



## Multi Resistance of *Staphylococcus aureus* isolated from the Nasal Cavity to Vancomycin and Some antibiotics

Wasan Dheyaa Fahem<sup>1\*</sup>, Shara Najmaldeen Abdullah<sup>2</sup>, Salah Salman Zain Alabden<sup>3</sup>

<sup>1,2,3</sup> Department of Biology, College of Education for Pure Sciences, University of Kirkuk, Kirkuk- Iraq.

**ABSTRACT:** *Staphylococcus aureus* is a common colonizer in approximately 30% of healthy individuals, yet it is one of the most frequently isolated species in community-acquired MRSA (CA-MRSA) infections. This study was conducted to isolate and identify *S. aureus* from nasal cavities of patients with the common cold attending medical clinics in the Al-Hajjaj neighborhood of Kirkuk city. Patients (age 1–60 years) were selected between November 1 and December 1, 2023. A total of 40 nasal swabs were collected, and diagnostic tests were performed using cultural, microscopic, and biochemical methods.

Out of 14 randomly selected samples, 8 isolates of *S. aureus* were identified (20% prevalence), and all showed complete resistance to vancomycin. Antibiotic susceptibility was tested using the disk diffusion method with 9 antibiotics. The isolates showed 100% resistance to vancomycin and piperacillin, and 62% resistance to ceftriaxone. In contrast, sensitivity was observed to imipenem and ofloxacin (100%), gentamicin (87.5%), amikacin and ceftazidime (75%), and ciprofloxacin (62.5%).

The study aims the prevalence of nasal *S. aureus* among cold patients and emphasizes the growing concern of multidrug resistance. The findings suggest that the misuse or overuse of antibiotics, particularly without medical supervision, plays a significant role in the emergence of resistant strains, which pose a challenge to conventional treatment approaches.

**KEYWORDS:** *Staphylococcus aureus*; Nasal Cavity; Vncomycin; antibiotics

### Introduction

*Staphylococcus aureus* is a saprophytic microorganism isolated from the skin and mucous membrane in human respiratory tract. Most often, this bacterium is found in people and causes infections such as bacteremia, pneumonia, skin and soft tissue infections including abscess and boils, post-operative wounds, Toxic Shock Syndrome (TSS) and Scalded Skin Syndrome [1, 2, 3]

The name *staphylococcus aureus* was given from the Latin language because of the arrangements of spherical cells of the bacteria. The word Staphylococci includes two parts: the first, Staphyle, in Latin, is again a term meaning a cluster of grapes and the part cocci which means spherical. The term aureus which in Latin can translate to gold aspect of the bacterium was earned by these bacteria due to the large yellow colonies they form [4]. They are facultative anaerobes that are chalked up to the category of Gram positive bacteria, which has spherical shaped cells agglomerated in clusters that look like grapes. [5, 6].

It is going to have spherical cells having smooth surface morphology as seen by scanning electron microscope. The size of the cells is from (0.5) to (1.0) micrometer, another unique feature for these cells consists of the thick cell wall, clear cytoplasmic membrane, and the amorphous cytoplasm [7]. The cocci are non-motile and does not produce spores. Normally isolated colonies of this bacterium are smooth, convex, and may be yellow to golden yellow in color [8]. The key positive tests that are observed in the bacterial culture are; catalase test which is positive, oxidase test which is negative, while *Streptococcus* spp. are catalase-negative and oxidase-positive [9].

Golden pigment in colonies of *Staph. aureus* has exhibited strong association with carotenoids, which have been categorized in this paper as a virulence factor owing to their ability to shield *Staph. aureus* from host immune system responses [10]. *Staph. aureus* is known to produce enzymes that can split mannitol into lactic acid hence the yellow coloration around the colonies on salt and mannitol agar medium it is halophilic because it can survive in sodium chloride salt [11, 12].

All *S. aureus* isolates that led to human disease produce coagulase, an enzyme that contributes to pathogenicity and is useful in identification [13]. Many of these bacteria have a variety of factors that make them capable of infecting host tissues by facilitating their ability to stick to tissues and avoid being neutralized or destroyed by the hosts immune system. Also, the ability to develop resistance to several classes of antibiotics makes *S. aureus* a relatively hard organism to combat. To our knowledge, almost all strains of *S. aureus* produce a variety of extracellular enzymes which could be supposed to be involved in tumour lysis or in

neutralization of antibacterial reactions of the host. Some of these enzymes are proteases, and nucleases, lecithinases, and lipases. Bacterial factors that contribute to bacterial disease include staphylokinase, hyaluronidase, and hyaluronidase because of the part they play in lipid metabolism. Lecithins split the glycerol-phosphate bond. Lecithin is a structural lipid of the cell membrane and as such leads to dismantling and lysis of the host cells. *Candida* species are opportunistic pathogens that rarely cause disease in a normal host but exhibit severe pathogenesis because of the rapid multiplication and spread in the host tissues and their ability to produce many extracellular substances such as enzymes and toxins that are genetically controlled [14, 15].

Which allowed bacteria to form various mechanisms for the ability to resist several antibiotics. The spread of multiple resistance to the Lactam, Tetracyclines, Chloramphenicol, Macrolides, Fluoroquinolones, and Aminoglycosides antibiotic class, makes a real danger to public health, which questioned the scientists' efforts to seek new antibiotics. However, bacteria are developing new mechanisms of resistance, in addition to the possibility of transferring resistance plasmids between bacteria, as well as the occurrence of mutations in the genes of beta-lactamase enzymes that work to convert antibiotics containing the beta-lactam ring from their active form to their inactive form [16].

The initial identification of vancomycin carried out in 1953 came from a strain that was made earlier as *Amiclatopsis orientalis* and was previously known as *Nocardia orientalis* found in the soil. The name vancomycin derives from the word Vanquish the name is because vancomycin can kill the infection causing Penicillin-resistant *Staphylococcus aureus*. After FDA approval in 1955 for the penicillin-resistant *Staph. aureus* strains [17] it was first used clinically. It is the first demonstrated glycopeptide antibiotic that is used in clinics and still the gold standard for the management of methicillin-resistant *Staphylococcus* infections. The case of vancomycin-intermediately resistant *Staphylococcus aureus* (VISA) was reported for the first time in 2019 [18].

In 2002 the first case of VRSA in the United States was reported in a patient with diabetes [19].

The mechanism of action of vancomycin and its antibacterial effect is through interference and prevention of cell wall formation in sensitive bacteria [20]. The hydrophilic vancomycin molecule binds with a hydrogen bond to the terminal region of the Dalanyl-Dalanine of the growing wall, which prevents the binding of the substrates in the growing peptide chain and glycan, and thus prevents the subsequent step of Transpeptidation, which leads to cell wall degradation and bacterial death [21].

Vancomycin has been an enduring first-line antimicrobial for the management of MRSA infections for several years [22]. In the recent past, Vancomycin resistance has become widespread among the *Staph. aureus* isolates itself and has been considered a major public health issue [19, 23]. Some of the VRSA strains that possess genes that make them resistant against vancomycin are those that are transferable by *vanA* and *vanB* plasmids. have said that the vancomycin resistance can cause thickening of the cell wall or can be by overproduction of D-ala caused by a sequence mutation.

## **MATERIALS AND METHODS**

### **1.Preparing the culture media**

The entire culture media employed in the experiment (from the blood agar and mannitol agar plates) were prepared from the manufacturer's instructions from the supplier company. All culture dishes were sterilized in an Autoclave at 121°C and 15 pounds/inch<sup>2</sup> for 15 minutes and then left to cool to 45–50°C before being poured into Petri dishes. All culture media were put in an Autoclave at 121°C and 15 pounds/inch<sup>2</sup> for 15 minutes, let them cool until 45–50°C and then added them to Petri dishes. Samples were put into 37°C incubators for 24 hours to check for contamination, then put back into refrigerators [24].

### **2.Specimens collection**

A total of 40 nasal swabs were obtained from randomly selected participants from Kirkuk city community for both for the period between 11/1/2023 to 12/1/2023. Swabs were obtained from a casual cold patient and data of the patient was also obtained. Cotton wool sterile tipped swabs were used and sampled on to a transport medium and sent to Microbiology Laboratory at the college of Education for Pure Sciences for culturing to develop confirmation.

### **3.Sample culture**

Both Blood agar (BA) and mannitol (MA) were used for isolation of nasal swab using streaking and were agape all in an incubator at 37 °C under aerobic conditions for 24 hrs of incubation.

## **4.DIAGNOSIS OF BACTERIAL ISOLATES**

### **4.1Culture diagnosis**

When the samples were sowed on the culture media, the colours, shapes, sizes, margin and height of the colonies that developed were observed before studying their viscosity and their ability to hemolys blood using beta, alpha or gamma haemolysis according to [25].

#### 4.2 Microscopic diagnosis

Identification at the Microscopic form was done by preparing smears from the suspected colonies and staining with the Gram stain for the intended first test on the bacteria, which are the Crystal-Violet stain, Iodin solution, 95% ethyl alcohol and Safranin stain. Observations of the morphology of the cells and nature of their reaction to Gram stain was done using a compound light microscope with an X100 oil lens as described by [26].

### 5. BIOCHEMICAL TESTS

#### 5.1 Catalase test

The method used for conducting the test was as follows. A little slice of a single colony of bacteria was pierced with a clean wooden saburo stick and spread out onto a clean glass slide. A drop of catalase reagent H<sub>2</sub>O<sub>2</sub> (3%) was placed in the sample, followed by shaking the glass. Indications that the test was positive were bubbles appearing in the device. This study helped them test the ability of bacteria to generate an enzyme called catalase which is needed to get rid of a harmful byproduct produced during cell respiration: hydrogen peroxide, resulting in oxygen gas, O<sub>2</sub> and water, H<sub>2</sub>O [27].

#### 5.2 Oxidase test

Only a small amount from a 24 hours old growing culture was placed on filter paper moistened with Tetramethyl- p-phenylene diamin dihydrochloride reagent. Having the colony change to deep purple within a minute indicates the test was positive [28].

#### 5.3 Coagulase test

This test was done by taking one solitary bacterial coloney and its streaked on the surface of a clean glass slide to a drop of normal saline solution was added and thoroughly mixed while to another streak of the bacterial coloney, a drop of blood plasma was added and also mixed well. An affirmative end product was affirmed by the aggregation in ≤15 seconds as stated by [29].

### 6. Antibiotic susceptibility testing

On the basis of [30], isolates were tested to determine their response to antibiotics.

To prepare the bacterial suspension, I put 4 to 5 young humor from a 16- to 24-hour old bacterial culture on a blood agar plate into a tube of physiological solution, mixed everything well and thumbed the mixture.

2 - the cloudiness of the prepared bacterial suspension was then evaluated against the standard McFarland turbidity which was similar to (0.5 McFarland) with about 1.5x10 bacteria /ml.

3- Brush the Mueller-Hinton agar plates which you have made to contain 1.5%, a double layer with the swab rotating overhead and touching the bottom of the tube. After washing the dishes, they were left at room temperature for 5-10 minutes.

5- Afterward, antibiotic discs were aseptically moved to the top of the dish using previously sterilized forceps: a total of 5-6 discs to a dish and the forceps were sterilized after each disc was picked up using flame.

5- Each dish was treated by incubation at 370 for 24 h after this, the rupture zone of each disc was measured and these isolates were categorized as either sensitive or resistant to those antibiotics. The below table includes diameter in mm to show typical inhibition zones caused by standard antibiotics.

**Table (2-1) shows the types of antibiotics used in the study**

Origin	Manufacturer	concentration	symbol	antibiotics	
Turkey	Bioanalyse	10	CRO	Ceftriaxone	1
Turkey	Bioanalyse	10	IPM	Imipenem	2
Turkey	Bioanalyse	30	VA	Vancomicin	3
Turkey	Bioanalyse	15	CN	Gentamicin	4
Turkey	Bioanalyse	10	AX	Amikacin	5
Turkey	Bioanalyse	10	CIP	Ciprofloxacin	6
Turkey	Bioanalyse	5	OF	Ofloxacin	7
Turkey	Bioanalyse	30	CAZ	Ceftazidim	8
Turkey	Bioanalyse	100	PRL	Peperacillin	9

### 3. RESULT AND DISSCUSSION

#### 3.1 Isolation

8 bacterial isolates were obtained which were (20%) *S. aureus* from the total forty nasal swabs from cold infected people from medical complexes of Al-Hajjaj neighborhood in Kirkuk city both in male & female participants aged between 1-60 years between

11/1/2023 to 12/1/2023 and 32 samples showed no bacterial growth or (80%) of total samples. The absence of bacterial growth may be explained by the fact that the pathogenic agent may be a virus, fungus or one of the anaerobic bacteria which in this study have not been grown with usual methods of aerobic bacteria culture, or because patients are taking doses of antibiotics [31].

### **3.2 Culture diagnosis**

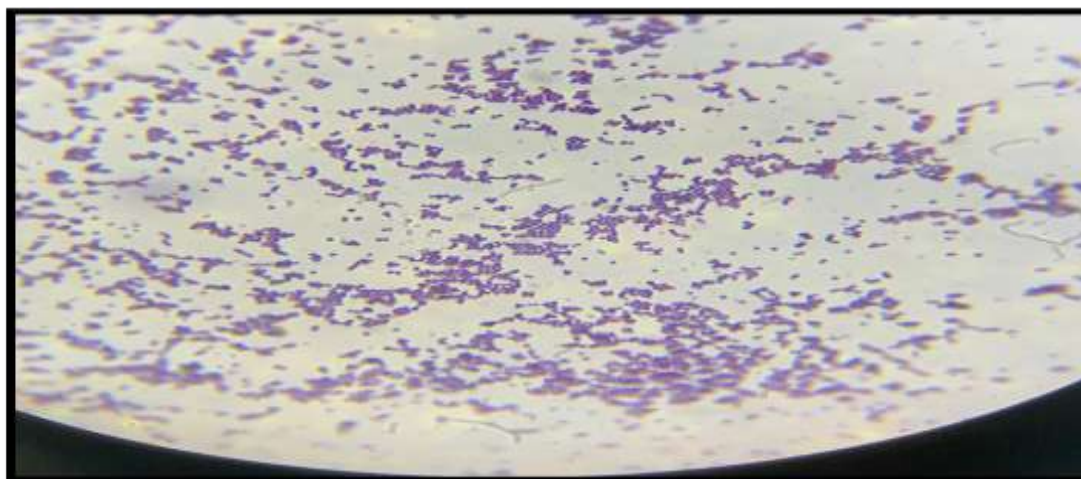
Primary identification of *Staphylococcus aureus* isolates was done by colony morphology after culturing the isolates on mannitol agar medium aerobically at 37°C for 24 hours characterized by yellow colonies due to fermentation of mannitol sugar present in the medium as indicated in Figure (3-1)



**Figure (3-1) *Staph.aureus* bacteria growing on mannitol saline agar medium.**

### **3.3 Microscopic diagnosis**

It was observed under the microscope that *Staphylococcus aureus* as an example of the Gram-positive cocci, had round shaped single cells of about 1-1.3 mm diameter tending to form clusters like grape- like clusters which gave evidence of their facility in the process of dividing as shown in Figure (3-2).



**Figure (3-2) The shape of *Staph.aureus* bacteria cells stained with Gram stain under a light microscope at a power of (100x).**

### **3.4 Biochemical tests and growth characteristics of staphylococci**

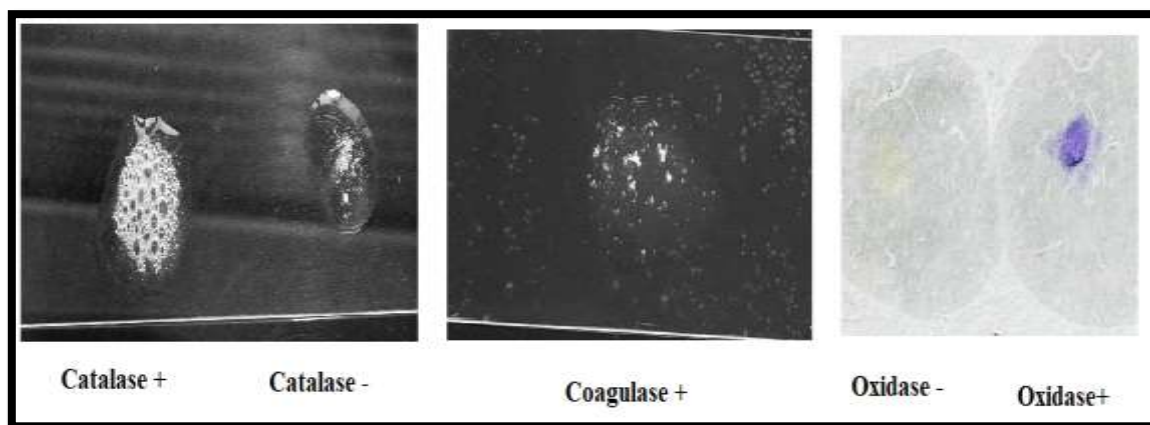
These exercises were conducted to identify *Staph. aureus* bacteria. The findings revealed that all isolates were hundred percent positive for catalase test [32]. all were hundred percent negative for oxidase test and plasma coagulase test. This affirmed the finding of [33]. who pointed out that *Staph. aureus* bacteria are oxidase positive. This Coagulase test is one of the most crucial differential tests between staphylococci because this enzyme coagulates blood plasma by converting fibrinogen to fibrin. They Coagulase enzyme



is of two types, the clumping factor that binds directly to fibrinogen causing clumping in the wall of bacterial cell when plasma is added to the bacterial suspension and is mentioned in table (3-2), figure (3-3).

**Table (3-2) shows the biochemical test for *Staph.aureus* bacteria**

Percentage positive isolates for testing	Total number of isolations	tests
% 100	8	Catalase
-	0	Oxidase
%100	8	Coagulase



**Figure (3-3) Biochemical test for *Staph.aureus* bacteria**

### 3.5 Antibiotic sensitivity and resistance of *Staphylococcus aureus* by the source method (disc method)

The test was done according to the paragraph (2-6) in the material methods because, we tested the resistance of the isolates under study which accounted for total eight isolates, against nine antibiotics and the diameter of inhibition was measured and isolates demonstrated 100% resistance against the antibiotic Vanomycin (VA) [34]. and against the antibiotic Pepercillin by 100%, Ceftriaxion by 62%, and the isolates also showed sensitivity to the antibiotic Imipenem by 100%, ofloxacin by 100%, Centamicin by 87.5%, Amikacin by 75%, Ceftazidime by 75%, Ciprofloxacin by 62.5%. As in Figure (3-4)

The researcher [35]. reported that although the vancomycin, which is heavily clinically used has shown remarkable effectiveness in preventing the emergence of resistance in *Staphylococcus aureus*, this antibiotic has displayed surprising effectiveness. Up until now, 16 cases of VRSA have been reported in the United States. A quick look at evidence that is available demonstrates the role played by the fitness cost as caused by the *vanA* operon, and it alone in contributing to the poor spread of VRSA.

This curative substance interacts in the final stages of peptidoglycan synthesis by binding to the routine D-Ala-D-Ala residues found in UDP-MurNAc pentapeptides from gram positive microbes and influences how peptidoglycan is put together. So, cells are unable to make cell walls and stay stuffed with pentapeptide complexes which make the bacteria highly resistant to vancomycin [36, 37] .

Vancomycin resistance mediated by the *vanA* operon requires two essential processes:

- 1-Hydrolysis of peptidoglycans precursors bearing the D-Ala-D-Ala dipeptide that can bind vancomycin.
- 2-Synthesis of novel precursors with D-Ala-D-lactate unable to bind to vancomycin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a critical challenge in clinical settings due to its association with elevated morbidity, mortality, and considerable healthcare costs. Recent meta-analytical evidence, encompassing randomized controlled trials and observational studies, indicates that vancomycin—despite its extensive and longstanding clinical use—may demonstrate comparatively reduced clinical efficacy and has been linked to a modest but statistically significant increase in mortality. Although no substantial differences were observed in microbiological eradication rates, these findings underscore the therapeutic limitations of vancomycin in the management of MRSA infections. Accordingly, these results emphasize the need for a more nuanced, patient-centered approach to antimicrobial therapy that considers the severity of infection, host-related factors, and clinical outcomes [38].



**Figure (3-4) Sensitivity and resistance of *Staph.aureus* bacteria to antibiotics**

## CONCLUSION

he results of this study indicate that *Staphylococcus aureus* isolated from the nasal cavity of patients with the common cold shows notable resistance to several antibiotics, including vancomycin, with 100% resistance observed. This highlights the potential impact of antibiotic use on the natural flora in the nasal cavity, as the isolates also exhibited resistance to other antibiotics such as penicillin and ceftriaxone. However, the isolates demonstrated full sensitivity to antibiotics such as imipenem and ofloxacin. These findings underscore the concern that antibiotic misuse, including the overuse of vancomycin, may contribute to the development of bacterial resistance. Continued monitoring of *Staphylococcus aureus* resistance patterns in the nasal cavity flora is essential to understand the potential long-term effects on public health. The results also emphasize the importance of targeted antibiotic use based on antimicrobial susceptibility testing to preserve the effectiveness of available treatments.

## RECOMMENDATION

Based on the findings, it is recommended to limit the use of antibiotics in cases where they are not necessary, especially for common viral infections such as the common cold, to prevent disruption of the natural flora in the nasal cavity and the development of resistance. Accurate diagnosis should guide the prescription of antibiotics, with careful selection based on susceptibility testing. Furthermore, public awareness on the rational use of antibiotics should be promoted as part of public health policies. Continuous monitoring of bacterial resistance in nasal flora should be prioritized, and further studies should explore the impact of antibiotic treatments on different bacterial strains in the community.

## REFERENCES

- Reddy, P. N., Srirama, K., & Dirisala, V. R. (2017). An update on clinical burden, diagnostic tools, and therapeutic options of *Staphylococcus aureus*. *Infectious Diseases*.
- Hallabjaiy, R. M., Darogha, S. N., & Hamad, P. A. (2014). Vancomycin resistance among methicillin-resistant *Staphylococcus aureus* isolated from clinical samples in Erbil City-Iraq. *Medical Journal of Islamic World Academy of Sciences*, 22(4), 168-174.
- ريم زهير شنشل, & رشا نزار حسون, رنا أبراهيم محمد. (2008). Antibacterial inhibition of three medical herbal extracts against *Staphylococcus aureus* and *Escherichia coli*. *College Of Basic Education Researches Journal*, 8(1).
- Licitra, G. (2013). History of antibiotics. *Clinical Microbiology and Infection*, 19(1), 5–10.
- Lakhundi, S., & Zhang, K. (2018). Methicillin-resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. *Clinical Microbiology Reviews*, 31(4). <https://doi.org/10.1128/cmr.00020-18>
- Edham, M. H. (2017). *Bacteriological study of urinary tract infection to children in Kirkuk city*. *Kirkuk Journal of Science*, 12(3).
- Gnanamani, A., Hariharan, P., & Paul-Satyaseela, M. (2017). *Staphylococcus aureus*: Pathogenesis, disease progression, and biocompatible interventions. *Critical Reviews in Microbiology*, 43(3), 318–336
- Turnidge, J., Chang, F. Y., Fowler, V. G., & Rao, N. (2008). *Staphylococcus aureus*. *Updated December. Guided Medline Search*.

9. Bitrus, A., Peter, O., Abbas, M., & Goni, M. (2018). *Staphylococcus aureus*: A review of antimicrobial resistance mechanisms. *Veterinary Sciences: Research and Reviews*, 4(2), 43-54. <http://dx.doi.org/10.17582/journal.vsr/2018/4.2.43.54>
10. Rasheed, N. A., & Hussein, N. R. (2021). *Staphylococcus aureus*: an overview of discovery, characteristics, epidemiology, virulence factors and antimicrobial sensitivity. *European Journal of Molecular & Clinical Medicine*, 8(3), 1160-1183.
11. Thakur, P., Nayyar, C., Tak, V., & Saigal, K. (2017). Mannitol-fermenting and tube coagulase-negative staphylococcal isolates: unraveling the diagnostic dilemma. *Journal of laboratory physicians*, 9(01), 065-066.
12. Khalf, S. H. (2012). Detection of siderophores from *Staphylococcus aureus*, *Klebsiella pneumonia* Isolated from rhinitis cases. *College of Basic Education Research Journal*, 11(2), 568-0.
13. Foster, T. J. (2005). Immune evasion by staphylococci. *Nature reviews microbiology*, 3(12), 948-958.
14. Jawetz, E., Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A., & Mietzner, T. A. (1991). *Jawetz, Melnick, & Adelberg's medical microbiology. (No Title)*.
15. Abod, H. A. (2017). The effect of silver nanoparticles prepared using *Aspergillus niger* in some pathogenic bacteria. *Kirkuk Journal of Science*, 12(1).
16. Fayaz, A. M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P. T., & Venketesan, R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine: Nanotechnology, Biology and Medicine*, 6(1), 103-109. <https://doi.org/10.1016/j.nano.2009.04.006>
17. Kest, H., & Kaushik, A. (2019). Vancomycin-resistant *Staphylococcus aureus*: Formidable threat or silence before the storm. *J Infect Dis Epidemiol*, 5(5), 93. DOI: 10.23937/2474-3658/1510093
18. Shariati, A., Dadashi, M., Moghadam, M. T., van Belkum, A., Yaslianifard, S., & Darban-Sarokhalil, D. (2020). Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis. *Scientific reports*, 10(1), 12689. <https://doi.org/10.1038/s41598-020-69058-z>
19. McGuinness, W. A., Malachowa, N., & DeLeo, F. R. (2017). Focus: infectious diseases: vancomycin resistance in *Staphylococcus aureus*. *The Yale journal of biology and medicine*, 90(2), 269.
20. Cong, Y., Yang, S., & Rao, X. (2020). Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. *Journal of advanced research*, 21, 169-176. <https://doi.org/10.1016/j.jare.2019.10.005>
21. 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. <https://doi.org/10.3389/fcimb.2020.00107>
22. Mermel, L. A., Allon, M., Bouza, E., Craven, D. E., Flynn, P., O'Grady, N. P., Raad, I. I., Rijnders, B. J., Sherertz, R. J., & Warren, D. K. (2009). Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, 49(1), 1-45. <https://doi.org/10.1086/599376>
23. Chang, S., Sievert, D. M., Hageman, J. C., Boulton, M. L., Tenover, F. C., Downes, F. P., Shah, S., Rudrik, J. T., Pupp, G. R., & Brown, W. J. (2003). Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. *New England Journal of Medicine*, 348(14), 1342-1347. DOI: 10.1056/NEJMoa025025
24. Tille, P. (2014). *Pasteurella and similar organisms*. *Bailey & Scotts Diagnostic Microbiology* (13th ed., pp. 393). St. Louis, MO: Elsevier Mosby.
25. Cheesbrough, M. *District Laboratory Practice in tropical Countries*, Part I. (2009).
26. Benson, H. J. (2002). *Microbiological Applications: Laboratory Manual in General Microbiology* (8th ed.). McGraw-Hill
27. Atlas, R. (2010). *Handbook of microbiological media*. 4th Edition. Library of Congress Cataloging-in-Publication Data. New York. USA.
28. Tille, P. M., & Bailey, S. (2014). *Diagnostic microbiology*. Missouri: Elsevier, 202-927.
29. Muktha, Z., Kabir, S., & Rahman, M. (2015). Isolation and identification of bacterial flora from respiratory tract of healthy horses. *Journal of the Bangladesh Agricultural University*, 13(452-2016-35864), 239-246 <https://doi.org/10.3329/jbau.v13i2.28785>
30. Vandamme, P., Vercauteren, E., Lammens, C., Pensart, N., Ieven, M., Pot, B., Leclercq, R., & Goossens, H. (1996). Survey of enterococcal susceptibility patterns in Belgium. *Journal of Clinical Microbiology*, 34(10), 2572-2576. <https://doi.org/10.1128/jcm.34.10.2572-2576.1996>
31. Balat, A. and Hill. (2003). genitourinary abnormalities is chi iron with urinary tract infection. *J. Med. Sciences*. 29:59-63. <https://dx.doi.org/10.21608/jbaar.2024.379934>

32. Manisha, D., Abdullah, A. M. S., Zobayda, F. H., Nanda, B., Amrita, P., & Sukumar, S. (2019). Characterization of *Staphylococcus aureus* isolated from human dental infection. *African Journal of Microbiology Research*, 13(14), 273-278. <https://doi.org/10.5897/AJMR2019.9076>
33. Rakotovoao-Ravahatra, Z. D., Randriatsarafara, F. M., Milasoanjara, R. N., Ranaivosoa, M. K., Rakotovoao, A. L., & Rasamindrakotroka, A. (2019). Assessment of the coagulase test in the identification of *Staphylococcus aureus* strains. *Journal of Biotechnology and Biomedicine*, 2(3), 105-111
34. Salih, R. M., Rafiq, S. N., & Hamad, P. A. (2017). Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolated from clinical samples in Erbil City, Iraq. *Kirkuk university journal for scientific studies*, 12(2), 108-20.
35. Blechman, S. E., & Wright, E. S. (2024). Vancomycin-resistant *Staphylococcus aureus* (VRSA) can overcome the cost of antibiotic resistance and may threaten vancomycin's clinical durability. *Plos Pathogens*, 20(8), e1012422.
36. Barna JC, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Annu Rev Microbiol.* 1984;38(1):339–357. doi: 10.1146/annurev.mi.38.100184.002011. [DOI] [PubMed] [Google Scholar]
37. Reynolds PE. Studies on the mode of action of vancomycin. *Biochim Biophys Acta.* 1961;52(2):403–405. doi: 10.1016/0006-3002(61)90698-9 . [DOI] [PubMed] [Google Scholar]
38. Dighriri, I. M., Alanazi, S., AlMutairi, K., Alhusayni, S. J., Balharith, F. M., Aljuwaie, R. A., ... & Jameel, O. (2025). Efficacy and Safety of Vancomycin, Linezolid, and Ceftaroline in the Treatment of Methicillin-Resistant *Staphylococcus aureus* (MRSA): A Systematic Review and Meta-Analysis. *Cureus*, 17(1).