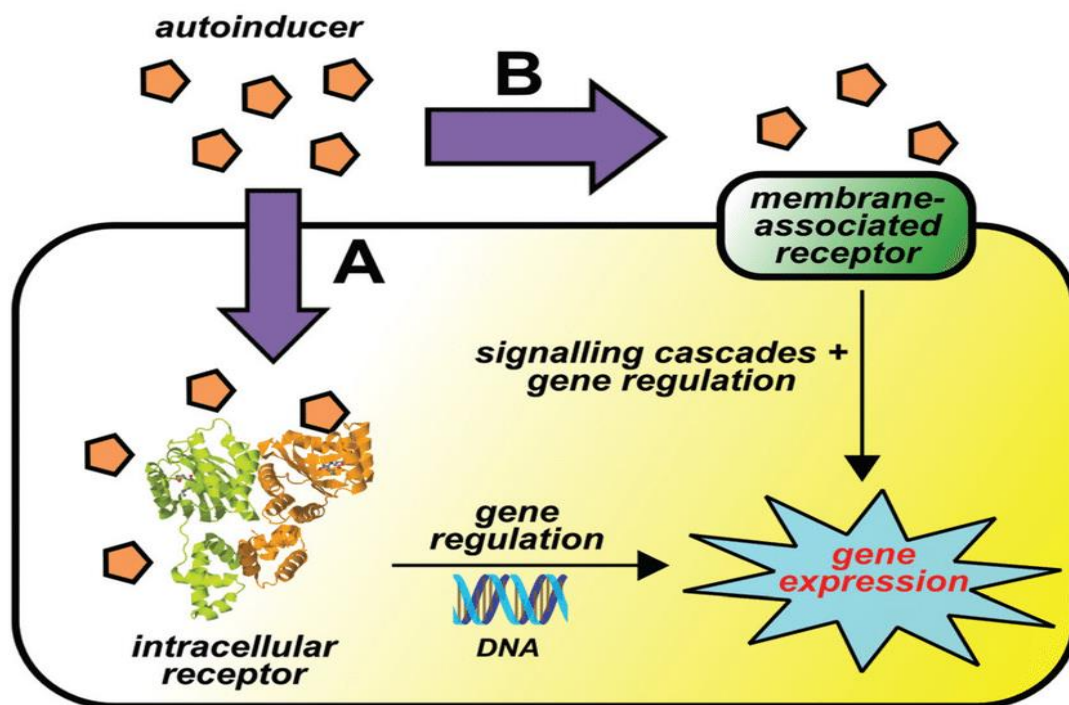


Figure (2-4): Diagram showing the intraspecies and interspecies quorum sensing for virulence factor synthesis in *P. aeruginosa* (Khatun, M. *et al* 2023) .

QS was first discovered in 1994 by Peter Greenberg in the bioluminescent marine bacterium *Vibrio fischeri*, which was composed of two regulatory proteins, lasL / lasR, the first protein is an auto-inducer synthase enzyme and the second is a promoter binding protein as shown in figure (2-5) (Singh & Bhatia, 2021). These systems are known to control the

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expression of a number of virulence genes in response to bacterial cell density, virulence elements such as elastase, protease, hemolysis, pyocyanin virulence, swimming and twitching, biofilm production (Parasuraman *et al.*, 2020). The general model for QS begins with the autoinducer, which is a diffusible molecule, being produced at a basal level at low cell densities. The quorum sensing mechanism regulates a variety of processes, including gene expression. There are species-specific auto-inducers and genus-specific auto-inducers. (Atique *et al.*, 2023)



Figure(2-5): The auto-inducers that bacterial species release let cells communicate with one another both inside and across species.

C. Enzymes

1-Hemolysin Production, is one of the important virulence factors of *P. aeruginosa*. Bacteria produce at two distinct hemolysins: heat-labile hemolysin, which is known as phospholipase C (Marchant and Banat, 2012), and the second type of hemolysin is Rhaminolipid, and it is heat

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stable and has a composition similar to detergents, so it helps to analyze and remove fats, (Truan *et al.*, 2013). The pathogenicity of this factor lies through the analysis of the red blood cells of the host, and this leads to the provision of iron for bacterial growth, which leads to necrotic infections in the skin (Ismail *et al.*, 2021).

2-Production of protease: protease enzyme is considered important virulence factor which cause damage in host tissues (Casilag *et al.*, 2015 ; Liao, *et al.*, 2022). Proteases destroy host tissues and interferes with the host's antibacterial defense mechanisms which is secreted from outside the cell and helps to destroy host tissues and infection. Many types of extracellular proteases enzymes were produced from *P. aeruginosa* including Alkaline protease (AprA), Elastase (LasA and LasB) and Protease IV. LasA and LasB are produced by *P. aeruginosa* to destroy elastin, an important component of the pulmonary tissue and blood vessels, impairing lung function and causing pulmonary hemorrhage (Everett *et al.*, 2023). An increased level of these proteolytic enzymes contribute to increased tissue destruction and bacterial invasion associated with *P. aeruginosa* otitis (Qin *et al.*, 2022) (Everett and Davies, 2021).

3-production of Lecithinase : is one of the most important virulence enzymes, as it degrades lipids that are stable in cell membranes, and then decomposes and the tissue dies. (AL-Samaraey and Jafar, 2021).

4-Metallo beta-lactamase(MBLs); enzymes are among the important clinical problems that raise concerns in all societies in the world because of their analysis and inhibition of almost all antibiotics containing the beta-lactam ring (Zakhour, *et al.*, 2015). MBL enzymes were first identified in 1966 from bacteria *Bacillus cereus* (Boyd *et al.*, 2020). The production of

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the MBL enzyme by *P.aeruginosa* were detection at 1991 in Japan, and after that it spread to other countries of the world (Peirano *et al.*, 2024). This have a broad spectrum of hydrolytic activity that includes every group, penicillin's, cephalosporin's, and carbapenem antibiotics (Furniss *et al.*, 2022).

E- Toxins

Exotoxin (Type III Secretion System) is important virulence factor of *P. aeruginosa*, shared with many other Gram-negative bacteria, T3SS are a complex “molecular syringe” able to inject different effectors in host cells, subverting cell machinery influencing immune responses, and increasing bacterial survival rates (Horna and Ruiz, 2021). As well as silence specific eukaryotic cell signals, to hinder or elude the immune response and cause apoptosis (Rahmatelahi *et al.*, 2021). Among the many functions of T3SS effectors are enhancing the bacterial invasion, inhibiting phagocytosis, inhibiting intracellular killing, promoting bacterial trafficking and activating cell death pathways (Lombardi *et al.*, 2019). Four effector toxins of *P. aeruginosa* identified: ExoS, ExoT, ExoU and ExoY. The four effector toxins are rarely all present in one strain, which assist *P. aeruginosa* in evading host defense systems (Wagener *et al.*, 2020).

2.3.2.5. Antibiotics Resistance of *P. aeruginosa*

Bacteria have high level of intrinsic antibiotic resistance due to decreased outer membrane permeability, active efflux mechanisms, and the production of antibiotic-inactivating enzymes (Verdial *et al.*, 2023). In addition to a remarkable intrinsic resistance to several antibiotics, *P. aeruginosa* can acquire resistance through chromosomal mutations and acquisition of AR genes *P. aeruginosa* has one of the largest bacterial

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genomes and possesses a significant assortment of genes acquired by horizontal gene transfer (HGT)(Botelho *et al.*, 2019). *P.aeruginosa* is frequently resistant to several antibiotics, being in the (critical) category of the WHO priority pathogens list for research and development of new antibiotics *P.aeruginosa* is one of the “ESKAPE” pathogens, emphasizing its impact on hospital infections and the ability of this microorganism to “escape” the activity of antibacterial drug (Boucher *et al.*, 2009; Mancuso *et al.*, 2021).

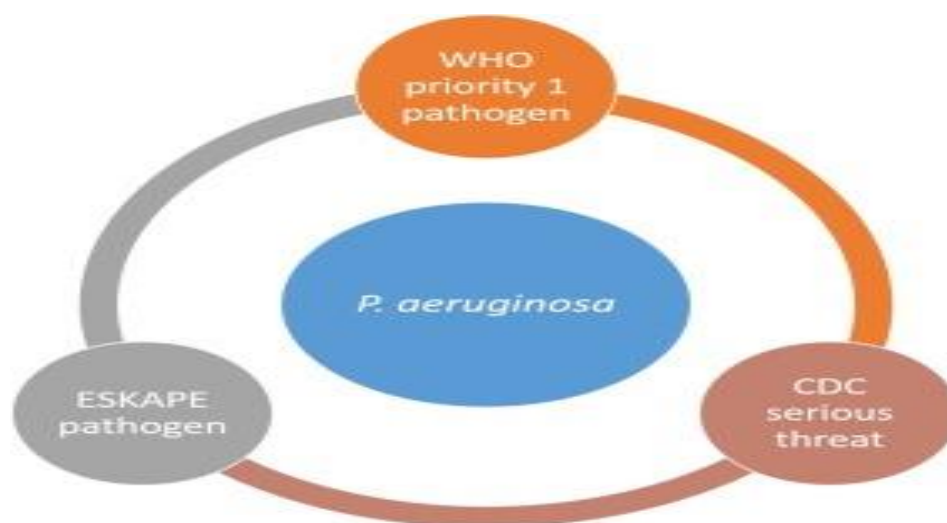


Figure (2-5) The scheme of *P. aeruginosa*. WHO classifies *P. aeruginosa* as a “critical” pathogen, which work to development of new antibiotics, whereas the US Center for Disease Control flagged multidrug-resistant *P. aeruginosa* as a serious threat and as one of the ESKAPE pathogens causing nosocomial infections worldwide(Botelho *et al.*, 2019).

2.4. Nanotechnology:

Nanotechnology is the engineering and manipulation of materials at the nanoscale. This field covers numerous fields of science, such as engineering, biology, chemistry, and physics (Suhag *et al.*, 2023). One contemporary technology that is crucial to daily living is nanotechnology. The creation, modification, and use of materials containing nanometer-

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sized particles are the focus of nanotechnology. (Thakur *et al.*, 2022). Nanotechnology is primarily concerned with 1-100 nm nanoparticles size in one dimension used in atomic physics, medical chemistry, and other developed fields (Khan *et al.*, 2022)

Biological synthesis is most acceptable than other methods for nanoparticle synthesis. It provides advancement over physical and chemical methods as it is costeffective, environment-friendly, and easily scaled up for large-scale synthesis. In this method, there is no need to use high pressure, temperature, energy, and toxic chemical.

The use of eco-friendly materials, such as plant extract (leave, flower, bark, seed, peels, etc.), bacteria, fungi, and enzymes for nanoparticle synthesis provides various advantages of ecofriendliness and versatility for pharmaceutical and other biomedical applications because they do not use harmful chemicals for the synthesis process as in figure(2-5) (Mughal *et al.*, 2021). Numerous formulations of nanoparticles have been developed to be safe and biocompatible for usage in a range of contexts, including medical. Biocompatible nanoparticles successfully fight microbial infections while lowering the possibility of negative health effects on humans(Damasco *et al.*, 2020). Presently using nanotechnological solutions, that include nanoparticles represents one of the possible ways to overcome the resistance to antibiotics in bacteria (AlMatar *et al.*, 2018) .

2.4.1. Nanotechnology Using as Antimicrobial Activity

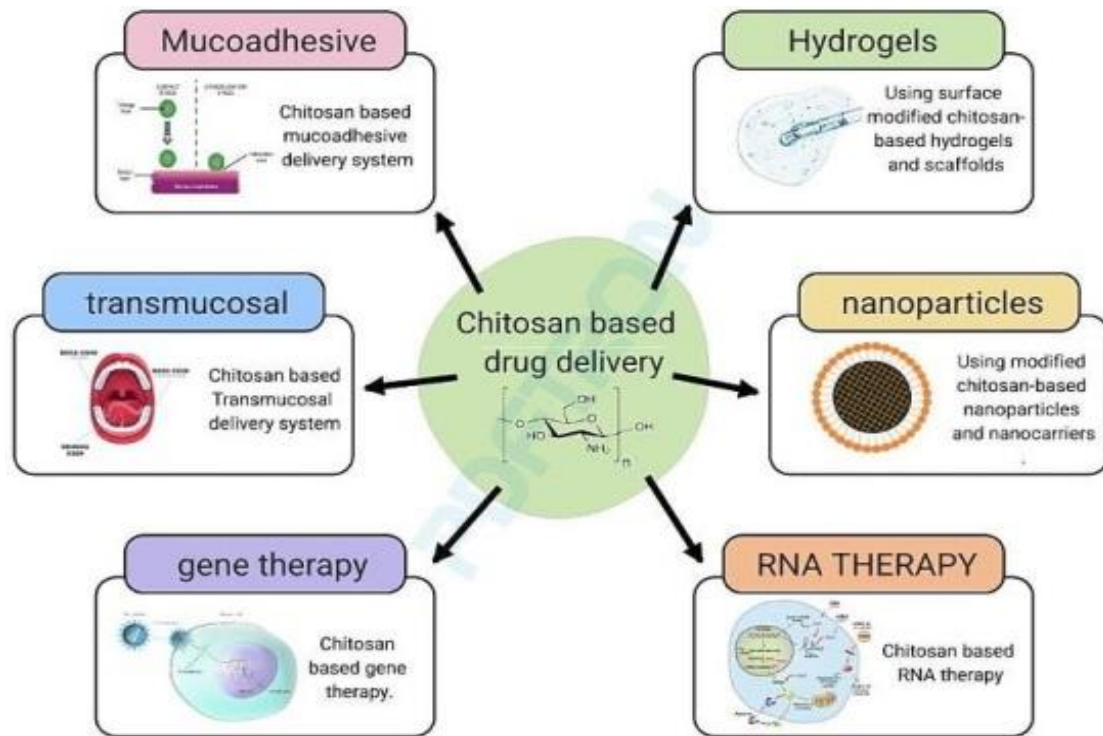
In place of antibiotics, nanoparticles (NPs) are being employed more frequently to target microorganisms. Nanotechnology might be useful for treating bacterial infections in particular (Mubeen *et al.*, 2021). The antibacterial properties of a variety of nanostructures have been investigated. In understanding the antibacterial properties of these

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nanostructures, it is important to recognize that while some metals, such as zinc, silver, and copper, exhibit antibacterial mechanisms in their bulk form, other materials, such as chitosan, are not antibacterial in their bulk form but may exhibit antibacterial properties in nanoparticles form (Seil and Webster, 2012) (Chandrasekaran, *et al.*, 2020). The mechanism of this antibacterial activity varies from nanoparticle to nanoparticle. For all varieties of nanoparticles, the antibacterial mechanism is not fully understood. While some proposed mechanisms relate to the physical structure of the nanoparticles, others relate to the enhanced release of antibacterial metal ions from nanoparticle surfaces. There are moreover, limited data on nanoparticles toxic effects and destiny in biological processes. Nanomaterials' possible exposure paths are inhalation, ingestion, and dermal penetration (Singh *et al.*, 2022).

2.4.2.Chitosan Nnoparticles

Chitosan nanoparticles, which have drawn a lot of interest in recent decades, are potential polymeric and bio-based NPs. These nanoparticles, which are easily obtained from natural sources, have enormous promise as nano carriers that may encapsulate materials like medications or active molecules and deliver them to a particular location (Naskar, *et al.*, 2019). Chitosan nanoparticles (CNP) include features and advantages of both chitosan and nanoparticles. Because it is inexpensive and widely accessible, chitosan has a broad range of uses, particularly in medicine, such as wound healing (Bashir *et al.* , 2022). and in the production of drug delivery system. Chitosan also has mucoadhesive characteristics, which enables ChNP to be delivered by transmucosal routes including intranasal, intraocular, intratracheal, intrapulmonary, and others. (Omidian *et al.*, 2024).



Figure(2-6): explicates chitosan nanoparticles, an approbative for the delivery CH-based drug delivery systems, (Haider *et al* 2024)

Chitosan is a biopolymer that consists of a straight chain and is a cationic polysaccharide. It is produced via the partial deacetylation of chitin as appears in Figure(2-9). Chitosan has been used in the fabrication of nanoparticles. CNPs are materials with unique physicochemical characteristics, biocompatible, biodegradable, less toxic, easy to prepare and have a wide range of applications in medicine, agricultural and pharmaceuticals. Due to their extremely small size, CNPs exhibit interesting interaction and surface characteristics. It is biocompatible and have been used in drug delivery, advanced cancer therapy and biological imaging and diagnosis (Iber, 2022)

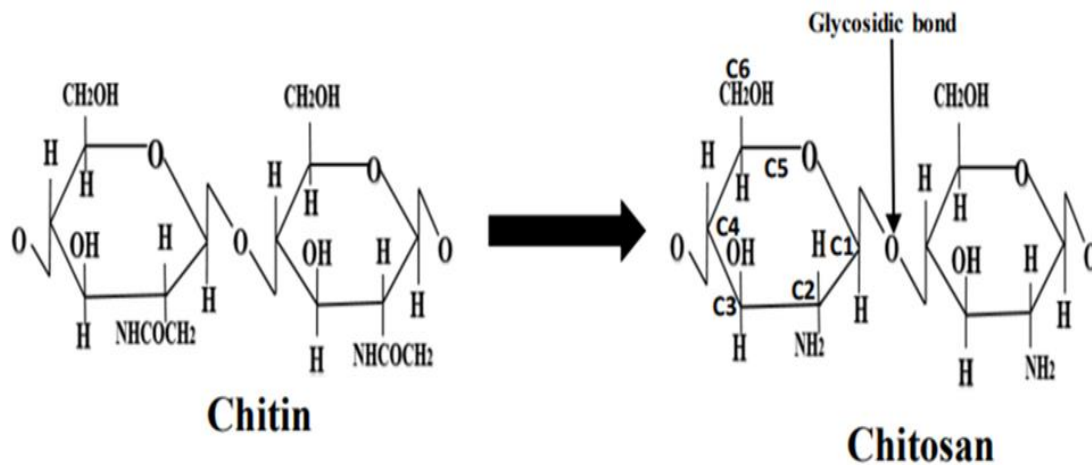


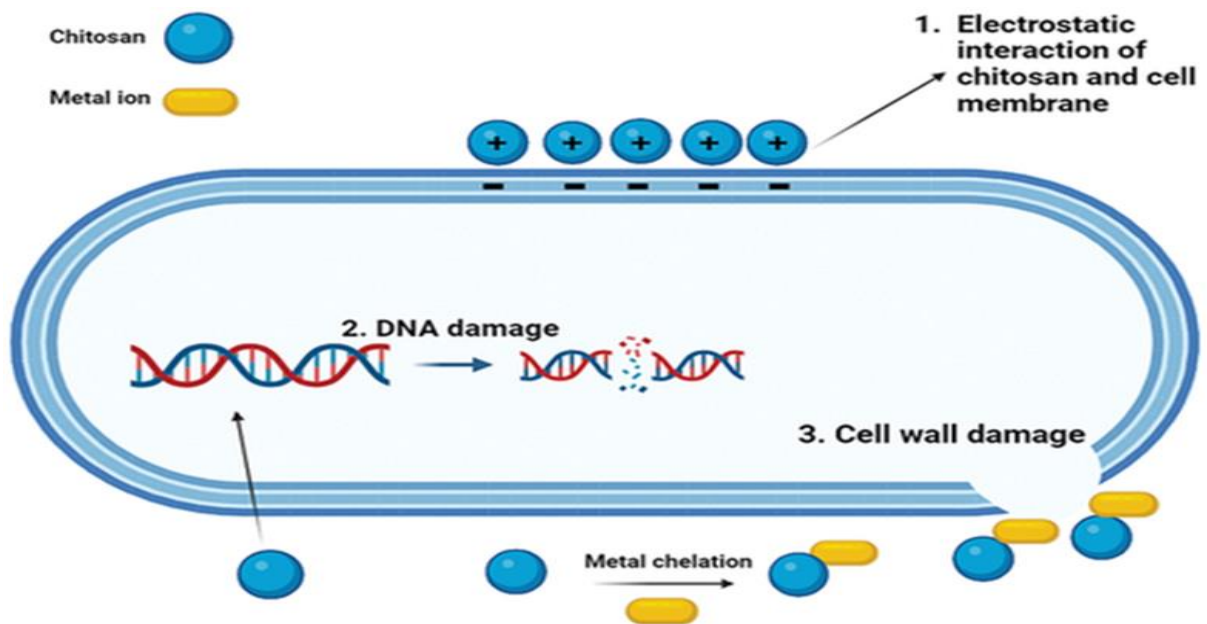
Figure (2-7): Show structure of Chitosan

2.4.3. Antimicrobial Mechanism of Chitosan

Numerous methods have been postulated to explain chitosan's antibacterial activity, including as electrostatic contact, interaction with bacterial DNA, and metal chelation, as seen in Figure(2-10). There is more than one mechanism behind chitosan's antibacterial action; the processes outlined are not mutually exclusive and most likely occur simultaneously or sequentially. Chitosan has demonstrated that it can be either bacteriostatic, which just prevents the bacteria from growing, or bactericidal, which means it may kill the germs (Marambio and Hoek 2010). Because of their different cell surface structures, Gram-positive and Gram-negative bacteria have different susceptibilities to chitosan. Unlike Gram-positive bacteria, which are composed of peptidoglycan and teichoic acid, Gram-negative bacteria's cell membranes include lipopolysaccharide, which is made up of anionic groups (phosphate and pyrophosphate groups) that give the cell surface more negative charges (Pasquina-Lemonche *et al.*, 2020). The question of which bacteria are more vulnerable to chitosan is up for debate. While some research indicates that Gram-positive bacteria are more sensitive to chitosan, other studies indicate that Gram-negative bacteria are more vulnerable(Guo *et al.*, 2019). while some demonstrate

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that both Gram-positive and Gram-negative bacteria are equally susceptible(Wang *et al.*, 2021). The most widely recognized method of chitosan's antibacterial activity involves electrostatically attaching itself to the negatively charged bacterial membrane. Through electrostatic interactions, the negatively charged bacterial cell membrane and the positively charged chitosan (the protonated NH_3^+ groups) change the permeability of the cells. When chitosan builds up on the cell surface, it disrupts the membrane, which leads to the membrane potential breaking down, allowing cellular components to flow out and ultimately killing the bacterium(Khan *et al.*, 2020).



Figure(2-8): Chitosan's several antibacterial mechanisms (Dhlamini *et al.*, 2024)

Chapter Three

Materials and Methods

Materials and Methods:

3.1. Materials:

3.1.1. Devices and equipment's

all devices and equipment that were used listed in table (3-1).

Table (3.1): Equipment and devices.

NO.	Devices and equipment	Company(origin)
1	Autoclave	Korea
2	Balance (Electrical)	Memmert /Germany
3	Burner	Amal / Turkey
4	Sensitive Balance	Germany
5	Micropipette	Humpitte/ Germany
6	Loop	India
7	Refrigerator	Concord(Korea)
8	Oven	Germany
9	Light Microscope	Novex(Holland)
10	Vortex mixer	Korea
11	Incubator	Korea
12	Hot plate magnetic stirrer	LABINCO / Korea
13	Water Bath	Korea
14	Eppendorf- centrifuge 1500g/1 min	china
15	Centrifuge	Korea
16	Cotton swap	AL-Rawbi / China
17	Cylinders (500, 250 ml)	HBG / England
18	Conical flasks (500 ml, 250 ml)	HBG / England
19	Disposable Petri Dishes	China
20	Automatic micropipette	Slammed / Germany
21	Microscope cover slides	Citogias / China
22	Microscope slides	Sail Brand / China
23	Transporter media swabs	Girenier / China
24	Plastic rack	Meheco / China
25	Disposable syringes (3cc, 10 cc)	UAE
26	Tips	Eppendorf / China
27	Distill Water	Iraq

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28	UV Gel documentation	Cleaver (UK)
29	Gel Electrophoresis system	Cleaver (UK)
30	Thermal Cycler (PCR)	Potimax / Germany
31	Microplate Reader — FlexA-200/FlexA-200HT	China \Hangzhou Allsheng Instruments Co., Ltd.
32	Micropepette Variable Volume 2-20 µl , 0.5-10 µl, 20-200 µl 10-100 µl ,100-1000 µl	Labnet\ USA

3.1.2 Chemical solutions

The chemicals, reagents, kits, solutions, and dyes that were used in laboratory experiments were listed in Table (3-2).

Table (3-2): Chemical, reagents, kits, solutions and dyes used in this study.

NO.	Chemical materials and Kits	Company- Origin
13	1-ethyl-3(3-dimethylamin propyl) carbodiimid Carboxylic acid	BDH / England
3	Acetic acid	India
14	Chitosan nanoparticle	Canada
10	Dextran sulfate	BDH / England
4	Ethanol 95%	India
2	Glycerol	Fluka / Switzerland
1	Gram stain kit	Sybio /S.A.R
7	Hydrogen peroxide (H ₂ O ₂)	BDH / England
9	Lactic acid	BDH / England
11	Methane sulfonic acid	BDH / England
12	n-hydroxy succinimide	BDH / England
5	Normal saline	SDI /Iraq
8	Pentetic acid	Korea
6	Sodium hydroxide (NaOH)	BDH/ England
15	Vitek-2 compact system \ kits	BioMérieux/France

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3.1.3. Synthetic Culture Media

The synthetic bacteria culture media used in this study were presented in the table(3-3)

Table (3-3): Synthetic media and Manufacturing Company.

NO.	Culture Media	Company
1	MacConkey agar	Oxoid\ India
2	Blood agar Base	Oxoid\ India
3	Mannitol salt agar	Oxoid\ India
4	Muller hinton agar	Oxoid\ India
5	Muller hinton broth	Himedia\ India
6	Brain heart broth	Himedia\ India
7	Brain heart agar	Himedia\ India
8	Nutrient agar	Himedia India
9	Nutrient broth	Himedia India

Table(3-4) The chemicals of DNA Extraction, PCR & Electrophoresis used in molecular analysis part .

No.	Chemical and Biotical materials	Company(origin)
1	DNA Extraction Kit	Geneaid (Taiwan)
2	Ethanol 95%	Chemik India)
3	Master mix 2X Amplicon	Denmark
4	Agarose	Bioner (South Korea)
5	Oligo Primers	Macrogen (Korea)
6	Loading Dye	Promega (USA)
7	DNA & Gene Dye "Safe- Green abm"	ABM (Canada)
8	TBE buffer	Bioneer (South Korea)
9	Nuclease free water	Promega (USA)

Chapter Three Materials and Methods

3.1.4 Antibiotic Discs

Antibiotic discs that were used in this study to test the antimicrobial sensitivity by the disc diffusion method were listed in Table (3-5), according to CLSI (Clinical and laboratory standards Institute, 2023)(Tamma, *et al.*,2023) .

Table (3-5): The antibiotics used for this study (Himedia / India)

No.	Antibiotic Disc	Code	Concentrations
1	Ampicillin	AMP	25
2	Ciprofloxacin	CIP	10
3	Ceftazidime	CAZ	30
4	Amoxicillin/ Clavulanic	AMC	30
5	Piperacillin	PRL	100
6	Rifampin	RA	5
7	Cephalexin	CL	30
8	Tobramycin	TOB	10
9	Erythromycin	E	10
10	Clindamycin	DO	10
11	Vancomycin	VA	30

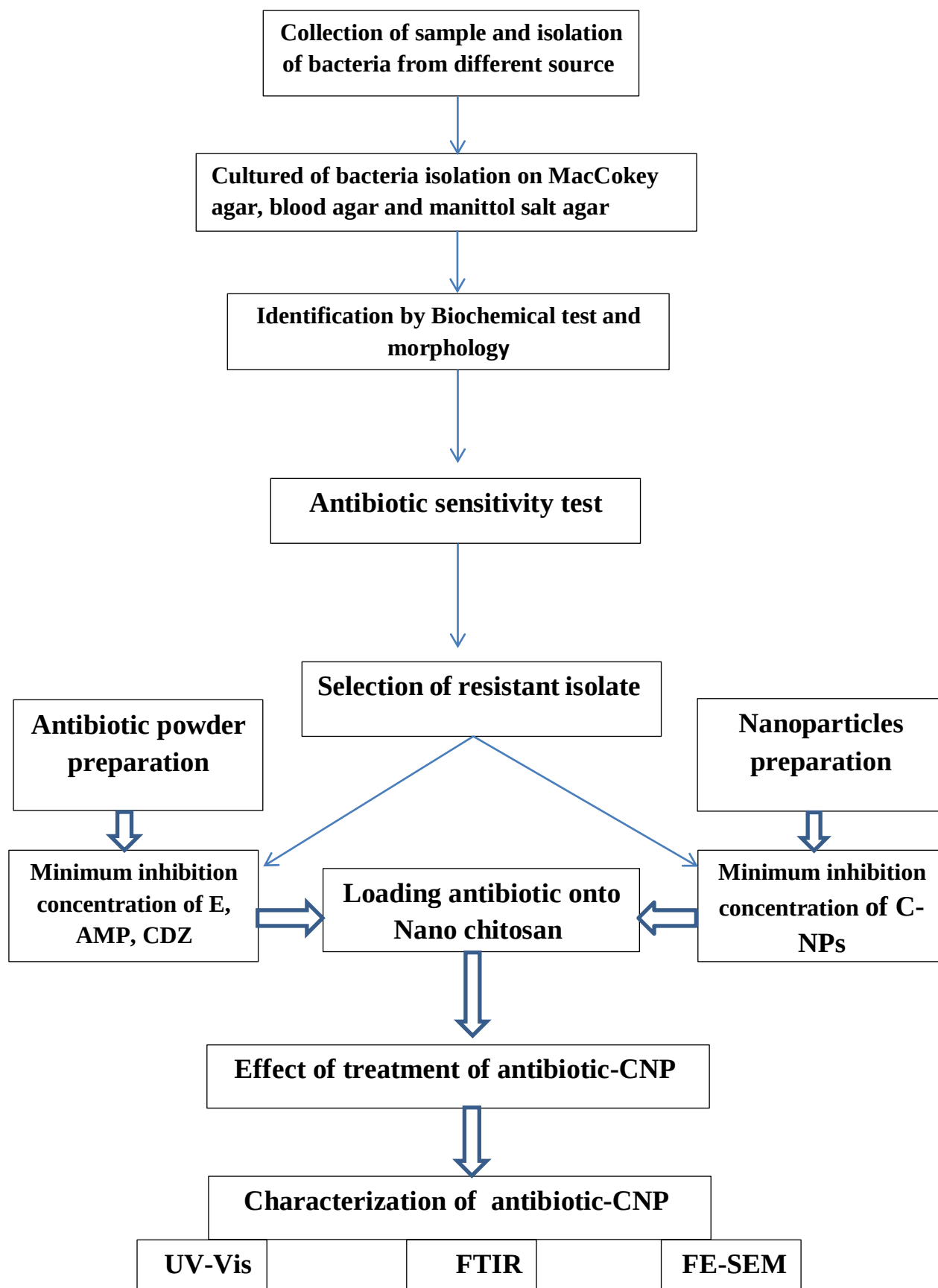
3.1.5. Antibiotic Powders

The antibiotic powder was used in this study.

Table (3-6): Antibiotic Powder, Their Company, and Origin.

No	Antibiotic powders	Manufacturing Company
1	Ceftazidime	Hyperchem / china
2	Erythromycin	Hyperchem / china
3	Ampicillin	Hyperchem / china

Study Design :



3.2. Methods:

3.2.1. Samples collection:

One hundred swab samples of burn injuries , wounds, bronchioalvilar lavage (BAL) and cerebro spinal fluid (CSF) were collected from patients admitted to hospitals in thi-qar province (Nasiriya City Hospitals, Al-shatrah Hospital, al- hussin teaching Hospital, Al-haboby Teaching Hospital). From the period from October, 2024 to April, 2025. The permission and ethical approval were received from the Thi-Qar health directorate (number 11415, on November 20, 2024). In appendix(1). Samples were collected in burn injuries of the first, second and third-degree, according to the doctors' diagnosis, as well as injuries of skin wounds, bedsores, and post-operative infection, and amputations. All 100 samples were collected by sterile cotton swabs and the samples which were transported to the laboratory without delay. The samples were inoculated on blood agar and MacC agar overnight at 37 °C under aerobic conditions. All growing bacterial isolates were subjected to preliminary diagnostic laboratory tests by using Gram stain, as well as some biochemical tests.

3.2.2. Preparation of cultural media

All media were prepared according to their manufacturer's instructions, then autoclaved at 121°C, 15 pounds/Inch² for 15 minutes and distributed into sterile Petri- dishes or tubes, and left to solidify at room temperature. The Petri- dishes were incubated at 37°C for 24 hours, to ensure the sterility. Some of these media need special additives. After that they were ready to use for isolation of bacteria or kept at 4 °C (Orekan, J. et al., 2021) :

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3.2.2.1.Blood agar (BA)

Blood agar is a general-purpose, enriched medium often used to grow fastidious bacteria and it is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria . It was prepared by adding of 40 g medium in to 1 L of D.W, then the medium heated for 1 minute, autoclave for 15 minutes at 121°C. Cooled to 45-50 and add sterile blood agar 5-7 percent .Finally, the media was poured in to petri dishes and store.

3.2.2.2. Mannitol Salt Agar (MSA)

Mannitol Salt Agar (MSA) is a selective and differential medium. The high concentration of salt (7.5%) selects for members of the genus *Staphylococcus* since they can tolerate high saline levels. It also contains the sugar mannitol and the pH indicator phenol red. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow. Most pathogenic staphylococci, such as *Staphylococcus aureus*, will ferment mannitol. It was prepared by solving 111.025 g medium into 1L of distilled water, Boiling to fully dissolved the media. After autoclaving, the medium cool down to 45-50°C and then pour into plates.

3.2.2.3.MacConkey agar medium

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. It was prepared according to their manufacturer's instructions then autoclaved, cooled to 50-55°C then poured into the sterile petri dishes and were stored at 4°C until utilized.

3.2.2.4. Mueller Hinton Agar (MHA)

Mueller Hinton Agar is a standardized solid medium recommended for the study of the susceptibility of bacteria to antimicrobial agents by the method of diffusion (Kirby-Bauer method) or dilution in agar. It was prepared according to its manufacturer's instructions then autoclaved, cooled to 50-55°C then poured into the sterile Petri dishes, and were stored at 4°C until utilized.

3.2.2.5. Brain- Heart infusion broth (BHIB)

Brain heart infusion (BHI) is a growth medium for growing bacteria. It is a nutrient-rich medium used to culture a variety of fastidious bacteria. It was prepared according to their manufacturer's instructions and poured into sterile test tubes, that were sterilized by autoclaved, the tubes were incubated at 37°C for 24 hours, to ensure sterilized after that they were stored at 4°C until utilized.

3.2.2.6. Nutrient Broth (NB)

Nutrient Broth is a liquid medium used for the cultivation of a wide variety of bacteria from clinical specimens and other materials. It was prepared in 1000 ml of distilled water by dissolving 13 g of nutrient broth, changing the pH to 7-7.2, and poured into sterile test tubes then autoclaved, after that they were stored at 4°C until utilized.

3.2.1. Sterilization Methods

A- Autoclave sterilization: All media were treated in an autoclave at 121 °C, 15 pounds/inch² for 15 minutes.

B-Filtration sterilization The temperature-sensitive solution was sterilized by filtrations through a 0.2µm Millipore filter. C- Dry heat sterilization: The glassware was sanitized in an electric oven using dry heat. at 160-180 °C for two to three hrs (Chauhan, and Jindal, T. 2020).

3.2.3. Identification of bacteria:

3.2.3.1. Morphological Examination

The morphological characteristics of colonies, including edges, texture, color, and form, were used to presumptive identification diagnosis. of the bacterial colonies growing on Mannitol salt agar, MacConkey agar, and Blood agar (incubated at 37°C for 24 hours) (Subhi *et al.*, 2017).

3.2.3.2. Microscopic Examination (Gram staining)

The colonies were stained by using the Gram staining technique. A bacterial smear was prepared by mixing a pure colony with water on a microscope slide. The cells were then fixed by moving slides through the flame. The slide was then immersed for 30 seconds in crystal violet and washed in tap water. Then, it was soaked in iodine for 30 seconds and washed with tap water. The slide was de-colored with 95% ethanol, and then sprayed for 30 seconds immediately with safranin, wash with tap water, and dry air. The slide was examined under a light microscope, with immersed oil (Tripathi and Sapra, 2020) .

3.2.4. Biochemical reaction identification

Bacterial isolates were subjected to biochemical tests. These tests facilitated the identification and characterization of the isolates. The biochemical tests used in this study were as following:

3.2.4.1-Catalase test. This test is used to differentiate those bacteria that produce the enzyme catalase from non-catalase-producing bacteria. A loop was used to transfer a small amount of colony on the surface of a clean dry glass slide, a drop of 3% H₂O₂ was placed on the slide, and bubbles were observed as a positive result. (Abdulhusein *et al.*, 2024).

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3.2.4.2- Oxidase test. This test was used to determine the ability of an organism to produce enzyme oxidase. By using a wooden stick to remove a small portion of a bacterial colony (18-24 hrs.) from the agar surface and rubbing the sample on the filter paper that was impregnated with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride), changing color was observed to deep blue or purple within 10 seconds as a positive result. (Abdulhusein *et al.*, 2024).

3.2.4.3. Identification of bacteria by Vitek 2 system:

The procedure was done according to Vitek-2 compact system method and worked according to the protocol (BioMerieux, Marcy L'Eyoily, France) (Putra *et al.*, 2020):

1. Inoculated aloopful of isolated colony to 3 ml of normal saline, which were placed in kahn tube and mixed by vortex.
2. The kahn tube was checked for standardization to McFarland standard (1.5×10^8 CFU/ml) by using DensiCHEK Plus meter (the density must be 0.6 – 0.8) and spectrophotometer 620 nm.
3. In kahn tube, ID card was inserted into the cassette and identification number of a sample entered via barcode.
4. In the filler module the cassette then placed, when the card was filled, transferred the cassette to the reader and incubator module.
5. The incubation temperature, the optical reading of the cards, and monitors were controlled by the instruments and data transfer to the computer for analysis. Then the system automatically ejected the cards into a waste container when the test cycle was completed.

3.2.5. Preservation and maintenance of bacteria by glycerol

The method of bacterial storage for three months in Brain Heart infusion broth (BHIB) containing 15% glycerol. This was done by

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adding 15ml of glycerol to 85ml of BHIB and sterile in 121° C for 15 minutes (Jana and Singh 2020).

3.2.6. Antibiotics susceptibility test (AST)

First: Mueller-Hinton plates need to be prepared

Mueller Hinton agar was made from a dried base, according to Himedia/India. The temperature of the incubator's medium was kept between 45 and 50°C. right before plating. Plates were put upright in the incubator and dried at 35 °C for 10 to 30 minutes as soon as the agar solidified.

Second: Preparation of inoculums (turbidity standard)

After putting 3-5 colonies into a tube of 5ml of sterile normal saline, mixed well, and then the tube was compared with the turbidity standard of McFarland 0.5, the inoculums were used within 30 minutes of preparation.

Third: Inoculation of the test plates

These excess inoculums were removed by pressing, and rotating the swab firmly against the side of the tube, then the swab was rubbed everywhere on the surface of the medium 3 times rotating the plate through at an angle of 60 after each application. Finally, the swab was passed around the edge of the agar surface. Next, the inoculum was left to dry for a few minutes at room temperature with the lid closed. Sterile Forceps were used to position the antibiotic disc on the inoculation plate (each plate with 4 discs). Within 30 minutes, the plates were incubated at 37 °C in an inverted position for 18–24 hours. After overnight incubation, the diameter of every zone including the diameter of the disc was measured in millimeters using a metric ruler. Clinical and laboratory standards institute guidelines assessed the inhibition zone width of each antibiotic disc for sensitive (S), intermediate(I), and resistant(R) groups

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and translated it appropriately (CLSI, 2022) (Nyuykonge *et al.*, 2021; Aguilera-Alonso *et al.*, 2022).

3.2.7. Antibiotics susceptibility test and (MIC) by Vitek 2 system

The procedure were done according to (Yoo *et al.* 2020) The 0.5 McFarland bacterial suspension was diluted to 1.5×10^8 CFU/ml by using DensiCHEK plus meter in 0.45% saline. Cards were automatically filled by bacterial suspension diluted and then Cards were loaded into the Vitek- 2 system for incubation and reading after 8h.

3.2.8. Genomic DNA extraction from Bacterial Culture

1. DNA extraction

Genomic DNA was extracted from bacterial culture isolates by using Gene aid Genomic DNA extraction Kit (Taiwan).

The bacterial culture has been inoculated in 10 ml nutrient broth medium and incubated at 37°C overnight in shacking incubator.

- 1- Transfer bacterial cells (up to 1×10^9) to a 1.5 ml micro centrifuge tube. Centrifuge for 1 minute at 15000g then discard the supernatant.
- 2- Transfer the required volume of Gram+ Buffer (200 µl/sample) to a 15 ml centrifuge tube. Add Lysozyme (0.8 mg/200 µl) to Gram+ Buffer (in the 15 ml centrifuge tube) then vortex to completely dissolve the Lysozyme. Transfer 200 µl of Gram-positive Buffer (make sure Lysozyme was added) to the sample in the 1.5 ml micro centrifuge tube then re-suspend the pellet by vortex or pipette. Incubate at 37°C for 30 minutes. During incubation, invert the tube every 10 minutes, as for Gram-negative bacteria, add 200 microliters of GT buffer, Subsequent steps were continued for both Gram-positive and Gram-negative bacteria.

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3-Add 20 µl of Proteinase K (make sure ddH₂O was added) then mix by vortex. Incubate at 60°C for at least 10 minutes. During incubation, invert the tube every 3 minutes.

4-Add 200 µl of GSB Buffer then mix by vortex for 10 seconds and incubated at 70°C for at least 10 min. to ensure the sample lysate is clear and during the incubation, invert the tube every 3 min. At this time, pre-heat the required Elution Buffer (100 µl per sample) to 70°C for using in step (8) DNA Elution.

5- Add 200 µl of absolute ethanol and mix immediately by shaking vigorously. If precipitate is appears, break it up as much as possible with a pipette then place the GD Column in a 2 ml Collection tube and transfer mixture (including any insoluble precipitate) to the GD Column and centrifuge at 15000 x g for 2 min . Discard the 2 ml Collection tube containing the flow-through and then place the GD Column in a new 2 ml Collection tube.

6- Add 400 µl of W1 Buffer to the GD Column and Centrifuge at 14-16,000 x g for 30 seconds; discard the flow-through then place the GD Column back in the 2 ml Collection tube.

7- Add 600 µl of Wash Buffer (with ethanol) to the GD Column then centrifuge at 15000 x g for 30 seconds, discard the flow-through and place the GD Column back in the 2 ml Collection tube and centrifuge again for 3 min. at 15000 x g to dry the column matrix.

8- Transfer the dried GD Column to a new 1.5 ml micro centrifuge tube and add 100 µl of pre-heated Elution Buffer into the center of the column matrix then Let stand for at least 3 min. to allow Elution Buffer to be

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completely absorbed and centrifuge at 15000 g for 30 seconds to elute the purified DNA.

Table (3-7) The specific primers used, annealing temperature and the products size

No.	Genus	GENE	Primer Sequence	Annealing temp	Product size
1	S. aureus	mecA-F	5CCTAGTAAAGCTCCGGAA3	54 °C	314 bp
		mecA-R	CTAGTCCATTCCGGTCCA		
2		blaZ-F	ACTTCAACACCTGCTGCTTTC	57 °C	240 bp
		blaZ-R	TGACCACTTTTATCAGCAACC		
3	P. aeruginosa	AmpC-F	GATGAAGGCCAATGACATTCCG	58 °C	742 bp
		AmpC-R	CATGTCGCCGA3CCTTGTAGTAA		
4			MexA-F	CTACGAGGCCGACTACCAGA	59 °C

Table (3-8) Preparation of PCR Reaction compounds

Reagents	Volume µl
Master Mix (Bione4	13 µl
Primer forward	1 µl
Primer reverse	1 µl
DNA template (30-100ng)	3 µl
Free water	7 µl
Total	25 µl

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Table (3-9): protocol PCR amplification for current Gene

Step		Temp.	Time	Cycle
Initial Denaturation		95 C	5.00 min	1 time
	Denaturation	95 C	30 sec.	35 cycle
	Annealing	51-59 C	40 sec.	
	Extension	72 C	45 sec.	
Final Extension		72 C	10.00 min	1 time

2. Agarose Gel Electrophoresis

gel 1.5 % was prepared by mixing 0.45 g agarose with 30 ml of 1 X TBE (Tris Boric EDTA) buffer in a beaker and heating the mixture to boiling point in microwave oven. The solution was left to cool down at 50 – 60 C and the comb was fixed carefully at one end was poured into the gel chamber and left to be solid at room temperature for 20 - 30 minutes.as neatly removed and the gel tray was placed electrophoresis chamber containing 1 X TBE buffer which approximetly reached it to 1 – 2 mm over the surface the gel.

The PCR reactions were tested by horizontal agarose gel electrophoresis. A 1.5% gel was prepared as previously descript. 4 µl of of PCR product mixed with 1 µl of Safe-Gree Dye (abm) then loaded into an insertion cavity (pocket). One pocket per row was used for the length standard (100 bp DNA ladder), volume 4µl of length standard was used to estimate the size and concentration of the PCR products. The electrophoresis was run at 60V and 85 mA when the electrophoresis run was completed the gel was placed on an UV screen To detect the success of the PCR product for studied genes and to know its size compared to the DNA ladder.

3. 2. 9. Preparation of Chitosan Nanoparticle Solution:

A commercially made nano-chitosan was acquired for the Chemazone@ corporation in Canada, as per the specifications provided in Annex No.(2) Several acids were used to create a nano-chitosan solution in order to assess its solubility and the stability of the resultant dispersion. Among the acids that were evaluated were lactic acid, pentetic acid, and hydrochloric acid (HCl). Winayu 2019) (Sapre *et al.*, 2024).

3.2.11. Preparation of Antibiotics Solution:

The antibiotic solutions were prepared using different solvents based on their solubility characteristics. Ceftazidime and erythromycin were dissolved in distilled water, whereas ampicillin required the addition of 1% ethanol to enhance solubility. Various concentrations of each antibiotic solution were prepared and tested for further experimental applications.

3.2.12. Antimicrobial activity of chitosan nanoparticle and antibiotics:

Mueller Hinton agar medium was used for this experiment. The *S. aureus* and *P. aeruginosa* were inoculated into nutrient agar media and incubated at 37°C overnight. The bacterial suspension was prepared by transferring fresh colony bacterial isolate to sterile tubes with 5 ml of normal saline and mixing by a sterile loop and adjusted to 0.5 standard McFarland tube. Agar plate surface was inoculated by spreading a volume of the bacterial inoculum over the entire agar surface using a sterile cotton swab. The agar was left to set at room temperature for 10 minutes. Well, was cut on each plate by using a sterile well borer of about 8 mm diameter. About 80µl of MIC of each chitosan NPs and antibiotics (ceftazedim, ampincillin, erythromycin) solutions were loaded and then incubated at 37°C for 24 h. the inhibition zone was

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observed surrounding the well and measured after incubation(Xiao. 2023).

3.2.13. Load the antibiotic onto the chitosan nanoparticles:

1.Direct method: The lowest non-inhibitory concentration of the chosen antibiotic—that is, a concentration that does not prevent bacterial growth—was mixed with the lowest concentration of chitosan solution. This method seeks to assess the antibiotic's and chitosan's synergistic effects at the lowest possible effective concentrations (Khan *et al.*, 2020).

2.Ionotropic gelation:

We used the following materials in this study; Chitosan, Acetic acid (0.5%), Sodium tripolyphosphate (TPP) –used as a crosslinking agent, Antibiotic (Amoxicillin, Erythromycin, Ceftazidime), Distilled water.

- Chitosan Solution Preparation: We dissolved 1 gram of chitosan in 100 mL of 0.5% acetic acid and stirred the mixture continuously for 1 to 2 hours at room temperature until a clear solution was obtained. Preparation of TPP Solution, we prepared the TPP solution by dissolving 0.5 grams of TPP in 100 mL of distilled water, reaching a concentration of approximately 0.5% (w/v). Antibiotic Loading, added the selected antibiotic (amoxicillin, Erythromycin and Ceftazidime) directly to the chitosan solution and stirred the mixture until the drug was either completely dissolved or homogenized.

-Nanoparticle Formation: We added the TPP solution dropwise to the chitosan–antibiotic solution under constant magnetic stirring. Nanoparticles formed spontaneously through ionic crosslinking between chitosan and TPP. Finally, Nanoparticle Isolation, we centrifuged the nanoparticle suspension at 12,000 rpm for 30 minutes. The resulting pellets were washed with distilled water to eliminate any unbound

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materials. Extraction of the final dry powder was done for antibacterial studies (Ashvini *et al* 2019) (Desai 2016).

3.2.13.Characterization of nanoparticles

The characterizations of the addressed materials were investigated using several analysis techniques such as ultra-violet visible light spectroscopy (UV-Vis), Fourier transforms infrared spectroscopy (FTIR), atomic force microscopy (AFM) and X-ray diffraction. The above-mentioned techniques are described in details in the following subsections .

3.2.13.1. Ultra-Violet Visible Light Spectroscopy (UV-Vis)

Absorbance measurements were carried out using the FlexA-200 Microplate Reader (Allsheng, china). The instrument was operated in absorbance mode across the UV-Visible range, with a wavelength scan set between 200 nm and 1000 nm depending on the assay requirements. Samples were loaded into standard 96-well microplates, and absorbance was recorded using the integrated monochromator. All measurements were performed at room temperature, and background correction was applied using blank wells.



Figure (3-1): UV-Visible Spectrophotometric by Microplate Reader – FlexA-200/FlexA-200HT

3.2.13.2.Fourier Transform Infrared Spectroscopy (FT-IR)

The analytical method known as FT-IR examines chemicals using infrared light.

Its primary goal is to detect the functional groups of molecules and determine their chemical structure . Principle,

Infrared Radiation: The sample is exposed to an infrared light beam.

Radiation Absorption: Each type of bond (C–H, O–H, C=O, etc.) absorbs infrared light at distinct wavelengths, and chemical bonds inside molecules vibrate at certain frequencies. Spectrum Signal Processing(Interferogram) :The FT-IR spectrometer does not directly measure absorption at each wavelength. Rather, it captures an interferogram, which is a composite signal.

This signal is then mathematically separated into an absorption spectrum using a Fourier Transform (Koczoń *et al.*, 2023).

3.2.13.3.Field emission scanning electron microscopy (FE-SEM)

(Hitachi Ltd./Japan) The images were taken with a Field emission scanning electron microscope (SEM) with a resolution of 500 nm (Hitachi s-3400N) and detectors containing a secondary electron; BSE semiconductors (quad-type) were used to study the size and shape nanoparticles , this approach is used to obtain detailed information about surface NPS. was used to characterize the average particle morphology and diameter of the nanoparticles. After using distilled water to sonicate the dried nanoparticles solution sample, a tiny drop of the sample was put on a microscope slide and left to dry. The samples were then coated with a thin layer of platinum to make them conductive.(El-Deeb *et al.*, 2018).

3.2.14. Statistical Analysis of Antibiotic-CNPs Results

Antibacterial activity of the antibiotic–CNP formulations (Ampicillin, Ceftazidime, and Erythromycin) was evaluated against *P. aeruginosa* and *S. aureus* using the agar well diffusion method. Inhibition zones were measured in millimeters.(IBM SPSS, Muijs, 2022) The results were statistically analyzed by using SPSS software and a Chi-square test was applied after categorizing the results into active (inhibition > 0 mm) and inactive (no inhibition). Also, one-way ANOVA was conducted on the inhibition zone diameters, followed by the Least Significant Difference (LSD) test at $p \leq 0.05$ to compare mean differences between treatments.

Results

4.Results

4.1. Isolation and Identification of Bacterial Isolates

Out of One hundred clinical samples 72 (72%) were found to yield bacteria growth, among these , there were 34 isolated identified as *P. aeruginosa* 43 (59.6%) and 23 isolates were *S. aureus* (40.35%). The other bacterial species were neglected in our study.

Samples were taken from burn injuries of the first- second -and third-degree, according to the doctors' diagnosis. as well as injuries of skin wounds and bacterial infections after operations, diabetic foot, and amputations. other samples from the bronchi (BAL) and cerebrospinal fluid (CSF), as mention in table (4-1):

Table (4-1): The distribution of clinical samples by type of injury

Injury type	Number of Isolates (%)
Burns	14
Wounds	32
BAL	9
CSF	2

The bacteria isolates were identified to species level based on traditional biochemical test and automatic system VITEK II.

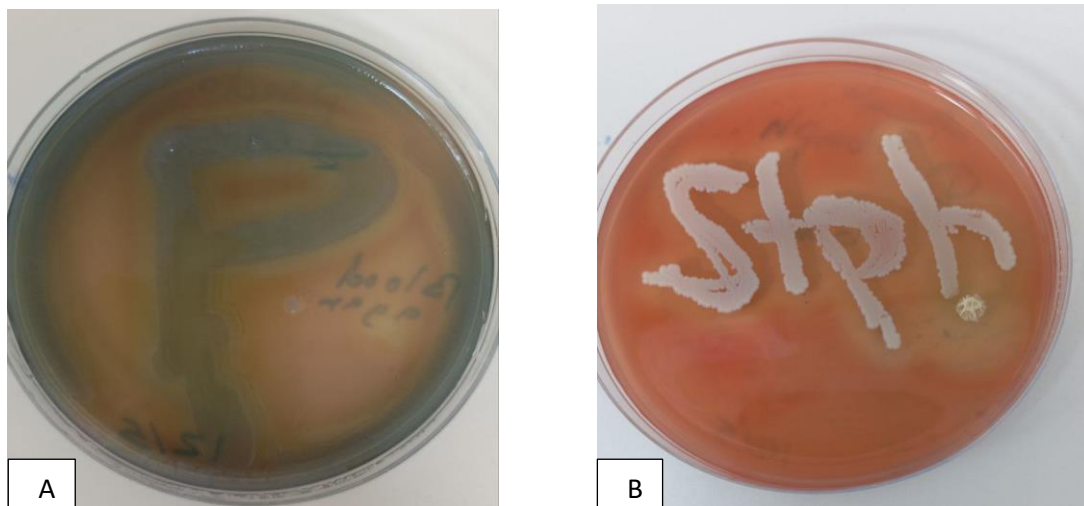
Out of 100 samples, 34 isolates were identified as *P. aeruginosa*, and 23 isolates were identified as *S. aureus* . These bacterial isolates were classified according to their source as presented in table(4-2) .

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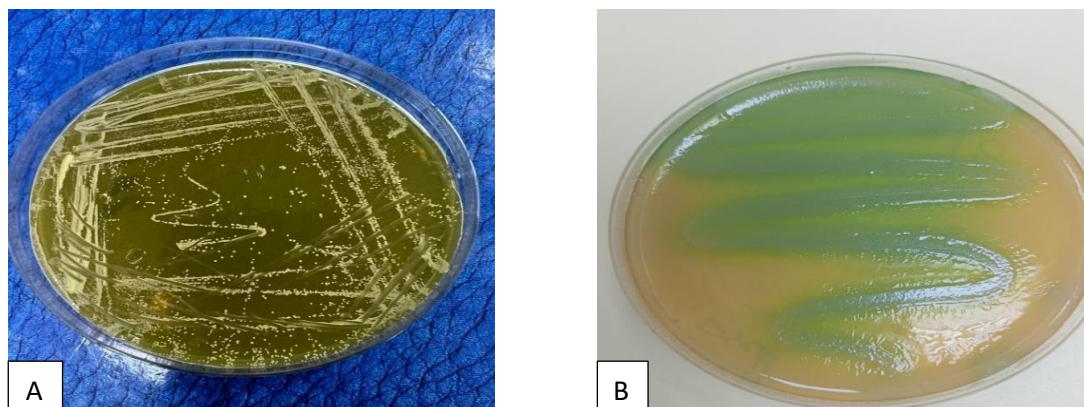
Table (4-2): Types of bacterial isolates by type of injury.

Type of bacterial isolate	Injury type	Number
Staphylococcus aureus	Wounds	14
Pseudomonas aeruginosa	Wounds	16
Staphylococcus aureus	Burns	7
Pseudomonas aeruginosa	Burns	10
Staphylococcus aureus	CSF	2
Pseudomonas aeruginosa	BAL	8
Total		57

The results of the Vitek-2system were in agreement with that obtained from the biochemical tests for used bacterial isolates.



Figure(4-1) shows hemolysis type Beta on blood agar media A- *P. aeruginosa* and B- *S. aureus*



Figure(4-2): A -*P. aeruginosa*, colonies appearance on nutrient agar, B- *S. aureus* on Mannitol Salt Agar

4.2 Antibiotic Sensitivity test

In the Kirby-Bauer method the results showed different levels of sensitivity against combo types of antibiotics commonly prescribed. guidelines A Set of 11 Tests were conducted on antibiotic discs from various classes. against *S. aureus* isolates which included : Ampicillin (25µg), Cefixime (5µg), Erythromycin (10µg), Cephalexin (30µg), Vancomycin (30µg), Clindamycin (10µg), Fusidic Acid (20µg), Rifampin (5µg), Tobramycin (10µg), Gentamicin (10µg), and Meropenem (10µg). However, Ampicillin (25µg), Cefixime (5µg), Erythromycin (10µg), Imipenem, Ceftazidime (5µg), Tetracyclin, Rifampin (5µg), Tobramycin (10µg), Amoxicillin-Clavulanate (30 µg), and Clindamycin (10µg) were used against *P. aeruginosa*.

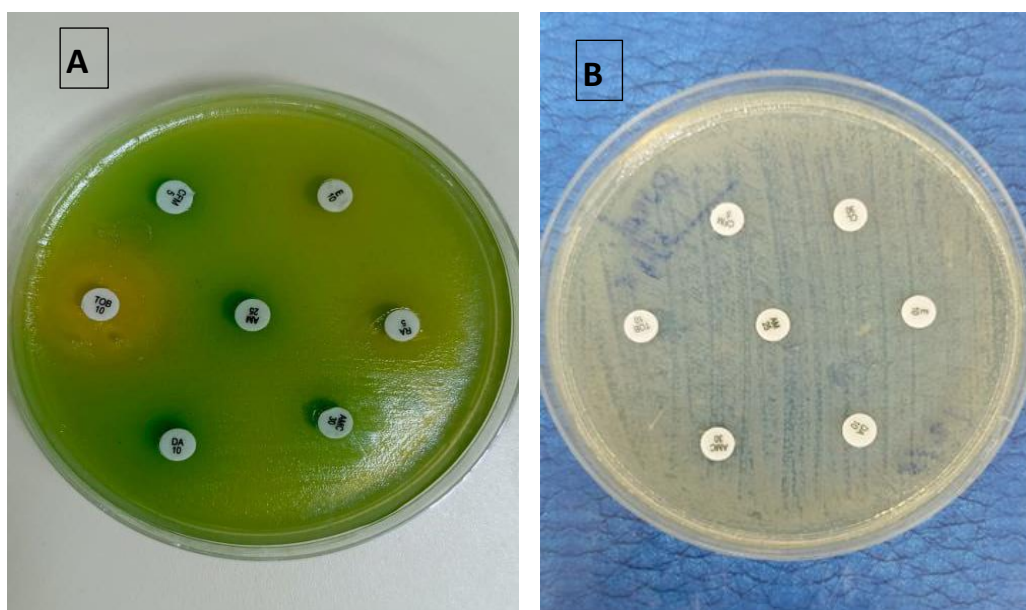


Figure (4-3); show the Antibiotic Sensitivity test on Muller-Hinton agar for A- *P. aeruginosa* and B- *S. aureus*

The results of antibiotic sensitivity test (AST) by using disk diffusion methods found that eight isolates of *S. aureus* (which are including isolates number 13, 14, 18, 19,20, 21, 22, and 23) exhibited resistance to all eleven tested antibiotics; consequently, it classified as MDR and XDR isolates at the same time, as show in figure (4-4). The resistance ration of isolates 1, 2, 4, and 12 was 55%. The least resistance was 27% by isolate number 8 although it was

Chapter Four.....Results

positive to the *blaZ* and *mecA* genes. Furthermore, isolate no.15 showed resistance to all antibiotics except vancomycin and it was intermediated sensitive (I) as shown in Figure (4-5).

The results showed that the highest resistance rate was against Ampicillin (AM) at 86.1%, Gentamicin (CN), and Tobramycin have resistance rates of 82.6%. These antibiotics were the least effective against *S. aureus*, indicating a significant challenge for treatment options involving these drugs.

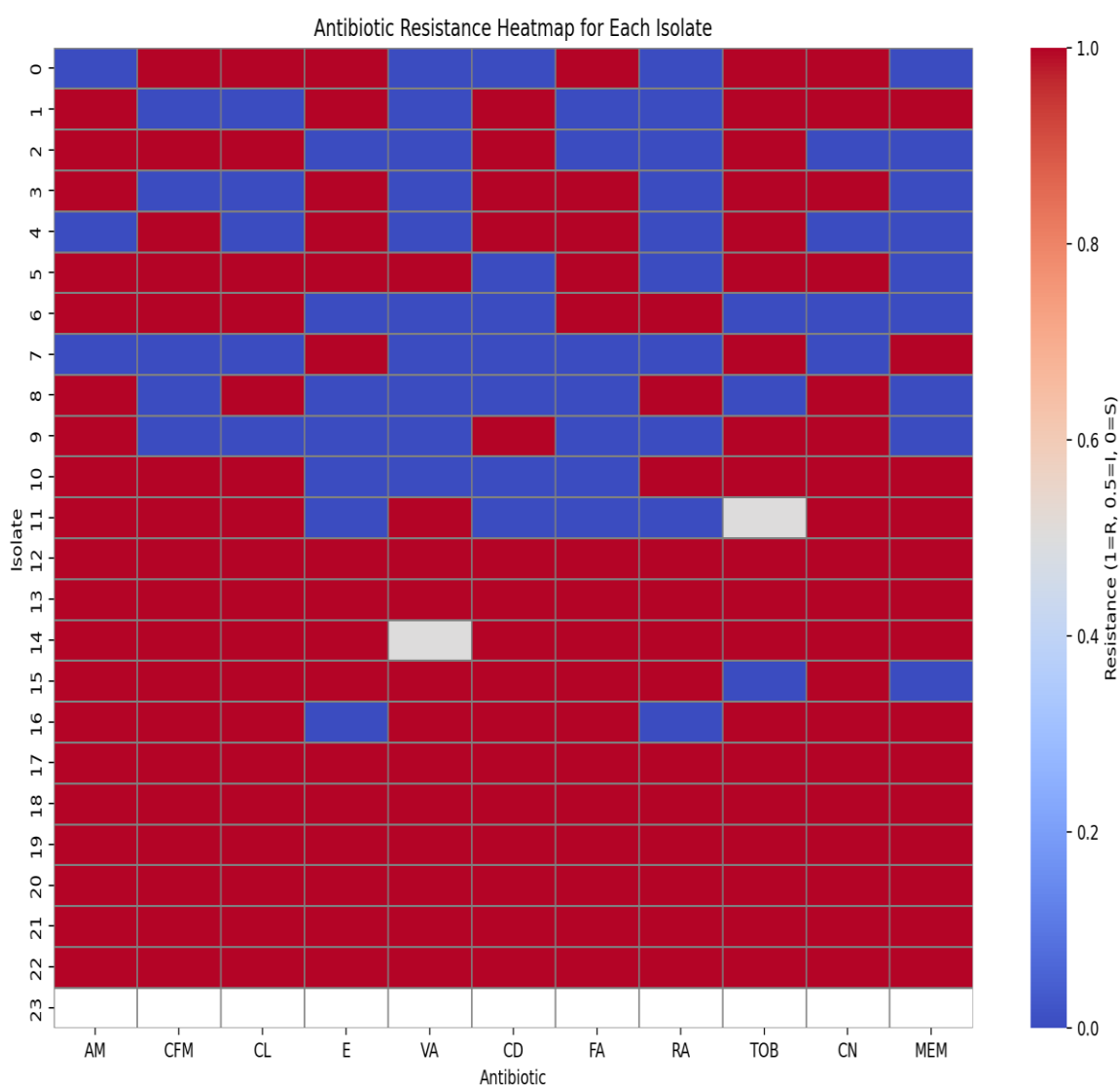


Figure 4-4. The heatmap showing antibiotics sensitivity test of *S. aureus* isolates using disc diffusion technique. S (Sensitive-blue), I (Intermediate), R (Resistance- Red)

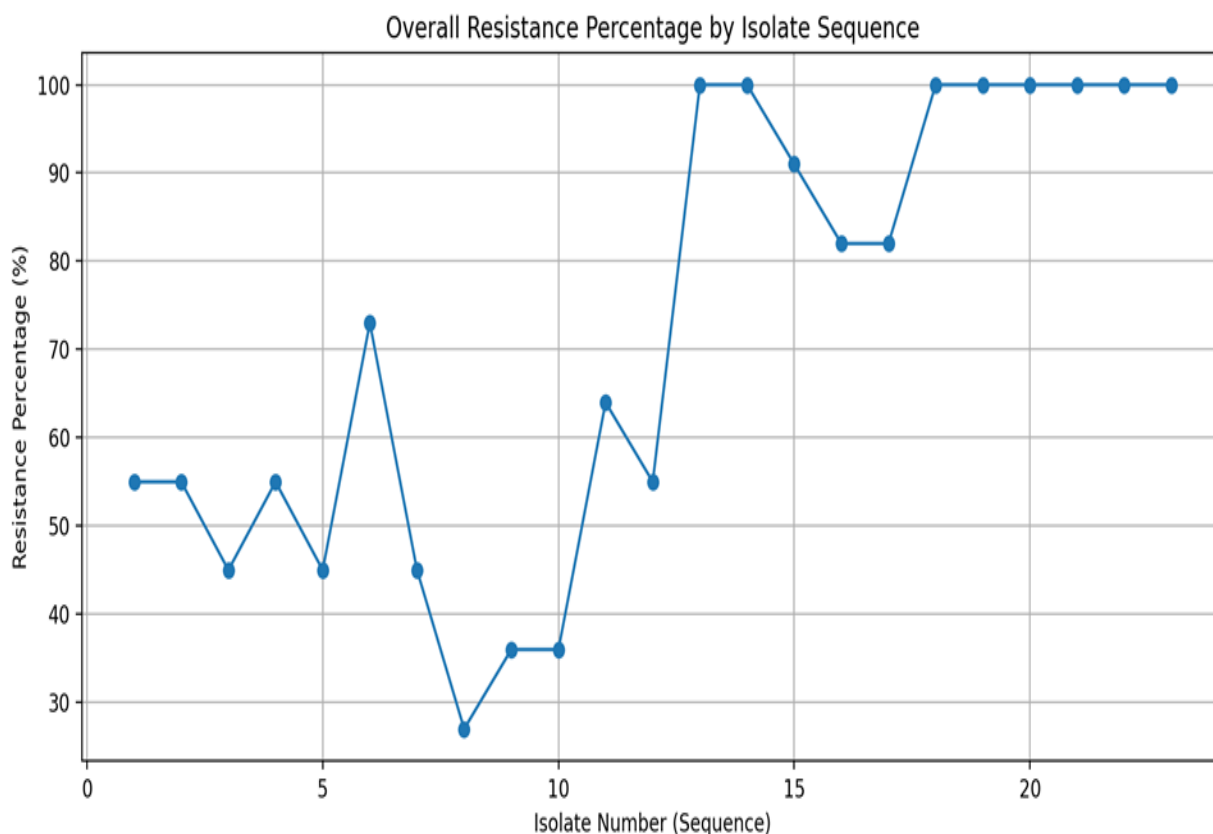


Figure (4-5): The chart shows the overall resistance percentage for each isolate in sequence.

The results observed that resistance percentages fluctuate among the early isolates; however, there is an obvious cluster of isolates toward the end with 100% resistance, as shown in Figure (4-3).

The antibiotics resistance of tested *S. aureus* isolates was 86.1% to Ampicillin, and Fusidic acid; while against all of Erythromycin, Clindamycin, Vancomycin, and meropenem was 60%. However, the results found that the antibiotic that worked best against the identical isolates was Rifampicin with 56.5% of resistance to antibiotics. Also, the percentage of antibiotic resistance for Topramycin and Gentamycin was 87% and represent the lowest effective antibiotics. Eight isolates were resistant to all used antibiotics, six isolates exhibited resistance to six out of 11 antibiotics, four isolate showed resistances to nine antibiotics, and two isolates demonstrated resistance to five antibiotics.

Chapter Four.....Results

The results elucidated that all of 34 isolates of *P. aeruginosa* exhibited varied levels of resistance to 10 used antibiotics. Mainly, 12 isolates were resistant to all used antibiotics, five isolates exhibited resistance to eight out of 10 antibiotics, seven isolate showed resistances to six antibiotics, four isolates demonstrated resistance to five antibiotics, and six isolates were resistance to four antibiotics, as shown in Figure (4-6).

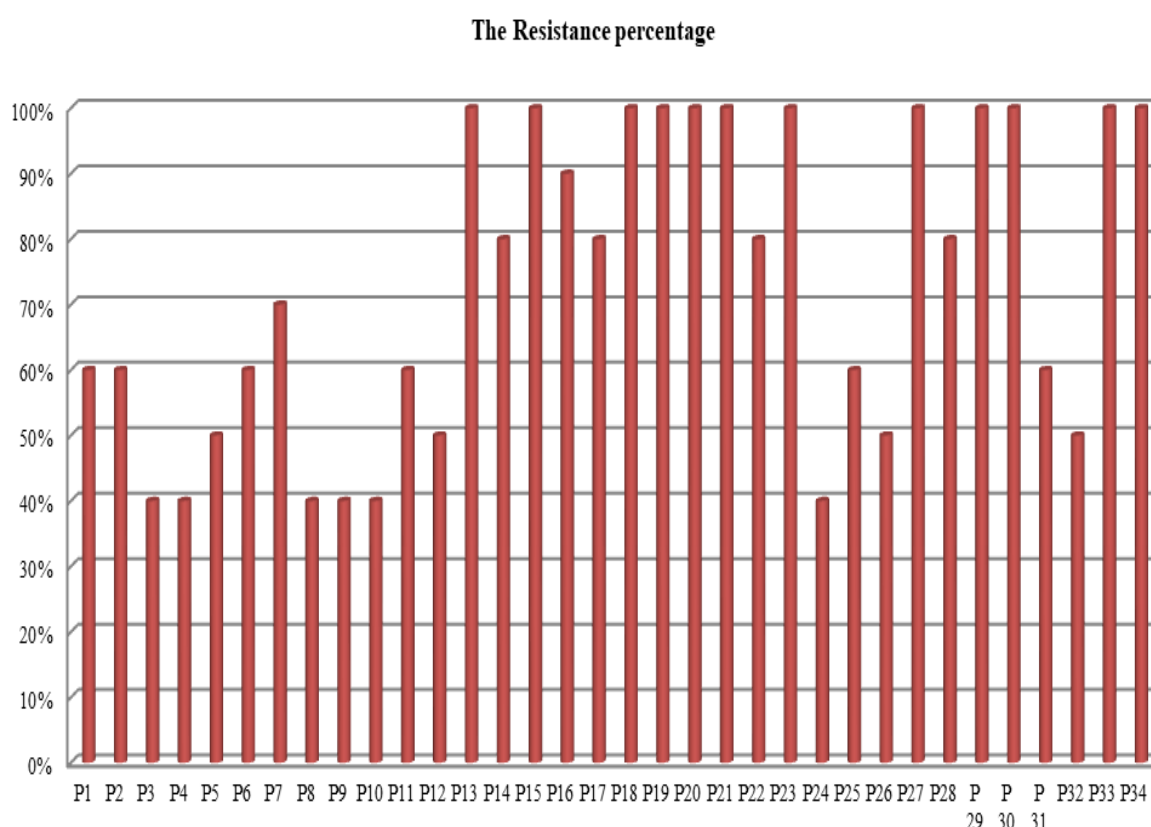


Figure 4-6). The resistance percentage of *P. aeruginosa* against 10 antibiotics

The findings of AST of *P. aeruginosa* elucidated that the Ampicillin (AM) has the highest resistance at 91% and was the least effective; whereas, the resistance rates of Clindamycin, Tobramycin, and Imipenem was 85%, 82%, and 79% respectively. Also, the results found that Ceftazidime was the most effective antibiotic against tested *P. aeruginosa*, as shown in Figure (4-7).

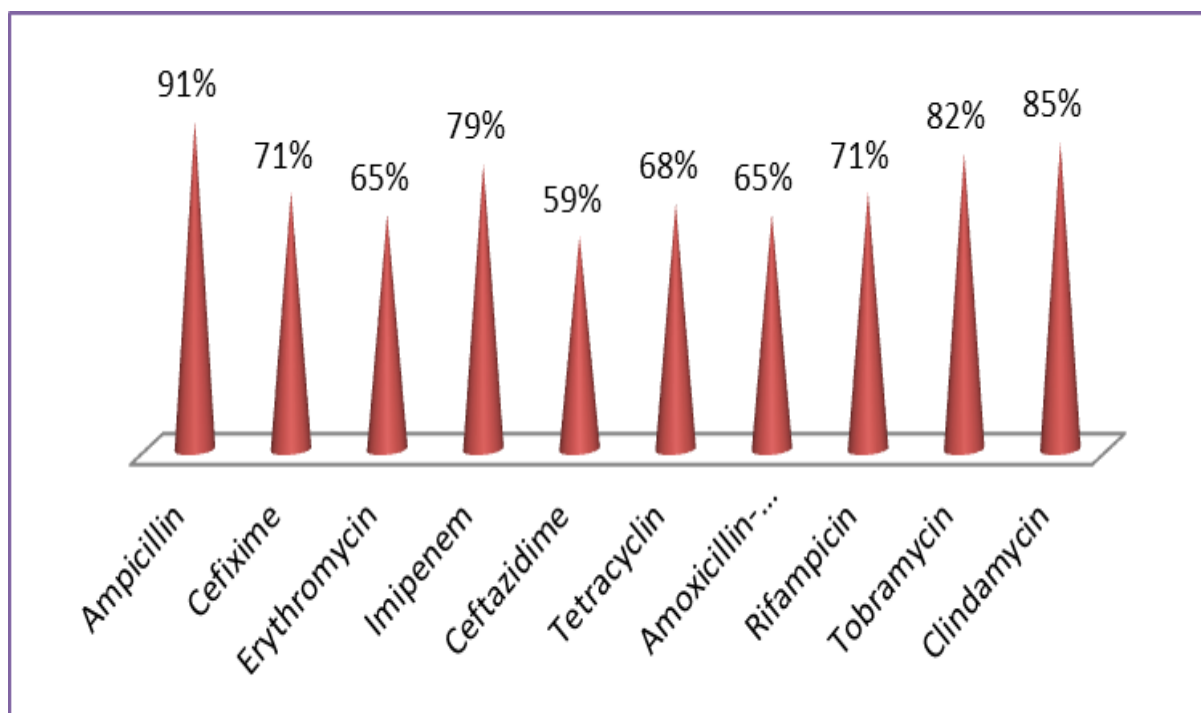


Figure 4-7. The resistance percentage of antibiotics by *P. aeruginosa*

The results of AST by using disk diffusion methods found that twelve isolates of *P. aeruginosa* (P13, P15, P18–23, P27, P29, P30, P33, P34) are fully resistant to every antibiotic tested. Mid-range isolates (≈ 55 – 78 %) retain sensitivity to only a few drugs. The least-resistant group (≈ 33 – 45 %) still has several effective treatment options; accordingly, it classified as MDR and XDR isolates at the same time. Furthermore, isolate no.16 showed resistance to all antibiotics except Tobromycin .

VITIK system

The MIC values to 10 antibiotics against 13 Isolates were varies. All Isolates under test showed resistance to Tigecycllin, Ceftazidime, and Imipenem. The MICs of AK for isolate no. 1, 4, 5, were $32_{\geq}$, $64_{\geq}$, and $64_{\geq}$ respectively. Also results found that isolates number one was the most affected one. Finally, the result revealed that the isolates 13, 12, 11, 10, 9, 7, 6, 2, 3 were resistant to tested antibiotic by using VITEK-II, as shown in table (4-3).

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Table (4-4): The Minimum Inhibition Concentration (MIC) of *P. aeruginosa* isolates

No. Isolates	TI	TZP	CAZ	FEP	IMP	MEM	AK	CN	TOP	CIP
1	12 ≥ R	8 ≥ S	64 ≥ R	64 ≥ R	16 ≥ R	8 ≥ S	32 ≥ S	16 ≥ R	16 ≥ S	4 ≥ R
2	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
3	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
4	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ S	16 ≥ R	4 ≥ S	4 ≥ S
5	12 ≥ R	64 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ S	16 ≥ R	16 ≥ R	4 ≥ R
6	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
7	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
8	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ S	16 ≥ R	16 ≥ S	64 ≥ R	16 ≥ R	16 ≥ S	4 ≥ R
9	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	4 R	4 ≥ R
10	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
11	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
12	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
13	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R

The 34 isolates of *P. aeruginosa* showed that were highly resistant to Cefazolin and Tigecycline with a percentage of (100%). While the resisting for Ampicillin, Ceftazidime, Erythromycin, Cephalexin, Clindamycin, Tobramycin, and Meropenem was (50%).

The Tigecyclin (TI), Oxacillin (OX), Vancomycin (VA), Cefepime (FEP), Rifampicin (RA), Erythromycin (E), Amikacin (AK), Gentamicin (CN), Tobramycin (TOP), Tetracycline (TE), and Ciprofloxacin (CIP) were used against 8 isolates of *S. aureus* and the results discovered that every isolate examined was resistant to Tigecyclin (TI). While the resisting for Cefepime (FEP), Gentamicin (CN), and Ciprofloxacin (CIP) was %88; Vancomycin (VA)

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2classified isolates number 6 and 8 are Extensive drug resistant (XDR), whereas the others are varying from multi-drug resistant (MDR) to Pun-drug resistant (PDR) isolates.

Table (4-4): Minimum inhibition concentration (MIC) to 11 antibiotics against clinical isolates, used VITEK2 system for *S. aureus*

No. Isolates	TI	OX	VA	FEP	RA	E	AK	CN	TOP	TE	CIP
1	12 ≥ R	8 ≥ R	4 ≥ S	64 ≥ R	16 ≥ S	8 ≥ R	32 ≥ S	16 ≥ R	16 ≥ R	16 ≥ S	4 ≥ R
2	12 ≥ R	12 ≥ R	64 ≥ S	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	16 ≥ S	4 ≥ R
3	12 ≥ R	12 ≥ S	64 ≥ S	64 ≥ S	16 ≥ S	16 ≥ R	64 ≥ R	16 ≥ S	16 ≥ R	16 ≥ R	4 ≥ S
4	12 ≥ R	64 ≥ R	64 ≥ S	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ S	16 ≥ R	4 ≥ R	16 ≥ S	4 ≥ R
5	12 ≥ R	64 ≥ S	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ S	16 ≥ R	16 ≥ R	16 ≥ S	4 ≥ R
6	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
7	12 ≥ R	12 ≥ R	64 ≥ S	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	16 ≥ S	4 ≥ R
8	12 ≥ R	12 ≥ R	64 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	64 ≥ R	16 ≥ R	16 ≥ R	16 ≥ R	4 ≥ R
R%	100	75	38	88	75	100	63	88	100	38	88

This study highlights how common antibiotic resistance is among microorganisms in Thi- Qar, Iraq, and showed that the prevalence of antibiotic resistance in patients with wounds and burns is alarmingly high. In the current study *P. aeruginosa*. is a significant contributor to both community-acquired and hospital-acquired infections because of its capacity to generate a number of virulence factors.

4.3.Molecular study

This section of the current study involved the genomic DNA extraction from clinical isolates then followed by detection of resistance genes (*S. aureus*: *mecA*, *blaZ* and *P. aeruginosa* *AmpC*, *mexA*)

Results showed *P. aeruginosa*, *S. aureus* isolates possess of these genes in all isolates, as shown in figure (4- 8), (4-9)

The found that *ampC* and *mexA* genes exist only in seven isolates of *P. aeruginosa* with 742bp and 772bp, respectively. Whereas, the *blaZ* and *mecA* genes were resented in all tested *S. aureus* isolates, respectively.

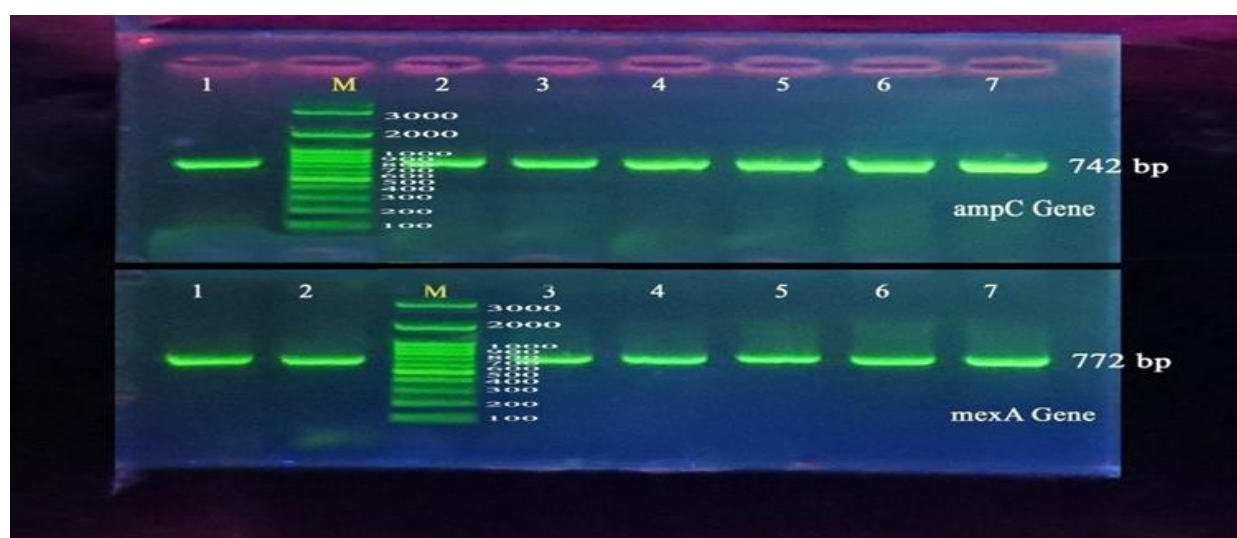


Figure 4-8. Gel Electrophoresis of amplified genes MexA(bp) and *ampC* genes in agarose gel (1%) for (time)under (MA and voltage)

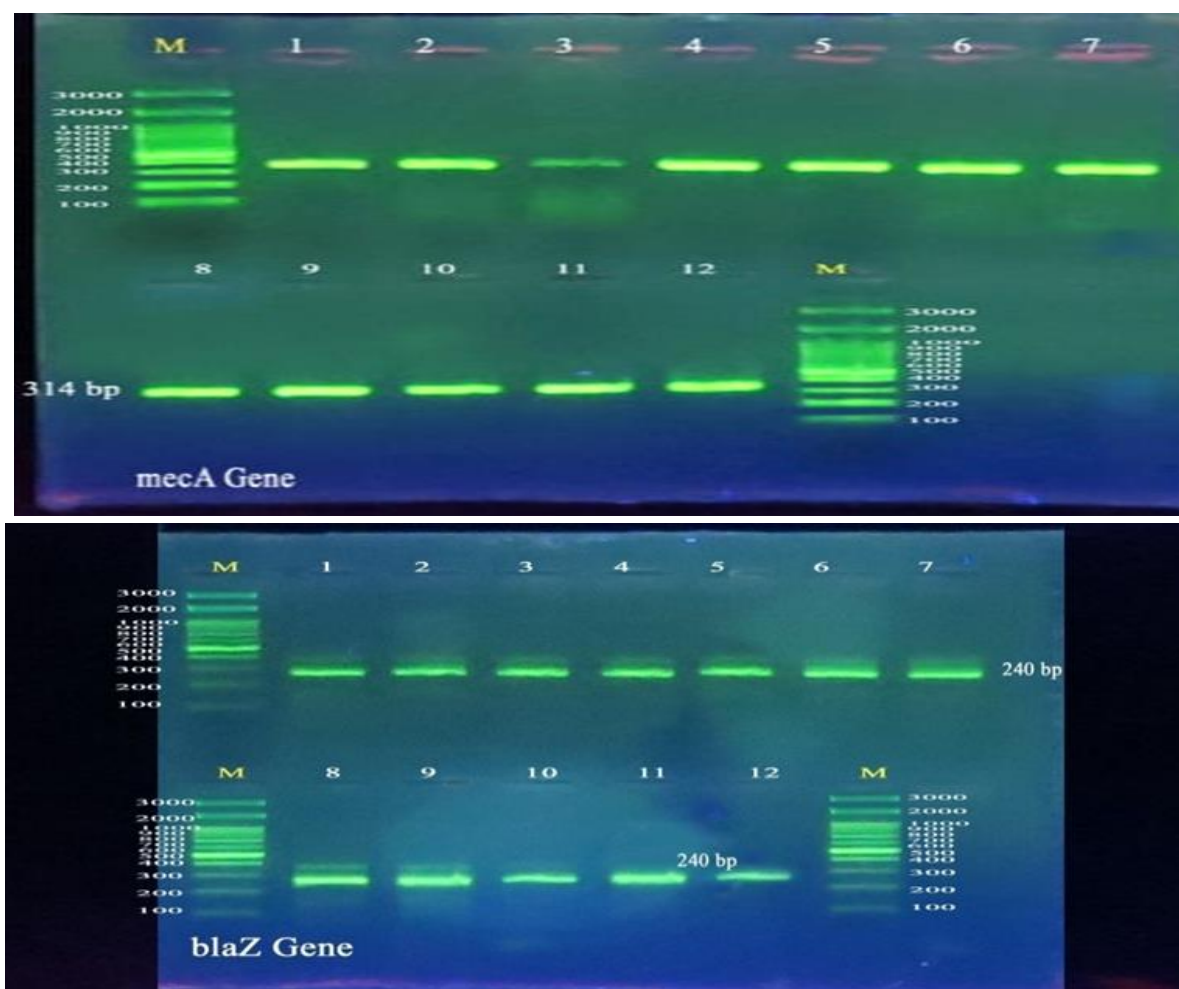
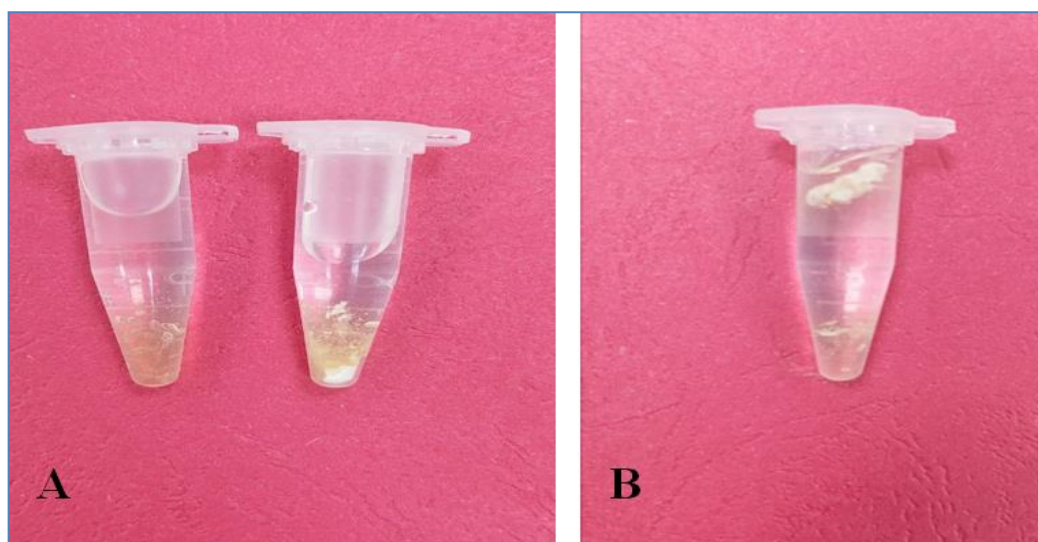


Figure 4-9. Gel Electrophoresis of amplified PCR product of *blaZ* gene in *S. aureus*

4.4. Enhancement The Activity of Antibiotic Using Chitosan Nanoparticles

Two of the most MDR-pathogenic isolates were isolated, diagnosed as *S. aureus* and *P. aeruginosa* and the AST for both of these isolates was investigated as well. The finds isolated bacteria were classified as MDR bacteria. The production of a nano-chitosan solution with varying acids showed notable differences in solubility and stability of the solution. Non-homogeneous, viscous, gel-like solutions or partial precipitation ,There types were used with different concentrations (0.5,1 and 2%) clear, complete dissolution of chitosan was obtained . however, both pentetic acid, and hydrochloric acid (HCl) were failed to dissolve the chitosan completely,as shown in Figure (4-10).



Figure(4-10). A- Shows how turbid, semi-gel layers or sediments create in lactic acid and HCl. B- Shows that pentetic acid contains massive, no dissolved chitosan particles.

Three types of solvent were used with different concentrations (0.5%, 1% and 2%), among these, only with acetic acid at concentrations 0.5%, 1%, and 2% , clear, complete dissolution of chitosan was obtained. However, both pentetic acid, lactic acid and HCL were failed to dissolve the chitosan completely. (shown in Figure 4-11).

According to their solubility characteristics, erythromycin, ampicillin, and ceftazidime were dissolved in the proper solvents . Erythromycin was found to dissolve more readily in distilled water when a modest quantity of ethanol (about 1% solution) was added. Similar results were seen with ampicillin, which became more soluble when ethanol was added. On the other hand, no co-solvents were required for ceftazidime to dissolve easily in distilled water. After preparation, the lowest doses that did not hinder the growth of bacteria were chosen for biological testing. The well diffusion approach was used to measure the antimicrobial activity, which made it possible to determine how effectively each antibiotic worked against bacterial isolates that were resistant.



Figure(4-11). show effectiveness of acetic acid in dissolving chitosan and preparing a homogeneous nano solution

Following the preparation of the chitosan solution using 0.5% acetic acid, various concentrations of chitosan (1%, 2%, 3%, 4%, and 5%) were tested against two resistant bacterial isolates: *S. aureus* and *P. aeruginosa*. The results demonstrated that 3% and 5% chitosan concentrations exhibited obvious antibacterial activity, as indicated by observable zones of inhibition around the wells and were therefore excluded. On the other hand, 1% and 2% concentrations did not show any significant inhibitory effect. As shown in Figure (4-12).

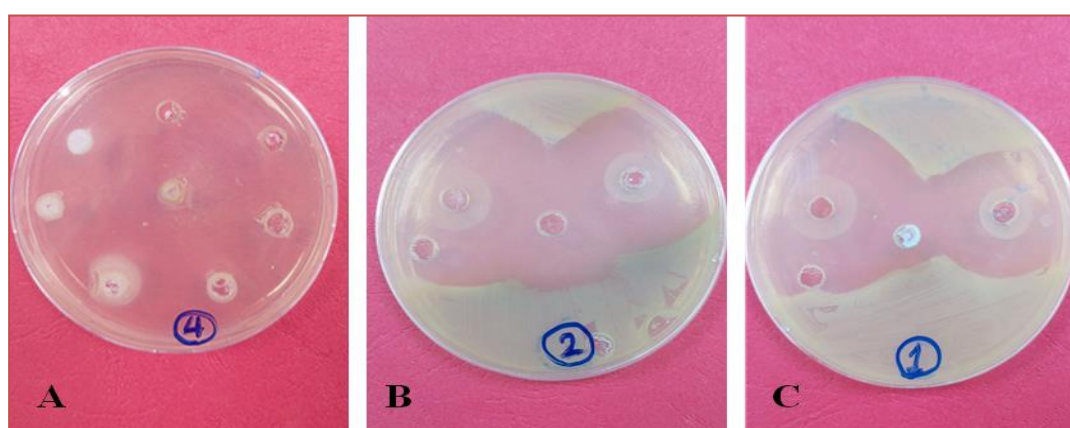


Figure (4-12). A-The antibacterial influences of Chitosan concentration 5%, B- 3% on *P. aeruginosa*, Whereas, C- illustrated the effects of Chitosan concentration 3% on *S. aureus*.

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The images above noticed that the 3% and 5% chitosan concentration has antibacterial impact against both of investigated pathogenic bacteria. Therefore, these concentrations were neglected and lower concentrations were prepared and tested which are including 1% and 2%. After the concentrations had been prepared mixed with the antibiotics at various concentrations and tested separately against both of isolates. The findings demonstrated that when the antibiotic concentration dropped, the width of the inhibition zones gradually shrank. The sub-inhibitory concentration (Sub- MIC) was determined to be the lowest concentration that failed to establish a distinct inhibition zone. As shown in Figure (4-13).

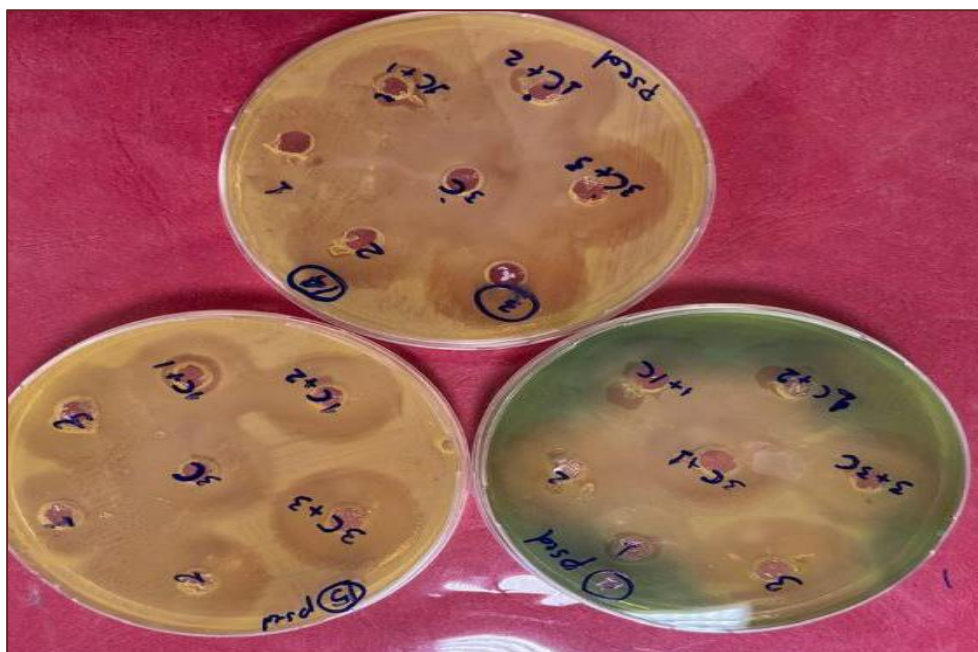


Figure (4-13): The range of antibiotic concentration mixed with 1% of chitosan concentration, tested and found to inhibit bacterial growth.1. Ampicillin, 2. Erythromycin, and 3. Ceftazidime

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In these above images the 3% concentration chitosan and the antibiotic showed inhibitory effects against the antibiotic –resistant bacteria. This indicated that these inhibition zones that produced by the effects of 3% chitosan concentration. However, the 1% and 2% concentrations of chitosan nanoparticles did not affect against *S. aureus* and *P. aeruginosa*. As shown in Figure (4-14).

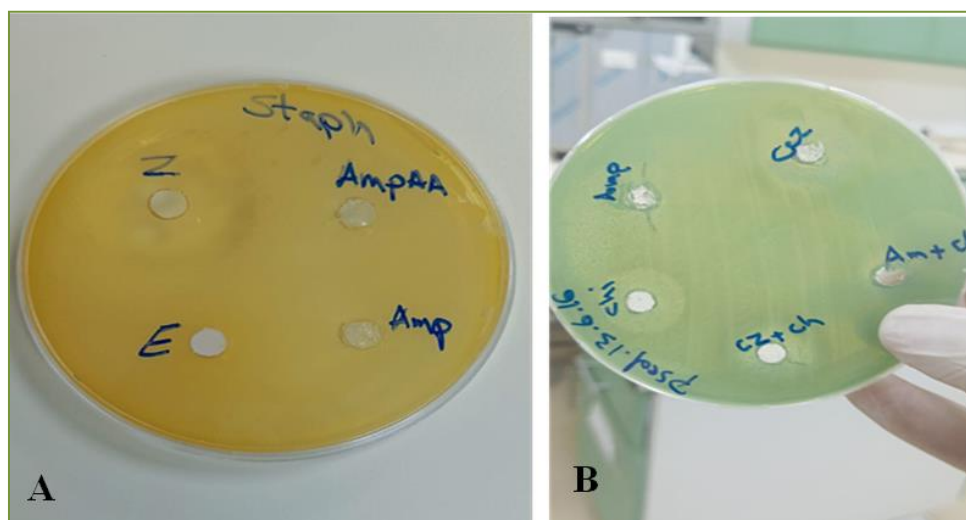


Figure (4-14). Illustrates the influence of 1% combination antibiotic-nanoparticles against (A). *S. aureus* and (B). *P. aeruginosa*. E: Erythromycin, Amp: Ampicillin, and Z: Ceftazidime

The direct method was applied by mixing equal volumes (1:1 ratio) of chitosan (1%) and ampicillin, Ceftazidime, Erythromycin at concentrations of 1% and 2% were separately combined to create a mixture included CNPs- Antibiotic, The results were remarkable, as this combination successfully inhibited bacterial growth. Suggesting a synergistic effect was noticed between the antibiotic and chitosan. Both of these isolates were significantly affected by Erythromycin and Ceftazidime and the inhibition zones were clearly visible, as depicted in Figure (4-15).

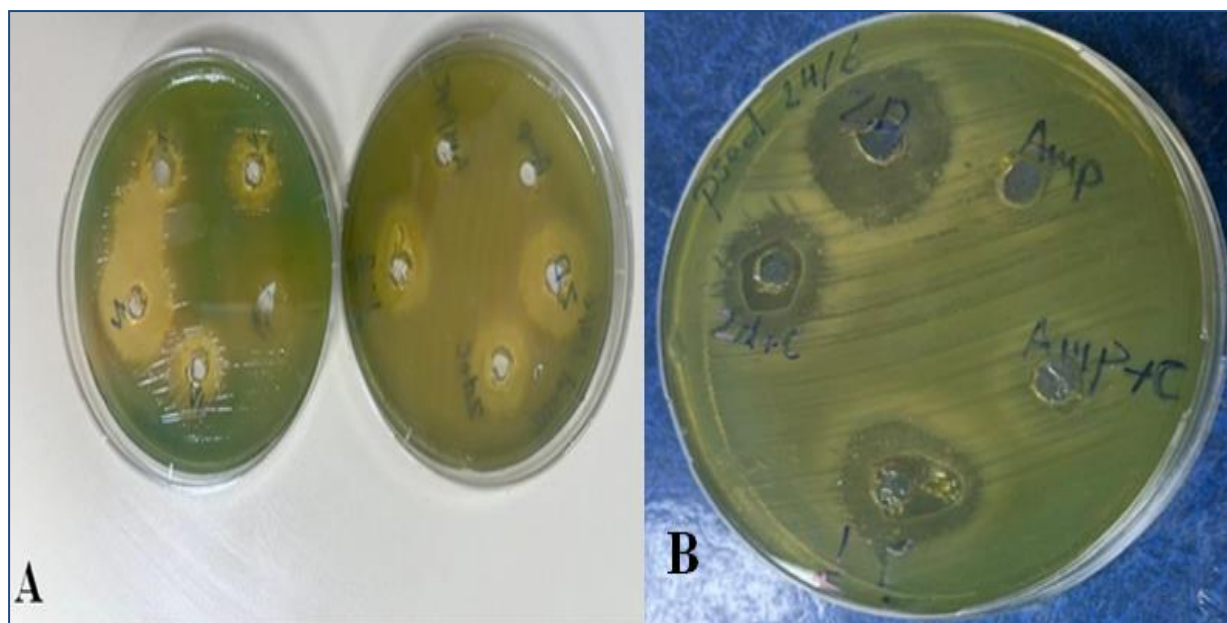


Figure (4-15): A Zone of bacterial growth inhibition appear as a result of the synergistic effect antibiotic-CNPs combination - *P. aeruginosa*, B- *S. aureus*

While, the combination a ratio of 1:2 of the Chitosan to antibiotic, i.e. 1 ml of Chitosan to 2 ml of the antibiotic, the results revealed that the effect was higher on bacterial growth and the areas of growth inhibition were greater. Furthermore, the ionic gelation approach was conducted to successfully create chitosan-based nanoparticles that were loaded with antibiotics. Using the well diffusion approach, the nanoparticle formulations demonstrated observable antibacterial activity, especially when ceftazidim, erythromycin, ampicillin-loaded chitosan nanoparticles were used. The findings indicated a possible synergistic impact, showing that chitosan nanoparticles and antibiotics, even at sub-inhibitory doses, might considerably reduce bacterial growth. The findings showed that, out of all the evaluated formulations, ceftazidime-loaded chitosan nanoparticles had the most antibacterial activity against *P. aeruginosa*, displaying the biggest inhibition zones. As shown in Figure (4-16). Erythromycin-loaded chitosan nanoparticles, on the other hand, were more efficient against *S. aureus*, suggesting that this strain was more susceptible to the erythromycin.

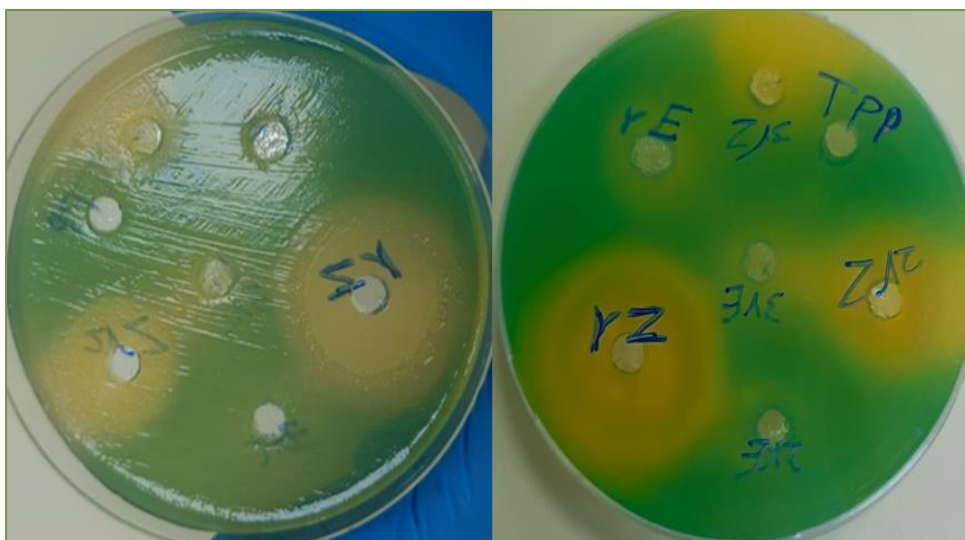


Figure (4-16). Showing the inhibition zones by antibiotic loaded nano-chitosan, rZ-Ceftazidime-loaded chitosan nanoparticles, E -Erythromycin- loaded chitosan nanoparticles

The results found that the CNPs-erythromycin combination at 2:1 concentration exhibited antibacterial effectiveness against *S. aureus*; whereas the CNPs-Ceftazidime combination at 2:1 concentration exhibited greater antibacterial effectiveness against *P. aeruginosa*. Following several tests and trials involving various antibiotic and nano-chitosan concentrations, this finding determined the lowest synergistic concentration that could effectively stop bacterial growth.

4.5The results of UV-Vis spectrum

The results of UV-Vis spectrum of the tested sample revealed a sharp absorption peak at approximately 280 nm ($\lambda_{\text{max}} \approx 0.106$), which was characteristic of aromatic compounds and proteinaceous materials containing tryptophan and tyrosine residues. Beyond this wavelength, the absorbance gradually decreased with no significant peaks observed in the visible region (400–800 nm), indicating the absence of colored pigments or chromophoric

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residuals. These results suggested that the sample shows high UV absorption with a typical protein-like spectral profile, reflecting relative purity and the dominance of aromatic functional groups. As shown in Figure (4-17).

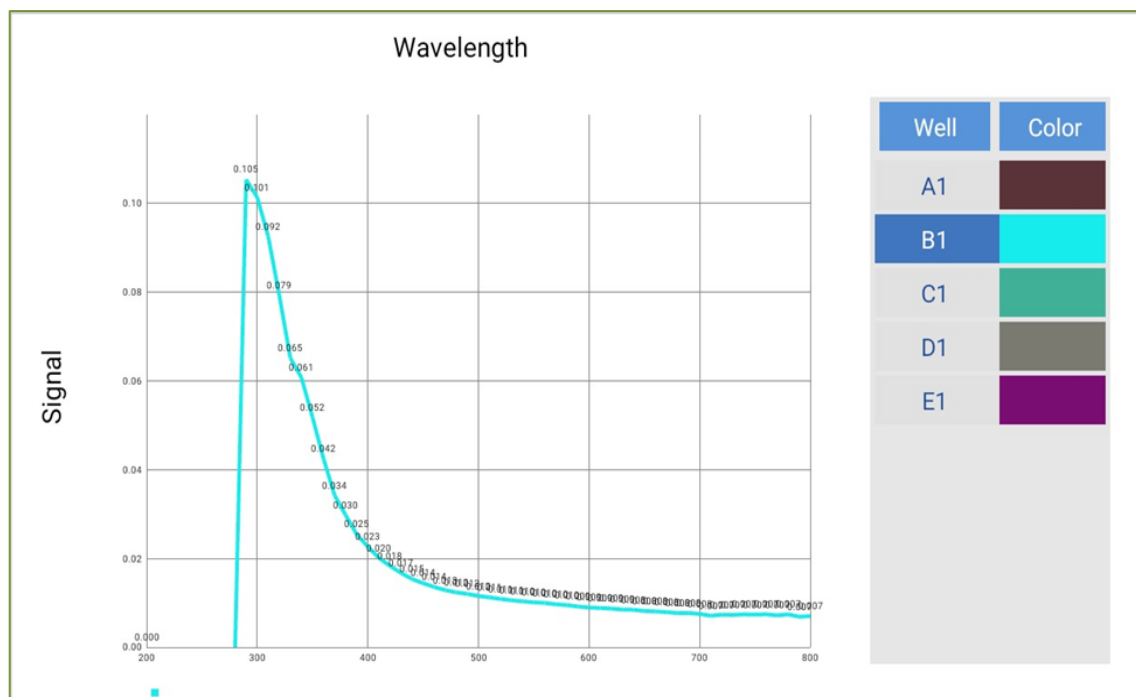


Figure (4-17) UV-Vis absorption spectrum of the tested Chitosan (Well B1)= 0.106

The Ampicillin–Chitosan mixture (C2) revealed a dominant UV absorption at 270–280 nm with $A \approx 0.267$ versus $A \approx 0.106$ for sample B1 (≈ 2.5 -fold increase). The essentially conserved λ_{max} indicated no major chromophore transformation, suggesting noncovalent association or additive absorption rather than chemical degradation. The monotonic decay across 400–800 nm in both spectra supports the absence of colored impurities. Overall, the mixture enhances UV absorbance while preserving spectral identity, consistent with synergistic complexation beneficial for formulation. As shown in Figure (4-18).

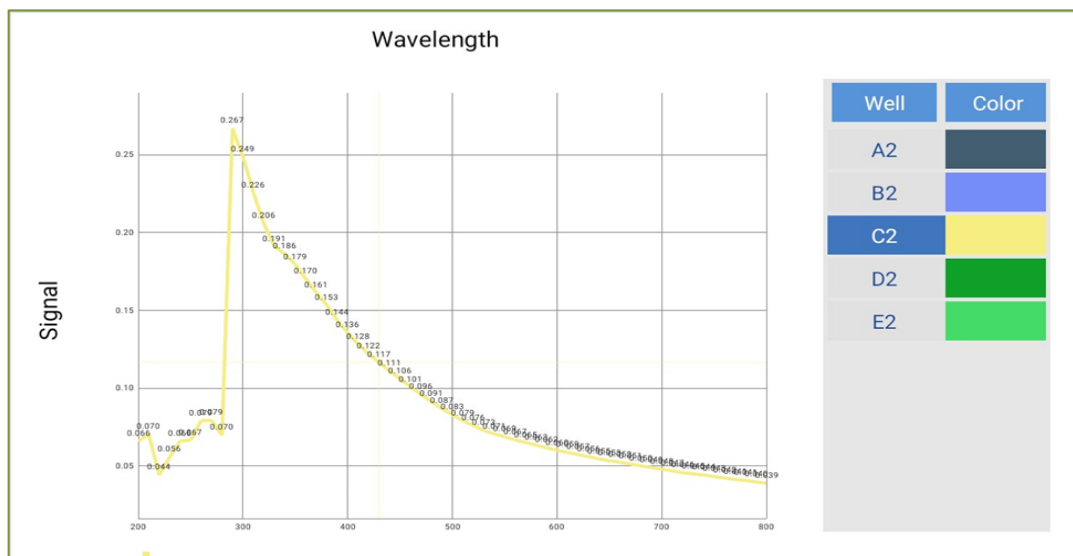
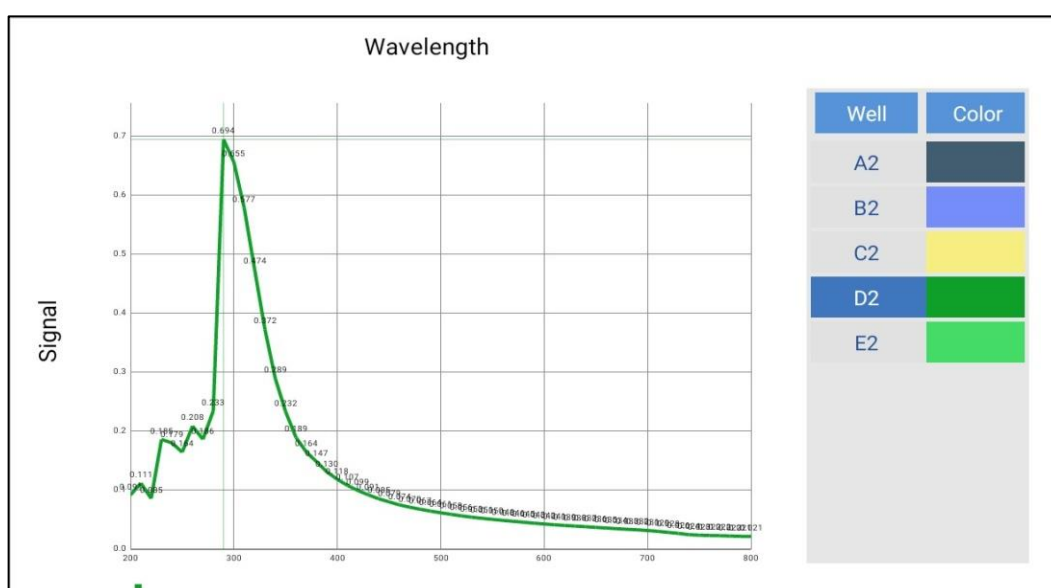


Figure (4-18) UV-Vis absorption spectrum of the tested Ampicillin-loaded Chitosan (Well C2)= 0.267

The Ceftazidime–Chitosan mixture (D2) showed a dominant UV absorption at 270–280 nm with $A \approx 0.694$. The basically conserved $\lambda_{\max}$ indicated no major chromophore transformation, suggesting noncovalent association or additive absorption rather than chemical degradation. The monotonic decay across 400–800 nm in both spectra supports the absence of colored residuals. Overall, the mixture enhances UV absorbance while preserving spectral identity, consistent with synergistic complexation beneficial for formulation. As shown in Figure4-19



Figure(4-19): UV-Vis absorption spectrum of the tested Ceftazidime-loaded Chitosan (Well C2)= 0.694

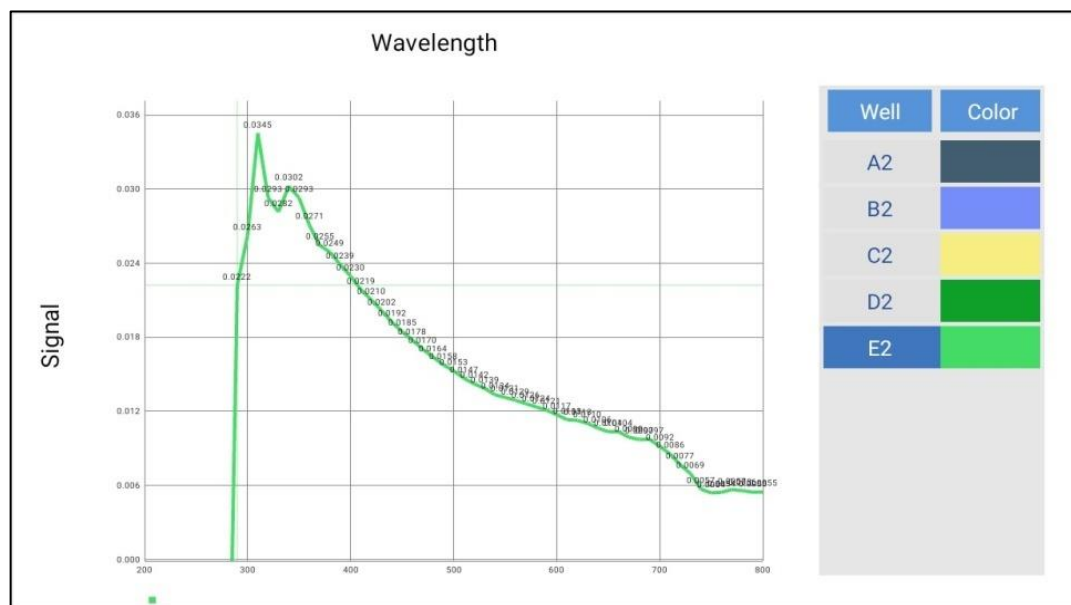
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The UV-Vis spectra of the tested samples revealed characteristic absorption behavior in the ultraviolet region (200–400 nm) with a dominant peak around 270–280 nm. For the single sample (B1), a relatively low absorbance was detected ($A \approx 0.106$ at 280 nm), indicating the presence of aromatic or conjugated functional groups with limited intensity. In contrast, the Ampicillin–Chitosan mixture (C2) exhibited a marked increase in absorbance ($A \approx 0.267$), reflecting enhanced interaction between the two components, most likely through non-covalent electrostatic or hydrogen bonding. The most significant response was observed for the Ceftazidime–Chitosan mixture (D2), which displayed the highest absorbance intensity ($A \approx 0.694$ at 280 nm), nearly 6.5-fold greater than that of B1. Despite these differences in intensity, the λ_{max} remained essentially unchanged across all spectra, suggesting that the underlying chromophores were not structurally altered but rather experienced changes in their microenvironment or additive contributions upon complex formation. The absence of additional peaks in the visible region (400–800 nm) for all samples indicates spectral purity and rules out the presence of colored impurities or degradation products. The observed fluctuations in the 200–250 nm regions of the D2 spectrum further support the involvement of multiple chromophoric groups within ceftazidime, which may account for its stronger interaction with chitosan.

The recorded spectrum of Erythromycin-loaded Chitosan showed a sharp peak at approximately 310 nm (absorbance ≈ 0.0345), followed by a rapid decline and a gradual decrease toward longer wavelengths (absorbance ≈ 0.009 at 750–800 nm). This sharp peak near 310 nm was likely an instrumental artifact commonly observed in plate reader scans, rather than a genuine absorption band. More than 330 nm, the curve displayed a monotonic decline without distinct peaks, suggesting the absence of any significant chromophoric interactions or the formation of a colored complex in the visible range. The

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pattern indicated light scattering rather than molecular absorption, which may originate from sample turbidity. At 600 nm, the absorbance was about 0.012–0.013, indicating minimal optical density and suggesting no substantial cell growth or visible aggregation under these conditions.



Table(4-5) Frequency of Absorption (cm^{-1}), Bonds, and Compound class of Functional Groups

	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
Chitosan	3433.06	O–H stretch H– bonded	Alcohol, phenols
	2927.74-2885.31	C-H stretch	Alkene
	1068.49-1029.92	C–N stretch	Aliphatic amines
	894.91	C-H bonded	Aromatics
	686.61-468.67	C–Br stretch	Alkyl halides

The spectrum of the antibiotic–nanoparticle sample displayed O–H stretching ($\sim 3400 \text{ cm}^{-1}$), aliphatic C–H ($2924/2854 \text{ cm}^{-1}$), a strong carbonyl C=O ($\sim 1717 \text{ cm}^{-1}$), amide I/II ($\sim 1635/1575 \text{ cm}^{-1}$), C–O–C/C–O ($\sim 1070 \text{ cm}^{-1}$), and M–O vibrations ($\approx 600\text{--}500 \text{ cm}^{-1}$). The presence and slight shifts/intensities of the carbonyl/amide/C–O–C bands relative to the pure components indicated successful conjugation/capping of the antibiotic on the nanoparticle surface, while the M–O region confirmed the nanoparticle core remains intact. As shown in Figure (4-21).

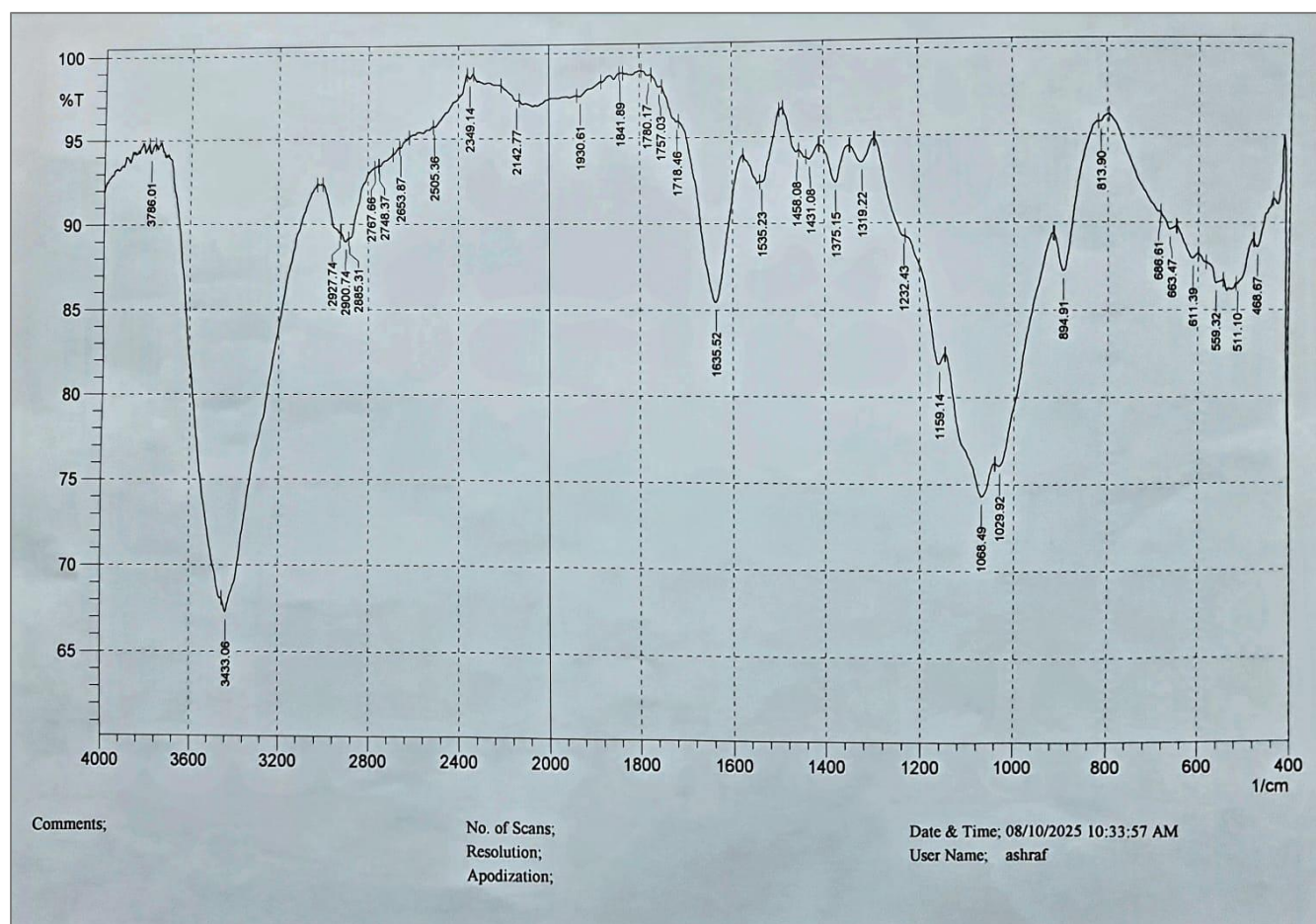


Figure (4-21): Showing functional group interactions and successful binding confirmation of antibiotic–nanoparticle conjugate by FTIR spectrum

Field emission scanning electron microscopy (FE-SEM) analysis

The FE-SEM analysis demonstrated that the antibiotic was efficiently concurrent with the nanoparticles, forming homogeneous and well-defined morphological structures. This structural modification illustrated a stable interaction between the two components, which may facilitate improved drug delivery, enhance antibacterial activity, and reduce the probability of resistance development compared to the free antibiotic. As shown in Figure (4-22).

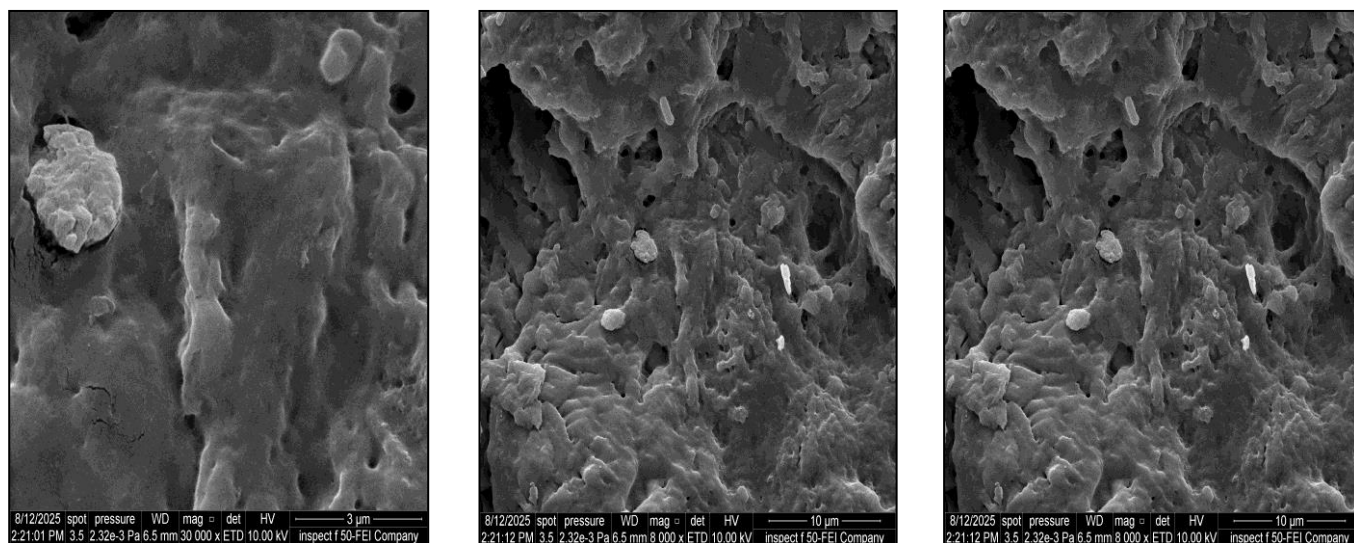


Figure (4-22): FE-SEM images confirm the successful binding of the antibiotic to nanoparticles, producing uniform and well-structured surfaces that indicate enhanced stability and potential antibacterial efficacy.

4.4.1. Results for bio statistical

Based on the inhibition zones of antibiotic- CNPs the statistical analysis was conducted and the results of antibiotics revealed clear differences in the antibacterial activity of the antibiotic–CNP formulations. Ampicillin–CNPs showed no inhibition against either bacterial strain, indicating complete inactivity. In contrast, both Ceftazidime–CNPs and Erythromycin–CNPs demonstrated significant antibacterial effects, with mean inhibition zones of 18.5 mm and 16.0 mm, respectively. There are clear significant differences at 0.05 level and the χ^2 value was 6.00, $df = 2$, and $p = 0.0498$. As shown in Table (4-6).

Chapter Four.....Results**Table(4-6):Inhibition zones (mm) of antibiotic–CNPs against tested bacteria**

Antibiotic–CNPs	<i>P. aeruginosa</i>	<i>S. aureus</i>	Mean ± SD
Ampicillin-CNPs	0	0	0.0 ± 0.0
Ceftazidime-CNPs	20	17	18.5 ± 2.1
Erythromycin-CNPs	18	14	16.0 ± 2.8
$\chi^2 = 6.00$, degree freedom = 2			

Moreover, LSD was investigated and the results indicated that both Ceftazidime-CNPs and Erythromycin-CNPs were significantly superior to Ampicillin-CNPs, while the difference between Ceftazidime-CNPs and Erythromycin-CNPs was not statistically significant. As shown in Table (4-7).

Table (4-7): LSD test results ($\alpha = 0.05$)

Comparison	Mean Difference	LSD (0.05)	Significance
Ceftazidime vs Ampicillin	18.5	2.8	Significant
Erythromycin vs Ampicillin	16.0	2.8	Significant
Ceftazidime vs Erythromycin	2.5	2.8	NS(Not Significant)
LSD test, $\alpha = 0.05$			

Discussion

Chapter Five Discussion

5. Discussion

The escalating crisis of antimicrobial resistance (AMR) represents one of the most formidable challenges to global public health in the 21st century. This study provides a critical snapshot of the AMR landscape in clinical settings within Thi-Qar Hospital, focusing on two of the most notorious opportunistic pathogens. The findings reveal alarmingly high rates of multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates, underscoring the urgent need for novel therapeutic strategies, such as nanoparticle-based drug delivery, to combat these resilient pathogens.

5.1. Prevalence of *S. aureus* and *P. aeruginosa*

The present study successfully isolated and identified *S. aureus* and *P.s aeruginosa* from a total of 100 clinical samples collected from patients suffering from burns, wounds, diabetic foot ulcers, postoperative infections, and other pathological sites. Seventy-two samples (72%) yielded bacterial growth, among which *P. aeruginosa* accounted for 34 isolates (59.6%) and *S. aureus* for 23 isolates (40.3%). These findings are consistent with previous reports emphasizing that these two pathogens are among the most common causative agents of nosocomial and burn-related infections worldwide (Rogers et al., 2022; Gupta et al., 2021).

The predominance of *P. aeruginosa* observed in this study can be attributed to its unique ecological adaptability and its remarkable ability to survive in hospital environments, including moist surfaces and medical devices (Moradali et al., 2021). Its high prevalence in burn and wound samples agrees with findings from Alharbi et al. (2022), who reported that *P. aeruginosa* constitutes more than 50% of bacterial isolates from burn patients in Middle Eastern hospitals. This microorganism's success as a pathogen is related to its multiple virulence factors such as elastases,

exotoxin A, and quorum-sensing systems that promote tissue colonization and antibiotic tolerance.

Staphylococcus aureus, identified in 40.3% of isolates, remains a major cause of both hospital-acquired and community-acquired infections. The recovery of *S. aureus* from wound and burn samples confirms its role as an opportunistic pathogen exploiting damaged skin barriers. This result is in line with previous studies that demonstrated *S. aureus* as one of the leading causes of wound infections due to its capacity to form biofilms and its possession of virulence genes (Aslam et al., 2023).

The identification methods used in the current work, conventional biochemical tests and automated VITEK II system, yielded consistent results, reinforcing the reliability of both techniques. This agreement aligns with previous validation studies that demonstrated over 95% concordance between biochemical identification and VITEK II results (Abdel-Moein et al., 2020).

Moreover, the high percentage of bacterial recovery from wound and burn samples highlights the serious risk of infection in patients with compromised skin integrity. Burns, in particular, destroy the protective epithelial barrier and create a moist, nutrient-rich environment favorable for bacterial colonization (Church *et al.*, 2022). The isolation rate (72%) in this study is comparable to reports from similar clinical settings in Iraq and neighboring regions,

In summary, the dominance of *P. aeruginosa* and *S. aureus* in this study indicates that these pathogens remain the most frequent and clinically significant organisms associated with skin and burn infections in the local hospital environment. Their co-existence in the same infection sites can further complicate treatment, emphasizing the need for continuous microbial surveillance and strict infection control strategies.

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Chapter Five Discussion

5-2 Antibiotic Sensitivity Test

The antimicrobial susceptibility testing (AST) performed in this study revealed alarming resistance patterns among both *S. aureus* and *P. aeruginosa* isolates. The disk diffusion and VITEK II analyses demonstrated that a high proportion of isolates exhibited multidrug resistance (MDR) and extensive drug resistance (XDR) profiles, confirming that antibiotic resistance remains a major therapeutic challenge in clinical settings in southern Iraq.

Among the *S. aureus* isolates, resistance to ampicillin (86.1%), gentamicin (82.6%), and tobramycin (87%) was most prevalent, while rifampicin and vancomycin remained relatively more effective. Eight isolates were resistant to all tested antibiotics, and several others showed intermediate resistance to vancomycin. This pattern aligns with numerous studies reporting high levels of β -lactam and aminoglycoside resistance in *S. aureus* due to the acquisition of the *mecA* and *blaZ* genes, which mediate penicillin-binding protein modification and β -lactamase production, respectively (Lakhundi & Zhang, 2020; Lee et al., 2023).

Vancomycin resistance in some isolates may indicate the emergence of vancomycin-intermediate *S. aureus* (VISA) or heterogeneous VISA phenotypes, which have been increasingly documented globally (Howden et al., 2023). The detection of isolates resistant to most antibiotic classes highlights the urgent need for continuous surveillance and rational antibiotic use policies.

In *P. aeruginosa*, resistance was even more pronounced. Ampicillin showed the highest resistance rate (91%), followed by clindamycin (85%), tobramycin (82%), and imipenem (79%). These findings are consistent with international reports describing *P. aeruginosa* as one of the most difficult Gram-negative pathogens to treat because of its intrinsic resistance mechanisms such as low membrane permeability,

efflux pump overexpression and production of *AmpC* β -lactamase (Lister *et al.*, 2021; Juan *et al.*, 2022).

The relatively higher susceptibility to ceftazidime observed in this study supports earlier findings that third-generation cephalosporins still retain partial activity against some *P. aeruginosa* isolates, particularly when extended-spectrum β -lactamase (ESBL) genes are absent (Shariati *et al.*, 2022). Nevertheless, 12 isolates of *P. aeruginosa* in this work were completely resistant to all tested drugs, fulfilling criteria for XDR and, in a few cases, PDR (pan-drug resistant) classification.

MIC results obtained through VITEK II confirmed these observations, revealing extremely high MIC values for tigecycline, imipenem, and ceftazidime, indicating enzymatic inactivation and reduced outer-membrane permeability. The finding that gentamicin and amikacin were ineffective in most isolates further supports previous reports of aminoglycoside-modifying enzymes (AMEs) being widely disseminated among *P. aeruginosa* and *S. aureus* isolates in the region (Bokhary *et al.*, 2023).

Since *S. aureus* was high resistant to the antibiotics Ampicillin and cefoxitin, as reported by the study in Iraq at Nasiriyah City (Al-Safi, M. T.2023). Then it is considered to be methicillin-resistant bacteria (MRSA), A study by Thamer and Alsammak (2021) in Erbil showed highly resistance (100%) to Methicillin, some isolate that the sensitivity to vancomycin. These studies support the results of the current study.

All *P. aeruginosa* isolates were multi resistant antibiotic, much higher than in previous studies in the region. According to our findings the results were dissenting with those of a study in Duhok (ALI, S. *et al.*, 2024), and compatible with a study in Najaf, Iraq. (Humady, I. K. *et al* 2024). The incidence of MRSA among patients increased over time as a result of antibiotics abuse (Hateet, Rahim 2021). Other previous

investigation that conducted in Misan / Iraq explained that *P. aeruginosa* and *S. aureus* isolates have increased resistance to most antibiotic, and the greatest prevalence of antibiotic resistance was noted in *S. aureus* to antibiotics was found to Tobromycin was shown to be one of the effective antibiotics (Hateet, Rahim 2021).

5.4. Molecular Detection of Resistance Genes:

The molecular analysis conducted in this study provided important insights into the genetic basis of antibiotic resistance among *S. aureus* and *P. aeruginosa* isolates. The detection of key resistance genes *mecA* and *blaZ* in *S. aureus*, and *AmpC* and *mexA* in *P. aeruginosa* confirms that the observed phenotypic resistance patterns are strongly correlated with well-established genetic mechanisms.

1–*S. aureus* resistance genes (*mecA* and *blaZ*)

All tested *S. aureus* isolates were positive for both *mecA* and *blaZ* genes. The *mecA* gene encodes an altered penicillin-binding protein, PBP2a, which has a low affinity for β -lactam antibiotics, conferring methicillin and oxacillin resistance — the molecular hallmark of MRSA strains (Lee et al., 2023). Similarly, *blaZ* encodes β -lactamase enzymes capable of hydrolyzing the β -lactam ring of penicillin and related antibiotics, thereby neutralizing their activity (Lakhundi & Zhang, 2020).

The coexistence of both *mecA* and *blaZ* in all isolates suggests a synergistic resistance mechanism in which the presence of β -lactamase activity complements PBP2a-mediated resistance, resulting in complete β -lactam inactivation. This dual resistance profile has been reported in several recent studies in the Middle East and Asia, where MRSA strains frequently carry multiple β -lactam resistance determinants (Almshawit et al., 2022; Ibrahim et al., 2021)

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The detection of these genes in all isolates also indicates a widespread dissemination of resistant clones within the clinical environment, possibly due to overuse of β -lactam antibiotics and horizontal gene transfer through mobile genetic elements such as SCCmec and plasmids (Shrestha et al., 2022). This observation aligns with global data showing that hospital-acquired MRSA (HA-MRSA) remains a major cause of wound and burn infections, particularly in resource-limited healthcare settings.

2-Pseudomonas aeruginosa resistance genes (AmpC and mexA).

Among *P. aeruginosa* isolates, the AmpC gene was detected in seven isolates, while the mexA gene was identified in the same isolates. AmpC encodes a chromosomally mediated cephalosporinase that provides resistance against most β -lactam antibiotics, including cephamycins and extended-spectrum cephalosporins (Lister et al., 2021). Overproduction of AmpC β -lactamase can result from mutations in the regulatory genes ampR or ampD, leading to constitutive expression (Juan et al., 2022).

The mexA gene is part of the mexAB-oprM efflux pump system — one of the most clinically significant efflux systems in *P. aeruginosa*. Its upregulation enables active extrusion of antibiotics such as fluoroquinolones, macrolides, and β -lactams, decreasing intracellular drug concentrations and facilitating multidrug resistance (Poole, 2020). The coexistence of AmpC and mexA in the same isolates demonstrates a multifactorial resistance strategy that combines enzymatic degradation with reduced intracellular accumulation of antibiotics.

Interestingly, not all *P. aeruginosa* isolates carried these genes, which may explain the variation in phenotypic resistance patterns observed in the AST results. This heterogeneity suggests that other mechanisms such as outer membrane porin loss and biofilm-associated tolerance may also contribute to resistance in isolates lacking AmpC or mexA (Bokhary et al., 2023).

Collectively, the molecular data from this study emphasize the genetic complexity underlying antibiotic resistance. The simultaneous presence of multiple resistance determinants within single bacterial isolates highlights the adaptive capacity of these pathogens and underscores the importance of molecular surveillance as a complement to traditional susceptibility testing.

5.5 Combination Effect of Chitosan with Antibiotics:

Chose to use chitosan in the current study to encapsulate antibiotics against bacteria that are resistant to many drugs. Chitosan is frequently used to deliver a variety of hydrophobic, hydrophilic, and unstable medications due to its biocompatibility, affordable, availability, and biodegradability (Khan, F.2020).

The current study demonstrated that the combination of antibiotics with chitosan nanoparticles (CNPs) significantly enhanced antibacterial activity against multidrug-resistant *S. aureus* and *P. aeruginosa* isolates. The synergistic interaction between the antibiotics (ceftazidime, erythromycin, and ampicillin) and the CNPs resulted in larger inhibition zones compared to the free antibiotics, with ceftazidime-loaded CNPs exhibiting the most pronounced effect against *P. aeruginosa* and erythromycin-loaded CNPs against *S. aureus*.

These findings are consistent with recent reports showing that the conjugation of antibiotics with chitosan nanoparticles improves bacterial uptake and bioavailability while protecting the drug from enzymatic degradation (El-Sherbiny & El-Baz, 2022; Divya *et al.*, 2022). Chitosan, a positively charged biopolymer, interacts electrostatically with the negatively charged bacterial cell wall, leading to cell membrane disruption, increased permeability, and enhanced drug penetration (Anbazhagan *et al.*, 2023). This explains the observed enlargement of

inhibition zones when antibiotics were combined with CNPs in the current work.

At the molecular level, chitosan nanoparticles can form non-covalent hydrogen-bond or ionic complexes with antibiotics, which stabilize the active drug molecules and facilitate sustained release (Abdel-Rahman *et al.*, 2023). The higher efficacy of ceftazidime-loaded CNPs compared with ampicillin-loaded CNPs may be attributed to ceftazidime's broader spectrum of activity and its β -lactam ring stability when incorporated into the chitosan matrix. The ampicillin–CNP complex, on the other hand, showed little or no inhibition, possibly due to degradation of the drug during formulation or weaker binding affinity to chitosan(Kaur, 2022).

Furthermore, the results indicated that higher chitosan concentrations (3–5%) exerted intrinsic antibacterial effects even without antibiotics. This is in agreement with earlier studies demonstrating that chitosan nanoparticles themselves possess antimicrobial activity through binding to microbial DNA and inhibiting mRNA synthesis (Panda *et al.*, 2022). However, excessive chitosan concentration may cause aggregation and reduce nanoparticle stability; therefore, the lower sub-inhibitory concentration (1–2%) was optimized for combination with antibiotics in this study (do AmaralSobral *et al.*, 2022).

The synergistic effect observed between CNPs and antibiotics can be explained by several mechanisms:

- Electrostatic attraction between cationic chitosan and anionic bacterial membranes, leading to cell leakage.
- Improved antibiotic delivery, as CNPs act as nanocarriers that promote sustained release and increase contact time at the infection site.

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- Biofilm disruption, as chitosan penetrates the extracellular polymeric matrix, allowing antibiotics to reach embedded bacterial cells (Kalia & Yadav, 2021).

-Inhibition of efflux pumps, which enhances intracellular antibiotic retention, as documented in recent in vitro studies (Mohanraj *et al.*, 2023).

The statistical analysis further confirmed significant differences ($p < 0.05$) between the inhibition zones of ceftazidime- and erythromycin-loaded CNPs compared to ampicillin-CNPs. Although ceftazidime- and erythromycin-CNPs did not differ significantly from each other, both were markedly more active than the free antibiotics, suggesting that chitosan acts as a potentiating agent.

These findings correspond with those of Sathiyaseelan *et al.*, (2023), who demonstrated that chitosan-ZnO nanocomposites loaded with ampicillin restored antibacterial activity against ampicillin-resistant pathogens through enhanced membrane interaction and controlled drug release. Similar synergistic enhancement of erythromycin when conjugated to chitosan was also reported by Jayakumar *et al.* (2022).

The current results indicate that the formulation of antibiotics with chitosan nanoparticles provides a promising strategy to overcome bacterial resistance by improving drug delivery, reducing the required dosage, and enhancing antibacterial efficacy. This synergy is particularly significant against MDR and XDR strains, where conventional therapy fails, positioning chitosan-based nanocarriers as a future cornerstone in antimicrobial nanomedicine.

The results substantiated that the ceftazidime-CNPs exhibited the most antibacterial activity against *P. aeruginosa*; Erythromycin-CNPs were more efficient against *S. aureus*. The successful formulation of chitosan-

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based nanoparticles loaded with the antibiotics ceftazidime and erythromycin using the ionic gelation method. The type of bacterial strain and the particular antibiotic utilized both affect how effective chitosan–antibiotic nanoparticles are against bacteria. These findings demonstrate that chitosan incorporation enhances the UV absorbance of Erythromycin and Ceftazidime, with a much more pronounced effect in the case of ceftazidime. This suggests the formation of a more stable and synergistic complex, which may have significant implications for improving the physicochemical stability and potential bioavailability of ceftazidime in drug delivery applications. The spectrophotometric data do not reveal any significant interaction between erythromycin and chitosan within the 300–800 nm range. The FTIR spectrum confirms the presence of hydroxyl, carbonyl, and amide groups, indicating successful conjugation or interaction of the antibiotic with the nanoparticle surface. Shifts in characteristic antibiotic peaks (C=O, N–H, C–N) compared to pure antibiotic spectrum would confirm binding; comparing with reference spectra is essential (Kaur and Kumar, 2019).

This results illustrated that the ionic gelation process was a straight forward and efficient way to create chitosan nanoparticles loaded with antibiotics. The cationic properties of chitosan, which break down bacterial membranes, and the antibiotic, which targets certain microbial activities, work in concert to provide the observed antibacterial activity, even at low doses. This study's direct loading approach, which uses sub-inhibitory doses, also aided in lowering the dosage of antibiotics, which may lessen adverse effects and the emergence of resistance. The obtained findings were in line with earlier research showing the benefits of nanocarrier systems in enhancing the effectiveness of antibiotics, particularly against bacteria that were resistant to them. Due to

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ceftazidime improved the capacity to target Gram-negative bacterial cell walls in conjunction with chitosan's permeabilizing qualities, ceftazidime-loaded chitosan nanoparticles demonstrated higher inhibitory effectiveness against *P. aeruginosa* (Campos 2024).

Conversely, *S. aureus* is commonly more susceptible to macrolides including erythromycin, which was more successfully combated by erythromycin-loaded chitosan nanoparticles. This differential activity supports the promise of chitosan nanoparticles as a synergistic delivery method to improve antibacterial effectiveness. Same findings were obtained by previous research (Hawi & Al-Ruaby, 2020), and emphasized the significance of choosing the right antibiotic-carrier combinations suited to the target disease; it was also consistent with another conducted study (Elshinawy *et al.* 2018).

Following several tests and trials involving various antibiotic and nano-chitosan concentrations, this finding determined the lowest synergistic concentration that could effectively stop bacterial growth. This concentration indicated a strong synergistic interaction between the two substances as shown by a research previously carried out (Lemke *et al.*, 2021). Since it is the lowest effective dosage at which the combined action of the antibiotic and chitosan caused a pronounced inhibitory effect. This resulted in chitosan nanoparticles loaded with antibiotics, which were subsequently evaluated for antibacterial activity utilizing techniques including MIC determination and well diffusion test (Xiao *et al.*, 2023).

By using specific UV-Vis the absorbency of chitosan and chitosan-antibiotics combinations separately were investigated in order to detect the presence of CNPs-Antibiotic combination and determine their purity

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and non-degradability. The absence of additional peaks in the spectrum demonstrated the absence of chromophore residuals. Determination of intermolecular interactions (Complex formation) indicated any change in absorption intensity or shift in λ_{max} indicated the occurrence of a bond or synergism between two substances. The UV-Vis examination of the chitosan nanoparticles revealed distinctive absorption spectra.

The FT-IR spectra further verified the successful binding between antibiotics and chitosan. The pure chitosan spectrum displayed broad absorption bands around 3430 cm^{-1} (O–H and N–H stretching), $2925\text{--}2880\text{ cm}^{-1}$ (C–H stretching), and 1070 cm^{-1} (C–N and C–O stretching). In the antibiotic-loaded samples, characteristic shifts and intensity changes were observed at $\sim 1717\text{ cm}^{-1}$ (C=O stretching of β -lactam carbonyl), 1635 cm^{-1} (amide I), and 1575 cm^{-1} (amide II), indicating hydrogen-bond formation and electrostatic interaction between the drug and polymer (Singh et al., 2022).

Moreover, new peaks at $600\text{--}500\text{ cm}^{-1}$ corresponding to metal–oxygen (M–O) or halogen-bond vibrations suggested that the nanoparticle core remained intact after antibiotic loading. The retention of these functional groups confirms that the chitosan matrix preserved its structural stability and functional reactivity during nanoparticle formation.

Field-emission scanning electron microscopy (FE-SEM) images revealed spherical to slightly irregular nanoparticles with smooth surfaces and uniform size distribution, indicating successful encapsulation of antibiotics and homogeneity of the nanoformulation. The surfaces of the antibiotic-loaded nanoparticles appeared denser and more compact than those of unloaded CNPs, reflecting drug adherence or surface adsorption (Hosseini et al., 2023).

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Such uniform morphology is crucial for predictable drug release and reproducible biological performance. Several similar investigations have confirmed that smooth-surfaced, nanosized chitosan particles (< 100 nm) enhance bioavailability and stability of encapsulated antibiotics by increasing surface area-to-volume ratio (Divya et al., 2022; Kumari et al., 2024).

Collectively, the UV–Vis, FT-IR, and FE-SEM findings corroborate the successful synthesis and stable conjugation of antibiotic–chitosan nanoparticles. The structural and morphological data confirm the preservation of chemical integrity, efficient drug loading, and potential for sustained release — essential features for biomedical application against MDR pathogens.

The statistical analysis revealed clear differences in the antibacterial activity of the antibiotic–CNP formulations. Ampicillin-CNPs showed no inhibition against either bacterial strain, indicating complete inactivity. In contrast, both Ceftazidime-CNPs and Erythromycin-CNPs demonstrated significant antibacterial effects, with mean inhibition zones of 18.5 mm and 16.0 mm, respectively. The Chi-square test confirmed significant variation among the treatments ($p < 0.05$). Furthermore, LSD analysis indicated that both Ceftazidime-CNPs and Erythromycin-CNPs were significantly superior to Ampicillin-CNPs, while the difference between Ceftazidime-CNPs and Erythromycin-CNPs was not statistically significant. These findings highlight the potential of nanoparticle-based formulations in enhancing the antibacterial activity of certain antibiotics.

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Study Limitations:

This study faced several limitations that should be considered when interpreting the results.

- Cytotoxicity Assessment: The cytotoxic or biocompatibility effects of chitosan nanoparticles were not evaluated in this study. Future research should include toxicity testing using suitable cell lines or animal models to confirm their safety for biomedical applications.
- Time Constraints: The study was conducted under limited time, which restricted the possibility of performing additional optimization experiments and long-term stability assessments of the prepared formulations.
- Financial Limitations: Due to limited financial resources, certain advanced characterization techniques and in vivo studies could not be carried out, which may have provided deeper insights into the nanoparticles' properties and therapeutic potential.

However, despite these limitations, the findings of this study provide valuable insights into the potential use of chitosan nanoparticles as effective carriers for antibiotics and form a strong basis for future research.

Conclusions and Recommendations

Conclusions and Recommendations.....

Conclusions

- 1- This study showed that the main causative agents of wound, burn infections were *P. aeruginosa* (gram negative group), while *S. aureus* was the main gram-positive bacteria.
- 2- The bacterial isolates of *Pseudomonas aeruginosa* and *S. aureus* showed highly resistance to most commonly antibiotics which used in wound infections treatment.
- 3- According the results of this study, chitosan nanoparticles showed a good antibacterial activity which was increased with concentration of NPs.
- 4- The synergistic effect of synthesized chitosan nanoparticles with antibiotics showed significant antibacterial activity against G-ve bacteria and G+ve, therefore these chitosan nanoparticles can be used in the treatment and reducing of microbial load.
- 5- The findings of this study revealed that the combination of chitosan nanoparticles with conventional antibiotics markedly enhanced their antibacterial efficacy against resistant and intermediate-resistant bacterial isolates. This synergistic interaction suggests that nano-chitosan can serve as an efficient drug delivery system, improving the therapeutic potential of antibiotics and offering a promising strategy for combating multidrug-resistant bacterial infections..

Conclusions and Recommendations.....

Recommendations:

The following points are suggested according to the data obtained from this research:

- 1- Study the antimicrobial activity of nano chitosan other various bacterial types from other clinical sites.
- 2- Evaluate the synergistic effect of chitosan nanoparticle with another type of antibiotic
- 3- Evaluation the protective activity of chitosan Nanoparticles in other non-infections disease like anti-tumor activity .
- 4- Considering the effectiveness of antibiotic loaded on Nanomaterials against multi-drug resistant bacteria in vitro recommends more research in vivo as a therapeutic alternative.
- 5- Study its toxicity in vitro and in vivo to ensure that it is safe when used as a drug.

REFERENCES

Abdelaziz, A. A.; Kamer, A. M. A.; Al-Monofy, K. B.; and Al-Madboly, L. A.(2023). *Pseudomonas aeruginosa* Greenish Blue Pigment Pyocyanin: Its Production and Biological Activities. *Microbial Cell Factories*, 22(110): 1–14.

Abdulhusein, Haider Sabah and Kadim, Baidaa M.(2024). Molecular Characterization and Antibiotic Sensitivity of *Staphylococcus* sp. Isolated from Animal Skin Wunds. *Journal of Education for Pure Science-University of Thi-Qar*, 14(4): 115-128 .

Abebe, A. A. and Birhanu, A. G. (2023). Methicillin Resistant *Staphylococcus aureus*: Molecular Mechanisms Underlying Drug Resistance Development and Novel Strategies to Combat. *Infection and Drug Resistance*, 16 (2) :7641–7662 .

Aguilera-Alonso, D.; Cantón, R., Giske, C.G.; Kahlmeter, G., Vasconcelos, M.K.; Papan, C. and Turnidge, J.(2022). Searching High and Low: Call for a Joint European Society for Paediatric Infectious Diseases-European Committee on Antimicrobial Susceptibility Testing Survey on Dosage of Antibacterial Agents in Children—Part One. *The Pediatric Infectious Disease Journal*, 41(4): 182-185

Ahmad-Mansour, N., Loubet, P.; Pouget, C., Dunyach-Remy, C.; Sotto, A.; Lavigne, J.-P. and Molle, V. (2021). *Staphylococcus aureus* Toxins: An Update on Their Pathogenic Properties and Potential Treatments. *Toxins*, 13(10): 677-689.

Alharbi, N. S.; Khaled, J. M. and Abdelrahman, M. A. (2022). Distribution of Bacterial Isolates and Antimicrobial Susceptibility Among

Burn Wound Infections in Saudi Hospitals. *Saudi Journal of Biological Sciences*, 29(5): 3198–3205.

Ali, S, and Assafi, Mahde. (2024) Prevalence and Antibigram of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Clinical Isolates from Burns and Wounds in Duhok City, Iraq. *The Journal of Infection in Developing Countries*, 18.01: 82-92.

Alkhulaifi, Z. M. and Mohammed, K. A. (2023). Prevalence and Molecular Analysis of Antibiotic Resistance of *Pseudomonas aeruginosa* Isolated From Clinical and Environmental Specimens in Basra, Iraq. *Iranian Journal of Microbiology*, 15(1): 45-54.

Almatar, M.; Makky, E. A; Var, I. and Koksai, F. (2018). The Role of Nanoparticles in the Inhibition of Multidrug-Resistant Bacteria and Biofilms. *Current Drug Delivery*, 15(4): 470-484.

AL-Samaraey, A. A. A.; and Jafar, N. A. H. (2021). Evaluation of Number of Virulence Factors and Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* Isolated From Wound and Burn Infections in Samarra City. *Tikrit Journal of Pure Science*, 26(6): 7-13.

Ambade, S. S.; Gupta, V. K., Bhole, R. P.; Khedekar, P. B.; Chikhale, R. V. (2023). A review on Five and SixMembered Heterocyclic Compounds Targeting the Penicillin-Binding Protein 2 (PBP2A) of Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Molecules*, 28(20): 708-7020.

Araos, R.; and D'Agata, E. (2019). *Pseudomonas aeruginosa* and Other *Pseudomonas* species. Chapter 219, *Infectious Diseases and Their Etiologic Agents*,.P:2186-2199

Ashvini, H. M.; Balla, A.; Mutta, S. K. ,(2019) .Clarithromycin-loaded Chitosan Nanoparticles: Preparation, Characterisation and Antibacterial Activity on *Streptococcus pneumonia*. *Indian Journal of Pharmaceutical Sciences*, 81(2):302-308

Church, D.; Elsayed, S, Reid, O.; Winston, B.; AND Lindsay, R. (2022). Burn Wound Infections. *Clinical Microbiology Reviews*, 35(1): 21-29.

Atique, R.; Saeed, H. A., Anwar, B.;Perveen, A., Sharif, J., Haidar, A. and Samad, A. (2023). Quorum Sensing in Bacteria; Cell-to-Cell Communication and Signaling Mechanism. *International Journal of Social, Humanities and Life Sciences*, 1(1): 1-13.

Bashir, S. M.; Ahmed Rather, G., Patrício, A.,, Haq, Z., Sheikh, A. A.; Shah, M. Z. U. H., Fonte, P. (2022). Chitosan Nanoparticles: a Versatile Platform for Biomedical Applications. *Materials*, 15(19), 6521.

Bassetti, M.; Vena, A.; Giacobbe, D. R. and Peghin, M. (2022). New Antibiotics for Difficult Gram-negative Infections. *Current Opinion in Infectious Diseases*, 35(6): 609–618.

Beza, C.; Gellerb, A. M., Levyb, A.; Venturihttps, V. (2023). Cell-Cell Signaling Proteobacterial LuxR Solos: a Treasure Trove of Subgroups Having Different Origins, Ligands, and Ecological Roles. *American Society For Microbiology*, 8(2): 1–18.

Bilyk, B. L.; Panchal, V. V.; Tinajero-Trejo, M., Hobbs, J. K. and Foster, S. J. (2022). An Interplay of Multiple Positive and Negative Factors Governs Methicillin Resistance in *Staphylococcus aureus*. *Microbiology and Molecular Biology Reviews*, 86(2): 15-21.

Botelho, J.; Gross, F. and Peix, L. (2019). Antibiotic Resistance in *Pseudomonas aeruginosa* – Mechanisms, *Epidemiology and Evolution*. *Elsvier*, 44(4): 26–47.

Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E., Gilbert, D., Rice, L. B.; Scheld, M.; Spellberg, B. and Bartlett, J. (2009). Bad Bugs, no Drugs: No ESKAPE! An Update from the *Infectious Diseases Society of America*. ` 48(1): 1–12.

Boyd, S. E.; Livermore, D. M.; Hooper, D. C. and Hope, W. W. (2020). Metallo -Lactamases: Structure, Function, Epidemiology, Treatment Options, and the Development Pipeline. *Antimicrobial Agents and Chemotherapy*, 64(10): 1–20.

Breijyeh, Z.; Jubeh, B. and Karaman, R. (2020). Resistance of Gram-negative Bacteria to Current Antibacterial Agents and Approaches to Resolve it. *Molecules*, 25(6): 1-23.

Callaghan, R.; Caballero, A.; Tang, A. and Bierdeman, M. (2019). *Pseudomonas aeruginosa* Keratitis: Protease IV and PASP as Corneal Virulence Mediators. *Microorganisms*, 7(9): 1–17.

Campo, C.; Villamizar-Gallardo, R. A.; Lopez-Jacome, L. E.; Gonz?alez, E. E. and Mu~noz-Carranza, S. (2022). Biologically Synthesized Silver Nanoparticles as Potent Antibacterial Effective Against Multidrug-Resistant *Pseudomonas aeruginosa*. *Letters in Applied. Microbiology*, 75(3): 680- 688.

Campos, L. A.; Neto, A. F.; Scavuzzi, A. M.; Lopes, A. C.; Stela, N. and Cavalcanti, I. M. (2024). Ceftazidime/Tobramycin Co-loaded Chitosan-Coated Zein Nanoparticles Against Antibiotic-Resistant and

Biofilm-Producing *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. *Pharmaceuticals*, 17(3): 1-30.

Casilag, F.; Lorenz, A.; Krueger, J., Klawonn, F.; Weiss, S.; and Häussler, S. (2016). The LasB elastase of *Pseudomonas aeruginosa* acts in Concert with Alkaline Protease AprA to Prevent Flagellin-Mediated Immune Recognition. *Infection and Immunity*, 84(1): 162-171.

Chadha, J.; Harjai, K.; And, and Chhibber, S. (2021). Revisiting the Virulence Hallmarks of *Pseudomonas aeruginosa*: a Chronicle Through the Perspective of Quorum Sensing. *Environmental Microbiology*, 6(4): 2630–2656.

Chandrasekaran; Murugesan, Ki Deok Kim; and Se Chul Chun. (2020): "Antibacterial Activity of Chitosan Nanoparticles: a review." *Processes* 8(9): 11-23.

Chauhan, A. and Jindal, T. (2020). Methods of Sterilization and Disinfection. In *Microbiological methods for environment, food and pharmaceutical analysis* , 14(7): 67-72.

Cheung, G. Y.; Bae, J. S.; and Otto, M. (2021). Pathogenicity and Virulence of *Staphylococcus aureus*. *Virulence*, 12(1): 547-569.

Chieffi, D.; Bongiorno, D.; Licitra, A.; Campanile, F. and Fusco, V. (2024). A Novel Approach Based on Real-Time PCR with High-Resolution Melting Analysis for the Simultaneous Identification of *Staphylococcus aureus* and *Staphylococcus argenteus*. *Foods*, 13(18): 30-54.

Chinemerem Nwobodo, D.; Ugwu, M. C.; Oliseloke Anie, C., Al-Ouqaili, M. T.; Chinedu Ikem, J.; Victor Chigozie, U. and Saki, M. (2022). Antibiotic Resistance: The Challenges and Some Emerging

Strategies for Tackling a Global Menace. *Journal of clinical laboratory analysis*, 36(9): 1-10.

Chiş, A. A.; Rus, L. L.; Morgovan, C.; Arseniu, A. M., Frum, A., Loredana, A.; Gligor, F. G., Mureşan, M. L.;and Dobrea, C. M. (2022). Microbial Resistance to Antibiotics and Effective Antibiotherapy. *Biomedicines*,10(5):11-21.

Christaki, E.; Marcou, M. and Tofarides, A. (2020). Antimicrobial Resistance in Bacteria Mechanisms, Evolution, and Persistence. *Journal of molecular evolution*, 88(1): 26-40.

Gupta, N.; Singh, A. and Dureja, C. (2021). Epidemiology and Resistance Pattern of Bacterial Isolates from Burn Wounds: A Global Perspective. *Journal of Infection and Public Health*, 14(10): 1526–1533.

Cieza, M. Y. R.; Bonsaglia, E. C. R.; Rall, V. L. M.; Santos, M. V. D. and Silva, N. C. C. (2024). Staphylococcal Enterotoxins: Description and Importance in Food. *Pathogens* , 13(8): 1-21.

Cong, Y., Yang, S. and Rao, X. (2019). Vancomycin Resistant *Staphylococcus aureus* Infections: A Review of Case Updating and Clinical Features. *Journal of Advanced Research*, 21: 169-176.

Damasco, J. A.; Ravi, S., Perez, J. D.; Hagaman, D. E. and Melancon, M. P. (2020). Understanding Nanoparticle Toxicity to Direct a Safe-By-design Approaching Cancer Nano medicine. *Nanomaterials*, 10(11): 218-236.

De Oliveira, D. M.; Forde, B. M.; Kidd, T. J.; Harris, P. N.; Schembri, M. A.; Beatson, S. A. and Walker, M. J. (2020).

Antimicrobial Resistance in ESKAPE Pathogens. *Clinical microbiology reviews*, 33(3): 10-128.

Dhlamini, K. S.; Selepe, C. T.; Ramalapa, B.; Tshweu, L. and Ray, S. S. (2024). Reimagining Chitosan-Based Antimicrobial Biomaterials to Mitigate Antibiotic Resistance and Alleviate Antibiotic Overuse: A Review. *Macromolecular Materials and Engineering*, 309(9):240-268.

Diggle, S. P.; and Whiteley, M. (2020). Microbe Profile: *Pseudomonas aeruginosa*: Opportunistic Pathogen and Lab Rat. *Microbiology*, 166(1):30-33.

Di Giuseppe, M.; Perry, J. C.; Prout, T. A. and Conversano, C. (2021). Recent Empirical Research and Methodologies in Defense mechanisms: Defenses as Fundamental Contributors to Adaptation. *Frontiers in psychology*, 12(4):80-92.

Divyakolu, S.; Chikkala, R.; Ratnakar, K. S. and Sritharan, V. (2019). Hemolysins of *Staphylococcus aureus*—an update on their biology, role in pathogenesis and as targets for anti-virulence therapy. *Advances in Infectious Diseases*, 9(2): 80-104.

do Amaral Sobral, P. J.; Gebremariam, G.; Drudi, F.; De Aguiar Saldanha Pinheiro, A. C., Romani, S.; Rocculi, P. and Dalla Rosa, M. (2022). Rheological and Viscoelastic Properties of Chitosan Solutions Prepared with Different Chitosan or Acetic Acid Concentrations. *Foods*, 11(17):26-92.

Edward, E. A.; Shehawy, M. R. El, Abouelfetouh, A. and Elsayed Aboulmagd. (2023). Prevalence of Different Virulence Factors and Their Association with Antimicrobial Resistance Among *Pseudomonas*

aeruginosa Clinical Isolates from Egypt. *BMC Microbiology*, 161(23): 1–13.

El-Amier, Y. A., El-hayyany, L. Y., Ahmed, I. K., Al-Rubaye, A. A., Fahmy, A. A., Khattab, Y. Z., and Sultan, M. S. (2024). Evaluation of the VITEK® 2 Cards for Rapid Direct Identification and Antimicrobial Susceptibility Testing of Human Pathogenic Bacteria. *Egyptian Journal of Chemistry*, 67(2):91-102.

Elbehiry, A.; Marzouk, E.; Abalkhail, A.; El-Garawany, Y.; Anagreyyah, S.; Alnafea, Y.; Almuzaini, A. M.; Alwarhi, W.; Rawway, M. and Draz, A. (2022). The Development of Technology to Prevent, Diagnose, and Manage Antimicrobial Resistance in Healthcare-Associated Infections. *Vaccines* , 10(12):21-100.

El-Deeb, B.; Al-Talhi, A.; Mostafa, N. and Abou-assy, R. (2018). Biological Synthesis and Structural Characterization of Selenium Nanoparticles and Assessment of their Antimicrobial Properties. *Am. Science. Res. Journal Technol. Sciences*, 45(1): 135-170.

El-Sherbiny, I. M. and El-Baz, N. M. (2022). Nanomaterials-Based Strategies for Combating Antibiotic Resistance. *International Journal of Biological Macromolecules*, 208: 659–674.

Elshinawy, M. I.; Al-Madboly, L. A.; Ghoneim, W. M. and El-Deeb, N. M. (2018). Synergistic Effect of Newly Introduced Root Canal Medicaments; Ozonated Olive Oil and Chitosan Nanoparticles, Against Persistent Endodontic Pathogens. *Frontiers in Microbiology*, 9(5): 1371.

Elshobary, M. E.; Badawy, N. K.; Ashraf, Y.; Zatioun, A. A.; Masriya, H. H.; Ammar, M. M., Mohamed, N. A.; Mourad, S. and Assy, A. M. (2025). Combating Antibiotic Resistance: Mechanisms,

Multidrug-Resistant Pathogens, and Novel Therapeutic Approaches: An Updated Review. *Pharmaceuticals*, 18(3):402.

Everett, M. J.; and Davies, D. T. (2021). *Pseudomonas aeruginosa* elastase 9 (LasB) as a therapeutic target. *Elsvier*, 26(9), 2108–2123.

Everett, M. J.,; Davies, D. T.; Leiris, S., Sprynski, N.; and Llanos, A. (2023). Chemical Optimization of Selective *Pseudomonas aeruginosa* LasB Elastase Inhibitors and Their Impact on LasB-mediated activation of IL-1 β in Cellular and Animal Infection Models. *Bio Infection*, 3 (7): 1-14.

Ezeador, C. O.; Ejikeugwu, P. C.;Ushie, S. N. and Agbakoba, N. R. (2020). Isolation, Identification And Prevalence Of *Pseudomonas aeruginosa* Isolates From Clinical And Environmental Sources In Onitsha Metropolis, Anambra State. *EJMED, European Journal of Medical and Health Sciences*, 2(2): 1–6.

Fayisa, W. O., and Tuli, N. F. (2023). Review on *Staphylococcus aureus*. *Int. J. Nurs. Care Res*, 1: 1-8.

Fazeli, H.; Akbari, R., Moghim, S.;Narimani, T.; Arabestani, M. R. and Ghoddousi, A. R. (2012). *Pseudomonas aeruginosa* Infections in Patients, Hospital Means, and Personnel's Specimens. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, 17(4): 332-353.

Flemming, H.; Van Hullebusch, E. D.; Neu, T. R., Nielsen, P. H.; Seviour, T.; Stoodley, P.; Wingender, J.; and Wuertz, S. (2023). The Biofilm Matrix: Multitasking in a Shared Space. *Nature Reviews Microbiology*, 21(2): 70-86.

Frieden, T.; and Buissonnière, M. (2021). Will a Global Preparedness Treaty Help or Hinder Pandemic Preparedness. *BMJ global Health*, 4(1):1–3.

Furniss, R. C. D.;Kaderabkova, N.; Barker, D.; Bernal, P.; Maslova, E. and Gonzalez, D. (2022). Breaking Antimicrobial Resistance by Disrupting Extracytoplasmic Protein Folding. *Microbiology and Infectious Disease*, 11(1): 1–37.

Fux, A. C.; Melo, C. C.; Michelini, S. and Benjamin J. Swartzwelter. (2023). Heterogeneity of Lipopolysaccharide as Source of Variability in Bioassays and LPS-Binding Proteins as Remedy. *Molecular Microbiology*, 24(4): 1 -21.

Ghafil, J. A.; and Flieh, M. (2021). Isolation and Identification of Bacterial Burn Wound Infection in Iraqi Patient. *Indian Journal of Forensic Medicine & Toxicology*, 15(4):1-14.

Ghanem, S. M.; El-Baky, R. M. A.; Abourehab, M. A.; Fadl, G. F.; and Gamil, N. G. (2023). Prevalence of Quorum Sensing and Virulence Factor Genes Among *Pseudomonas aeruginosa* Isolated from Patients Suffering from Different Infections and Their Association with Antimicrobial Resistance. *Infection and Drug Resistance*, 16(2): 2371–2385.

Ghareeb, N. H.; Obaid, S. S.; and Jumaah, I. A. M. (2023). The Prevalence of Multidrug-Resistant *Staphylococcus aureus* Isolated from Different Clinical Samples. *Medical Journal of Babylon*, 20(Supplement 1): 185-187.

Ghosh, S.; Lahiri, D.; Nag, M.; and Dey, A. (2022). Phytocompound Mediated Blockage of Quorum Sensing Cascade in ESKAPE Pathogens. *Mdpi*, 11(6): 43- 61.

Grandy, S.; Scur, M.; Dolan, K.; Nickerson, R.; and Cheng, Z. (2023). Using Model Systems to Unravel Host–*Pseudomonas aeruginosa* Interactions. *Environmental Microbiology*, 10(1): 1–20.

Guo, Y.; Chen, X.; Yang, F., Wang, T.; Ni, M.; Chen, Y.; and Wang, S. (2019). Preparation and Characterization of Chitosan-Based Ternary Blend Edible Films with Efficient Antimicrobial Activities for Food Packaging Applications. *Journal of Food Science*, 84(6): 1411-1419.

Guo, Y.; Song, G.;Sun, M., Wang, J.; & Wang, Y. (2020). Prevalence and Therapies of Antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in cellular and infection microbiology*, 10, 107:1-11

Gupta, N.; Singh, A.; and Dureja, C. (2021). Epidemiology and Resistance Pattern of Bacterial Isolates From Burn Wounds: A global perspective. *Journal of Infection and Public Health*, 14(10):1526–1533.

Haider, A.; Khan, S.; Iqbal, D. N.; Shrahili, M.; Haider, S., Mohammad, K.; and Mustafa, G. (2024). Advances in Chitosan-Based Drug Delivery Systems: A Comprehensive Review for Therapeutic Applications. *European polymer journal*, 210(13): 112983.

Hateet, Rahim. 2021. Isolation and Identification of Some Bacteria Contemn in Burn Wounds in Misan, Iraq. *Archives of Razi Institute*, 7(6): 1665.

Hawi, A. A. H.; and Al-Ruaby, K. J. (2020). Antibacterial Activity Study Of Erythromycin-Loaded Chitosan Nanoparticles and Chitosan

Nanoparticles Against Identified MRSA and MSSA Bacteria Isolated from Mastitis Cow Milk. *EurAsian Journal of Biosciences*, 14(2): 1–10.

Hidalgo, A.; Libby, P.; Soehnlein, O.; Aramburu, I. V.; and Papayannopoulos, V. (2023). Neutrophil Extracellular Traps: From physiology to Pathology. *European Society of Cardiology*, 118(1): 2737–2753.

Horna, G.; & Ruiz, J. (2021). Type 3 Secretion System of *Pseudomonas aeruginosa*. *Elsevier*, 246(3): 1–17.

Howden, B. P.; Giulieri, S. G.; Baines, S. L.; Sharkey, L. K.; Lee, J. Y.; Hachani, A.; Monk, I. R.; and Stinear, T. P. (2023). *Staphylococcus aureus* Host Interactions and Adaptation. *Nature Reviews Microbiology*, 21(6): 380-395.

Humady, Ihsan K.; Hadi, O. (2024). Molecular Study of ESBL Genes and Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from The Burn Patients in AL-Najaf AL-Ashraf, Iraq. *Journal of Scientific Research in Medical and Biological Sciences*, , 5(3): 120-128.

Humphries, R.; Bobenchik, A. M., Hindler, J. A. and Schuetz, A. N. 2021. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100. *Journal of Clinical Microbiology*, 59, (2):13-21.

Huszczynski, S. M.; Lam, J. S.; and Khursigara, C. M. (2020). The Role of *Pseudomonas aeruginosa* Lipopolysaccharide in Bacterial Pathogenesis and Physiology. *Pathogens*, 9(1): 6–22.

Iber, B. T.; Kasan, N. A.; Torsabo, D.; and Omuwa, J. W. (2022). A Review of Various Sources of Chitin and Chitosan in Nature. *Journal of Renewable Materials*, 10(4):1097.

Ismail, Y. M.; Fayed, S. M.; Elesawy, F. M.; El-Halim, N. Z. A.; and El-Shimi, O. S. (2021). Phenotypic and Molecular Characteristics of *Pseudomonas aeruginosa* Isolated from Burn Unit. *Egyptian Journal of Medical Microbiology*, 30(1): 19–28.

Issa, A. A.; Fawzy, M. A.; & El-Deeb, B. (2016). Uptake of Cadmium by the Green alga *Scenedesmus Quadricauda* in the Presence of Selenium Nanoparticles. *Int JNano Chem*, 4 (2):47-52.

Jacob, M. E.; Keelara, S.; Aidara-Kane, A.; Matheu Alvarez, J. R.; and Fedorka-Cray, P. J. (2020). Optimizing a Screening Protocol for Potential Extended-Spectrum β -lactamase *Escherichia coli* on MacConkey Agar for Use in a Global Surveillance Program. *Journal of Clinical Microbiology*, 58(9), 10-28.

Jana, A. M.; and Singh, P. (2020). Bacterial Preservation. *International Journal of Life Sciences and Technology*, 13(1).127-169

Jiang, J. H.; Cameron, D. R.; Nethercott, C.; Aires-de-Sousa, M.; and Peleg, A. Y. (2023). Virulence Attributes of Successful Methicillin-Resistant *Staphylococcus aureus* Lineages. *Clinical microbiology reviews*, 36(4): 148-22.

Jin, T. (2024). Exploring the Role of Bacterial Virulence Factors And Host Elements in Septic Arthritis: Insights from Animal Models For Innovative Therapies. *Frontiers in Microbiology*, 15(5): 76- 82.

Juan, C.; Peña, C.; and Oliver, A. (2022). Host and Pathogen Biomarkers for *Pseudomonas aeruginosa* Infections. *Microbiology Spectrum*, 10(4): 12-22.

Kadim, Baidaa M, 2024. Distribution of Antibacterial Resistance among Pathogenic Bacterial Isolates from Patient in Al-Shatrah General

Hospital. *University of Thi-Qar Journal of agricultural research*, 13(2): 679-690.

Kaur, M.; Cohen, Y.; Poverenov, E.; and Eltzov, E. (2022). Synergistic Antimicrobial Effect of the Combination of Beta-Lactam Antibiotics and Chitosan Derivative on Multidrug-Resistant Bacteria. *International Journal of Biological Macromolecules*, 223(17): 1107-1114.

Khan, F.; Pham, D. T. N.; Oloketuyi, S. F., Manivasagan, P.; Oh, J.; and Kim, Y. M. (2020). Chitosan and Their Derivatives: Antibiofilm Drugs Against Pathogenic Bacteria. *Colloids and Surfaces B: Biointerfaces*, 185(6): 110-122.

Khan, Fazlurrahman; YU, Hongsik; Kim, Young-Mog. Bactericidal Activity of Usnic Acid-Chitosan Nanoparticles Against Persister Cells of Biofilm-Forming Pathogenic Bacteria. *Marine Drugs*, 18(5): 270.

Khan, Y.; Sadia, H.; Ali Shah, S. Z.; Khan, M. N.; Shah, A. A.; Ullah, N.; and Khan, M. I. (2022). Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in Various Fields of Nanotechnology: a review. *Catalysts*, 12(11): 1386.

Khatun, M. A.; Hoque, M. A.; Koffas, M.; and Feng, Y. (2023). Reducing the Virulence of *Pseudomonas aeruginosa* by Using Multiple Quorum-Quenching Enzymes. *Journal of Industrial Microbiology and Biotechnology*, 50(1): 1-16.

Kim, D. W.; and Cha, C. J. (2021). Antibiotic Resistome from the One-Health Perspective: Understanding and Controlling Antimicrobial Resistance Transmission. *Experimental and Molecular Medicine*, 53(3):301-309.

Koczoń, P.; Holaj-Krzak, J. T.; Palani, B. K.; Bolewski, T.; Dąbrowski, J.; Bartyzel, B. J.; and Gruczyńska-Sękowska, E. (2023). The Analytical Possibilities of FT-IR Spectroscopy Powered by Vibrating Molecules. *International Journal of Molecular Sciences*, 24(2): 1013.

Kulkarni, D.; Sherkar, R.; Shirsathe, C.; Sonwane, R.; Varpe, N.; Shelke, S.; More, M. P.; Pardeshi, S. R.; Dhaneshwar, G.; Junnuthula, V.; and Dyawanapelly, S. (2023). Biofabrication of Nanoparticles: Sources, Synthesis, and Biomedical Applications. *Frontiers in Bioengineering and Biotechnology*, 11(7): 1159193.

Lade, H.; and Kim, J.-S. 2021. Bacterial Targets of Antibiotics In Methicillin-Resistant *Staphylococcus aureus*. *Antibiotics*, 10(8): 284-298.

Lakhundi, S. and Zhang, K. (2020). Methicillin-resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. *Clinical Microbiology Reviews*, 33(4): 12-19.

Langendonk, R. F.; Neill, D. R. and Fothergill, J. L. (2021). The Building Blocks of Antimicrobial Resistance in *Pseudomonas aeruginosa*: Implications for Current Resistance-Breaking Therapies. *Frontiers in Cellular and Infection Microbiology*, 11 (3): 1-11.

Lansbury, L.; Lim, B.; Baskaran, V.; and Lim, W. S. (2020). Co-Infections in People with COVID-19: a Systematic Review and Meta-analysis. *Journal of Infection*, 81(1): 266–275.

LeBlanc, M.-A.; Fink, M. R.; Perkins, T. T.; and Sousa, M. C. (2021). Type III secretion system effector proteins are mechanically labile. *Biophysics and Computational Biology*, 118(12): 1–8.

Leboffe MJ; Pierce BE.(2021). A Photographic Atlas for the Microbiology Laboratory. *Morton Publishing Company*; 21(5):122-141.

Lee, A. S.; Huttner, B.; and Harbarth, S. (2023). Antimicrobial resistance in *Staphylococcus aureus*—Emerging Trends and Control Strategies. *Nature Reviews Microbiology*, 21(2): 123–136

Lemke, P.; Jünemann, L.; and Moerschbacher, B. M. (2021). Synergistic Antimicrobial Activities of Chitosan Mixtures And Chitosan–Copper Combinations. *International Journal of Molecular Sciences*, 23(6): 3345.

Liao, C.; Huang, X.; Wang, Q.; Yao, D.; and Lu, W. (2022). Virulence Factors of *Pseudomonas aeruginosa* and Antivirulence Strategies to Combat its Drug Resistance. *Frontiers in cellular and infection microbiology*, 12(4): 1-17.

Linz, M. S.; Mattappallil, A.; Finkel, D.; and Parker, D. (2023). Clinical Impact of *Staphylococcus aureus* Skin and Soft Tissue Infections. *Antibiotics*, 12(3): 557.

Lister, P. D.; Wolter, D. J. and Hanson, N. D. (2021). Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Therapeutic Options Regulation of Chromosomally Encoded Resistance Mechanisms. *Clinical Microbiology Reviews*, 22(4), 582-610.

Liu, C.; Shen, Y.; Yang, M.; Chi, K. and Guo, N. (2022). Hazard of *Staphylococcal* Enterotoxins in Food and Promising Strategies for Natural Products Against Virulence. *Journal of Agricultural and Food Chemistry*, 70(8): 50-65.

Lombardi, C.; Tolchard, J.; Bouillot, S.; Signor, L.; Gebus, C.; Liebl, D., and Job, V. (2019). Structural and Functional Characterization of the Type Three Secretion System (T3SS) Needle of *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, 10:573.

Mancuso, G.; Midiri, A.; Gerace, E. and Biondo, C. (2021). Bacterial Antibiotic Resistance: The Most Critical Pathogens. *Pathogens*, 10(10): 1310.

Marambio-Jones, C.; and Hoek, E. M. (2010). A review of the Antibacterial Effects of Silver Nanomaterial's and Potential Implications for human health and the environment. *Journal of Nanoparticle Research*, 12(5): 1531-1551.

Marchant, R.; and Banat, I. M. (2012). Biosurfactants: a Sustainable Replacement For Chemical Surfactants?. *Biotechnology letters*, 34(9): 1597-1605.

Mashruwala, A. A.; and Bassler, B. L. (2020). The Vibrio Cholerae Quorum-Sensing Protein VqmA Integrates Cell Density, Environmental, and Host-derived Cues Into the Control of Virulence. *Mbio*, 11(4): 10-1128.

Mehata, A. K.; Priya, V.; Malik, A. K.; Setia, A.; Suseela, M. N. L.; and Muthu, M. S. (2022). Bimetallic Au–Ag Nanoparticles: Advanced Nanotechnology for Tackling Antimicrobial Resistance. *Molecules*, 27(20): 7059.

Mekonnen, H.; Seid, A., Molla Fenta, G.; and Gebrecherkos, T. (2021). Antimicrobial Resistance Profiles and Associated Factors of *Acinetobacter* and *Pseudomonas aeruginosa* Nosocomial Infection Among Patients Admitted at Dessie Comprehensive Specialized Hospital, North-East Ethiopia. A cross-sectional study. *Plos one*, 16(11): 25-38.

Mikušová, V.; and Mikuš, P. (2021). Advances in Chitosan-Based Nanoparticles For Drug Delivery. *International journal of molecular sciences*, 22(17):46-52.

Miragaia, M. (2018). Factors Contributing to The Evolution of Meca-Mediated B-Lactam Resistance in Staphylococci: Update And New Insights from Whole Genome Sequencing (WGS). *Frontiers in microbiology*, 9(5): 2723.

Mirghani, R.,; Saba, T.; Khaliq, H.; Mitchell, J.; Do, L.; Chambi, L.; Diaz, K.; Kennedy, T.; Alkassab, K.; Huynh, T.; Elmi, M.; Martinez, J.; Sawan, S.; and Rijal, G. (2022). Biofilms: Formation, Drug Resistance and Alternatives to Conventional Approaches. *AIMS Microbiology*, 8(3): 226-239.

Mlynarczyk-Bonikowska, B.; Kowalewski, C.; Krolak-Ulinska, A.; and Marusza, W. (2022). Molecular mechanisms of drug resistance in *Staphylococcus aureus*. *International journal of molecular sciences*, 23(15): 80-88.

Mohammed, K. A.;Abdulkareem, Z. H.; Alzaalan, A. R.; and Yaqoob, A. K. (2021). Spa Typing of *Staphylococcus aureus* Isolated from clinical specimens from outpatients in Iraq. *Polish Journal of Microbiology*, 70(1): 60-79.

Moradali, M. F., Ghods, S., & Rehm, B. H. (2017). *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. *Frontiers in Cellular and Infection Microbiology*, 7:(39):1-16.

Mubeen, Bismillah,. (2021) "Nanotechnology as a Novel Approach in Combating Microbes Providing An Alternative to Antibiotics." *Antibiotics* 10(12): 1473.

Mughal, B.; Zaidi, S. Z. J.; Zhang, X. and Hassan, S. U. (2021). Biogenic Nanoparticles: Synthesis, Characterisation and Applications. *Applied Sciences*, 11(6): 2598.

Mulani, M. S.; Kamble, E. E.; Kumkar, S. N.; Tawre, M. S.; and Pardesi, K. R. (2019). Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Frontiers in Microbiology*, 10(3): 40-57.

Murray, P.; Maestrelli, F.; Cirri, M. and Mennini, N. (2022). Multiple Roles of Chitosan in Mucosal Drug Delivery: An Updated Review. *Marine Drugs*, 20(5): 335.

Muteeb, G.;Rehman, M. T.; Shahwan, M.; and Aatif, M. (2023). Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals*, 16(11): 1615.

Naik, P.; Pandey, S.; Gagan, S. et al. (2021). Virulence Factors in Multidrug (MDR) and Pan-Drug Resistant (XDR) *Pseudomonas aeruginosa*: a Cross-Sectional Study of Isolates Recovered from Ocular Infections in A High-Incidence Setting in Southern India. *J Ophthalm Inflamm Infect* 11(1): 21-36 .

Naji Hasan, R.; and Abdal Kareem Jasim, S. (2021). Detection of Pantone-Valentine leukocidin and MecA Genes in *Staphylococcus aureus* Isolated from Iraqi Patients. *Archives of Razi Institute*, 76(4):1054-1059.

Naji Hasan, R.; and Abdal Kareem Jasim, S. (2021). Detection of Pantone-Valentine leukocidin and MecA Genes in *Staphylococcus aureus* isolated from Iraqi Patients. *Archives of Razi Institute*, 76(4): 1054-1059.

Naskar, S.; Kuotsu, K.; and Sharma, S. (2019). Chitosan-Based Nanoparticles as Drug Delivery Systems: A Review on Two Decades Of Research. *Journal of Drug Targeting*, 27(4): 379-393.

Nelson, K. S.; Baltar, F.; Lamare, M. D.; and Morales, S. E. (2020). Ocean Acidification Affects Microbial Community and Invertebrate Settlement On Biofilms. *Scientific Reports*, 10(1): 1-9.

Nikolic, P.; and Mudgil, P. (2023). The Cell Wall, Cell Membrane and Virulence Factors of *Staphylococcus aureus* and Their Role in Antibiotic Resistance. *Microorganisms*, 11(2): 259.

Nyuykonge, B.; Van Amelsvoort, L.; Eadie, K.; Fahal, A.H.; Verbon, A. and Van De Sande, W.; 2021. Comparison of Disc Diffusion, Etest, and a Modified CLSI Broth Microdilution Method for *In Vitro* Susceptibility Testing of Itraconazole, Posaconazole, and Voriconazole against *Madurella mycetomatis*. *Antimicrobial Agents and Chemotherapy*, 65(9): 33-21

Omidian, H.; Gill, E. J.; Dey Chowdhury, S.; and Cubeddu, L. X. (2024). Chitosan Nanoparticles for Intranasal Drug Delivery. *Pharmaceutics*, 16(6): 746.

Orekan, J.; Barbé, B.; Oeng, S.; Ronat, J. B.; Letchford, J.; Jacobs, J. and Hardy, L. (2021). Culture media for Clinical Bacteriology in Low-and Middle-Income Countries: Challenges, Best Practices for Preparation and Recommendations For Improved Access. *Clinical Microbiology and Infection*, 27(10): 1400-1408.

Organization, W. H. 2017. Antibacterial Agents in Clinical Development: An Analysis of The Antibacterial Clinical Development Pipeline, Including Tuberculosis. *World Health Organization*.

Paracini, N.; Schneck, E.; Imberty, A. and Micciulla, S. (2022). Lipopolysaccharides at Solid and Liquid Interfaces: Models for

Biophysical Studies of the Gram-negative Bacterial Outer Membrane. *Advances in Colloid and Interface Science*, 301(1) :1–20.

Parasuraman, P.; Devadatha, Sarma, V. V. and Ranganathan, S. (2020). Anti quorum Sensing and Antibiofilm Activities of *Blastobotrys parvus* PPR3 against *Pseudomonas aeruginosa* PAO1. *Elsvier*, 138(1): 1–13.

Pasquina-Lemonche, L.; Burns, J.; Turner, R. D.; Kumar, S.; Tank, R.; Mullin, N. and Hobbs, J. K. (2020). The Architecture of the Gram-Positive Bacterial Cell wall. *Nature*, 582(7811): 294-297.

Peirano, Gisele, et al. (2024). "Population-Based Genomic Surveillance of *Pseudomonas aeruginosa* Causing Bloodstream Infections in a Large Canadian Health Region." *European Journal of Clinical Microbiology and Infectious Diseases* 43(3): 501-510.

Pitout, Johann DD, (2005). "Detection of *Pseudomonas aeruginosa* Producing Metallo- β -lactamases in a Large Centralized Laboratory." *Journal of clinical microbiology* 43(7): 3129-3135.

Plata, K.; Rosato, A. and Węgrzyn, G. (2009). *Staphylococcus aureus* as an Infectious Agent: Overview of Biochemistry and Molecular Genetics of Its Pathogenicity. *Acta Biochimica Polonica*, 56(4): 597-612.

Putra, A. R.; Effendi, M. H.; Koesdarto, S.; Tyasningsih, W. and Pangestie, S. E. (2020). Detection of The Extended Spectrum β -Lactamase Produced By *Escherichia Coli* From Dairy Cows By Using The Vitek-2 method in Tulungagung Regency, Indonesia. *Iraqi Journal of Veterinary Sciences*, 34(1):1-13.

Qin, S.; Xiao, W.; Zhou, C., Pu, Q.; Deng, X., Lan, L.; and Wu, M. (2022). *Pseudomonas aeruginosa*: pathogenesis, Virulence Factors,

Antibiotic Resistance, Interaction With Host, Technology Advances And Emerging Therapeutics. *Signal Transduction and Targeted Therapy*, 7(1): -181-199.

Rahmatelahi, H.; El-Matbouli, M.; and Menanteau-Ledouble, S. (2021). Delivering the Pain: an Overview of The Type III secretion System with Special Consideration for Aquatic Pathogens. *Veterinary Research*, 146(52): 1–18.

Rasheed, N. A.; and Hussein, N. R. (2021). *Staphylococcus aureus*: an Overview of discovery, Characteristics, Epidemiology, Virulence Factors and Antimicrobial Sensitivity. *European Journal of Molecular and Clinical Medicine*, 8(3): 1160-1183.

Rezić, I. (2022). Nanoparticles for Biomedical Application and Their Synthesis. *Polymers*, 14(22): 4961.

Rogers, K. L.; Baines, S. L. and Howden, B. P. (2022). Antimicrobial Resistance in *Staphylococcus aureus*: Evolution, mechanisms, and Control. *Nature Reviews Microbiology*, 20(6): 373–388

Salam, M. A.; Yusuf, M.; Salam, M. T.; Pawar, J. S.; Akhter, N.; Rabaan, A. A.; and Alqumber, M. A. (2022). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*, 11(13): 1946.

Sami Awayid, H.; Qassim Mohammad, S. 2022, Prevalence and Antibiotic Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* Isolated from Iraqi Hospitals. *Archives of Razi Institute*, 77(3):1-16.

SAPRE, Nidhi, et al. 2024, Optically Tuneable Chitosan Nanoparticles for Biomedical Imaging Application. *Sensing Technology*, 2(1): 242-250.

Sauer, K.; Stoodley, P.; Goeres, D. M.; Hall-Stoodley, L.; and Stewart, P. S. (2022). The Biofilm Life Cycle– Expanding The Conceptual Model of Biofilm Formation. *Author Manuscript*, 20 (10): 608–620.

Seil, J. T.; and Webster, T. J. (2012). Antimicrobial applications of nanotechnology: methods and literature. *International Journal of Nanomedicine*, 7(1): 2767-2781.

Shah, H. H. (1995). Oral Microbiology and Immunology, 2nd edition. *Journal of Dentistry*, 7 (5): 313-324.

Shariati, Aref,. (2020). Evaluation of Nano-Curcumin Effects on Expression Levels of Virulence Genes and Biofilm Production of Multidrug-Resistant *Pseudomonas aeruginosa* isolated from Burn Wound Infection in Tehran, Iran 7(3)123-140.

Shariati, A.; Dadashi, M.; and van Belkum, A. (2022). Global Prevalence and Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa*. *Frontiers in Cellular and Infection Microbiology*, 12(4): 912103

Sharma, D.; Misba, L.; and Khan, A. U. (2019). Antibiotics Versus Biofilm: an Emerging Battleground in Microbial Communities. *Antimicrobial Resistance and Infection Control*, 76(8): 1–10.

Sharma, S.; Datta, S.; Chatterjee, S.; Dutta, M., Samanta, J.; Vairale, M. G.; and Dwivedi, S. K. (2021). Isolation and Characterization of a Lytic Bacteriophage Against *Pseudomonas aeruginosa*. *Scientific Reports*, 11(1): 19393.

Shatti, Hanan Hameed¹; Al-Saeed, Wisam M.²; Nader, 2022. Mohammed I.³. Effect Biofilm Formation in *Pseudomonas aeruginosa* Resistance To Antibiotic. *Mustansiriya Medical Journal* 21(1): 13-17

Shahbazi, M. A.; Abbaraju, P. L.; and Santos, H. A. (2023). Nanotechnology Approaches for Antibiotic Delivery: Overcoming Multidrug Resistance. *Advanced Drug Delivery Reviews*, 198 (2): 114934

Shoaib, M.; Aqib, A. I.; Muzammil, I.; Majeed, N.; Bhutta, Z. A.; Kulyar, M. F. E. A.; and Pu, W. (2023). MRSA Compendium of Epidemiology, Transmission, Pathophysiology, Treatment, and Prevention within One Health Framework. *Frontiers in Microbiology*, 13(2): 106-114.

Shree, P.; Singh, C. K.; Sodhi, K. K.; Surya, J. N.; and Singh, D. K. (2023). Biofilms: Understanding the Structure and Contribution Towards Bacterial Resistance in Antibiotics. *Medicine in Microecology*, 16 (3): 10-24.

Shui, Jian, (2021). Relationship of biofilm-forming ability of with Swimming Motility, Twitching Motility and Virulence Gene Distribution. *Zhejiang Da xue xue bao. Yi xue ban= Journal of Zhejiang University. Medical Sciences*, 50(3): 345-351.

Silva, V.; Araújo, S.; Monteiro, A.; Eira, J.; Pereira, J. E.; Maltez, L.; Igrejas, G.; Lemsaddek, T. S.; and Poeta, P. (2023). *Staphylococcus aureus* and MRSA in Livestock: Antimicrobial Resistance and Genetic Lineages. *Microorganisms*, 11(1): 124.

Singh, C.; Mehata, A. K.; Priya, V.; Malik, A. K.; Setia, A.; Suseela, M. N. L.; and Muthu, M. S. (2022). Bimetallic Au–Ag Nanoparticles:

Advanced Nanotechnology For Tackling Antimicrobial Resistance. *Molecules*, 27(20): 70-89.

Singh, S.; and Bhatia, S. (2021). Quorum Sensing Inhibitors: Curbing Pathogenic Infections through Inhibition of Bacterial Communication. *Iranian Journal of Pharmaceutical Research*, 20(2): 486–514.

Soe, P. E.; Han, W. W.; Sagili, K. D.; Satyanarayana, S.; Shrestha, P.; Htoon, T. T. and Tin, H. H. (2021). High Prevalence of Methicillin-Resistant *Staphylococcus aureus* among Healthcare Facilities and Its Related Factors in Myanmar (2018–2019). *Tropical Medicine and Infectious Disease*, 6(2): 70-87.

Subhi, S. A.; Risan, M. H. and Al-Wattar, W. M. (2017). Collection, Isolation and Identification of Pathogenic Bacteria from Blood Clinical Specimens in Baghdad. *European Journal of Biomedical and Pharmaceutical Sciences*, 4(12): 1-8.

Suhag, D.; Thakur, P. and Thakur, A. (2023). Introduction to Nanotechnology. In *Integrated nanomaterials and their applications* . 13(2):1-17.

Tam K.Torres VJ.(2019). *Staphylococcus aureus* Secreted Toxins and Extracellular Enzymes. *MicrobiolSpectr*. Microbiolspec 7(10): 3-39.

Tamma, P. D.; Harris, P. N.; Mathers, A. J.; Wenzler, E. and Humphries, R. M. (2023). Deconstructing the 2023 Clinical and Laboratory Standards Institute revised piperacillin-tazobactam breakpoints against *Pseudomonas aeruginosa*. *Clinical Infectious Diseases*, 76(10): 1868-1870.

Tavassoli, S.; Nayar, G.; Darcy, K.; Grzeda, M.; Luck, J.; Williams, O. M. and Tole, D. (2019). An 11-Year Analysis of Microbial Keratitis

in the South West of England using brain–heart infusion broth. *Eye*, 33(10):1619-1625.

Thakur, P. and Thakur, A. (2022). Introduction to Nanotechnology. *Synthesis and Applications of Nanoparticles*, 12(4): 1-17.

Thambiliyagodage, C.; Jayanetti, M.; Mendis, A.; Ekanayake, G.; Liyanaarachchi, H. and Vigneswaran, S. (2023). Recent Advances in Chitosan-Based Applications—a review. *Materials*, 16(5): 2073.

Todar, K. (2011). Bacterial Resistance to Antibiotics (page 3). *Todar's Online Textbook of Bacteriology*, 4(1):1-3.

Tripathi, Nishant, and Amit Sapra. (2020). "Gram staining." *International Journal of Research and Review*, 10(9): 336-341

Truan, D.; Vasil, A.; Stonehouse, M.; Vasil, M. L. and Pohl, E. (2013). High-level over-expression, Purification, and Crystallization of a Novel Phospholipase C/sphingomyelinase from *Pseudomonas aeruginosa*. *Protein Expression and Purification*, 90(1): 40-46.

Tuon, F. F.; Dantas, L. R.; Suss, P. H.; and Ribeir, victoria S. T. (2022). Pathogenesis of the *Pseudomonas aeruginosa* Biofilm: A Review. *Pathogens*, 11(300):1–19.

Urban-Chmiel, R.; Marek, A.; Stępień-Pyśniak, D.; Wieczorek, K.; Dec, M., Nowaczek, A. and Osek, J. (2022). Antibiotic Resistance in Bacteria—A review. *Antibiotics*, 11(8): 1079.

Urgancı, N. N.; Yılmaz, N.; Alaşalvar, G. K. and Yıldırım, Z. (2022). *Pseudomonas aeruginosa* and its Pathogenicity. *Turkish Journal of Agriculture-Food Science and Technology*, 10(4): 726-738.

Verdial, C.; Serrano, I.; Tavares, L. and Gil, S. (2023). Mechanisms of Antibiotic and Biocide Resistance That Contribute to *Pseudomonas aeruginosa* Persistence in the Hospital Environment. *Biomedicines*, 11(4), 1–19

Vinshia, Jebadass Jasmine; Kesavaram Padmavathy; and Baskaran Sathyapriya. (2024). "Comparison of the VITEK® 2 System with Conventional Methods For Species Identification and Antimicrobial Susceptibility Pattern Of Staphylococcal Carrier Isolates." *International Research Journal of Multidisciplinary Scope (IRJMS)*, 5(2): 940-948.

Wagener, B. M.; Anjum, N.; Christiaans, S. C.; Banks, M. E.; Parker, J. C.; Threet, A. T.; Walker, R. R.; Isbell, K. D.; Moser, S. A.; Stevens, T.; Alexeyev, M. F.; Audia, J. P.; Richter, W., Hardy, K. S.; Saleh, L. A.; Morgan, C. and Pittet, J. F. ois. (2020). Exoenzyme y Contributes to End Organ Dysfunction Caused by *pseudomonas aeruginosa* Pneumonia in Critically ill patients: *An exploratory study*. In *Toxins*, 12 (6), 1-17.

Wang, M.; Bi, S.; Qin, D.; Su, C.; Wang, H. and Chen, X. (2021). Quantitative Evaluation of The Antibacterial Effectiveness and Efficiency of Chitosan Considering The Effect of Neutralization. *Carbohydrate Polymers*, 265(13): 117918.

Wagner III, W. E. (2019). Using IBM® SPSS® Statistics for Research Methods and Social Science Statistics. *Sage Publications*.5(60): 208

Winayu, I. J., Ekantari, N., Puspita, I. D., Budhijanto, W., and Nugraheni, P. S. (2019, October). The Effect of Reduced Acetic Acid Concentration on Nano-Chitosan Formulation as Fish Preservative. In *IOP Conference Series: Materials Science and Engineering* 633(1): 120-140).

Xia, G. and Wolz, C. (2014). Phages of *Staphylococcus aureus* and their impact on host evolution. *Infection, Genetics and Evolution*, 21, 593-601.

Cheung, G. Y., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1): 547-569.

Xiao, G.; Li, J. and Sun, Z. (2023). The Combination of Antibiotic and Non-Antibiotic Compounds Improves Antibiotic Efficacy Against Multidrug-Resistant Bacteria. *International Journal of Molecular Sciences*, 24(20): 15493.

Xiao, Gang; LI, Jiyun; Sun, Zhiliang. 2023, The combination of antibiotic and non-antibiotic compounds improves antibiotic efficacy against multidrug-resistant bacteria. *International Journal of Molecular Sciences*, 24(20): 15493.

Yin, W.; Wang, Y., Liu, L. and He, J. (2019). Biofilms: The Microbial “Protective Clothing” in Extreme Environments. *International Journal of Molecular Sciences*, 20(14): 3423.

Yoo, I. Y.; Han, J.; Ha, S. I., Cha, Y. J.; Pil, S. D. and Park, Y. J. (2021). Clinical performance of ASTA Sepsiprep kit in direct bacterial identification and antimicrobial susceptibility test using MicroIDSys Elite and VITEK-2 system. *Journal of Clinical Laboratory Analysis*, 35(6): 44-56.

Yougbaré, S.; Mutalik, C.; Okoro, G.; Lin, I. H.; Krisnawati, D. I.; Jazidie, A. and Kuo, T. R. (2021). Emerging Trends in Nanomaterials for Antibacterial Applications. *International Journal of Nanomedicine*, 16(7): 5831-5867.

Zakhour, J.; El Ayoubi, L. E. W. and Kanj, S. S. (2024). Metallo-betaLactamases: Mechanisms, Treatment Challenges, and Future Prospects. *Expert Review of Anti-Infective Therapy*, 22(4): 189-201.

الخلاصة

عد ظهور المقاومة للمضادات الحيوية (AMR) بين البكتيريا ذات الأهمية السريرية أحد التحديات الخطيرة التي تواجه أنظمة الرعاية الصحية الحديثة على مستوى العالم. وتُعدّ كلٌّ من *Staphylococcus aureus* و *Pseudomonas aeruginosa* من أكثر العزلات شيوعاً من العينات السريرية، وغالباً ما ترتبط بإصابات حادة يصعب علاجها. وتمتلك هذه البكتيريا قدرة عالية على تطوير آليات مقاومة متعددة، مما يحد من فعالية المضادات الحيوية التقليدية، ويستدعي البحث عن استراتيجيات علاجية بديلة أكثر فاعلية.

وفي هذا السياق، حظيت الجسيمات النانوية المعتمدة على البوليمرات الطبيعية باهتمام متزايد كخيار واعد لتعزيز النشاط المضاد للميكروبات والحد من المقاومة البكتيرية. ويُعدّ الكيتوسان، وهو سكاريات متعددة متوافق حيويًا وقابل للتحلل الحيوي مشتق من الكيتين، من المواد التي تمتلك خصائص مضادة للبكتيريا بطبيعتها. كما أن تحضيره بصيغة نانوية يمنحه نشاطاً سطحياً واستقراراً أعلى، فضلاً عن تفاعلات محسّنة مع الخلايا البكتيرية. إن دمج جسيمات الكيتوسان النانوية مع المضادات الحيوية التقليدية من شأنه أن يوفر تأثيرات تآزرية تسهم في تحسين الفعالية العلاجية.

هدفت هذه الدراسة إلى تقييم الفعالية المضادة للبكتيريا لجسيمات الكيتوسان النانوية، منفردة أو عند دمجها مع ثلاثة مضادات حيوية مختارة، ضد عزلات سريرية من *S. aureus* و *P. aeruginosa*. حيث جُمعت (100) عينة من مرضى راقدين في مستشفيات محافظة ذي قار خلال المدة من تشرين الأول/أكتوبر 2024 إلى شباط/فبراير 2025. خضعت العينات لعمليات الزرع البكتيري، وأُجريت الدراسات اللاحقة في مختبر الأحياء المجهرية بكلية الطب البيطري/ جامعة الشطرة. استُخدمت أنظمة VITEK I و VITEK II لإجراء التشخيص، كما نُفذ اختبار الحساسية للمضادات الحيوية بطريقة الانتشار بالقرص.

تمت دراسة الجينات المسؤولة عن المقاومة لمجموعة البيتا لاكتام على المستوى الجزيئي من خلال الكشف عن وجود الجينات (*mecA* و *blaZ*) في عزلات *S. aureus*، والجينات (*AmpC* و *MexA*) في عزلات *P. aeruginosa*. كذلك جرى تحضير محلول جسيمات الكيتوسان النانوية وتوصيفها من حيث الحجم والشكل والاستقرار. كما جرى اختيار مضادات الإريثروميسين، الأمبيسلين، والسيفتازيديم ومزجها مع جسيمات الكيتوسان النانوية تحت ظروف مختبرية مضبوطة.

أظهرت نتائج الدراسة أن من أصل (100) عينة، جرى التعرف على (34) عزلة تابعة لـ *P. aeruginosa* و (23) عزلة تابعة لـ *S. aureus*. وقد بيّنت الفحوص الجزيئية وجود الجينات (*mecA* و *blaZ*) في (12) عزلة من *S. aureus*، بينما وُجدت الجينات (*mexA* و *ampC*) في (8) عزلات من

P. aeruginosa. وأكدت النتائج أن جميع العزلات قيد الدراسة أظهرت مقاومة للمضادات الحيوية، كما كشفت عن ارتفاع معدلات المقاومة لدى مرضى الحروق والجروح في محافظة ذي قار بشكل مقلق. كما أوضحت النتائج أن المحاليل المائية لكل من الكيتوسان أو المضادات الحيوية منفردة أبدت فعالية محدودة، في حين أن المزج بينهما لتكوين مزيج ثنائي (كيتوسان-مضاد حيوي) أدى إلى تأثير تآزري ملحوظ. وأظهرت التجارب أن مزيج CNPs-Ceftazidime كان الأكثر فعالية ضد *P. aeruginosa* من خلال إحداث أكبر مناطق تثبيط، بينما كان مزيج CNPs-Erythromycin أكثر فاعلية ضد *S. aureus*.

وقد تم بنجاح تحضير جسيمات الكيتوسان النانوية المحملة بالمضادين سيفتازيديم و إريثروميسين باستخدام طريقة التجلط الأيوني. وأسهم الكيتوسان في تعزيز الامتصاصية الضوئية لكل من السيفتازيديم والإريثروميسين، مع تأثير أوضح في حالة السيفتازيديم. أما نتائج التحليل الطيفي (UV-Vis) فلم تُظهر وجود تفاعلات ملحوظة بين الإريثروميسين والكيتوسان ضمن المدى (280-300 نانومتر). كما أكدت نتائج طيف FTIR وجود مجموعات الهيدروكسيل والكربونيل والأميد، مما يدل على نجاح الارتباط أو التفاعل بين المضاد الحيوي و سطح الجسيم النانوي.

تُبرز هذه النتائج الإمكانيات الواعدة لجسيمات الكيتوسان النانوية كاستراتيجية مساعدة يمكن أن تسهم في تعزيز الفعالية المضادة للبكتيريا للمضادات التقليدية، الأمر الذي قد يساعد في تقليل الجرعات العلاجية، والحد من الآثار الجانبية، وإطالة العمر الافتراضي للمضادات الحيوية الحالية. وتوصي الدراسة بضرورة إجراء المزيد من البحوث المستقبلية لتقييم السمية الخلوية، ودراسة الفعالية داخل الجسم الحي (*in vivo*)، والتحقق من إمكانية التطبيق السريري لهذه التقنية .

Appendices



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة الشطرة

كلية الطب البيطري / فرع الاحياء المجهرية

تعزيز نشاط المضادات الحيوية ضد البكتريا المقاومة باستخدام جسيمات نانوية محددة في المختبر

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري- جامعة الشطرة وهي جزء من
متطلبات الحصول على شهادة الماجستير في اختصاص علوم الطب البيطري (الاحياء
المجهرية)

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