Diagnosis of Staphylococcus aureus bacteria from wound Infections and their resistance to some antibiotic

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Abstract:

In this study, it is clear that the most common types of bacteria that were isolated and diagnosed from patients who were coming to Al-Sharqat General Hospital in Salah al-Din Governorate, which is represented by Staphylococcus aureus bacteria, at a rate of ١٦٥ Staphylococcus included about ٧٣ samples of the total compared to other types of bacteria that have a major role in causing many different diseases, such as wound infections, burns, blood poisoning, and other diseases that threaten the lives of patients in hospitals and health centers, as the presence of Staphylococcus aureus bacteria was recorded in areas of various wounds, which are represented by wounds resulting from surgical operations of various types and simple and medium wounds, as the percentage of the presence of S.aureus bacteria reached ٤٩ samples out of ٧٣ samples of Staphylococcus samples at a rate of (٦٧,١٪), followed in second place by the presence of Staphylococcus epidermids bacteria, as its presence rate in wounds reached (١٦) samples out of a total of ٧٣ samples At a rate of (٢١,٩٪), while the presence of S.saprophyticus bacteria in wounds was at a rate of (٥) samples out of ٧٣ samples, at a rate of (٦,٨٪), while S.hemolyticus bacteria was the least present in the types of staphylococci, at a rate of (٣) samples, at a rate of (٤,١٪), as these types of bacteria were diagnosed from other types by using Gram stain and observing the spherical shapes of the bacteria and their presence in the form of clusters gathered in positive purple color. After that, the Catalase test and the Coagulase test were performed, which distinguishes and differentiates between these types of bacteria, as the Coagulase test distinguishes S.aureus bacteria from other types of staphylococci that are negative for this test. After that, the antibiotic sensitivity test was performed for S.aureus bacteria, as (١٠) antibiotics were used at different concentrations, and these bacteria showed great resistance to β-lactam antibiotics, which are represented by Penicillin antibiotic (٩٩٪) and methicillin antibiotic (٩٦٪). These antibiotics are represented in the Penicillins family, followed by Cefotaxime antibiotic (٨١٪). This antibiotic belongs to the

Cephalosporins family within the third-generation antibiotics. While the bacteria were highly sensitive to Meropenem antibiotic, S.aureus bacteria were resistant to this antibiotic (٦٪), as this antibiotic belongs to the Carbapenems antibiotic family, followed by Vancomycin antibiotic, as S.aureus bacteria were resistant to this antibiotic (٨٪). This antibiotic belongs to the Glycopeptides antibiotic family. As for Amikacin antibiotic, the bacteria were resistant to it (٤٣٪), Gentamycin antibiotic (٢٠٪), and Streptomycin antibiotic (١٢٪). These antibiotics belong to the Aminoglycoside antibiotic family, while their resistance to Ciprofloxacin antibiotic was (٤٤,٢٪). This antibiotic belongs to the Fluroquinolones, while its resistance to Azithromycin was (٦٠٪). This antibiotic belongs to the Macrolides family, as these bacteria showed great resistance to these antibiotics, which explains the reason for the spread and ferocity of these bacteria, which is due to their resistance to many different antibiotics.

Introduction:

Studies have shown that there are ٤٧ species and ٢٤ subspecies in the genus Staphylococcus, where ١٧ species are specialized in causing many diseases in humans, while the rest of the other species of Staphylococcus are found in animals (Kot rt al., ٢٠١٨). Staphylococcus aureus is a common organism and is present opportunistically, which leads to causing a large group of different diseases and multiple infections associated with hospitals, such as blood bacteria, sepsis, endocarditis, pneumonia, osteomyelitis, arthritis, and many skin diseases (Dayan et al., ٢٠١٦). S.aureus bacteria have the ability to cause various wound infections, which range from simple wounds to causing many systemic diseases that are a major cause of life-threatening diseases due to the ease of invasion and spread of these bacteria in the various tissues of the body. In addition, they possess many virulence factors that increase the severity of their pathogenicity. These virulence factors are: With β-lactamase enzymes, enterotoxin, coagulase enzymes, and enzymes that work to break down blood (Hemolysin) (Ferry et al., ٢٠٠٥), S.aureus bacteria have shown their diversity and acquisition of many virulence factors and the establishment of resistance to many antibiotics. Many studies and researches have been conducted to clarify

the importance of molecular determinants that determine virulence factors in S. bacteria. Aureus and the regulatory systems that control the expression of virulence factors with the aim of developing new therapeutic approaches against various infectious diseases produced by S.aureus bacteria (Saleem et al., ٢٠١٦). In ٢٠٠١, the complete genome sequence of S.aureus bacteria was determined, and the ongoing molecular and genetic analysis of the bacteria and its great ability to adhere to many different tissue surfaces and produce enzymes, secretions and toxins that facilitate the process of bacterial invasion of these tissues and cause many diseases (Monteiro et al., ٢٠١٥). These bacteria have recently gained their bad reputation due to their resistance to antibiotics, especially Methicillin (Onyango et al., ٢٠١٨). This is due to the possession of this bacteria of the mecA gene, which expresses the resistance of S.aureus bacteria to Methicillin and many antibiotics belonging to the penicillin family. These bacteria that contain the mecA gene are called (MRSA) MRSA produces the protein (PBP٢a) pencillin binding protein ٢a, which is encoded by the mecA gene. This gene is located on the (SCCmec) Staphylococcal Cassette Chromosome mec, which contains at least six different types (Humphreys, ٢٠١٢). In addition, these bacteria possess the blaZ gene, which encodes the production of the bacterial enzyme β-lactamase, which works to destroy the β-lactam ring and thus inhibit β-lactam antibiotics. This gene is mostly found on the chromosomes or plasmids of Staphylococcus aureus bacteria, so these bacteria result in resistance to many antibiotics (Bagcigil et al.,٢٠١٢).

Methodology:

Collection of samples and Diagnosis

In this study, about (١٦٥) clinical samples were collected from patients lying in Al-Sharqat General Hospital in Salah al-Din Governorate from ١\٧\٢٠٢٤ to ١٦\١٠\٢٠٢٤. An autoclave was used to sterilize the agricultural media, whether these media were liquid or solid, in addition to sterilizing tools and solutions that are not affected by the high temperature produced by the autoclave. After

taking bacterial isolates using Gel cotton swab swabs from the affected areas, which are represented by various wound infections in the body areas, these swabs were then cultured on appropriate agricultural media using Petri dishes. These media are represented by (Blood base agar) and MacConkeey agar at the same time. After that, the Petri dishes are placed in the incubator at a temperature of ٣٧ degrees Celsius for ١٨-٢٤ hours. After that, a Gram stain and a Catalase test are performed to differentiate between Staphylococci and Streptococci bacteria in addition to the Coagulase test that distinguishes and differentiates between S.aureus bacteria from other types of staphylococci bacteria, which are represented by S.epidermis, S.sapropgyticus and S.hemolyticus bacteria that are negative for the Coagulase test. After that, they were cultivated on a medium (mannitol salt agar), which is a selective medium for these bacteria, as it allows the growth of S.aureus bacteria over other types of bacteria that cannot grow in it because it contains a percentage of sodium salts at a rate of ٧-١٠٪, which inhibits the growth of many other types of bacteria, as S.aureus bacteria ferment mantol sugar and change the color of the medium from red to yellow. Sometimes the Catalase test and Oxidase test are used (Ekta et al., ٢٠٢٢) to differentiate between staphylococci bacteria that are negative for oxidase from other negative types. After diagnosis The bacteria were cultured using culture media and biochemical tests, and then the bacteria were diagnosed using the VITEK ٢ device to ensure the purity of the diagnosed bacterial isolates and to exclude other types of bacteria. After that, the bacteria were preserved and the results were recorded.

Results and discussion

Biochemical diagnosis of bacterial species Staphylococcal under study

The bacterial species that cause many diseases and lead in many cases to wound and burn infections, blood poisoning and many other life-threatening diseases were diagnosed. These bacteria were isolated and Gram stain was done for all types of bacteria and many biochemical tests were done according to Table (١).

S.aureus bacteria were identified by doing a Catalase test for all types, as all types of bacteria produced this test and formed bubbles. In addition, a Coagulase test was done to separate S.aureus bacteria from other types of staphylococci. In addition, a Urease test was done and all types also produced this enzyme. In addition, a Novobiocin antibody test was done, which distinguishes between S.saprophtycus bacteria and S.epiderms bacteria, as it formed bacteria. S.saprophyticus is resistant to this bacteria, while S.epiderms bacteria are sensitive to this antibiotic that distinguishes between these two types. The Oxidase test was performed to determine the ability of these bacteria to produce this enzyme. All types of bacteria were negative for this test.

Table (١) shows the chemical diagnosis of the different types of staphylococci under study and Figure (١) ,the rest of the other types of bacteria that were diagnosed and identified.

Type bacteria	Catalase test	Coagulase test	Oxidase	Motility	Gram stain
S.aureus	+	+	–	non motile	Gr +
S.epidermids	+	–	–	non motile	Gr+
S.saprophyticus	+	–	–	non motile	G+
S.hemolyticus	+	–	–	non motile	Gr+

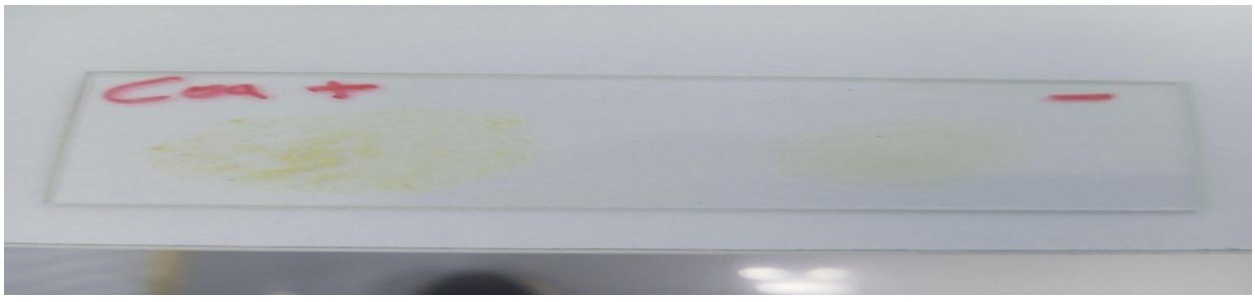


Figure (١) Coagulase test positive reaction for Staphylococcus aureus bacteria

Isolation of Staphylococcus aureus aureus

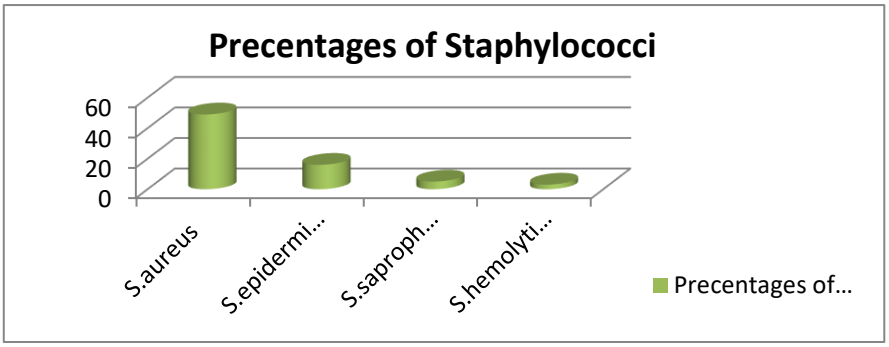
In this study, the most common and widespread types of bacteria were found, as about ١٦٥ samples were collected from various areas of wounds and burns in patients, and among these isolates were about ٧٣ samples of staphylococcus bacteria, as shown in Table (٢), and among these isolates were (٤٩) samples of S.aureus, at a rate of (٦٧,١). The result of this study agreed with the result of the

researcher (Al-Samaraey, ٢٠٢١), as the percentage of the presence of these bacteria in wounds was (٦٥٪). The result of this study also agreed with the results of the study of the researcher (Ahmed AL-Salman, ٢٠٢٢), as the percentage of the presence of these bacteria in wounds reached (٦٥٪). Many previous studies indicated that Staphylococcus aureus S.aureus had the highest percentage among all bacterial isolates that were collected from different wound areas that were contaminated with these bacteria or the source of contamination was external, represented by various germs, or the source of contamination was in the environment of operating rooms and people working in Operations or surgical tools and materials in the operating room (Monistero et al., ٢٠١٨) While the percentage of the presence of S.epidermids bacteria was in second place, the rate of presence of these bacteria was about (١٦) samples out of (٧٣) samples for staphylococci, at a rate of (٢١,٩٪), and in third place was the percentage of the presence of S.sapropgyticus bacteria, at a rate of (٥) samples, at a rate of (٦,٨٪), and in fourth place was S.hemolyticus bacteria, at a rate of (٣) samples, at a rate of (٤,١٪). The results of this study agreed with what the researcher (Maha Qusay., ٢٠٢١) reached, as the percentage of the presence of S.epidermids bacteria in wounds reached (١١٪), while the percentage of the presence of S.saprophyticus bacteria reached (٤٪) and S.hemolyticus bacteria reached (٨٪). These percentages indicate that S.aureus bacteria are one of the most common types that cause infections in humans. It is considered one of the most important factors causing infection in wound injuries and other infections, including (impetigo, infective endocarditis, wound infection, blood contamination, meningitis, toxic shock) and other diseases (Taylor and Unakal, ٢٠٢١) in addition to respiratory infections, pneumonia, surgical sites, artificial joints, and cardiovascular inflammation (Cheung et al., ٢٠٢١). The highest percentage of its presence in this study was S.auerus bacteria, while the lowest presence was S.hemolyticus bacteria. This is most likely due to the virulence of S.aureus bacteria and its enzymes and toxins that destroy the host's defenses, in

addition to its ability to resist antibiotics. Table (٢) shows the numbers of isolates from different wound areas and their percentages. Specimens

Specimens	Sampling ferquency	Percentage (%)
S.aureus	٤٩	٦٧,١٪
S.epidermids	١٦	٢١,٩٪
S.saprophyticus	٥	٦,٨٪
S.hemolyticus	٣	٤,١٪
Totale	٧٣	٩٩,٩٪

Figure (٢) shows the percentages of staphylococci isolated from wounds



Distribution of the study sample according to samples and gender

This study shows, as shown in Table (٣), a significant difference in the presence and distribution of staphylococcal isolates and their sources in both sexes, in addition to their resistance to antibiotics. This study showed that Staphylococcus aureus S.aureus is the most prevalent in (٤٩) samples. The presence of this bacteria was recorded in furniture more than in males, as (٣٢) samples out of (٤٩) samples were recorded in females, at a rate of (٦٥,٣٪), while only (١٧) samples were recorded in males, at a rate of (٣٤,٦٪). This is mostly due to the fact that men are more resistant to infection with this bacteria than females, as a result of their frequent contact with health institutions and people. In addition, the antibiotics used may play a major role in infection, and these antibiotics may be ineffective in resisting these bacteria, thus leading to the spread of bacteria and infection, while the presence of S.epidermids bacteria was at a rate of (١٦) samples, (٩) samples were recorded in Females and at a rate of (٥٦,٢٪), while in males (٧) samples were recorded at a rate of (٤٣,٧٪), while the presence of S.saprophyticus bacteria was recorded in men higher than in females, as (٣) samples were recorded at a rate of (٦٠٪), while in females (٢)

samples at a rate of (٤٠٪), and this is mostly due to the property of these bacteria to be present in abundance in injuries to prosthetic limbs, medical parts, etc., etc. In addition to that, the presence of these bacteria is opportunistic, as is the case with S.hemolyticus bacteria, as these bacteria may be present opportunistically, and when the appropriate conditions are available, they become pathogenic, as (٣) samples were recorded, (٢) appeared in females at a rate of (٦٦,٦٪), while (١) sample was found in males at a rate of (٣٣,٣٪), as this study showed that females are more susceptible to infection than males, and this study agreed with what the researcher (Hindy et al., ٢٠٢٢) and other researchers reached, who indicated that the infection appears in females more From males and in general this may be due to the physiological difference between females and males as in Figure (٣)

Specimens	Male	Percentage(%)	Female	Percentage(%)
S.aureus	١٧	٣٤,٦٪	٣٢	٦٥,٣٪
S.epidermids	٧	٤٣,٧٪	٩	٥٦,٢٪
S.saprophyticus	٢	٤٠٪	٣	٦٠٪
S.hemolyticus	١	٣٣,٣٪	٢	٦٦,٦٪
Totale	٢٧	٣٣,٦٪	٤٦	٦٣٪

Table (٣) shows the distribution of staphylococcal samples isolated from different wound areas for females and males

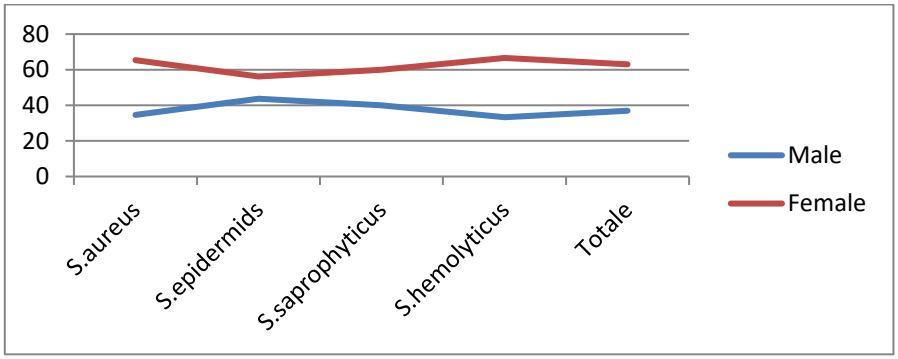


Figure (٣) shows the percentage distribution of staphylococcal samples from wound areas for females and males

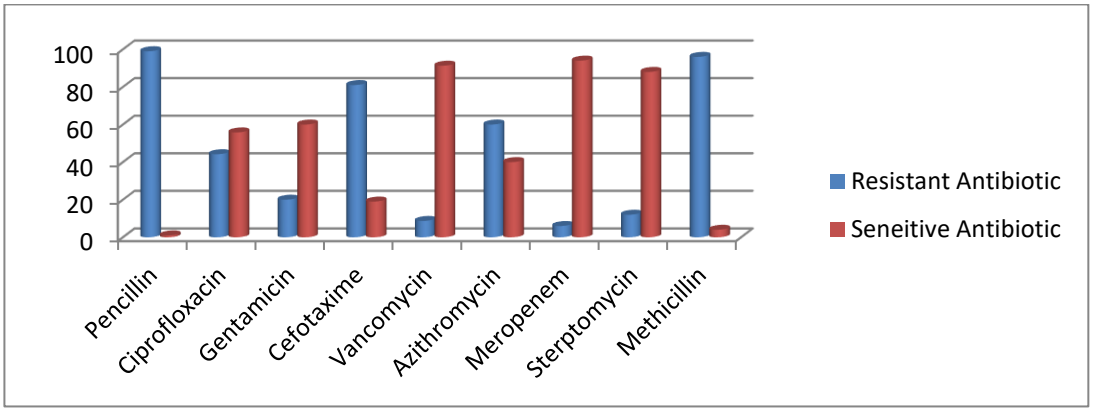
Sensitivity of bacteria to common antibiotics

under study According to Table (٤), which notes the antibiotics that were resistant and those that were sensitive among S.aureus isolates that were measured using the VITEK ٢ method, which shows the result of ٤٤ antibiotics,

as ١٠ antibiotics were used to observe their effect on S.aureus bacteria and compare them with the results that appeared using the VITEK device, as these bacteria showed great resistance to β-lactam antibiotics, which include Pencillins antibiotics, which are represented by Pencillin at a rate of (٩٩٪) and methicillin antibiotic at a rate of (٩٦٪) in addition to Cefotaxime antibiotic at a rate of (٨٦٪), which belongs to the third generation Cephosporins antibiotics, while these bacteria showed great sensitivity to Meropenem antibiotic, which belongs to Carbapenems antibiotics, with a resistance rate of (٦٪). The bacteria also showed great sensitivity to Vancomycin antibiotic among the Glycopeptide antibiotics, with a resistance rate of (٨٪), then Aminoglycosides antibiotics, which Streptomycin includes (١٢٪), Amikacin antibiotics (٤٣٪), Gentamicin antibiotics (٢٠٪), then Macrolides antibiotics, which include Azithromycin antibiotics (٦٠٪), and Ciprofloxacin antibiotics among Fluroquinolones antibiotics (٤٤,٢٪). The results of this study agree with the results of both the researcher (Ahmed AL-salman ., ٢٠٢٢) and the researcher (Maha Qusay., ٢٠٢١) and the researcher (Hindy et al., ٢٠٢٢) who confirmed the ability of S.aureus bacteria that carry virulence genes that help them resist antibiotics, as in Figure (٤), which shows the percentages of antibiotics. Table (٤), which shows the resistance of bacteria to antibiotics and their sensitivity. P-value

Isolated	Antibiotic	Sample	Resistant Antibiotic	Sensitive Antibiotic	P-Value
١	Penicillin	P	٩٩٪	١٪	**٠,٠٠٠١
٢	Methicillin	Me	٩٦٪	٤٪	**٠,٠٠٠١
٣	Cefotaxime	CTX	٨١٪	١٩٪	**٠,٠٠٠١
٤	Azithromycin	AZM	٦٠٪	٤٠٪	**٠,٠٠٠١
٥	Ciprofloxacin	CIP	٤٤,٢٪	٥٥,٨٪	**٠,٠٠٠١
٦	Vancomycin	VA	٨,٧٪	٩١,٣٪	**٠,٠٠٠١
٧	Streptomycin	S	١٢٪	٨٨٪	**٠,٠٠٠١
٨	Gentamicin	CN	٢٠٪	٨٠٪	**٠,٠٠٠١
٩	Meropenem	MEM	٦٪	٩٤٪	**٠,٠٠٠١
١٠	Amikacin	AK	٤٣٪	٥٧٪	**٠,٠٠٠١

Figure (٤) shows the percentage of bacterial sensitivity and resistance to antibiotics



The spread of antibiotic resistance

Currently, one of the biggest threats to human health is the emergence of resistance to various antibiotics, as reports of drug-resistant bacteria increase every year. However, the number of new antibiotics being developed is declining, so the use of antibiotics in health care is being reduced as a means of stopping the spread of antibiotic resistance by bacteria and its spread (AL-Zobaidy et al., ٢٠١٩) As in Table (٥)

Pharmacological family	Category	Drug Classification	Trade Name	The scientific name
Pencillin	Infection diseases	Antibacterial	Amoxil	Amoxicillin
Aminoglycosides	Sexual diseases	Antibacterial	Gramycin	Gentamicin
Tetracyclin derivatives	Infection diseases	Antibacterial	Rocephin	Ceftriaxon
Felin new-burn	Infection diseases	Antibacterial	Azith	Azithromycin

Table (٥) shows some common types of antibiotics and their most important uses The scientific name

Conclusion:

According to this study and the isolates collected from different wound areas and for different ages and both sexes, it was found that the most common bacterial species in wounds is Staphylococcus bacteria, as its percentage reached (٧٣) samples out of (١٦٥) different samples, where it was found that S.aureus bacteria is present in the first place in wounds with a percentage of (٤٩) samples out of (٧٣) and it is the most widespread, while it was found that

the least common samples in this study are S.hemolyticus bacteria with a percentage of (٣) isolates. In addition, these bacteria showed great resistance to various antibiotics, which indicates the danger of these bacteria and the many diseases they cause, as they showed great resistance to Pencillins, including Pencillin at a percentage of (٩٩٪) and Methicillin (٩٦٪). In addition, the bacteria also showed great resistance to Cephosporins, which include Cefotaxime at a percentage of (٨١٪), while these bacteria showed sensitivity The effect of anti-Carabenems is great, which is represented by Meropenem (٦٪) and Vancomycin (٨,٧٪), which is one of the anti-Glycopeptides.

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