Original Research Article

Prevalence of Methicillin Resistance S.aureus (MRSA) and Vancomycin Resistance S.aureus (VRSA) among Iraqi Patients: a Comparative Study

Mohammed Salim Mohammed[1*], Shadman Tariq Sadiq[1], Dawood Salim Mohammed[2]

[1]Department of Life Sciences, College of Science, University of Tikrit, Tikrit, Iraq
[2]Department of Applied Chemistry, College of Applied Sciences, Samarra University, Tikrit, Iraq

*Corresponding Author: Mohammed Salim Mohammed
Department of Life Sciences, College of Science, University of Tikrit, Tikrit, Iraq

Abstract: It is clear from this study the prevalence of Staphylococcus aureus resistant to methicillin and *Staphylococcus aureus* resistant to vancomycin in different regions of Iraq. According to this study, which was conducted in the Iraqi city of Tikrit, on 285 samples, 131 of them were infected with various bacterial infections, of whom only 80% were infected with S. aureus bacteria. According to the chemical tests, including the DNA test (PCR) for the Staphylococcus bacteria isolates, the sensitivity of the isolates under study against a group of different antibiotics was tested by using the disc diffusion method. Gentamicin and Chloramphenicol (30, 28, 23, 22, and 21 mm), respectively, for all samples under study, while the percentage of bacteria resistance to Methicillin was 100%, while the percentage of Staphylococcus resistance to Vancomycin was 70% for all samples under study, where it was recorded The rate of inhibition reached (13 mm). As a result of the investigation on resistance genes, it was found that most of the virulence genes are present in methicillin-resistant samples, while the virulence genes in the Vancomycin-resistant samples recorded two types of genes, namely (Van A) and (Van B), which are responsible for building the cell wall protein. A) In samples isolated from bacterial strains, the percentages of (Van B) gene were higher. By conducting a comparison with some studies in some Iraqi cities, it was found that 71% of the isolates were resistant to Vancomycin in the city of Hilla out of a total of 200 infected isolates, and the city of Baghdad recorded infections estimated at 66.6% resistant to Vancomycin out of a total of 250 infected samples. 50% of the 20 samples infected with S. aureus, Finally, the city of Kirkuk recorded a percentage of Vancomycin resistance to isolates of S. aureus bacteria 100%, and it is also clear that all isolates in all Iraqi cities were 100% Methacillin resistant.

Keywords: MRSA, VRSA, Antibiotics resistance.

INTRODUCTION

A gram-positive strain of staphylococcus aureus that cannot be treated by common antibiotics is called methicillin-resistant *Staphylococcus aureus* (MRSA) and is. MRSA and even more resistant S. aureus are becoming more common, especially in hospitals (Andela *et al*., 2022), and these infections are difficult to treat. This germ can cause infection ranges from minor skin infections to severe skin infections, to deep tissue infection and all soft tissues in the body (Benjamin *et al*., 2022).

The virulence of *Staphylococcus aureus* bacteria lies in its ability to multiply and spread rapidly and widely in fragile tissues. It can achieve this by producing many extracellular substances such as enzymes and proteins that are involved in the formation of the cell wall, in addition to having a capsule. These proteins, along with the small capsule, lead to tissue damage. And the death of macrophage cells contributes to the phenomenon of abscess formation, after *Staphylococcus aureus* bacteria became resistant to the antibiotic (Methicillin MRSA) until it was named by this name (Siham *et al*., 2023). The antibiotic Vancomycin appeared and was very effective against this bacteria more than two decades ago, until this resistance developed in some strains of S. aureus of Vancomycin (VRSA) infection cases were

recorded in 2002 in the United States of America, and since then, good strains began to appear resistant to Vancomycin (Weigel *et al*., 2007; Kim *et al*., 2000).

The aim of this study was to know the proportions of Iraqi patients infected with Staphylococcus aureus of the (MRAS) and (VRAS) aureus strains, and to make a comparison of the extent and rates of their prevalence in Iraqi cities.

METHOD OF WORK

About 285 samples were collected for patients attending the health departments of the Iraqi city of Tikrit, and a control sample without bacterial infection was also collected for the patients and patients for the period from (1/11/2022), and the collection continued in the period (3/2/2023). The patient's information was recorded in a special form, which included general clinical symptoms, such as abdominal pain, fever, vomiting, inflammation of the lymph nodes, infections of the respiratory system, and areas of wounds and skin.

The biochemical tests were performed for the bacterial samples, where the colonies of the bacterial isolates were purified by re-growing them on blood agar medium, MacConky agar medium, and the nutritious agar medium using the planning method and incubating them for 24 hours at a temperature of 37 °C. Bacterial colonies were initially diagnosed based on their morphological and culture characteristics on the culture media, including the size, color, rims, and height of the colonies, their ability to ferment the sugar lactose, and their ability to produce mucus and hemolysin. This test is used to distinguish between Staphylococcus aureus (positive) that produces a blood clotting enzyme from Staphylococcus (negative). Staphylococcus aureus bacteria produce two types of blood clots, free blood clots and blood clots associated with the conversion of fibrinogen into fibrin by the coagulase enzyme in the slide). For the detection of free blood clotting using the method (tube test clotting in the test tube) the positive result in both methods is interpreted when there is a lump or clot in the sample, either when no lump or clot is observed in the sample then the result is negative, the tests were conducted according to (Leber, 2016).

All antibiotics were prepared in the form of pure powder with constant weights according to Table (1). Tests were carried out using the disc brushing method on all samples under study. Weight concentrations were calculated according to the equation $N_1 + V_1 = N_2 + V_2$, and the results were recorded.

Table 1: Weights of pure antibiotics used under study

Weight of antibiotics	Antibiotics Powder
5MG	Chloro
5MG	Gento
5MG	Azithro
5MG	Levofio
5MG	Cipro
10MG	Methacilin
30MG	Vancomycin

RESULTS AND DISCUSSION

It is clear from Table (2) that the highest rate of inhibition or killing of staphylococcal bacteria for the pure antibiotic Levofloxacin, where the highest rate was recorded for all samples under study reached (30 mm). It is effective against a large group of pathogenic gram-positive and negative bacteria. Levofloxacin works to stop Bacterial growth by inhibiting the DNA gyrase enzyme, which is important for the growth of bacteria and the manufacture of bacterial DNA. The least pure antibiotic inhibiting staphylococcal bacteria was Chloramphenicol, where its inhibition rate was (21 mm), as it is one of the toxic organic compounds that are used and its derivatives manufactured from it to control bacteria and fungi, because of its properties, it is considered a broad-spectrum antimicrobial compound, this study has It is similar to the study (Ekta *et al*., 2022) that confirmed the sensitivity of Staphylococcus aureus to these antibiotics.

It was also shown, according to Figure (2), that the most types of bacteria diagnosed for the study samples with various bacterial infections were staphylococcal bacteria, which amounted to 80% of the total number of 131 infections, and this represents clear evidence of the extent of the seriousness and spread of these bacteria among the infected in Iraqi health institutions As well as the extent of the resistance of this bacteria to various antibiotics, compared to other types also diagnosed in the same form, and this is consistent with the study (Eun *et al*., 2021), which proved the extent of the danger of this bacteria and its spread among patients lying in hospitals and health institutions.

It is also noticeable in Figure (3) the difference in the rates of inhibition or killing in the bacteria isolates under study. There is a significant and clear difference between the previously mentioned antibiotics under study and between the antibiotics Methicillin and Vancomycin. The two antibiotics recorded the lowest inhibition rates, which are 0.0 and

13 mm, respectively. In comparison, the difference between (MRAS) and (VRAS) bacteria was superior to (VRAS) by up to 30% over (MRAS), and this result may be similar to many studies on the level of Iraq and the world in this field, but when looking at Figure (1 We note that the most patients in Iraqi cities infected with Staphylococcus aureus resistant to the two types of bacteria (MRAS) and (VRAS) were the city of Kirkuk (Siham *et al*., 2023), with a clear significant difference with the rest of the Iraqi cities in a different way, where the Iraqi city of Dhi Qar recorded the lowest rates of infection With the type (VRAS), where the infection rates were 50% for this type only, according to the aforementioned form. This may be due to the nature of the infected patients, the influence of the environment, or the nature of the health services provided in these institutions.

We conclude from the foregoing the extent of the danger of staphylococci of all kinds to the health of Iraqi society in particular and the global community in general. Studies must be intensified in order to reach the best way to limit the spread of staphylococcal infection by working towards discovering new antibiotics or resorting to antibiotic synergy to eradicate these dangerous bacteria.

Table 2: Shows the average inhibition ratios for all antibiotics under study

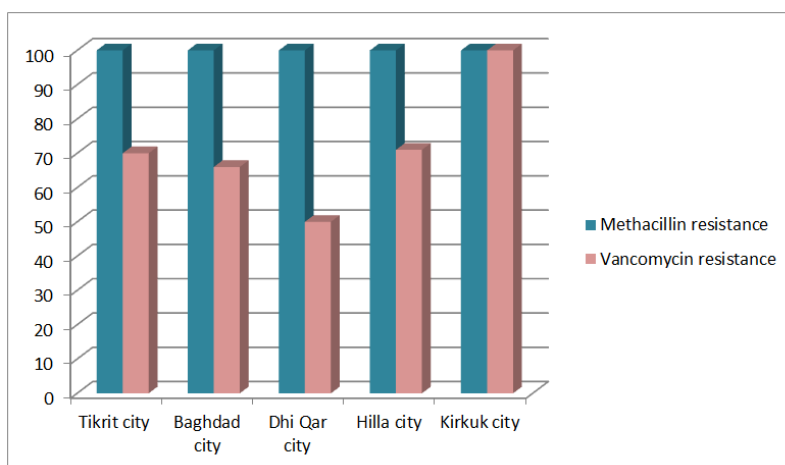| The rate of bacteria inhibition rate for all samples under study	Standard counterweight	Antibiotics Powder
21mm	5MG	Chloro
23mm	5MG	Gento
28mm	5MG	Azithro
30mm	5MG	Levofio
22mm	5MG	Cipro
mm 0.0	10MG	Methacilin
13mm	30MG	Vancomycin

Figure 1: Shows the percentage of patients infected with staphylococcus in some Iraqi cities
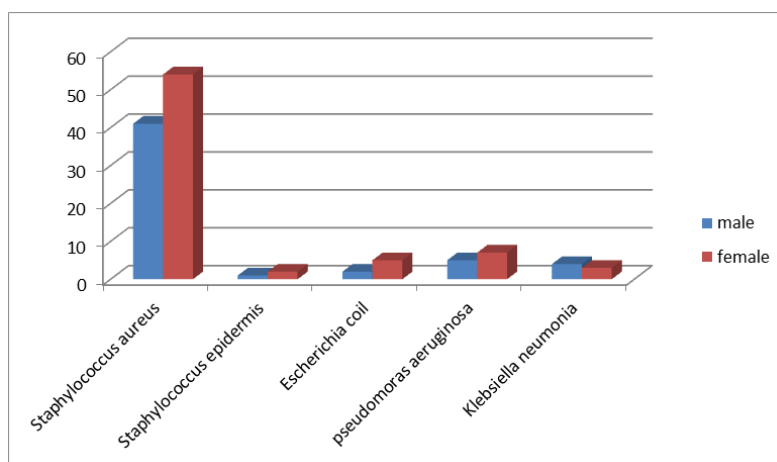

Figure 2: A chart showing the percentage of Staphylococcus bacteria in addition to other types of pathogenic bacteria diagnosed in the Iraqi city of Tikrit
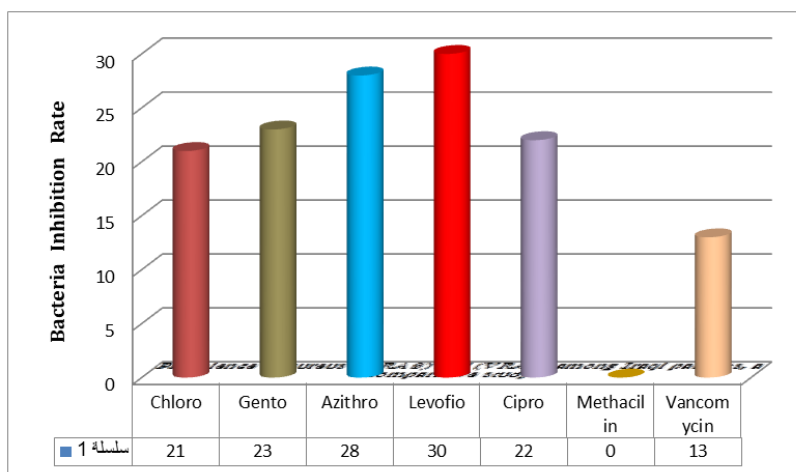
Figure 3: A schematic diagram showing the inhibition rate of all the antibiotics under study for Staphylococcus bacteria, and also shows the ratio of (MRAS) and (VRAS)

RESOURCES

- Martinović, A., Cocuzzi, R., Arioli, S., & Mora, D. (2020). Streptococcus thermophilus: to survive, or not to survive the gastrointestinal tract, that is the question! *Nutrients*, *12*(8), 2175. doi: 10.3390/nu12082175.
- Howden, B. P., Giulieri, S. G., Wong Fok Lung, T., Baines, S. L., Sharkey, L. K., Lee, J. Y., ... & Stinear, T. P. (2023). Staphylococcus aureus host interactions and adaptation. *Nature Reviews Microbiology*, 1-16.
- Kamble, E., Sanghvi, P., & Pardesi, K. (2022). Synergistic effect of antibiotic combinations on Staphylococcus aureus biofilms and their persister cell populations. *Biofilm*, *4*, 100068. doi: 10.1016/j.bioflm.2022.100068.
- Jeong, E. T., Park, S. K., Jo, D. M., Khan, F., Choi, T. H., Yoon, T. M., & Kim, Y. M. (2021). Synergistic antibacterial activity of an active compound derived from sedum takesimense against methicillin-resistant Staphylococcus aureus and its clinical isolates. *Journal of Microbiology and Biotechnology*, *31*(9), 1288-1294. doi:10.4014/jmb.2105.05015.
- Kim, M. N., Pai, C. H., Woo, J. H., Ryu, J. S., & Hiramatsu, K. (2000). Vancomycin-intermediate Staphylococcus aureus in Korea. *Journal of clinical microbiology*, *38*(10), 3879-3881.
- Leber, A. L. (2016). Clinical Microbiology Procedures Handbook, 4th ed, vol 2. Washington DC: ASM Press.
- AL-Salihi, S. S., Karim, G. F., Al-Bayati, A., & Obaid, H. M. (2023). Prevalence of Methicillin-Resistant and Methicillin Sensitive Staphylococcus aureus Nasal Carriage and their Antibiotic Resistant Patterns in Kirkuk City, Iraq. *Journal of Pure & Applied Microbiology*, *17*(1), 329-337. doi.org/10.22207/JPAM.17.1.22.
- Weigel, L. M., Donlan, R. M., Shin, D. H., Jensen, B., Clark, N. C., McDougal, L. K., ... & Patel, J. B. (2007). High-level vancomycin-resistant Staphylococcus aureus isolates associated with a polymicrobial biofilm. *Antimicrobial agents and chemotherapy*, *51*(1), 231-238.