

Bacteriological profiles of multidrug resistance bacteria isolated from skin and soft tissue infections

Abstract

In present study thirty one of bacterial isolates were isolated from twenty six samples collected from different patients suffered with skin infections. The bacteria isolated on culture media (Mannitol salt agar, MacConkey agar and blood agar), identified and determined the antibiotic resistance by Vitek 2 system. The results showed that most frequent infections were impetigo, boil and chronic folliculitis 5 (19%) at age group (2-17), (18-55) and (18-38) years respectively. According to the gender the infections were more in females 15 (57.6%) than males 11 (42.3%), of all those infections, impetigo and boil were more common in females 4 (15%), while skin cancer and chronic folliculitis were more common in males 3 (12%). The results of bacterial isolations showed that 26 (83.87%) of isolates were Gram positive bacteria while 5 (16.13%) were Gram negative. The high frequent genus identified by Vitek 2 system was *Staphylococcus* sp. followed by *Acinetobacter* sp., *Aeromonas* sp., and *Sphingomonas* sp., the most dominant species was *Staphylococcus aureus* 11 (35.5%) followed by *Staphylococcus epidermidis* 8 (25.8%). The results of antibiotic resistance to (48) antibiotics from different groups revealed that out of (31) isolates (23) were multidrug resistance bacteria, the more resistance isolates were *Staphylococcus aureus* (R3) was resistant to (12) classes of antibiotics followed by *Staphylococcus hominis* (R16) and *Staphylococcus epidermidis* (R18) (9) classes. There has been a concerning rise in the antibiotic-resistant pattern of skin infection-causing *Staphylococcus aureus*. These days, it is imperative to utilize antibiotics wisely and to put antimicrobial stewardship into practice.

Keywords: Skin infection, Bacteria, Drug resistance

Introduction

Skin is the largest organ in human body and basic barrier, serving as the first line of defense against bacterial infections. It's not only blocking the pathogens from entry to body from the environment, but also providing a large-scale biological niche for a wider range of bacteria (Ibrahim *et al.*, 2015). Skin and soft tissue infections (SSTIs) are a major cause of morbidity and mortality, they are characterized by a microbial invasion of the skin layers and underlying soft tissues which range in severity from mild to life-threatening, the infections can occur from the uncomplicated cellulitis, to

abscesses, deep tissue necrosis and necrotizing fasciitis (Esposito *et al.*, 2016; Allaw *et al.*, 2023). Bacteria ISSTIs are mainly caused by Gram-positive bacteria like *Staphylococcus aureus* and β -hemolytic streptococci and many coagulase-negative *Staphylococcus*, Gram-negative Enterobacteriaceae, non-fermentative bacteria such as *Pseudomonas* spp. and *Acinetobacter baumannii* can also cause (Ramirez *et al.*, 2020; Allaw *et al.*, 2023). Necrotizing fasciitis, scarlet fever, erysipelas, erythrasma, abscesses, folliculitis, furunculosis, and impetigo have all been related to a number of bacterial skin infections (Al-Kahfaji, 2022).

Cellulitis is an acute bacterial infection that is inflaming the subcutaneous tissue around it as well as the deep dermis (Brown and KL, 2019). The bacteria that most commonly involve do cellulitis are *S. aureus* and group A streptococci, also Gram-negative bacteria *Pseudomonas aeruginosa* and Enterobacteriaceae group (Sari *et al.*, 2022; Joseph, *et al.*, 2022). Erysipelas is a severe streptococcal infection of the skin primarily spreading through the lymphatic vessels (Jendoubi *et al.*, 2019). It's a soft tissue infection involving the upper dermis, it has more distinct margins when compared with other soft tissue skin infections. Impetigo is a superficial soft tissue skin infection that infects the epidermis. It is a most common bacterial skin infection in children two to five years of age. It is caused by *S. aureus* or *S. pyogenes* (Lin *et al.*, 2023). Currently, folliculitis is a common skin condition, which is an inflammation of the tiny pockets in the skin of hair growth (hair follicles) and the perfollicular tissue that can affect the healthy people of any age group or any sex. It occurs when bacteria infect hair follicles. Some of it resolves spontaneously, but others progress to furuncles (boils), involving adjacent tissue. That is distinguished with red swelling, hard and painful lumps filled with pus. *S. aureus* are the most common bacteria cause of these infections (Nasr, 2018; Jappa, and Kutre, 2018 ; Lin *et al.*, 2021).

Resistance to antibiotics is one of the crucial issues related to public health and one of the most vital threats to the healthcare sector is the rise of antibiotic-resistant microbes. Multidrug-resistant bacteria (MDR) that are deadly pathogens are rising day by day and pose a very serious threat to human health. Whereas earlier, such antibiotic resistance was only found in nosocomial infections, but it is now become a common phenomenon (Jube *et al.*, 2020; Bharadwaj *et al.*, 2022). The spread of "superbugs" that are now resistant to several antibiotics is a serious issue (Davies and Davies, 2010). These include the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Khan and Khan, 2016). The total deaths from methicillin-resistant *S. aureus* (MRSA) are now comparable to those caused by HIV, and it is predicted that by 2050, antimicrobial resistance would be responsible for at least 10 million annual deaths (De Kraker *et al.*, 2016). So the present study aimed to evaluate the clinical and bacteriological profiles of bacterial isolates in skin infections, including the number and type of potential bacteriological pathogens as well as antibiotic susceptibility and pattern of isolates because of limited data are available regarding skin and soft tissue infection, mortality rate, and antibiotic susceptibility of bacteria in our hospital settings.

Material and Methods

Sample collection

A total of 26 samples were collected from patients undergoing skin infections including cellulitis, erysipelas, impetigo, folliculitis, furuncles and acne (it's a very common skin infection that causes pimples) in Al-Sadr Teaching Hospital, Al-Fayhaa Teaching Hospital, Al-Mauana Teaching Hospital and Tumor center in Basrah provinces south of Iraq at the period from the beginning of October 2022 to the end of December 2022. The age of patients ranged between 3-70 years old. It should be mentioned that all patients in this study were selected depending on their history and clinical examination by specialist doctors. Samples that were taken were collected from deep parts of the infection (pus) by a cotton sterile media swab. Then all samples were collected in sterilized containers and transported to a laboratory for isolation.

Culturing of bacteria

All swabs were streaked on the media of blood agar, MacConkey agar and Mannitol salt agar plates incubated at 37°C for 24 hrs. Then observed for the presence of isolated colonies. re-cultured on nutrient agar plates for purification and subsequent experiments.

Identification of bacterial isolates

The Vitek 2 system was performed at Al-Bayan laboratory in Basrah city by using Vitek 2 kit (NGREF21 341) (Pincus, 2006).

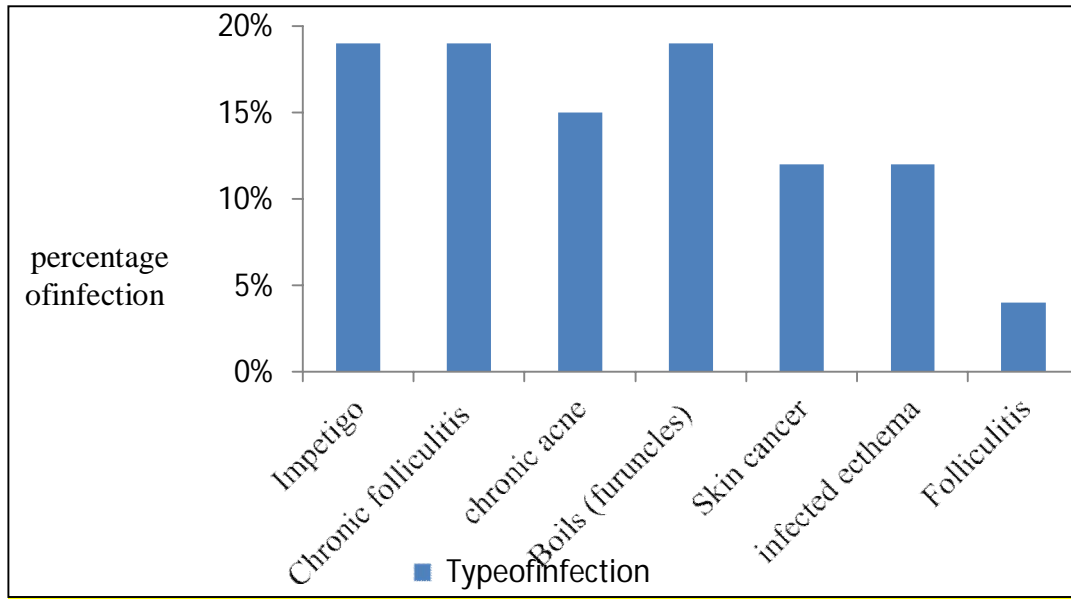
Antibiotic sensitivity test

The sensitivity and resistance of isolates were tested by using VITEK 2 system (Piddok, 1991).

Results

Skin infection frequency

The result of skin infection frequency showed that impetigo was the most frequent infection with percentage 5 (19%) followed by chronic folliculitis and boils (furuncles) 5 (19%), while folliculitis infection recorded the lowest frequent 1 (4%) figure (1).



Figure(1):Percentage of skin infection.

Skin infection percent according to the age

Table(1) revealed the results of skin infection according to the age. Where the percentage of impetigo, boils (furuncles) and chronic folliculitis was 5 (19%) at (2-17), (18-55) and (18-38) years respectively. Folliculitis infection showed the lowest percentage 1 (4%) at the age of 73 years old.

Table(1): Percentage of skin infections according to the age.

Type of infection	No. of infections per (%)	Age of patients
Impetigo	5 (19%)	2-17
Chronic folliculitis	5 (19%)	18-38
Skin cancer	3 (12%)	25-35
Boils (furuncles)	5 (15%)	18-55
Folliculitis	1 (4%)	73
infected ecthema	3 (12%)	9-50
chronic acne	4 (15%)	27-25
Total	26	

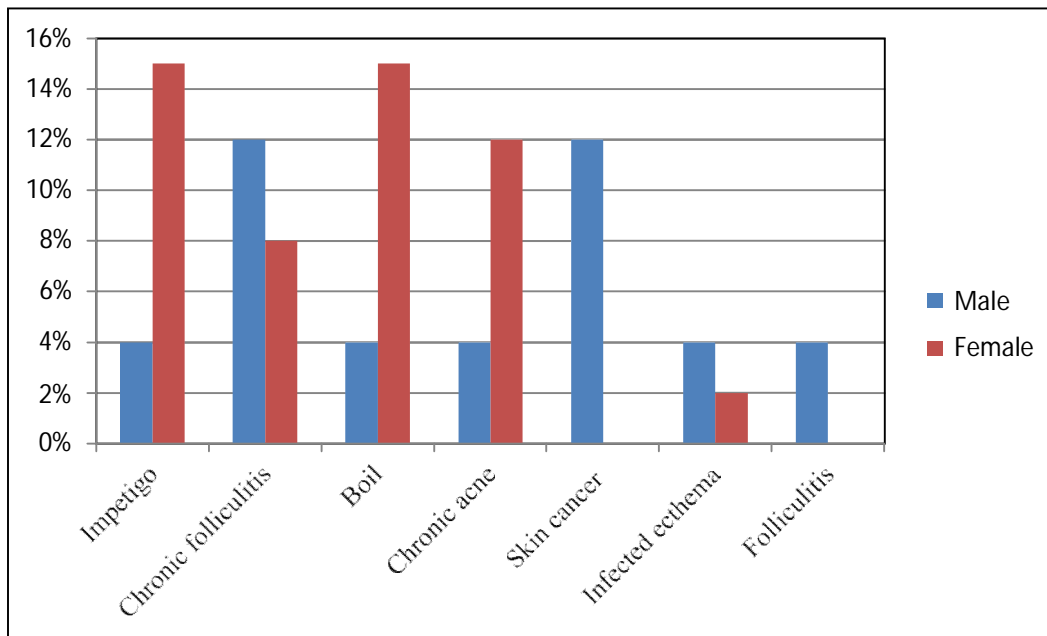
Skin infection percent according to the gender

The results showed that the frequent of skin infection in female (15) was more than male (11), the impetigo and boils (furuncles) showed the highest percentage.

4(15%) in female compared to the male where the skin cancer and chronic folliculitis recorded 3(12%) table(2) and figure(2).

Table(2):percentage of skin infection according to the gender.

Type of infection	Male	Female
Impetigo	1(4%)	4(15%)
Boils(furuncles)	1(4%)	4(15%)
Skin cancer	3(12%)	0
Chronic folliculitis	3(12%)	2(8%)
Folliculitis	1(4%)	0
infected ecthema	1(4%)	2(8%)
chronic acne	1(4%)	3(12%)
Total	11(42.3%)	15(57.6%)

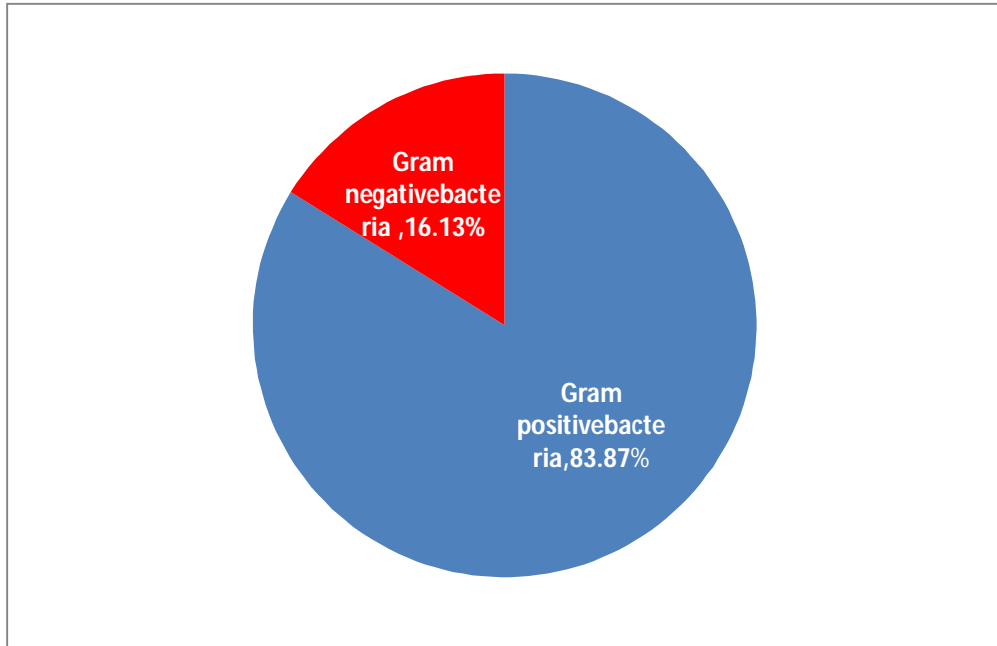


Figure(2):percentage of infections according to the gender.

Culturing of samples and isolated of bacteria

All samples were cultured on blood base agar, Mannitol salt agar, and MacConcy agar media with duplicate. Out of 26 samples, 25 samples showed bacterial growth while one sample showed no. The total of isolates that obtained were 31 and depending

on Gram staining, the percentage of Gram positive was high 26 (83.87%) compared to Gram negative 5 (16.13%) figure(3).



Figure(3):percentage of Gram positive and Gram negative bacteria isolated from skin infections.

Identification of bacterial isolates

Thirty-one isolates were identified by Vitek2 system analysis. The results showed identified 4 genera and 10 species with identity percent about (99-86%) table(3).

Table(3): Identification of bacterial isolates by Vitek2 system.

Sample No	Type of bacteria	Identity
R1	<i>S.hominis</i> <i>sphominis</i>	94%
R2	<i>A.baumannii</i> complex	99%
R3	<i>S.aureus</i>	86%
R4	<i>S.aureus</i>	95%
R5	<i>S.aureus</i>	91%
R6	<i>S.aureus</i>	92%
R7	<i>S.aureus</i>	87%
R8	<i>S.epidermidis</i>	96%
R9	<i>S.aureus</i>	92%
R10	<i>S.aureus</i>	99%

R11	<i>S.aureus</i>	95%
R12	<i>S.aureus</i>	92%
R13	<i>S.aureus</i>	99%
R14	<i>S.aureus</i>	99%
R15	<i>S.epidermidis</i>	92%
R16	<i>S.hominissphominis</i>	95%
R17	<i>S.epidermidis</i>	92%
R18	<i>S.epidermidis</i>	99%
R19	<i>A.baumannii</i> complex	99%
R20	<i>A.baumannii</i> complex	99%
R21	<i>S.warneri</i>	87%
R22	<i>S. lentus</i>	94%
R23	<i>S.epidermidis</i>	95%
R24	<i>S.epidermidis</i>	95%
R25	<i>S.epidermidis</i>	99%
R26	<i>S.epidermidis</i>	99%
R27	<i>S.hominissphominis</i>	92%
R28	<i>S.xylosus</i>	99%
R29	<i>Sphingomonaspaucimobilis</i>	97%
R30	<i>S. lugdunensis</i>	ND
R31	<i>A.hydrophila/Punctate(caviae)</i>	98%

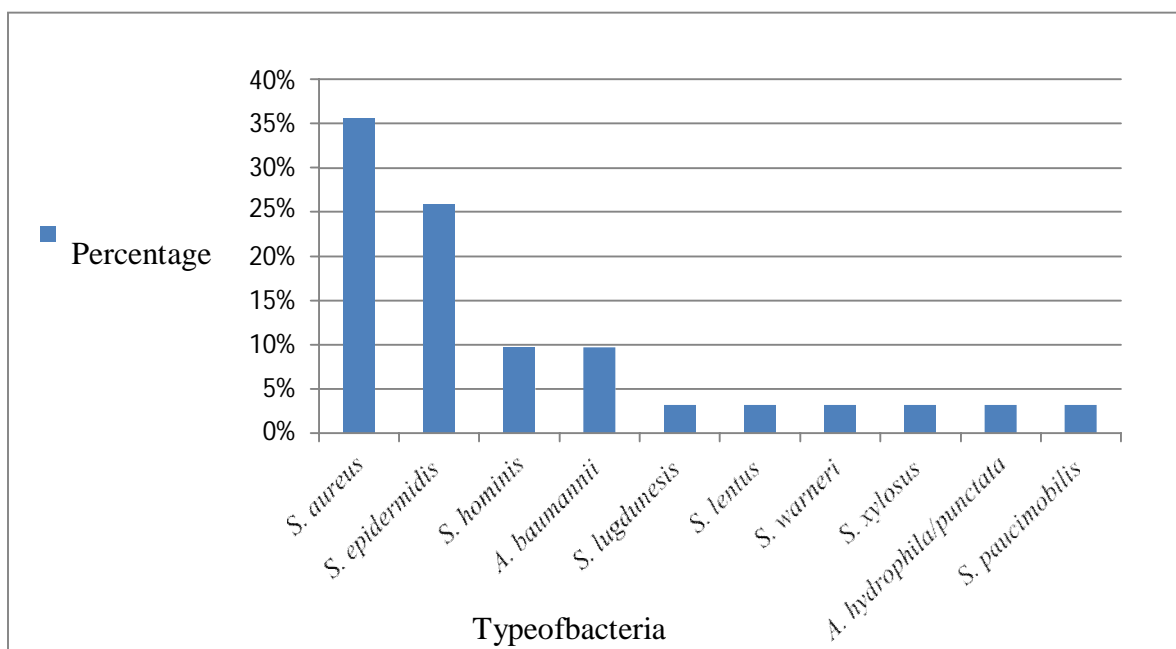
*ND=notedone

The species of *S. aureus* showed significantly superior percentage 11(35.5%) followed by *S.epidermidis* 8(25.8%), *S.hominis* and *A.baumannii* were 3(9.7%) table (4) and figure (4).

Table(4): frequency and percentage of bacterial species identified by vitek2 system.

Vitek2system	NO	Per(%)
<i>Staphylococcus aureus</i>	11	35.5 %
<i>S.epidermidis</i>	8	25.8 %
<i>S.hominissphominis</i>	3	9.7 %

<i>S. lugdunensis</i>	1	3.2 %
<i>S. lentus</i>	1	3.2 %
<i>S. warneri</i>	1	3.2 %
<i>S. xyloso</i>	1	3.2 %
<i>Acinetobacterbaumanniicomplex</i>	3	9.7 %
<i>Aeromonashydrophila/punctate(caviae)</i>	1	3.2 %
<i>Sphingomonaspaucimobilis</i>	1	3.2 %



Figure(4):Percentageofbacterialspeciesidentified byvitek2system.

Antibiotic Susceptibility test by Vitek2 System analysis

Antibiotic Susceptibility of all bacterial isolates was determined by using Vitek2 Compact System analysis. Bacterial isolates were tested for susceptibility to different antibiotics from different groups. Out of 31 bacterial isolates 23 were shown to be multiple drug resistant (MDR), were resistant to more than three classes of antibiotics and were classified as MDR as shown in table (5). The isolate of R3 (*S. aureus*) was resistance for 12 classes of antibiotics followed by R16 (*S. hominis*) and R18 (*S. epidermidis*) resist for 9 classes of antibiotic, table (5).

Table(5):Numberofantibiotics classesthatresistancebybacteriaisolatedfromskininfections.

Bacterialspecies	NO.ofantibiotics classes	Classesofantibiotics
<i>S.hominissphominis</i> R1	7	Cephalosporins, Penicillins,Carbapenems,Quinolones, Macrolides, Lincosamides,andTetracyclines
<i>A.baumannii</i> complex R2	6	Penicillins, Cephalosporins,Carbapenems, Tetracyclines, polymyxineandAntifolate
<i>S.aureus</i> R3	12	Cephalosporins, Penicillins,Carbapenems, Aminoglycosides,Quinolones,Macrolides, Lincosamides,Tetracyclines,Antifolate,Rifamycins, FusidaneandSulphonamides
<i>S.aureus</i> R4	5	Cephalosporins,Penicillins, Carbapenems,Macrolidesand Lincosamides
<i>S.aureus</i> R5	7	Cephalosporins, Penicillins,Carbapenems,M acrolides, TetracyclinesFusidaneand Lincosamides
<i>S.epidermidis</i> R8	7	Cephalosporins, Penicillins,Carbapenems,Amino glycosides,Macrolides,Tetracyclinesand Fusidane
<i>S.aureus</i> R9	3	Cephalosporins, Penicillins, andCarbapenems
<i>S.aureus</i> R10	3	Cephalosporins,Penicillins,and Carbapenems,
<i>S.aureus</i> R11	6	Cephalosporins, Penicillins,Carbapenems,,Amino glycosides, Quinolones,andMacrolides
<i>S.aureus</i> R12	3	Cephalosporins,Penicillins,and Carbapenems
<i>S.aureus</i> R13	3	Cephalosporins,Penicillins,and Carbapenems
<i>S.aureus</i> R14	3	Cephalosporins,Penicillins,and Carbapenems
<i>S.epidermidis</i> R15	7	Cephalosporins, Penicillins,Carbapenems,,Aminoglycosides,Lincosamides,Glycopeptidesand

		Fusidane
<i>S. hominis ssp hominis</i>	9	Cephalosporins, Penicillins, Carbapenems, Glycopeptides,

R16		Quinolones,Lincosamides,Fusidane, Rifamycins,andMacrolides
<i>S.epidermidis</i> R17	7	Cephalosporins, Penicillins,Carbapenems, Macrolides,Lincosamides,Tetracyclinesand Fusidane
<i>S.epidermidis</i> R18	9	Cephalosporins, Penicillins,Carbapenems,Quinolones, Macrolides, , Tetracyclines, Antifolate,Sulphonamidesand Fusidane
<i>S.warneri</i> R21	5	Cephalosporins, Penicillins,Carbapenems,Macrolides,and Fusidane
<i>S.lentus</i> R22	6	Cephalosporins, Penicillins,Quinolones,Macrolides, SulphonamidesandFusidane
<i>S.epidermidis</i> R23	7	Cephalosporins, Penicillins,Carbapenems,,Aminoglycosides, Lincosamides,Glycopeptidesand Fusidane
<i>S.epidermidis</i> R24	6	Cephalosporins,Penicillins, Macrolides, LincosamidesGlycopeptidesandFusidane
<i>S.epidermidis</i> R25	4	Cephalosporins,Penicillins, Lincosamides,andGlycopeptides
<i>S.epidermidis</i> R26	3	Cephalosporins,Penicillins,and Carbapenems
<i>S. hominis ssp hominis</i> R27	6	Cephalosporins, Penicillins,Lincosamides,Macrolides, GlycopeptidesandFusidane

Discussion

In the current study, Out of twenty six samples collected from various skin infections, Impetigo and chronic folliculitis were the most frequent infection 5 (19%) figure (1), according to the age the result showed that most of impetigo infections were between 2-17 years, table (1) this was agreed with previous studies that showed impetigo common in children under 18 years (Loadman *et al.*, 2019; Bowen *et al.*, 2015). Children between the ages of two and five are typically affected by impetigo, a highly contagious superficial skin illness that comes in two varieties: nonbullous impetigo and bullous impetigo (Brown *et al.*, 2003 ;Manje *et al.*, 2023).

Skin infection is a prevalent illness that affects children.

Geographical

location, climate and season, social status, and personal hygiene all have an impact (Kelbore *et al.*, 2019; García *et al.*, 2020). Alkubaisi *et al.* (2020) suggests that because this age group of "school children" has access to the same health care services, and practices almost identical personal hygiene, they are more likely to be exposed to infectious skin

diseases. In a developing country like Iraq, it is a complicated big health issue. Temperature, humidity, poor personal hygiene, a lack of good water sources, and low levels of education allow the disease to spread among children in the community. One of the contributing reasons to skin infections and infectious diseases is personal cleanliness (Gauchan *et al.*, 2015; Aggarwal *et al.*, 2021). For chronic folliculitis the infection was between (18-38) year stable (1), this was compatible with Srinivas and KA, (2020) that observed most commonly in the age group of (21-40) years.

The infections were significantly higher in female 15 (57.6%) compared to male 11 (42.3%), table (2), this finding is consistent with previous studies conducted in Brazil and Egypt (Ferreira *et al.*, 2011; Abdel Hafez, *et al.*, 2003) and disagreement with other study conducted in Iraq by Alkubaisi *et al.* (2020).

According to the gender the results showed that prevalence of impetigo infections were significantly higher among female (Little girls (4 (15%) than male (Little boy) table (2) and figure (2), while in chronic folliculitis the results showed that the infections were high in male 3 (12%) compared to the female 2 (8%) this was in agreement with Srinivas and KA, (2020). Immunomodulation is influenced by progesterone, testosterone, and estradiol. They impact a wider range of cells, such as lymphocytes, macrophages, and dendritics (Muenchhoff and Goulder, 2014). It is well known that testosterone suppresses the immune system, which may lower interferon- γ levels (IFN- γ), Conversely, estradiol has the ability to increase T-helper 1 (Th1) immunity (Jacobsen and Klein, 2021). So, the difference in the level of estradiol and testosterone between males and females could be affected.

Depending on Gram staining, 26 (83.87%) were Gram positive, whereas 5 (16.13%) Gram negative. This result comes in agreement with Ahmed *et al.* (2020) where Gram positive cocci recorded (65.4%) while 68 (34.5%) was Gram negative bacilli. The bacteria was identified biochemically by an automated microbiology vitek2 system, it's used for microbial identification provide highly accurate and reproducible results that approved by multiple independent studies. The result revealed that isolated bacteria with probability range between (99-86%) the frequency showed high percentage of *S. aureus* 11 (35.5). This outcome is consistent with research conducted by previous studies (Matiny *et al.*, 2012; Marko *et al.*, 2012; Sanaa, 2017), which used the Vitek2 method to identify a variety of bacterial species.

The results showed that *Staphylococcus* genus was the most frequent with high percent (35.5%) of *S. aureus* this compatible with previous studies (Mohanty *et al.*, 2018; Zhao *et al.*, 2021; Manje *et al.*, 2023). Major Gram-positive bacterial pathogen *S. aureus* is responsible for a broad range of clinical illnesses, from endocarditis and localized soft-tissue infection to potentially fatal bacteremia (Mohanty *et al.*, 2018). Human skin is frequently home to the commensal bacteria *S. epidermidis*. In the present study this species showed the percent of (25.8%). This bacteria viewed as a key member of the skin microbiota, involved in the fight against pathogens, influencing the immune system, and implicated in wound repair. Concurrently, it is the second source of nosocomial infections, and skin conditions including atopic dermatitis have been linked to an overgrowth of this bacteria (Landemaine *et al.*, 2023).

Antibiotic susceptibility testing was performed using the Vitek-2 Compact system analysis, (48) antibiotics were used. The results revealed that bacterial isolates had different patterns of resistance to antibiotic stable (5). (23) isolates of bacteria were MDR out of (31) isolates. The spectrum of resistance was observed for *S. aureus* (R3) among the Gram positive bacterial isolates, this isolate showed more resistance to antibiotics than any other where resist (12) classes of antibiotics such as aminoglycosides, penicillins, macrolides, quinolones, lincosamides, tetracyclines, antifolate and sulphonamide. The result was compatible with previous studies in Botswana (Truong *et al.*, 2011; Alteret *et al.*, 2019). The bacteria of *S. epidermidis* (R18) was resistant for (9) classes of antibiotics include penicillins, cephalosporins, tetracyclines, macrolides, antifolate and sulphonamide. This result agreed with previous studies (Chabi and Momtaz, 2019; Siciliano *et al.*, 2023; Leili *et al.*, 2024).

Conclusion

According to the current investigation, *S. aureus* and *S. epidermidis* are the two Gram-positive bacterial isolates most frequently associated with skin infections. The concerning increase in Gram-positive bacterial infections that are resistant to many drugs highlights the critical need to determine the sensitivity pattern and epidemiology of the bacteria causing infections of the skin and soft tissues. Adhering to antibiotic stewardship can assist in preventing the development of drug resistance and minimizing the use of ineffective antibiotics.

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Details of the AI usage are given below:

- 1.
- 2.
- 3.

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