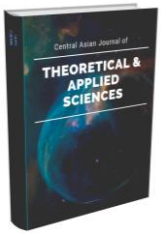

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Article

Comparing the Effects of Nanoparticles on *Staphylococcus aureus* Bacteria Isolated from Pathological Samples with Some Antibiotics Effects

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Abstract: The staphylococcus bacteria that causes infections of the skin and soft tissues, including abscesses, as they show in burns on the skin's surface, as well as upper respiratory tract and urinary tract infections in our isolates, was found in this investigation, which included sixty samples from Fallujah City. Samples from nose infections, burn infections, otitis infections, wound infections, and urinary tract infections were taken from the bodies of patients and auditors at Fallujah Teaching Hospital. The extended time for collection was set for September 2022 through November 2022. The outcomes of The study's findings showed that whereas 22 samples (26%) tested negative for bacterial culture, 38 samples (74%) tested positive. and (23) isolates that met the criteria for Staphylococcus classification using culture and biochemical tests; moreover, the findings of phenotypic detection using Mannitol Salt Agar revealed the presence of S. aureus (8) in the coagulase test. The show, the interaction, the results, and the study are all crucial to this episode. The effects of around ten different antibiotic types—Trimethoprim, Azithromycin, Rifampin, Gentamicin, Norfloxacin, Chloramphenicol, Nalidixic acid, Vancomycin, Oxacillin, and Piperacillin—on this particular kind of bacteria were most often studied. Because nanoparticles are suitable for use in the food and agriculture industries, they have little negative effects on human cells, are selectively poisonous to S. aureus, and were used to study their effect on the bacteria. Cobalt oxide nanoparticles were used at many concentrations (2000, 5000, 10000 mg/ml). Using nanoparticles, we were able to apply zones of inhibition on our isolates that measured 26±0.1 mm and 20±0.15 mm, respectively, at concentrations of 10,000 µg/ml and 5000 µg/ml of Co NPs. concentration displayed larger inhibition zones than a number of widely used antibiotics, with the exception of NORFLOXACIN and chloramphenicol, which exhibited superior antibacterial action against the bacterial isolates used in this investigation. It is noteworthy that the most resistant antibacterial isolates utilized in this investigation were employed to evaluate the impact of nanoparticles on them.

Keywords: Nanoparticles, *Staphylococcus aureus*, Antibiotics Effects

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1. Introduction

Staphylococcus bacteria are a type of parasitic bacteria that can infect humans and other animals. Initially, they were divided into two groups based on their coagulase reaction, which is responsible for the clotting of blood plasma [1]. Staphylococcus ranks among the most potent pathogenic bacteria involved in both community- and hospital-acquired infections; it is present in about one-third of healthy individuals. The ability of

pyogenic staphylococcus to synthesise many classes of antibiotics easily also makes them more dangerous, hence more likely to worsen diseases.

In many cases, MRSA infection is difficult to treat because of the huge resistance capabilities of the bacterium; this is further promoted by S. aureus biofilms. Together, these unique resistances continue to make standard therapies ineffective. An example might include endocytic nanoparticles with formal modes of transport capable of carrying drugs and antigens across bacterial membranes—allowing for targeted delivery against these diseases [2]. The use of nanoparticles exhibited a greater percentage inhibition of bacterial growth than when both antibiotics and nanoparticles were used, signifying increased rates of antibiotic resistance and hence the need for more research in finding other agents as well as a cure for bacterial infections. The present study has been undertaken with the following specific objectives: i) To isolate a large number of antibiotic-resistant Staphylococcus aureus, particularly MRSA and VRSA, from various clinical specimens ii) To understand the genetic and phenotypic relationships among these isolated strains iii) To evaluate their resistance against drugs and nanoparticles iv) To find out the lowest minimum bactericidal concentration of zinc oxide (an antibiotic) at various particle sizes The synergistic effect between antibiotics and nanoparticles can be summarized in the following protocols [3].

The aim of this study:

1. Separating and characterizing Staphylococcus aureus bacteria from clinical specimens with pathologies.
2. Investigation the spread of *S. aureus* bacteria and comparing their isolation among the studied samples.
3. Examining how various antibiotics affect S. aureus germs that have been isolated.
4. Examining how nanocobalt oxide affects isolated S. aureus bacteria
5. Contrasting the impact of antibiotics and nanoparticles on the microorganisms under study.

Genus *staphylococcus:*

Staphylococcus aureus is a Gram-positive bacterium that is cocci-shaped and occurs in clusters (like grapes). When stained by the Gram method, these organisms appear purple. They can grow on medium with up to 10% salt, and colonies are golden or yellow. The word "aureus" means "golden or yellow." These organisms can grow either aerobically or anaerobically and are facultative anaerobes. They are also catalase positive, non-indole producers: Gram positive cocci capable of growing at temperatures ranging from 18 to 40 degrees Celsius. Some biochemical tests for these organisms include coagulase, novobiocin susceptibility, and mannitol fermentation. MRSA strains possess the mec gene, which is part of staphylococcal chromosomal cassette mec (SCCmec) elements that can encode resistance to multiple classes of antibiotics, depending on the type of SCCmec. The aim of this article is to give an account of the role played by the mec gene and the protein it codes for, PBP-2a (Penicillin binding protein 2A). Peptidoglycan synthesis in the bacterial cell wall is accomplished by PBP-2a, an enzyme otherwise known as penicillin-binding protein (PBP) that is essential to the bacterial cell wall. PBP-2A has comparably minimal affinity among other PBPs to bind with beta-lactams and other penicillin-derived antibiotics; therefore, PBP-2A is free to synthesize the bacterial cell wall during the administration of these antibiotics. Consequently, antibiotics are unable to stop S. aureus strains that produce PBP-2A from multiplying, hence the many antibiotic resistances of these MRSA bacteria. Methicillin, nafcillin, oxacillin, and cephalosporins are examples of common antibiotics that MRSA strains become resistant to [4].

Clinical Manifestations

It is known to be a common pathogen of humans, causing skin and other infections, such as boils, furuncles, styes, impetigo, and other more serious systemic diseases, particularly in individuals with chronic disease, or those burned, traumatized, immunosuppressed; deep abscesses, pneumonia osteomyelitis, endocarditis phlebitis mastitis meningitis— many associated with hospital patients rather than lay people. The most frequent infections related to indwelling devices (e.g., joint prostheses or cardiovascular devices and artificial heart valves) are S. aureus and S. epidermidis. Staphylococcus aureus is able to cause localized abscesses at other sites of the body as well as superficial skin infections such as boils and styes. More serious skin conditions plus localized and chronic infections (e.g., osteomyelitis or endocarditis) are caused by S. aureus [5]. S. aureus is one of the leading causes of nosocomial infections in surgical wounds, while S. epidermidis is responsible for infections acquired from implanted medical devices or equipment. Since Staphylococcus aureus produces enterotoxins in food, it can cause food poisoning. It has been known to cause toxic shock syndrome through the release of superantigens into the bloodstream. S saprophiticus causes urinary tract infections, especially in females. Other staphylococci species are less common pathogens and include S lugdunensis, S haemolyticus, S warneri, S schleiferi, and S intermedius [6].

Staphylococcus aureus virulence factors

Staphylococcus aureus possesses several surface proteins that allow it to bind host proteins such as laminin and fibronectin, which are components of the extracellular matrix. Some of the virulence factors of S. aureus are as follows: antigens (capsule, adhesins), enzymes (coagulase, lipase, hyaluronidase, staphylokinase, nuclease), and toxins (β-toxin, δ-toxin, P-V Leukocidins, Enterotoxin, Exfoliative Toxin, Toxic Shock Syndrome Toxin) [7].

1. Capsular Polysaccharide
2. Adherence factors
3. Toxin
4. HEMOLYSINS
5. Lipase
6. Nuclease

Pathogenicity of *Staphylococcus aureus*

One of the most prevalent bacterial infections in humans, S. aureus is the cause of many illnesses in humans, such as meningitis, urinary tract infections, bacteremia, infective endocarditis, skin and soft tissue infections, gastroenteritis, ocular infections, osteoarticular infections, and toxic shock syndrome [8]. These bacteria can result in invasive infections and/or toxin-mediated illnesses, depending on the strains involved and the infection location.

1. Osteoarticular infections
2. Rhinosinusitis
3. Osteoarticular Infection
4. Ocular Infections
5. Pneumonia
6. Staphylococcal Scalded Skin Syndrome

TOXIN-MEDIATED ILLNESS

1. Food Poisoning
2. Toxic Shock Syndrome

Techniques used by Staphylococcus aureus to elude the host's acquired immunity

In the reports, it is stated that the most frequent cause of bacterial infections in the bloodstream, skin and soft tissues, and lower respiratory tracts in industrialized countries is Methicillin-resistant Staphylococcus aureus acquired outside healthcare settings or community-acquired MRSA; this has increased interest in S. aureus pathophysiology in recent studies. A considerable body of research over many years has identified and characterized about 40 proteins—also called immune evasion proteins—that can hinder various immune system processes of both the innate and adaptive arms. Between 100 and 200 proteins are released by S. aureus, based on proteomics studies, many of which are of unknown function [9]. These unrevealed released proteins might also be molecules of evasion, indicating that the discovery of new evasion proteins is a process that is still ongoing and will likely continue in future years.

1. Aureolysin
2. Staphylococcus chemo taxis inhibitory protein
3. Collagen adhesion of S. aureus
4. Extracellular adhesion protein and its homologue
5. Complement-binding protein outside of cells

Host Defenses

Neutrophils are the most common leukocytes, making up 60% of leukocytes in blood, and they are important in combating S. aureus infection. They have different granules with different contents for the killing action against both Gram-positive and Gram-negative bacteria. After their development and maturation in the bone marrow, neutrophils are released into the circulation. It is at this site that chemotactic signals generated by bacteria as well as host cells act to cause these end stage cells to circulate and then migrate to the site of infection. Many investigations have demonstrated an association between staphylococcal infections and neutrophil function impairment. Take, for example, individuals who have congenital neutropenia; they are often prone to very serious infections like potentially lethal staphylococcal infections. Following this initial response, the acquired immune system comes into play; however, its contribution in acute infection is minimal. Although most adult humans do have high levels of circulating antibodies against various staphylococcal proteins, these are not protective immunologically [10]. Nonetheless, antibodies are important in the long run with recurrent staphylococcal infections, but antibodies against staphylococcal proteins do not show effective protection.

Intrinsic Antibiotic Resistance

Clinical anti-infective therapies are becoming more challenging due to the increasing rates of resistance in S. aureus infections and multidrug-resistant strains. There are three main components to the endogenous resistance mechanisms (Figure 1) [11].

1. Outer Membrane Permeability

Drug resistance occurs because there is reduced drug absorption due to the bacterial energy metabolism alteration that arises from decreased cell membrane permeability. For example, a resistance of S. aureus to aminoglycosides is as a result of reduction in membrane permeability, which leads to reduced intake of the drug (Figure 1B).

2. Efflux Systems

Ball and McMurry, in 1980, discovered bacterial active efflux systems while studying tetracycline resistance in Escherichia coli. According to the results of subsequent research, an active efflux system is a typical physiological structure found in bacteria, even in sensitive strains. Resistance occurs when the induction of substrates in the environment extends over a long period; this induction leads to the activation and expression of genes that encode the efflux system, thereby increasing the host's ability to efflux drugs tremendously. Active drug efflux mechanisms influence drug resistance to multiple

medications. Three different types of multidrug-pumping proteins are present in the cell membrane of Staphylococcus aureus: QacA, NorA, and Smr. It has been suggested that QacA has a major role to play in MRSA. All proton kinesins are multidrug pumping proteins; that is, material exchange takes place based on an electrochemical gradient of H+ ions on both sides of the cell membrane rather than on ATP hydrolysis alone as a source of energy release. This is usually a reversible process in which intracellular toxic substances, such as dyes and antibiotic drugs, are removed from the cell and H+ ions move from the extracellular to the intracellular space (11).

3. Excessive Production of β-Lactamase

The hydrolysis of β-lactam antibiotics, including carbapenems, is facilitated by the enzyme β-lactamase. The genes encoding this enzyme are widespread in bacterial chromosomes and can be transferred among different bacteria species. Recent research has shown that β-lactam antibiotics kill bacteria mainly through two different mechanisms.

The process entails binding to penicillin-binding proteins (PBPs), which are involved in the production of cell wall peptidoglycan. Binding to these proteins inhibits the synthesis of peptidoglycan, which weakens the cell wall and results finally in bacterial growth and lysis.

The main cause of MRSA resistance to antibiotics is the secretion of β-lactamase. This is an enzyme that reduces the activity of antibiotics in two main ways: hydrolyzing β-lactam antibiotics (which means breaking them down) and pinching (holding on tightly) to extracellular antibiotics, preventing their entry into intracellular spaces where they can reach their target sites effectively and trigger bacterial autolytic enzyme activity, eventually leading to autolysis and death (Figure 1D).

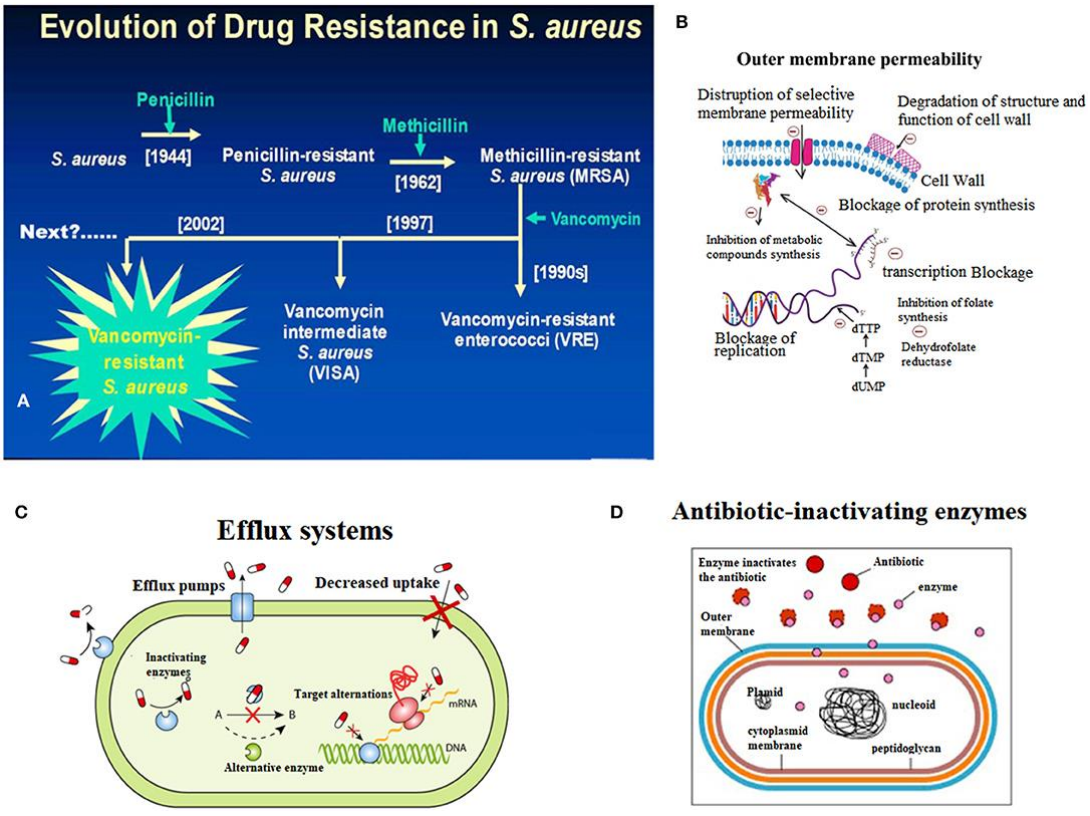


Figure 1. The Staphylococcus aureus endogenous resistance mechanism. (A) A succinct overview of S. aureus drug resistance evolution. (B) Diagram showing how drug resistance in S. aureus is caused by reduced outer membrane permeability. (C) How MRSA resistance is influenced by active efflux mechanisms. (D) The part cellular enzymes play in S. aureus drug resistance

Guo, Yunlei, et al. "Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus."

Acquired Antibiotic Resistance

1. Resistance by Mutations

Genetic changes that alter the target DNA gyrase or reduce outer membrane proteins can confer resistance in Staphylococcus aureus by preventing drug accumulation. For example, resistance to clindamycin and erythromycin is due to a modification in ribosomal RNA methylase [11].

2. Biofilm-Mediated Resistance

Complex structures known as bacterial biofilms form when communities of microorganisms adhere to surfaces and encase their internal microorganisms in a highly hydrated outer polymer matrix. Bacteria use this as a defensive survival strategy that helps them adapt to their environment. Most bacteria in nature live as biofilms due to their high adhesion and drug-resistant properties, which allow them to withstand host immune responses and evade the effects of antibiotics. They can be up to 1,000 times more resistant to antimicrobial drugs than planktonic cells. Currently, attempts to control biofilms at the national and global levels are focused on the development of new antimicrobial drugs; however, many of the synthetic drugs and antibiotics currently in use have harmful side effects. As biofilm-forming bacteria become more resistant to these traditional therapies, drug-resistant strains are on the rise [11].

Nanotechnology

The production, analysis and use of materials with nanoscale dimensions (1 to 100 nm) for scientific purposes is called nanotechnology. Nanoparticles have extraordinary special properties compared to conventional macroscopic materials. The latest development in nanoscience and nanotechnology is the use of nanoparticles in materials and structures. Nanoparticles, also known as nanoparticles, have a variety of uses in several fields such as energy, electronics, medicine, healthcare and solar energy applications. Due to the requirements of the applications, methods for producing nanoparticles - physical, chemical and biological - have become indispensable in research. Nanoparticles can be produced through physical and chemical processes using top-down and bottom-up approaches. In the top-down approach, the metal is first mechanically ground and then the resulting nano-sized metal particles are stabilized in sequence using colloidal protective agents. On the other hand, probe combinations, electrochemical techniques and metal reduction are examples of bottom-up approaches. With the emergence of infectious diseases caused by various harmful bacteria and antibiotic resistance, pharmaceutical companies and researchers are looking for new antimicrobial drugs. Nanoparticle materials are innovative antimicrobial agents due to their unique chemical and physical properties and high specific surface area. Recently, the use of metal nanoparticles in combination with certain antibiotics has been investigated as a means of improving antibiotic effectiveness and overcoming drug resistance. In terms of antimicrobial properties, different nanoparticles have different abilities to kill bacteria; metal nanoparticles in particular are known to utilize multiple modes of action to achieve this goal. Nanoparticles have been shown to penetrate bacterial cell walls and form pores in the membrane surface. This releases free radicals that damage the cell membrane and make it porous, ultimately leading to cell death.

Reactive oxygen species (ROS) and disruption of enzyme synthesis are two possible effects of NP ionization. Studies have also shown that DNA transcription is impaired. Silver nanoparticles are one of the most promising metal nanoparticles (NPs) due to their

unique properties, including high electrical conductivity, chemical stability, catalytic activity, and antimicrobial activity [12]. Multidrug-resistant microorganisms (MROs) are a growing public health problem that make many healthcare-related diseases difficult to treat with existing drugs. Therefore, new approaches are needed to combat bacteria, as nanotechnology has shown promising results [13].

1. Applications of NPs as Antimicrobial Agents

To evade antimicrobial effects, nanoparticles have antimicrobial activity that circumvents traditional resistance mechanisms such as reduced cell permeability, target site alteration, increased efflux through overexpressed pumps, and enzyme inactivation (Figure 2). In addition, conjugated nanoparticles and antibiotics have synergistic effects on bacteria, inhibit biofilm formation, and can be used to combat multidrug-resistant bacteria [13].

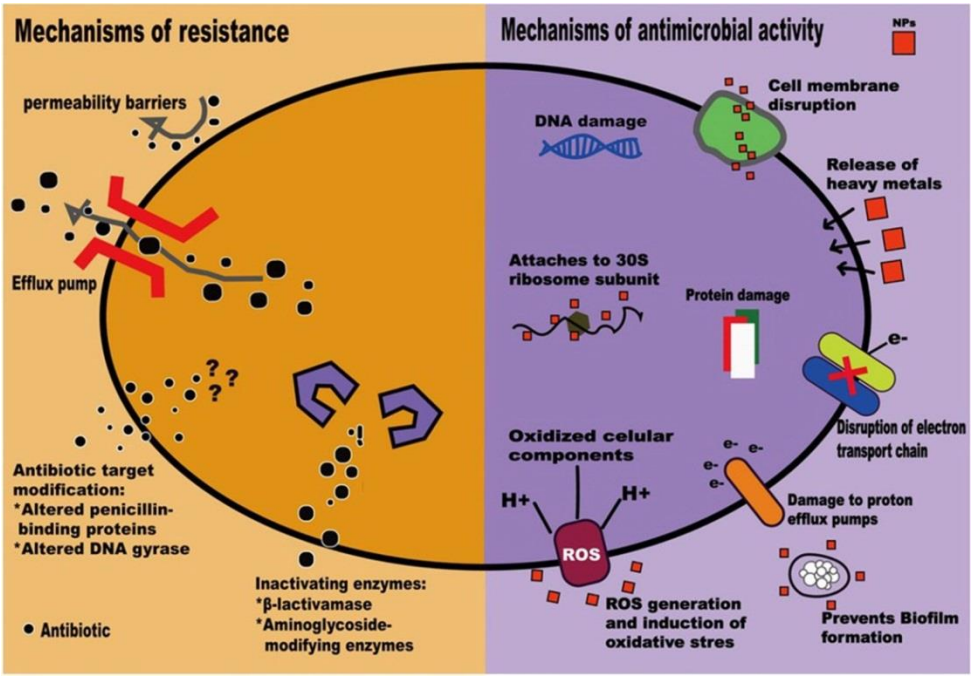


Figure 2. Mechanisms of nanoparticle activity and resistance to antibiotics [22]. [Ko, W. C., Hsueh, P. R., & Lee, N. Y. (2019). Using nanoparticles to treat infections

2. Several characteristics of NPs make them viable alternatives to traditional antibiotics

At first, the expanded contact area with the target organism due to the high surface-to-volume ratio of nanoparticles (NPs) allows them to get in through cell membranes and affect biochemistry processes.

Second, NPs might enhance the inhibitory actions of antibiotics. For example, gold nanoparticles have been shown to decrease the minimum inhibitory concentrations (MICs) against gram-positive and gram-negative bacteria of ampicillin, streptomycin, or kanamycin. However, several physicochemical variables such as size, shape, chemical changes, solvent, and method of preparation among environmental factors may influence the antibacterial activities of NPs [14].

2. Materials and Methods

Laboratory Method

1. Bacterial isolation

Since positive bacteria cannot grow on MacConkey agar, pathological samples are grown on blood agar and MacConkey agar plates to separate the two bacteria. The plates are incubated at 37°C for a whole day. After necessary identification tests are performed and confirmed as Staphylococcus spp., colonies are found on the slanted medium [15].

2. Morphological Examination

S. epidermidis shows white pigmentation on blood agar, S. saprophyticus may be pale yellow or white, and S. aureus typically shows pale yellow to golden pigmentation. However, pigmentation is not usually a reliable characteristic. While S. epidermidis and S. saprophyticus are almost always non-hemolytic, S. aureus is usually beta-hemolytic. S. aureus colonies are round, convex, and have well-defined borders. They range in diameter from 1 to 4 mm. Clear zones of beta-hemolysis typically surround S. aureus colonies. The word "Staphylococcus aureus" means "golden" in Latin, referring to the golden hue of some strains, although this specific colony color may not always be recognizable.[16]

Identification of the bacterial isolates

Biochemical tests

1. Catalase Test

This test detects the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H2O2). It is used to differentiate between bacteria that do not produce catalase (e.g., Streptococcus) and those that do (e.g., Staphylococcus). 3% H2O2 is typically used for normal cultures, while 15% H2O2 is used to detect catalase in anaerobic bacteria.

2. Gram Stain

A popular laboratory test that aids in the prompt diagnosis of bacterial infections is the Gram stain. In this test, certain bacteria are classified as "gram-positive" or "gram-negative" depending on which of the two color sets (purple to blue or pink to red) they take on after a particular staining procedure. Bacteria are distinguished by the chemical and physical characteristics of their cell walls through the use of gram staining. But not all microbe can be examined in this way. The bacteria Staphylococcus aureus is categorized as gram-positive.

3. Coagulases test

Enzymes called coagulases cause blood plasma to clot using a method akin to normal coagulation. The ability of an organism to create this exoenzyme, which clogs blood's plasma component, is assessed by the coagulase test. Coagulase is a good predictor of the pathogenic potential of Staphylococcus aureus since it is the only important disease-causing bacteria in people that generates it [17].

Cultural identification

1. Mannitol salt agar for isolation of staphylococcus aureus

Staphylococcus aureus is isolated and identified from both clinical and non-clinical material using mannitol salt agar (MSA), which is a selective and differential medium. It follows Chapman's rule of separating presumed pathogenic staphylococci by allowing the growth of some bacteria while inhibiting the development of others. Besides, it provides essential nitrogenous constituents, vitamins, minerals, and amino acids required for the growth of microorganisms due to the presence of peptones and beef extract in the medium. Other than staphylococci, other bacteria are inhibited by 7.5% sodium chloride, which provides an adequate supply of essential electrolytes needed for osmotic balance and

transport. The fermentable carbohydrate in this medium is mannitol. Production of acid during fermentation can be detected by an indicator such as phenol red, and this helps in differentiating various species of staphylococci. Coagulase-negative staphylococci will form red colonies without any color change in the phenol red indicator surrounding the colonies, while coagulase-positive staphylococci like Staphylococcus aureus will form yellow colonies with a surrounding yellow medium. The solidifying agent is agar [18].

2. Indole test

The indole test determines if a given organism is capable of breaking down the amino acid tryptophan and producing indole. It is a component of the IMViC protocols, a series of examinations intended to differentiate between Enterobacteriaceae family members.

3. Urease Test

The ability of an organism to hydrolyze urea into ammonia and carbon dioxide is determined by the urease test. Its main purpose is to differentiate Enterobacteriaceae other than urease-positive bacteria. The medium turns pink when the urease level is positive, while the negative stays yellow [19].

4. Citrate test

The capacity of a bacterial isolate to use citrate as a source of carbon and energy is assessed by the citrate test. The generation of alkaline byproducts from citrate metabolism raises the pH of the medium, as demonstrated by a color shift in the pH indicator, indicating a successful outcome. After preparing the medium as previously mentioned, the slant is injected with a bacterial suspension, and it is then left to incubate for 24 hours.

If the findings are citrate-positive, the medium will become a deep Prussian blue and growth will be apparent on the slant surface. The medium's pH is raised by the alkaline carbonates and bicarbonates that are created during the catabolism of citrate [20].

5. Voges–Proskauer (VP) Test

In a bacterial broth culture, metabolic activity is found using the Voges–Proskauer test. Alpha-naphthol and potassium hydroxide are added to Voges-Proskauer broth, a glucose-phosphate broth that has been infected with bacteria, as part of the test. The medium is mildly infected using organisms from a pure culture that is left for 18 to 24 hours.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing (AST) is a crucial part of infection control that helps choose which antimicrobial treatments to use. When "immunocompetent patients with monomicrobic bacterial infections treated with a single antimicrobial agent which is administered parenterally in circumstances in which the penetration of drug to the site of infection is predictable," according to the literature, AST is the best predictive of the clinical response [21].

Cobalt Nanoparticles

1. Preparation and Characterization of Cobalt Nanoparticles (Co NPs)

Cobalt sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$) and hydrazine monohydrate were utilized as precursors to create Co NPs. Ten milliliters of a 0.1 M aqueous cobalt sulfate solution were mixed with a 0.2 M sodium citrate dehydrate solution. For sixty to one hundred and twenty minutes, the mixture was kept at a certain temperature and allowed to react. The solution was centrifuged for an hour at 4000 rpm after the reaction. The suspensions that resulted were collected, repeatedly cleaned with distilled water, and then dried at 80 °C in a vacuum drier. The nanoparticles were positioned on a glass slide and subjected to an accelerating voltage of 12.5 kV using a scanning electron microscope (SEM) to examine the particle morphology (Inspect S50, FEI Company, Netherlands). Additionally, the phase structure was described [22].

2. Antimicrobial Activity Measurements of Co NPs

Cobalt nanoparticles (Co NPs) were tested for their antibacterial efficacy against isolates of Staphylococcus aureus using the agar well diffusion technique. Sterile water was used to create bacterial suspensions, which were diluted to 1×10^8 CFU/ml. Using a sterile cotton swab, the suspension was equally distributed over Mueller-Hinton agar. Using a sterilized cork borer, wells (8 mm) were made in the agar after 15 minutes. The wells were filled with 100 µl of Co NPs at concentrations of 1000, 5000, and 10,000 µg/ml. The Petri plates were incubated for 24 hours at 37°C, and the antibacterial activity was assessed by measuring the inhibitory zones surrounding each well. The clear region that surrounds the wells and shows the antibacterial activity of Co NPs is known as the inhibition zone. Sterile water was used to set up the negative controls, and no inhibition zone was seen for these controls. The National Committee for Clinical Laboratory Standards' standards were followed in measuring the widths of the inhibition zones to assess antibacterial activity. Greater antibacterial activity of CoNPs is shown by a broader inhibition zone. To obtain an average outcome, each measurement was done three times [23].

3. Results

Isolation and Identification of *Staphylococcus aureus*

In order to differentiate between Gram-positive and Gram-negative bacteria, the obtained samples were grown on MacConkey and Blood agar media. Some samples only grew on Blood agar, according to the results, indicating that they are Gram-positive bacteria (Figure 3A), which is why they were chosen for this investigation. S. aureus emerged as smooth, tiny, round, elevated colonies with a gray-yellow tint after 24 hours on Blood agar. After being streaked onto Mannitol Salt Agar, a S. aureus-selective medium, this bacterial colony produced tiny to large yellowish colonies on MSA plates (Figure 3B). Furthermore, the colonies developed into sizable yellow or white colonies on nutrient agar; the yellow hue resulted from the organism's production of carotenoids (Figure 3C). Catalase assays were performed on pure cultures to get biochemical confirmation; the presence of gas bubbles suggested a favorable outcome. There was agreement on these morphological characteristics [24]. Under a microscope, S. aureus bunches like grapes and forms spherical, often golden-yellow colonies on nutritional agar.

A B C

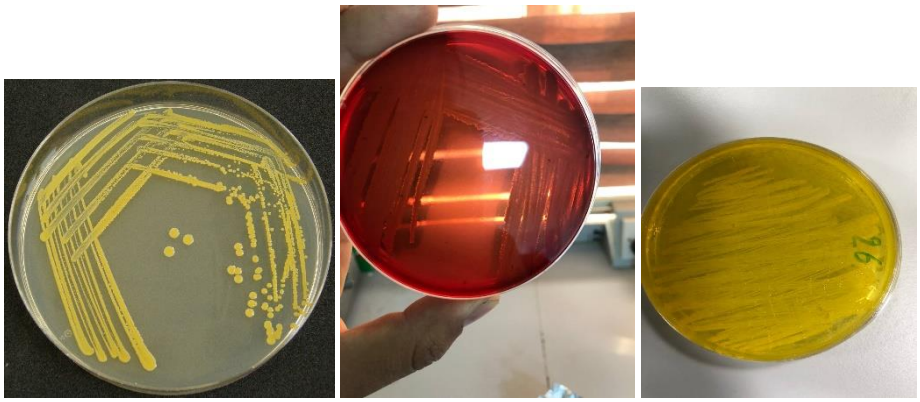


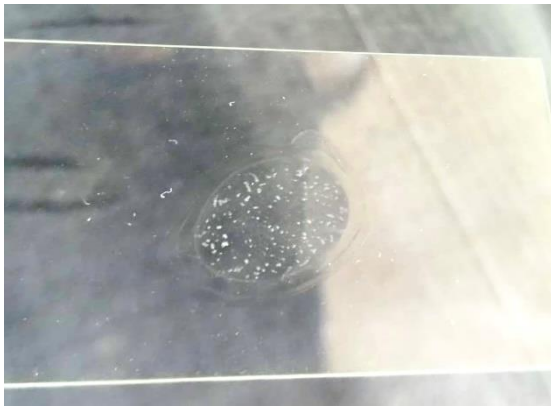
Figure 3. Colony morphology *of staphylococcus aureus* isolates through 24hrs of incubation at 41 ºC in: A BLOOD AGAR. B Mannitol salt agar. C Nutrient agar

Microscopic and Biochemical Diagnostic test:

Gram-positive (purple when stained with Gram stain) cocci-shaped facultatively anaerobic bacteria that do not move or create spores. These bacteria are called Staphylococcus aureus. All of the isolates tested positive for catalase, according to the biochemical test findings (see Table 1). The findings of the IMViC test revealed that all isolates tested positive for citrate as the only carbon source, negative for indole, and positive for methyl red (MR) and Voges-Proskauer (VP). At 42ºC, all isolates could grow, but at 4ºC, none of them could. These results align with previously published data [25].

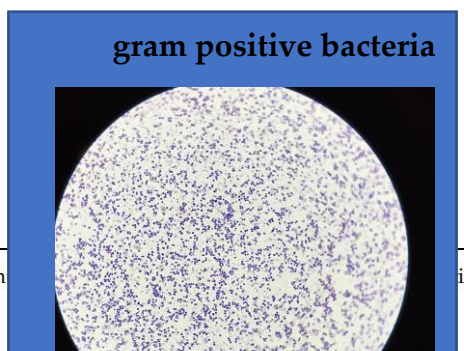
Table 1. Results of the Biochemical Tests and Culture of S. aureus isolates

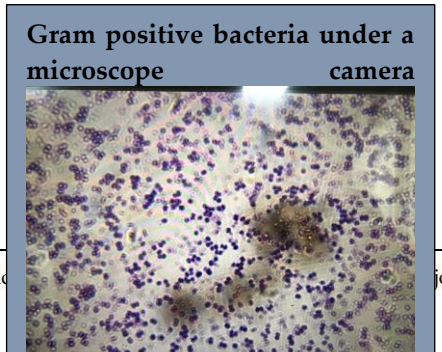
Test	Result
nutrient agar	as smooth, small, circular raised with gray yellowish color colony
Mannitol salt agar	Growth +
Hemolysin	β-hemolysis
Oxidase	-
Catalase	Babbles (+)
Indole	-
Methyle red	+
coagulase test	+
Vogas-proskauer	+
Citrate utilization	+
Growth at 42ºC	Growth (+)
Growth at 4ºC	Growth (-)



coagulase test

catalase test





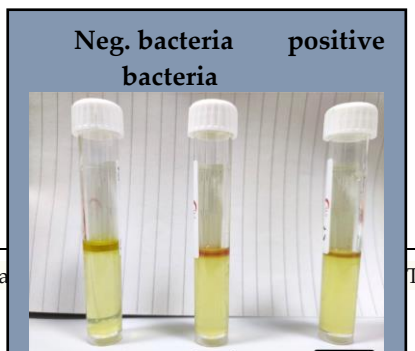


Figure 4. Microscopic and Biochemical Diagnostic test of staphylococcus aureus :

1. Gram stain 3. Urease test 5. Citrate test
2. Indole test 4. Voges proskauer test
6. Catalase test 7. Coagulase test

Prevalence of *S. aureus* isolates in Clinical samples

After all confirmation test for identification of *S. aureus*, total of 60 isolates were obtained as showed in following Table (2).

Table 2. Test for identification of S. aureus

Sample	Sample number	*Staphylococcus*	Other Bacteria	*S. aureus*	*No growth*	Male	Female
Urine	16	9	2	2	5	10	6
Wound infection	20	10	6	2	2	9	11
Otitis	13	3	7	1	3	7	6
Sinusitis	6	1	2	1	3	4	2
Burn	5	2	2	2	1	2	3

According to the current study's data, wound infections account for the largest percentage of clinical isolates (20/60, or 12%), followed by urinary tract infections (16/60, or 9%). These findings concur with those of [26], who discovered that the most frequent bacterium isolated from chronic wounds is Staphylococcus aureus. Staphylococcus aureus has the ability to express surface proteins and virulence factors that affect wound healing. S. aureus co-infections are frequently more pathogenic than S. aureus single infections. Antibiotic resistance in S. aureus is noteworthy because it can be acquired or intrinsic, making therapeutic management of infections more difficult, particularly in patients with co-occurring diseases. Therefore, a complete understanding of the bacterial flora on the skin is necessary for the correct and prompt identification of chronic wound infections.This is an essential precondition for symptom improvement, side effect reduction, and antibiotic resistance, as well as for customized pharmaceutical therapy.Of all clinical isolates, otitis media accounts for 13/60 7% of infections, sinusitis for 6/60 3%, and burn infection for 5/60 3%. Table 3.2's findings show that various samples' S. aureus isolation

rates vary from one another. This variance may be explained by a number of things, one of which being the different sample collecting sites, such as different hospitals. The quantity and variety of samples obtained, the distribution of isolates, and the site of infections are some of the variables affecting this variance. The degree of hygiene, the kinds of disinfectants and sterilizers utilized, and the sterilization techniques used in hospitals are other significant variables. It is well known that S. aureus is resistant to several antibiotics and sterilizers. Hospitals are a major source of hospital-acquired illnesses because they frequently play a role in the creation of novel bacterial strains that are resistant to medications [27].

Hemolysin production

According to the hemolysin production test findings, 20 out of 60 (33%) S. aureus isolates did not show hemolytic activity, whereas 40 out of 60 (67%) isolates were able to generate hemolysin (beta-hemolysis) on blood agar. These results are in line with earlier research showing that the majority of S. aureus isolates generate hemolysin. Hemolysin damages tissue and releases resources from the host, such iron, that help the bacteria proliferate and develop inside the host cells [28, 29].

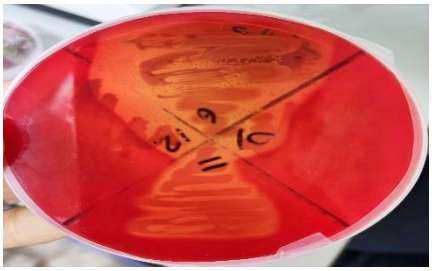


Figure 5. Hemolysin production by S. aureus

Antibiotic susceptibility profile

Table 3. Antibiotic susceptibility profile

Sample Num.	Measurement of the antibiotic in millimeters									
Staph.	TMP10	NOR30	RA5	NA30	ox 5	PRL100	C10	AV30	CN10	AZM15
1	16(S)	0(R)	36(S)	0(R)	22(R)	18(S)	32(S)	0(R)	8(R)	0(R)
2	40(S)	15(R)	6(r)	7(R)	28(s)	24(S)	28(S)	22(S)	26(S)	0(R)
5	38(S)	34(S)	0(R)	0(R)	0(R)	9(R)	20(R)	17(s)	24(S)	0(R)
7	15(S)	28(S)	0(R)	0(R)	30(s)	11(R)	10(R)	16(s)	26(S)	5(R)
9	30(S)	26(S)	22(S)	18(S)	28(S)	10(R)	18(s)	14(R)	22(S)	24(S)
11	0(R)	26(S)	0(R)	33(s)	0(R)	10(R)	30(s)	0(R)	18(s)	0(R)
12	0(R)	30(S)	20(s)	0(R)	0(R)	20(S)	26(S)	20(S)	10(R)	0(R)
13	14(S)	33(s)	0(R)	40(S)	30(S)	2(R)	0(r)	16(s)	22(s)	28(S)

14	12(R)	30(S)	0(R)	0(R)	0(R)	0(R)	22(S)	21(S)	20(S)	22(S)
16	0(R)	34(S)	0(R)	30(S)	0(R)	8(r)	18(s)	22(S)	22(S)	30(S)
18	30(s)	22(S)	36(S)	10(R)	0(R)	0(R)	32(S)	16(s)	26(S)	0(R)
20	0(R)	20(S)	10(R)	18(S)	0(R)	10(R)	0(R)	18(s)	18(S)	26(S)
21	26(S)	36(S)	5(R)	24(S)	0(R)	20(S)	8(R)	15(r)	11(r)	10(R)
22	36(S)	30(S)	40(S)	18(S)	30(S)	28(S)	30(S)	20(S)	0(R)	0(R)
26	20(S)	10(r)	6(R)	22(S)	4(r)	8(R)	19(S)	5(r)	33(s)	30(s)
36	5(r)	26(S)	2(r)	14(s)	2(R)	18(S)	32(S)	1(r)	0(r)	17(R)
38	28(S)	0(R)	32(S)	14(s)	12(R)	10(R)	30(S)	22(S)	0(R)	12(R)
45	0(R)	45(S)	22(S)	18(S)	33(s)	14(R)	18(S)	26(S)	23(S)	0(R)
52	0	36(S)	10(R)	18(S)	20(R)	12(R)	0(R)	16(s)	8(R)	10(R)
57	25(S)	38(S)	0(R)	28(S)	0(R)	18(S)	18(s)	14(R)	18(S)	10(R)

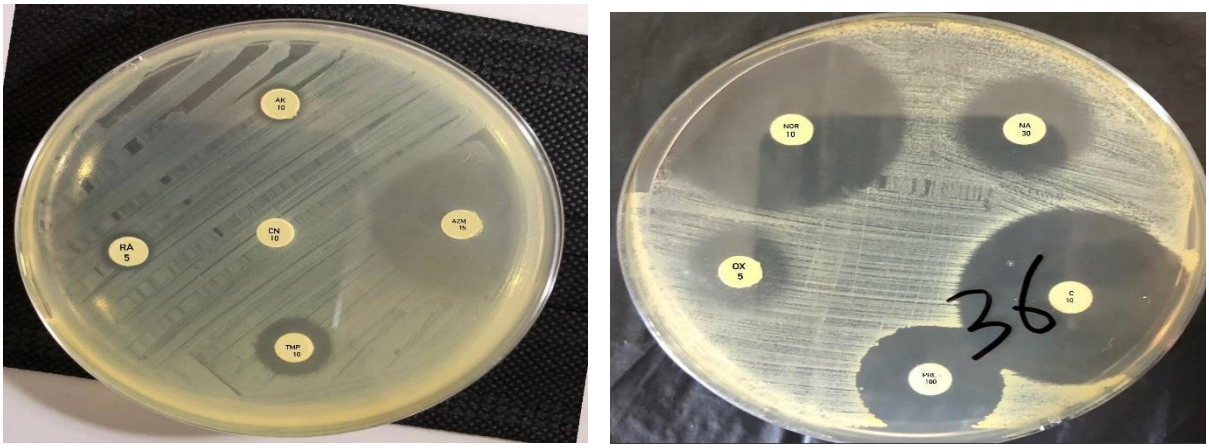


Figure 6. Antibiotic susceptibility test

Natural Antagonist

The development of alternative antimicrobials to manage bacterial infections is gaining traction as a result of bacteria's rising resistance to several antibiotic classes. Furthermore, bacteria that create biofilms are protected from human defenses and frequently show signs of antibiotic resistance. The application of cobalt nanoparticles (Co NPs) as a natural antagonist against certain virulence factors and the production of biofilms is investigated in this work. These nanoparticles are known for being readily available and having antibacterial qualities.

Effect of nanoparticles cobalt oxide on *staphylococcus aureus*

On Staphylococcus aureus, the effects of nanoparticles at different doses were investigated. Standard procedures were followed in the synthesis and characterization of the nanoparticles. Co NPs' antibacterial efficacy against S. aureus was evaluated at five different concentrations: 500, 1000, 2000, 5000, and 10,000 μg/ml. Concentrations of 500, 1000, and 2000 μg/ml were shown to have no impact on Staphylococcus aureus. This defies [30], who demonstrate It was discovered that at 500 μg/ml, Co NPs' inhibitory zones against S. aureus measured 21.17 mm. The inhibition zones were 20±0.15 mm at 5000 μg/ml and 26±0.1 mm at 10,000 μg/ml at higher dosages. Clear zones around the wells, indicating that Co NPs, at these different concentrations, had antibacterial effects. With a few popular antibiotics showing greater antibacterial activity against the studied bacterial isolates, chloramphenicol and norfloxacin displayed bigger inhibition zones than the Co NPs. The spherical, smooth-surfaced nanoparticles had a crystalline structure. The antibacterial resistance of Co NPs against bacteria, including Staphylococcus aureus, was evaluated in this work. Contrary to findings from [31], where Co NPs at 100 μg/ml displayed the strongest antibacterial activity against Staphylococcus aureus with inhibition zones of 22.2 mm and 20.3 mm, respectively, the study found that Co NPs at 10,000 μg/ml gave a similar or stronger antibacterial impact. With the exception of Ciprofloxacin, which exhibited stronger antibacterial action against the bacterial isolates in that investigation, Co NPs had larger inhibition zones at 100 μg/ml than a number of widely used antibiotics. Notably, the efficacy of the nanoparticles was evaluated using the toughest bacterial strains.

Bactericidal ability of Cobalt Oxide Nanoparticles and there Effect on *staphylococcus aureus*

When bacteria or compounds interact with cobalt oxide nanoparticles, both gram-positive and gram-negative bacteria are significantly reduced in number. Cobalt oxide nanoparticles are thought to function similarly to other nanoparticles in that they may cause oxidative stress, release toxic metal ions, and damage to the bacterial cell membrane, which would disrupt the functions of the cell. However, the precise mechanism by which they exert their antibacterial activity is still unknown.

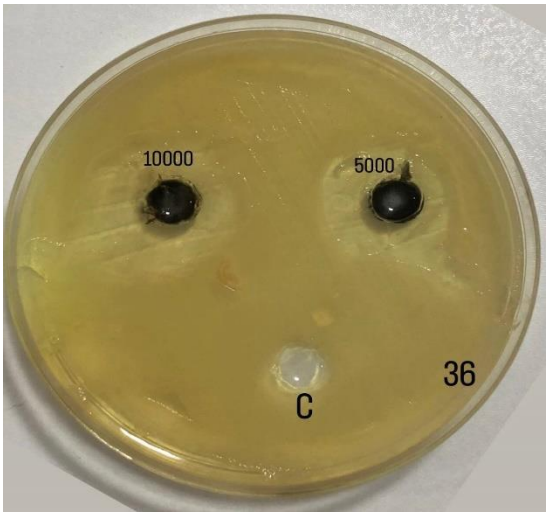


Figure 7. Zone of inhibition of bacterial growth by nanoparticles

4. Conclusion

1. S. aureus is a highly drug-resistant bacterium and a major human disease.
2. These bacteria have a reputation for quickly spreading clones that are more virulent and resistant to several drugs.

3. One unique feature of these bacteria is their ability to quickly proliferate clones, which are more virulent and resistant to a wide range of medications.
4. One of the main causes of bacteremia, infective endocarditis, osteoarticular infections, infections of the skin and soft tissues, pleuropulmonary infections, and infections linked to medical devices is Staphylococcus aureus.
5. The majority of antibiotics lose their ability to fight Staphylococcus aureus
6. The appearance of methicillin-resistant strains, which are identified by the insertion of the mec cassette into the bacterial genome, has been one of the most important advances in S. aureus resistance.
7. Constant observation of novel bacterial variations is required due to the quick establishment and dissemination of resistance in S. aureus, which is frequently accompanied by pathogenicity alterations.
8. Results of bacterial inhibition demonstrated by norflexisin suggest that the antibiotic is still efficacious.
9. Compared to several antibiotics, the water-prepared nanoparticles were more effective.

5. Recommendation

1. Staphylococcus aureus's increasing antibiotic resistance is a serious problem that emphasizes the need to investigate the use of nanoparticles to either directly kill the germs or increase the efficiency of medicines.
2. Finishing the tests on schedule is important since running late might contaminate and harm the material.
3. To ensure that the condition is positive, it's critical to consider the sample size and run many tests.
4. Reducing the amount of antibiotics from more recent generations used to treat mild illnesses in order to stop the spread of resistant bacteria.
5. The patient's age and gender should be taken into consideration as they may have an impact on the outcomes. It has been shown that women are more prone than males to contract Staphylococcus aureus.
6. Find out if the patient has a family history of immunological disorders, as those who do have a history are twice as likely to get the illness as healthy individuals.

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