

Diagnosis of Staphylococcus aureus isolated from different body sites of patients and assessment of their resistance to certain antibiotics.

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Abstract:

The study showed that Staphylococcus aureus was the most prevalent in (٣٥) samples. The presence of this bacteria was recorded more in furniture than in males, as (٢٠) samples out of (٣٥) samples were recorded in females, at a rate of (٥٧,١٤%), while only (١٥) samples were recorded in males, at a rate of (٤٢,٨٥%). This is mostly due to the fact that males are more resistant to infection with this bacteria than females, as a result of their frequent contact with health institutions and individuals. The results of the sensitivity of bacterial isolates (resistant and sensitive) to antibiotics among Staphylococcus aureus isolates using the VITIK^٢ method showed that the bacteria were resistant to most antibiotics at rates ranging between ٩٠% and ١٠٠%, namely: cefixime, cefoxitin, benzylpenicillin, amoxicillin, ampicillin, amoxicillin/clavulanic acid, piperacillin, oxacillin, cefuroxime, cefpodoxime, cefotaxime, cefepime, and imipenem. The most sensitive antibiotics to the bacteria under study, with varying rates ranging from ٦١% to ٨٠%, were trimethoprim/sulfamethoxazole, rifampicin, vancomycin, ofloxacin, and tetracycline, with rates of ٧٤,٢٨%, ٦٨,٥٧%, ٧٤,٢٨%, ٦٨,٥٧%, and ٧١,٤٢%, respectively. According to this study, the bacteria were sensitive to a group of antibiotics that may not exceed ٨ out of a total of ٤٤ antibiotics. This means they were resistant to approximately ٨٠% of the antibiotics and sensitive to approximately ٢٠% of the antibiotics.

١.Introduction

Staphylococcus aureus is a Gram-positive bacterium that is naturally present on human and animal skin and in mucous membranes (Otarigho & Falade, ٢٠٢٣). Approximately ٣٠% of humans naturally carry *Staphylococcus aureus* in their noses. They are more susceptible to internal infections (Akhtar Danesh et al., ٢٠٢٠).

Staphylococcus aureus lives symbiotically with other bacterial species in the human body. These bacteria can also become opportunistic pathogens and infect other tissues (Otarigho & Falade, ٢٠١٨)

Staphylococcus aureus can be divided into methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA).

In recent decades, due to bacterial evolution and increased antibiotic use, the resistance of *Staphylococcus aureus* to antibiotics has gradually increased. The incidence of MRSA has increased worldwide, and clinical treatment for MRSA infections has become more challenging (Bo et al., ٢٠٢١). These Gram-positive bacteria are also known to cause skin infections, pneumonia, bacteremia, endocarditis, and toxic shock syndrome due to their production of various toxins (Otto, ٢٠١٨)

In addition, *S. aureus* can easily acquire resistance to antimicrobials and is a major causative agent of hospital-acquired infections (Nakaminami et al., ٢٠١٤).

Penicillin was discovered in ١٩٢٨ and became commercially available in the ١٩٤٠s, significantly reducing the incidence of *S. aureus* infections. Unfortunately, penicillin-resistant *S. aureus* soon emerged by acquiring the gene for penicillinase, which is encoded on a large plasmid (Kurukulasooriya et al., ٢٠٢٤). The discovery of beta-lactamase-resistant penicillins (such as nafcillin, oxacillin, cefoxitin, and methicillin) provided temporary relief (Kadhum & Abood, ٢٠٢٢). However, later strains of methicillin-resistant *Staphylococcus aureus* evolved the *mecA* gene located on the *Sc* *mecA* cassette chromosome. This gene encodes the penicillin-binding protein ٢a (PBP٢a), cell wall-synthesizing enzymes with low affinity for penicillin (Alghammal et al., ٢٠٢٠). MRSA strains are resistant to almost all antibiotics containing the beta-

lactam ring, including penicillins, cefpodoxime, and carbapenems, because PBP^{2a} has very low affinity for beta-lactams

٩٥% of resistant *S. aureus* isolates produce the beta-lactamase enzyme (penicillinase) encoded by the *blaZ* gene, which hydrolyzes the beta-lactam ring of penicillin (Lowy, ١٩٩٨). Two mechanisms contribute to resistance to Pc penicillin G in *Staphylococcus aureus*. First, it involves the production of penicillinase encoded by *blaZ*, which can inactivate Pc by hydrolyzing the β -lactam ring. Second, it involves the variable Pc-binding protein, PBP^{2a}, encoded by *mecA* (Dos Santos et al., ٢٠١٦).

blaZ is an ٨٤٦-base-pair gene controlled by two regulatory genes (the anti-repressor gene *blaR*^١ and the repressor gene *blaI*). After exposure to a beta-lactam, *blaR*^١ (a transmembrane sensor-adaptor) undergoes autocatalytic cleavage, which promotes cleavage of *blaI* and leads to transcription of *blaZ* (Katayama et al., ٢٠٠٤)

BlaZ proteins are serologically classified into types A to D. Types A, C, and D of *blaZ* genes are located on the plasmid, while type B is located on the chromosome. Substrate specificity against β -lactams varies slightly between species. Each type of *BlaZ* (Nomura et al., ٢٠٢٠).

Antibiotic resistance is subject to direct and indirect regulation by environmental signals, as mentioned above, with the two-component signal transduction system (TCS) being the main mediator of this process. The membrane-bound TCS detects the presence of antibiotics in the environment, which leads to the activation of transcriptional regulators

٢. Methodology:

٢.١ Sample Collection and Diagnosis

In this study, approximately (١٦٠) clinical samples were collected from patients at Al-Sharqat General Hospital in Salah al-Din Governorate and from the Medical City Hospital in Baghdad Governorate between July ١, ٢٠٢٤, and October ١٦, ٢٠٢٤. An autoclave was used to sterilize liquid and solid culture media, as well as tools and solutions, without being affected by the high heat generated by the autoclave. After collecting bacterial samples using cotton swabs from the affected areas, these areas

represented by various wounds and burns throughout the body, as well as from the urinary tract, sputum, and V.S., these swabs were cultured on appropriate culture media using Petri dishes, represented by blood base agar and MacConkey agar simultaneously. The Petri dishes were then placed in an incubator at 37°C for 18–24 hours. Gram staining and catalase testing were then performed to differentiate between staphylococci and streptococci. The coagulase test distinguishes *Staphylococcus aureus* from other staphylococci, namely coagulase-negative (*S. epidermis*) and coagulase-negative (*S. epidermis*). Samples are then cultured on mannitol salt agar (MSA), a selective medium for these bacteria. This medium allows *S. aureus* to grow, while other bacteria cannot, as it contains 4–10% sodium salts. *S. aureus* ferments the sugar mannitol, which changes the color of the medium from red to yellow. Catalase and oxidase tests (Ekta et al., 2022) are sometimes used to distinguish oxidase-negative staphylococci from other negative species. After diagnosis, bacteria are cultured using culture media and biochemical tests, then identified using the VITEK 2 to ensure the purity of the identified bacterial isolates and exclude other species. The bacteria are then stored, and the results are recorded.

٣. Results and Discussion

٣.١ Biochemical Identification of the Staphylococcal Species Under Study

Bacterial species that cause numerous diseases, often leading to wound and burn infections, sepsis, and many other life-threatening diseases, were identified. These bacteria were isolated, all species were Gram-stained, and several biochemical tests were performed according to Table (1). *Staphylococcus aureus* (*S. aureus*) was identified by performing a catalase test for all species, as all bacteria produced this test and formed blisters. In addition, a coagulase test was performed to separate *S. aureus* from other staphylococcal species. A urease test was also performed, as all species also produced this enzyme. Furthermore, a novobiocin antibody test was performed, which distinguishes between *S. saprophyticus* and *S. epidermis*, as these bacteria are bacteriocins. *Staphylococcus aureus* is resistant to this antibiotic, while *Staphylococcus albicans* is sensitive to it, which distinguishes between the two types. An oxidase test was performed to determine the ability of these bacteria to produce this enzyme, and all

bacteria tested negative. Table (١) shows the chemical identification of the various types of staphylococci under study, and Figure (١) shows the remaining types of bacteria that were identified

٣,٢ Isolation of *Staphylococcus aureus*

In this study, the most common and widespread bacterial species were identified. Approximately ١٦٠ samples were collected from various areas of patients' wounds and burns. Of these, approximately ٦٢ were *S. aureus*, as shown in Table (٢). Of these isolates, (٣٥) were *S. aureus*, with a mean of (٥٦,٤٥). This study is consistent with the findings of researcher (Alkhafaji & Alsaimary, ٢٠٢٠), who found that the percentage of these bacteria in wounds was (٤٢,٨٥%). The results of this study also agree with the results of researcher (Salman & Abd Al-Mayahi). Many previous studies have indicated that *Staphylococcus aureus* *S.aureus* had the highest percentage among all bacterial isolates collected from different wound areas that were contaminated with this bacteria, or the source of contamination was external, represented by various germs, or the source of contamination was in the operating room environment, the people working in operations, or the surgical tools and materials in the operating room (Monistero et al., ٢٠١٨), while the percentage of the presence of *S.epidermids* bacteria came in second

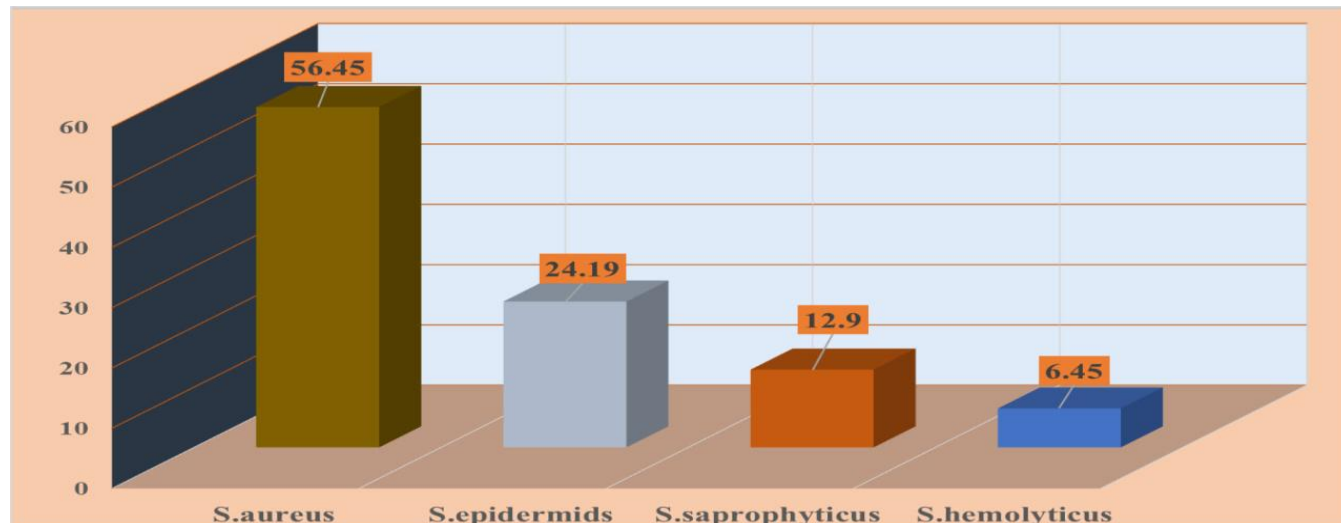
Type bacteria	Catalase test	Coagulase test	Oxidase	Motility	Gram stain
S.aureus	+	+	–	non motile	Gr +
S.epidermids	+	–	–	non motile	Gr+
s. saprophyticus	+	–	–	non motile	G+
s. hemolyticus	+	–	–	non motile	Gr+

place, as the rate of the presence of this bacteria reached about (١٥) samples out of (٦٢) samples of staphylococci, at a rate of (٢٤,١٩%), and in third place was the percentage of the presence of *S.sapropgyticus* bacteria, at a rate of (٨) samples, at a rate of (١٢,٩%), and in fourth place was *S.hemolyticus* bacteria, at a rate of (٤) samples, at a rate of (٦,٤٥%). The results of this study are consistent with the findings of the researcher (Muhammed et al., ٢٠٢٢). These proportions indicate that *S.aureus* is one of the most common pathogens in humans. It is considered a major causative agent of infections in

wounds, burns, and other infections, including impetigo, infective endocarditis, wound infections, sepsis, meningitis, and toxic shock (Pines), as well as respiratory tract infections, pneumonia, surgical sites, artificial joints, and cardiovascular infections (Cheung et al., ٢٠٢١). The highest prevalence in this study was *S.aureus*, while the

Specimens	Sampling frequency	Percentage (%)
<i>S.aureus</i>	٣٥	٥٦,٤٥
<i>S.epidermids</i>	١٥	٢٤,١٩
<i>S.saprophyticus</i>	٨	١٢,٩٠
<i>S.hemolyticus</i>	٤	٦,٤٥
Totale	٦٢	٩٩,٩

lowest prevalence was *S. hemolyticus*. This is likely due to the virulence of *S.aureus*, its enzymes and toxins that destroy host defenses, and its potential for antibiotic resistance. Table (٢) shows the numbers and percentages of isolates from different regions.

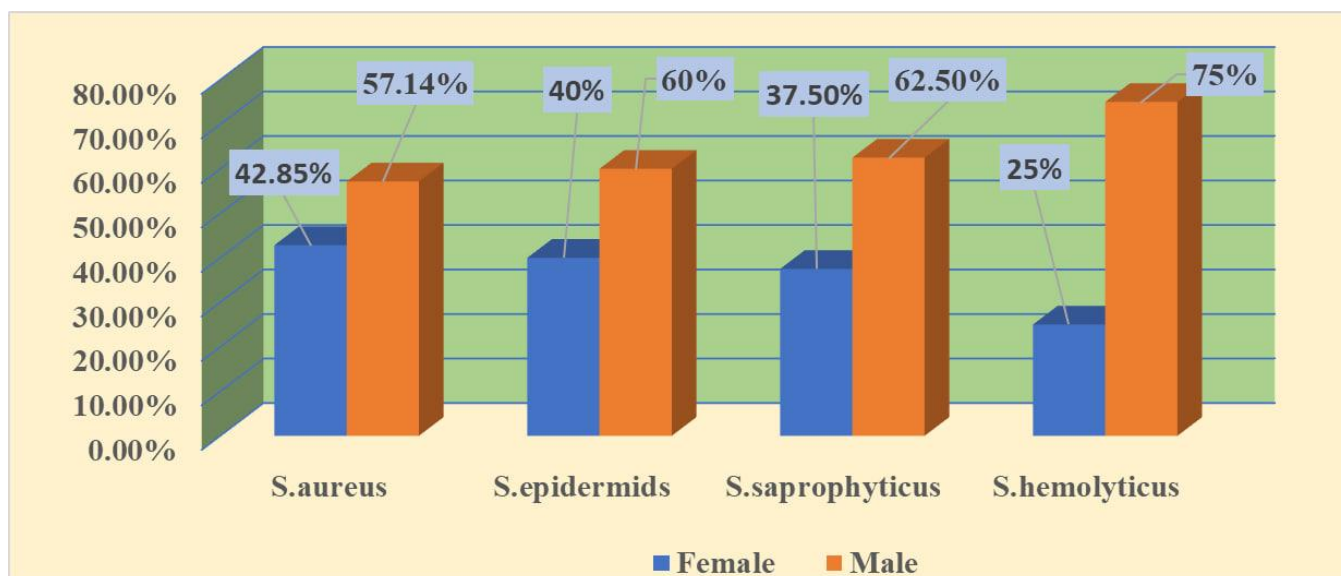


٣,٣ Distribution of the Study Sample by Sample and Gender

This study, as shown in Table (٣), demonstrated significant differences in the presence and distribution of staphylococcal isolates and their sources across both sexes, as well as their resistance to antibiotics. The study showed that *Staphylococcus aureus* was the most prevalent bacteria in (٣٥) samples. The presence of this bacteria was more prevalent in furniture than in men, with (٢٠) of the (٣٥) samples recorded in women,

representing (٥٧,١٤%), while only (١٥) samples were recorded in men, representing (٤٢,٨٥%). This is likely due to the fact that men are more resistant to infection with this bacteria than women, due to their frequent contact with health facilities and individuals. In addition, the antibiotics used may play a major role in infection, and these antibiotics may be ineffective in resisting these bacteria, leading to the spread of bacteria and infection. While the presence of *S. epidermids* bacteria was at a rate of (١٥) samples, (٩) samples were recorded in females at a rate of (٦٠%), while in males (٦) samples were recorded at a rate of (٤٠%), while the presence of *S. saprophyticus* bacteria was recorded in men higher than in females, as (٥) samples were recorded at a rate of (٦٢,٥%), while in females (٣) samples at a rate of (٣٧,٥%). This is mostly due to the property of these bacteria to be found in abundance in injuries of prosthetic limbs, medical parts, etc. In addition, 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 (٤) samples were recorded, (٣) of which appeared in females at a rate of (٧٥%), while (١) sample was found in males at a rate of (٢٥%). This study also showed that females are more susceptible to infection. This study agreed with the findings of the researcher (Hindi et al., ٢٠٢٢) and other researchers, as they indicated that infection appears more in females than in 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	١٥	٤٢,٨٥%	٢٠	٥٧,١٤%
S.epidermids	٦	٤٠%	٩	٦٠%
S.saprophyticus	٣	٣٧,٥%	٥	٦٢,٥%
S.hemolyticus	١	٢٥%	٣	٧٥%
Totale	٢٥	٤٠,٣٢%	٣٧	٥٩,٦٧%



٣,٤ Antibiotic sensitivity of bacterial isolates in this study:

According to Table (٤), the results of the antibiotic sensitivity of bacterial isolates (resistant and susceptible) among S. aureus isolates using the VITIK^٢ method show ٤٤. All antibiotics used to test S. aureus strains from different infections using the VITIK^٢ method were resistant, and S. aureus strains differed in their antibiotic resistance rate when using the probability value ($**p \leq 0.01$) as an indicator. No statistically significant differences were found for most of the antibiotics studied, as ٤٤ antibiotics were used according to VITIK^٢ measurements. Bacteria were found to be resistant to most antibiotics at rates ranging between ٩٠% and ١٠٠%. We note, according to the table, that the bacteria were ٨٠% to ١٠٠% resistant to the following antibiotics: cefixime, cefoxitin, benzylpenicillin, amoxicillin, ampicillin, amoxicillin/clavulanic acid, piperacillin, oxacillin, cefuroxime, cefpodoxime, cefotaxime, cefepime, imipenem. This is a large number of antibiotics that bacteria have become resistant to. They are completely unaffected by them, but the antibiotics to which the bacteria were sensitive, at varying rates between ٣٠% and ٦٠%, were: gentamicin, azithromycin, amikacin, tobramycin, ciprofloxacin, levofloxacin, clarithromycin, erythromycin, clindamycin, and doxycycline. The most sensitive antibiotics to the bacteria under study, with varying rates ranging from ٦١% to ٨٠%, were trimethoprim/sulfamethoxazole, rifampicin, vancomycin, ofloxacin, and tetracycline, with rates of (٧٤,٢٨%), (٦٨,٥٧%), (٧٤,٢٨%),

(٦٨,٥٧%), and (٧١,٤٢%), respectively. According to Table and Figure (٤), we note that the bacteria were sensitive to a group of antibiotics that may have exceeded ٨ antibiotics out of a total of ٤٤ antibiotics. This means that they were resistant to approximately ٨٠% of the antibiotics and sensitive to approximately ٢٠% of the antibiotics, according to these results, which may be consistent with some studies such as (Hindi et al., ٢٠٢٢) and (Onyango et al., ٢٠١٨).

This confirms the ability of *S. aureus* bacteria carrying virulence genes to resist the most common antibiotics.

Isolate	Antibiotic	Sample	R No. (%)	S No. (%)	P-value
١	Cefoxitin	FOX	٣٢(٩١,٤٢%)	٣(٨,٥٧%)	٠,٠٠٠١ **
٢	Benzylpenicillin	PENPEN	٣٣(٩٤,٢٨%)	٢(٥,٧١%)	٠,٠٠٠١ **
٣	Amoxicillin	AMX	٣٣(٩٤,٢٨%)	٢ (٥,٧١%)	٠,٠٠٠١ **
٤	Ampicillin	AMP	٣٣(٩٤,٢٨%)	٢ (٥,٧١%)	٠,٠٠٠١ **
٥	Amoxicillin/Clavulanic acid	AMC	٣٣(٩٤,٢٨%)	٢ (٥,٧١%)	٠,٠٠٠١ **
٦	Ampicillin/ Sulbactam	AM /SCF	٢٩(٨٢,٨٥%)	٦ (١٧,١٤%)	٠,٠٠٠١ **
٧	Piperacillin/Tazobactam	PTZ	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
٨	Oxacillin MIC	OX/ MIC	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
٩	Oxacillin	OX	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
١٠	Cefuroxime	CXM	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
١١	Cefixime	CFM	٣٥(١٠٠%)	٠ (٠,٠٠%)	٠,٠٠٠١ **
١٢	Cefpodoxime	CPD	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
١٣	Cefotaxime	CTX	٢٩(٨٢,٨٥%)	٦(١٧,١٤%)	٠,٠٠٠١ **
١٤	Ceftriaxone	CRO	٢٧(٧٧,١٤%)	٨(٢٢,٨٥%)	٠,٠٠٠١ **
١٥	Cefepime	FEP	٢٨(٨٠%)	٧ (٢٠%)	٠,٠٠٠١ **
١٦	Imipenem	IMP	٢٨(٨٠%)	٧ (٢٠%)	٠,٠٠٠١ **
١٧	Meropenem	MEM	٢٧(٧٧,١٤%)	٨(٢٢,٨٥%)	٠,٠٠٠١ **
١٨	Amikacin	AK	٢٢(٦٢,٨٥%)	١٣(٣٧,١٤%)	٠,٠٠٠١ **
١٩	Gentamicin	GN	٢٣(٦٥,٧١%)	١٤(٤٠%)	٠,٠٠٠١ **
٢٠	Tobromycin	TO	١٩(٥٤,٢٨%)	١٦(٤٥,٧١%)	٠,٠٠٠١ **

٢١	Ciprofloxacin	CIP	١٧(٤٨,٥٧٪)	١٨(٥١,٤٢٪)	٠,٠٠٠١ **
٢٢	Levofloxacin	LFV	١٧(٤٨,٥٧٪)	١٨(٥١,٤٢٪)	٠,٠٠٠١ **
٢٣	Moxifloxacin	MXF	١٢(٣٤,٢٨٪)	٢٣(٦٥,٧١٪)	٠,٠٠٠١ **
٢٤	Norfloxacin	NOR	١٢(٣٤,٢٨٪)	٢٣(٦٥,٧١٪)	٠,٠٠٠١ **
٢٥	Ofloxacin	OFL	١١(٣١,٤٢٪)	٢٤(٦٨,٥٧٪)	٠,٠٠٠١ **
٢٦	Inducible clindamycin Resistance	I/CD/R	٢٣(٦٥,٧١٪)	١٢(٣٤,٢٨٪)	٠,٠٠٠١ **
٢٧	Azithromycin	AZM	٢٤(٦٨,٥٧٪)	١١(٣١,٤٢٪)	٠,٠٠٠١ **
٢٨	Clarithromycin	CLR	٢٤(٦٨,٥٧٪)	١١(٣١,٤٢٪)	٠,٠٠٠١ **
٢٩	Erythromycin	E	٢٤(٦٨,٥٧٪)	١١(٣١,٤٢٪)	٠,٠٠٠١ **
٣٠	Clindmycin	CD/DA	٢٣(٦٥,٧١٪)	١٢(٣٤,٢٨٪)	٠,٠٠٠٢ **
٣١	Lincomycin	LCM	٢٥(٧١,٤٢٪)	١٠(٢٨,٥٧٪)	٠,٠٠٠١ **
٣٢	Linezolid	LZD	٩(٢٥,٧١٪)	٢٦(٧٤,٢٨٪)	٠,٠٠٠١ **
٣٣	Teicoplanin	TEC/TEI	٩(٢٥,٧١٪)	٢٦(٧٤,٢٨٪)	٠,٠٠٠١ **
٣٤	Vancomycin	VAN	٩(٢٥,٧١٪)	٢٦(٧٤,٢٨٪)	٠,٠٠٠١ **
٣٥	Doxycycline	DOX	١٦(٤٥,٧١٪)	١٩(٥٤,٢٨٪)	٠,٠٠٠١ **
٣٦	Minocycline	MIN	١٦(٤٥,٧١٪)	١٩(٥٤,٢٨٪)	٠,٠٠٠١ **
٣٧	Tetracycline	TE	١٠(٢٨,٥٧٪)	٢٥(٧١,٤٢٪)	٠,٠٠٠١ **
٣٨	Tigecycline	TIG	١٢(٣٤,٢٨٪)	٢٣(٦٥,٧١٪)	٠,٠٠٠١ **
٣٩	Fosfomycin	FOS	١٢(٣٤,٢٨٪)	٢٣(٦٥,٧١٪)	٠,٠٠٠١ **
٤٠	Nitrofurantoin	NI/F	١٢(٣٤,٢٨٪)	٢٣(٦٥,٧١٪)	٠,٠٠٠١ **
٤١	Fusidic Acid	FD	١٨(٥١,٤٢٪)	١٧(٤٨,٥٧٪)	٠,٠٠٠١ **
٤٢	Mupirocin	MUP	١٨(٥١,٤٢٪)	١٧(٤٨,٥٧٪)	٠,٠٠٠١ **
٤٣	Rifampicin	RA	١١(٣١,٤٢٪)	٢٤(٦٨,٥٧٪)	٠,٠٠٠١ **
٤٤	Trimethoprim/sulfametho xazole	TS/SXT	٩(٢٥,٧١٪)	٢٦(٧٤,٢٨٪)	٠,٠٠٠١ **
P-value		—	٠,٠٠٠١ **	٠,٠٠٠١ **	—
** (P≤٠,٠١).					

٣,٥ The spread of antibiotic resistance:

The spread of antibiotic resistance currently poses a significant threat to human public health. Cases of multidrug-resistant bacteria are reported annually, while the development of new antibiotics is declining. Focus has been placed on reducing the spread of antibiotic resistance by limiting their use in healthcare, which reduces the exposure of pathogens to antibiotics and thus limits the selection of resistant strains (Table ٥) (Al-Zubaidi et al., ٢٠١٩).

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

Conclusion:

According to this study, the most common bacterial species diagnosed in the healthcare facilities under study was *Staphylococcus aureus* (٢١,٨٧%), compared to other pathogenic bacteria. Wounds and burns were the most common sites of its spread, while pus was the least common. In general, the infection rate of this pathogenic bacteria was higher in males than in females. It was also found that *Staphylococcus aureus* bacteria are resistant to common antibiotics at rates that may exceed ٨٠% of the antibiotics under study, which total ٤٤ antibiotics, as it was found to be resistant at a rate of ٨٠% to ١٠٠% to cefixime, cefoxitin, benzylpenicillin, amoxicillin, ampicillin, amoxicillin/clavulanic acid, piperacillin, oxacillin, cefuroxime, cefpodoxime, cefotaxime, cefepime, imipenem, and sensitive to a small group of antibiotics at varying rates, namely trimethoprim/sulfamethoxazole, rifampicin, vancomycin, ofloxacin, and tetracycline, which reached (٧٤,٢٨%), (٦٨,٥٧%), (٧٤,٢٨%), (٦٨,٥٧%), and (٧١,٤٢%), respectively.

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