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Isolation and Identification of some Bacterial isolates causing Skin Infections in Humans and detect Their Resistance to Antibiotics

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Abstract

In this study, swabs were taken from 80 patients with skin diseases including swabs from hair follicles, impetigo and boils. The patients included males and females with their ages ranging from (5-60) years from the outpatient clinic in Baghdad. The results showed that 35 (43.7%) of the isolates were Gram-positive bacteria, and 45 (56.2%) were Gram-negative bacteria. The rates of Gram-negative bacteria isolates were as follows: 22 (48.8%) isolates were *P. aeruginosa*, 10 (22.22%) isolates were *E. coli*, 7 (15.5%) isolates were *Proteus mirabilis*, 4 (8.8%) isolates were *Klebsiella pneumoniae*, and 2 (4.4%) isolates were *Morganella morganii*. Gram-positive bacteria rates were as follows: 14 (40%) isolates were S. aureus, 9 (25.7%) isolates were S. epidermidis, and 4 (11.4%) for each of *Strept pyogenes*, strept viridans and *Strept agalactiae* (Strept). The results showed a significant difference (P > 0.01) between infected males (68.7%) compared to females (31.2%), and the infection rate was higher in the age group 5-20 years for male patients (50.9%) and females (36%), with a significant difference (P>0.05) compared to other age groups of the same sex. The majority of the isolated *S. aureus* isolates showed sensitivity to vancomycin, ofloxacin, gentamicin/norfloxacin, imipenem, and meropenem, and resistance to trimethoprim, ceftriaxone, and penicillin G. On the other hand, they showed sensitivity to ofloxacin and imipenem, while they were resistant to aztreonam, trimethoprim, ceftriaxone, and ampicillin.

Introduction

Bacterial skin infections are important health problems that many patients suffer from, especially those in hospitals, which range from simple inflammatory conditions that do not eliminate good antibodies, which end with the patient's death, especially when the pathogen reaches the blood and causes cases of septicemia. Among the bacterial skin infections that affect the skin are boils, contagious impetigo, hair folliculitis, and cellulitis. *Staphylococcus aureus* is one of the most common causes [1]. These bacteria are responsible for superficial skin infections, as they constitute 60% of all bacterial skin infections, which may be due to their possession of many virulence factors, such as their ability to secrete enzymes such as plasma coagulase enzyme, the enzyme hyaluronidase, the enzyme haemolysin, DNase, leucocidin, and protease [2]. The other bacteria is *P. aeruginosa* as the second pathogen isolated from most cases of burns and open skin wounds because it is one of the dangerous bacterial species that is characterized by its resistance to many antibiotics [3].

Antibiotics have had a major impact since their discovery in reducing the rates of various infections, but the indiscriminate use of these antibiotics in treatment has led to the emergence of bacterial strains resistant to one or more antibiotics. The failure of antibiotics to kill skin pathogens leads to recurrence of infection due to the resistance of microorganisms to these antibiotics [4]. The mechanisms of resistance to antibiotics are under the control of genetic factors carried on chromosomes, plasmids, or transposons. One of the mechanisms used by bacteria to resist beta-lactam antibiotics is the production of beta-lactamase enzymes. These enzymes attack the beta-lactam ring found in the nucleus of cephalosporins and penicillins, turning the antibiotic into an inactive compound. These enzymes are encoded by genes carried on the chromosome or plasmid. Among other mechanisms of resistance is to change the location of the target and change the permeability barrier, and all of these mechanisms are encoded by genetic factors possessed by bacteria [5].

Materials and Methods

The 80 in this study, 80 skin swabs were collected from patients suffering from several skin infections (boils, impetigo, and hair follicles). These samples were transferred by transport media swabs to the laboratory of microbiology.

Bacteria were isolated after culturing them on blood agar, MacConkey agar, and mannitol salt agar and incubated at 37°C for 24 hours. Then, the bacterial isolates were diagnosed based on phenotypic characteristics and microscopic examination. Several biochemical tests were used, including catalase, oxidase, indole production detection, and mannitol fermentation test.

Antibiotic susceptibility test

The disk diffusion method according to (Kirby Bauer) was performed to check the sensitivity of the most prevalent bacteria to most conventional antibiotics as follows: Four or five isolated colonies of the bacteria to be tested were transferred by a sterile loop and suspended in 2 ml of normal saline and then the tube was vortexed to create a fine suspension. The turbidity of this suspension was adjusted to McFarland standard 0.5. A sterile swab was dipped into the inoculation tube and inoculated onto the dried surface of a Mueller-Hinton agar plate. After preparing and sterilizing it according to the manufacturer's instructions, the medium was cooled to 50 C and poured into sterile plates (2.5 ml) by smearing the swab over the entire surface of the agar in a back-and-forth motion very close to each other to distribute it evenly and then the plate was left at room temperature for 15 minutes, until the surface of the agar plate was dry. Appropriate antimicrobial discs were placed on the agar surface with sterile forceps and plates were incubated for 18–24 h at 37°C, and the sizes of the inhibition zones were measured to the nearest millimeter using a ruler.

Statistical analysis

The effect of different factors on the studied traits was studied, and the numerical variables were described using the arithmetic mean, while the descriptive variables were described in the form of a number and percentage and were compared using the 2X chi-square test.

Results and Discussion

Types of bacteria isolated from skin infections

Swabs were taken from 80 patients with different skin infections, including follicular swabs, impetigo, and boils, from outpatient clinics in Baghdad; their ages ranged between 5 and 60 years for both sexes. The results of culture and diagnostic tests of the samples showed that 45 isolates, representing 56.2%, belonged to the *P. aeruginosa*, *E. coli*, *Proteus mirabillis*, *Klebsiella pneumonia*, *Morganella morganiiout* of a total of 80 bacterial isolates, which are the most common skin infection isolates. 35 isolates, representing 43.7%, belonged to the *S. aureus*, *S. epidermidis*, *Strep. Pyogenes*, *Strep. Viridans*, *Strep. agalactiae* as shown in Figure 1. The predominance of Gram-negative bacterial isolates in skin infections may be due to their high resistance to antibiotics and the virulence factors they possess, such as endotoxins, which are toxic substances present in their cells within their outer walls and which are secreted after the bacteria enter the host's bloodstream.

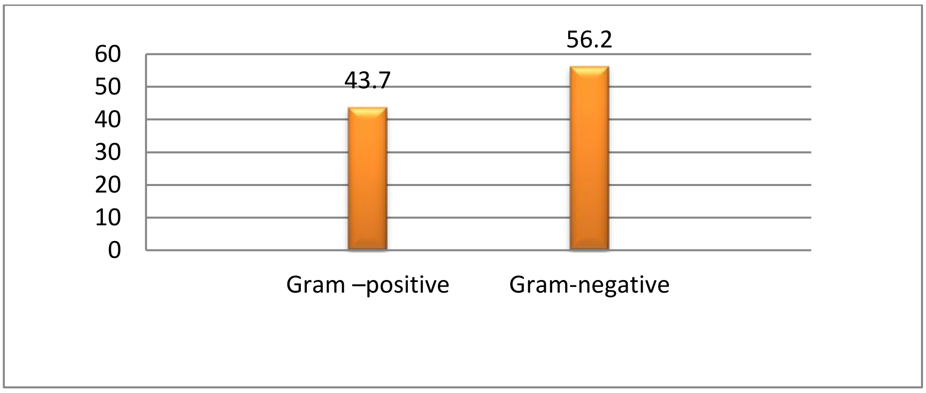


Fig. 1 *Types of bacteria isolated from the skin from people with skin infection Distribution of bacterial isolates according to Gram staining*

As shown in table 1, the most isolated bacteria was *S. aureus*, which was isolated at a rate of 40% of the total 35 isolates. It was the dominant one in skin infections, and this is consistent with what was shown by [6], who reported that most skin infections were caused by this bacteria that infect both the skin and the host's body. It also agreed with the results of [7] who recorded that *S. aureus* bacteria had the highest (40%) isolation rate. The dominance of *S. aureus* may be attributed to its possession of many virulence factors, as infections occur through contact with the surface of the host's skin tissues or through scratches or wounds, and it secretes a number of enzymes, including the lipolytic enzyme Lipase and the enzyme Hyaluronidase, which helps in the spread and destruction of the basic material of the connective tissues, causing disruption and disorder in these tissues.[8]. Our results demonstrated that 9 (7.25%) of *S .epidermidis* bacteria were isolated, and this is attributed to the fact that it is an opportunistic bacteria that causes infection when the appropriate conditions are available and possesses several virulence factors represented by its ability to produce a biofilm and a slim layer in addition to its secretion of toxins and enzymes, and the efflux pump and exoenzyme pumps [9]. However, it does not agree with the fact that it was isolated at a rate of 9.3% of the total isolates [10]. Also, 4 (11.4%) of *S.*

pyogenes bacteria were isolated from skin infections, and this is consistent with the findings of [11]. The reason for its ability to cause a wide range of diseases is due to its possession of many virulence factors, such as the capsule and surface proteins of the cell wall.

Table 1: Gram-positive bacterial species isolated from skin infections and their percentages

Bacterial isolates	Impetigo		Boils		Folliculitis		المجموع	
	no	%	no	%	no	%	no	%
S. aureus	7	50	4	33.3	3	33.3	14	40
S. epidermidis	3	21.4	3	25	3	33.3	9	25.7
Strep. pyogenes	1	7.1	2	16.6	1	11.1	4	11.4
Strep. viridans	1	7.1	2	16.6	1	11.1	4	11.4
Strep. agalactiae	2	14.2	1	8.3	1	11.1	4	11.4
Total	14	100	12	100	9	100	35	100

The Gram-negative isolates included 45 bacterial isolates (2.56%), 22 *Pseudomonas aeruginosa* isolates (48.8%), 10 *Escherichia coli* isolates (22.22%), 7 *Proteus mirabilis* isolates (15.5%), 4 *Klebsiella pneumoniae* isolates (8.8%), and 2 *Morganella morganae* isolates (4.4%), as shown in the same table 2. The results of the current study showed that *P. aeruginosa* was the most abundant among the Gram-negative bacteria (48%). The study agreed with [12] who reported that the bacteria reached the highest isolation rate of 44%. This may be due to the virulence of these bacteria, which are invasive pathogens that invade the skin and soft tissues and cause skin infections and possess many different virulence factors that enable them to resist antibiotics, such as the production of enzymes and toxins. Enzymes such as broad-spectrum beta-lactamase and metallo-beta-lactamase, in addition to the production of antibiotic-resistant beta-lactamase and phagocytosis, are responsible for retaining nutrients and water in harsh environmental conditions [10]. *E. coli* isolates were 22.22% of the total isolates, which is consistent with study conducted by [13], who isolated 21% of the total isolates, and it does not agree with [14] who isolated only 8.13%. The pathogenicity of *E. coli* is due to its possession of many virulence factors, such as the cell wall that contains lipopolysaccharide threads and a capsule, in addition to the adhesion members Fimbriae that play a role in adhesion to the surface of the host cell and invasion of the host tissues. Although some species exist as natural flora, they become opportunistic when suitable conditions are available, such as weak host immunity and malnutrition. In addition, 7 (5.15%) isolates of Proteus mirabilis bacteria were isolated from skin infections. This result did not agree with what was reached by another study, who isolated 2.3%, and showed that the reason for its pathogenicity and infection is due to its possession of efflux pumps in addition to its ability to produce broad-spectrum beta-lactamase enzymes and its possession of the phenomenon of reproduction. While 4 (8.8%) isolates belonged to *Klebsiella pneumoniae* of the total isolates. This result was consistent with the study [15] who isolated 8.13%. The reason for its virulence is due to its ability to produce the urease enzyme in addition to its possession of a capsule that gives it the property of viscosity and resistance against the host's defense mechanisms. Our mother bacterium, *Morganella morganae*, was isolated from only 2 (5%) isolates. This result was close to that shown by study [16], who isolated 4.4%. The ability of *Morganella morganae* to cause disease is attributed to its ability to adapt to changes in environmental conditions, to compete with other microorganisms, and to its resistance to antibiotics.

Table 2: Gram-negative bacterial species isolated from skin infections and their percentages

Bacterial isolates	impetigo		Boils		Folliculitis		Total	
	no	%	no	%	no	%	no	%
P. aeruginosa	5	22.7	2	9	15	68,1	22	48.8
E. coli	1	10	6	60	3	30	10	22.22
Proteus mirabillis	2	28	3	42,8	2	28,5	7	15.5
Klebsiella pneumoniae	1	25	2	50	1	25	4	8.8
Morganella morganii	0	0	1	50	1	50	2	4.4
Total	9		14		22		45	100

Isolation and identification

The bacteria isolated from the skin infections were identified based on their morphological characteristics on culture media, microscopic classifications, Gram staining and biochemical tests. Gram-positive bacteria were isolated based on their microscopic characteristics by observing the arrangement and shapes of the cells. Staphylococcus spp. appeared round on microscopic examination and its cells were arranged in a grape-like manner [17]. Colonies appeared on solid blood agar medium with a pericolonic zone of hemolysis with medium to large in size. Staphylococcus aureus appeared on mannitol-salt medium with a golden yellow color due to its ability to ferment mannitol [18]. Therefore, it is considered as a distinguishing medium between *S. aureus* and *S. epidermidis*, which do not ferment mannitol. Biochemical test results showed that all *S. aureus* isolates gave positive results for the catalase test, *S. pyogenes* appeared as spherical with long or short chains or pairs on microscopic examination and gave a complete colony analysis on open culture and showed a negative result on the catalase test, while *S. viridans* appeared as chains of round balls containing a capsule on microscopic examination, and *S. pneumoniae* had a negative result on the catalase test.

Gram-negative isolates were identified based on morphological characteristics and microscopic and biochemical tests. The colonies were identified based on their shape and texture, in addition to their ability to ferment lactose. *P. aeruginosa* colonies appeared to be large in size and had a distinctive odor resembling the odor of fermented grapes, with a gray color on monads, and did not ferment lactose into monads [19]. They gave positive results for the oxidase and catalase tests and were negative for Indol test [20]. *E. coli* colonies were medium-sized, dry, firm, convex, and appeared pink on solid MacConkey medium, which is attributed to their ability to ferment lactose in the medium. They gave positive results for the catalase and Indole tests [21]. Colonies of *Klebsiella pneumoniae* were identified on MacConkey agar as pink colonies due to their ability to ferment lactose on MacConkey medium and were mucilaginous due to the presence of a capsule [22].

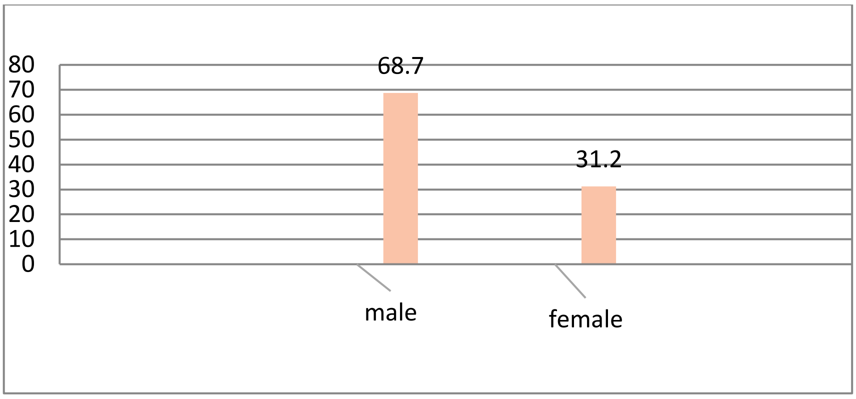
The results of biochemical tests of *Morganella morganii* colonies gave positive results for the Indole test. The table 3 shows the results of biochemical tests for Gram-negative isolates.

Table 3: Biochemical tests for Gram-positive and Gram-negative isolates

Type of bacteria / Test	Gram stain	Catalase test	Oxidase test	mannitol fermentation test	Indole production test
S. aureus	+	+	-	+	
S. epidermidis	+	+	-	--	
Strep. pyogenes	+	-	-	-	
Strep. viridans	+	-	-	-	
Strep. agalactiae	+	-	-	-	
P. aeruginosa	-	+	+		
E. coli	-	+	-		+
Proteus mirabillis	-	+	-		-
Klebsiella pneumoniae	-	+	-		-
Morganella morganii	-	+	-		-

Positive test (+), Negative test (-)

The results of the current study showed that the percentage of males infected with skin infections was greater than the percentage of females, as the number of infected males was 55 (68.7%), while the number of females was 25 (31.2%) of the total infections as shown in Figure 2. This result is consistent with the results obtained by [23], who showed that the percentage of infected males was 68% and 30% for females. The findings of [24] agreed with us. The reason for the difference in the infection rate between males and females may be due to the amount and type of natural growth in the bodies of both sexes, in addition to the difference in the method of collecting samples, or to males' daily exposure to environmental pollutants according to the nature of work, hormonal and physical activity, psychological state such as anxiety and stress, health condition, poor personal hygiene, genetic makeup of males, sharing personal tools such as razors with barbers, and type of nutrition. All of these factors help increase the number of infected males compared to females. However, the results of the current study did not agree with [25], where the percentage of infected males was 42.2% and the percentage of females was 42.2%.



(*) means there is a significant difference at the significance level (P>0.01)

Figure 2: Distribution of patients with skin infections according to gender

The present study showed that the age group 5 to 15 years had the highest infection rate 28 (50.9%) among males as compared with other age groups and also among females at 9 (36%). This may be due to the fact that patients aged 5–15 years, which includes school-age children, have increased physical and hormonal activity more than other age groups, in addition to economic factors, poor personal hygiene, educational level, and income factors, in addition to crowding of family members or the use of poor quality medical injections for treatment, all of which explain the high infection rate in this age group. The results of the current study agreed with what was shown by another study who found that the age group (5-18) years is the most susceptible to infection, at a rate of 50.9% compared to other age groups. As showed in table 4.

Table 4: Distribution of skin infection patients according to age groups for both sexes

sex	Age/ %					Total/%
	5-20 *	21-30	31-40	41-50	51-60	
male	28(50.9)	10(18.18)	8(14.5)	5(9)	4(7.2)	55(68.7)
female	9(36)	6(24)	5(20)	4(16)	1(4)	25(31.2)
total	37(46.2)	16(20)	13(16.2)	9(11.25)	5(6.25)	80(100)

(*) means there is a significant difference at the significance level (P>0.05)

Sensitivity of bacterial isolates to antibiotics

The sensitivity of 14 Gram-positive *S. aureus* isolates and 22 Gram-negative *P. aeruginosa* isolates, which were isolated from 80 skin infections, to 7 antibiotics was studied according to the Kirby-Bauer method. The sensitivity was determined based on measuring the diameter of the zone of inhibition. The antibiotics used in the study were compared with those stated in CLSI (2023). The sensitivity of *S. aureus* bacteria was tested against 7 antibiotics, as shown in figures 3 and 4. All isolates showed 100% high resistance to penicillin G, which is consistent with the findings of [26]. The high resistance is attributed to the bacteria possessing beta-lactamase enzymes that degrade the penicillin group, whose genes are either chromosomal or zymogenic. These bacteria also produce penicillin-binding proteins (PBPs) located in the cytoplasmic membrane attached to the cell wall [27]. These proteins are the target of both penicillin and cephalosporin antibiotics, as they change the target site of beta-lactam antibiotics, leading to bacterial resistance to them. The amount of penicillin-binding proteins is not entirely related to resistance to beta-lactam antibiotics, because of the presence of the mecA gene that encodes the production of penicillin-binding proteins (PBPs), which reduce the ability to bind to beta-lactam antibiotics, which is the main factor in increasing the rate of bacterial resistance to beta-lactam antibiotics [28].

While the majority of isolates showed sensitivity to Gentamicin and Ofloxacin antibiotics by 92.8 and 85.7%, respectively, these results agreed with what was reached by [29] who reported that all tests showed 100% sensitivity to imipenem and vancomycin. The results of the current study are consistent with those of [30], as vancomycin is a broad-spectrum antibiotic that is effective against many types of Gram-positive bacteria. In addition, imipenem and meropenem, which belong to the carbapenem group of broad-spectrum antibiotics against Gram-positive and Gram-negative bacteria, are effective against many skin pathogens by destroying beta-lactamase enzymes.

The sensitivity of *P. aeruginosa* to 7 antibiotics was tested as shown in figure 4. *P. aeruginosa* isolates showed high resistance to beta-lactam antibiotics, with a sensitivity rate of 100% to ampicillin. A change in the composition of penicillin-binding proteins (PBPs) or the production of beta-lactamase enzymes that enable the isolates to attack a wide range of beta-lactam antibiotics [31]. The results of the study agreed with [32], as their isolates showed different resistance to trimethoprim, and the results of the study agreed with [33], which means that resistance is multifactorial and that resistance genes are transmitted. It indicates that the resistance is multifactorial, and the resistance genes are transmitted annually to the recipient cell by means of special elements of DNA known as integrons, which are located on the plasmid or chromosome and can capture genes that encode resistance through the process of recombination.[34]. Integrons can attach to one or more cassette genes within their binding sites, thus forming groups of genes for resistance to antibiotics and beta-lactam antibiotics. While the majority of isolates showed sensitivity to Ofloxacin antibiotics by 85.7%, which is consistent with what was reached by [35], as all isolates showed high sensitivity to the antibiotics meropenem and imipenem by 100%, and the results of the study were consistent with what was reached by [36], and this is due to the fact that they are among the most effective antibiotics against Gram-positive and Gram-negative bacteria, in addition to the fact that these antibiotics are newly used and their use is limited to patients in the hospital only because their circulation is avoided by intravenous normal saline solution for a specific period of time determined by the treating physician, and this is one of the reasons for the sensitivity of bacteria to them.

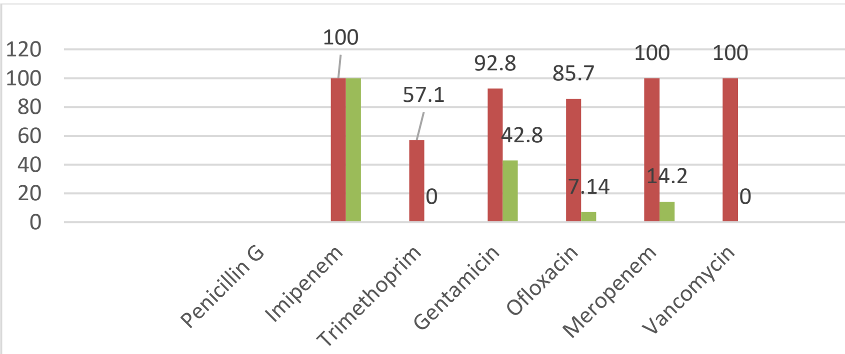


Figure 3: Sensitivity of S. *aureus* isolates to antibiotics

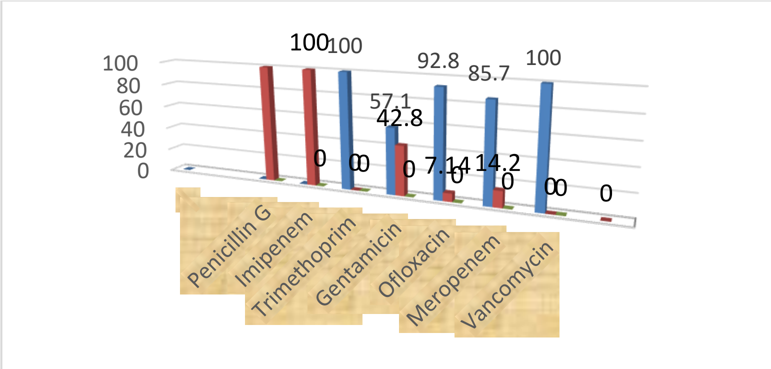


Figure 4: Sensitivity of P. *aeruginosa* isolates to antibiotics

Conclusions

It was found that Gram-negative bacteria were more prevalent in skin infections than Gram-positive bacteria, and *Staphylococcus aureus* was more prevalent in skin infections. The percentage of males infected with skin infections was higher compared to the percentage of females, and the age group 5-20 years was more infected than other age groups. The bacterial isolates *S. aereus* and *P. aeruginosa* have the characteristic of multi-resistance to antibiotics, which poses a great threat to society.

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Conflict of Interest: Nothing to declare.

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عزل وتشخيص بعض العزلات البكتيرية المسببة لالتهابات الجلد لدى الانسان والكشف عن مقاومتها للمضادات الحيوية

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الخلاصة:

تم اخذ مسحات من 80 مريض مصاب بامراض جلدية مختلفة منها مسحات من بصيلات الشعر (التهاب الجريبات) والقوباء والدمامل ولكلا الجنسين وباعمار تتراوح من (5-60) سنة من العيادة الخارجية في بغداد وتم تشخيص 80 عزلة بكتيرية من مسحات مرضى مصابين بامراض جلدية منها 35 عزلة بنسبة 43.7% لمجموعة البكتيريا سالبة الجرام والتي كانت قريبة من مجموعة البكتيريا موجبة الجرام والتي كانت 45 عزلة بنسبة 56.2%. وتضمنت مجموعة البكتيريا سالبة الجرام 22 عزلة بنسبة 48.8% لبكتيريا *P. aeruginosa*، و10 عزلات بنسبة 22.22% لبكتيريا *E. coli*، و7 عزلات بنسبة 15.5% لبكتيريا *Proteus mirabilis*، و4 عزلات بنسبة 8.8% لبكتيريا *Klebsiella pneumoniae*، وعزلتين بنسبة 4.4% لبكتيريا *Morganella morganii*. وتضمنت مجموعة البكتيريا موجبة الجرام 5 عزلات بنسبة 48.14% لبكتيريا S. aureus (40.0%))، و9 عزلات بنسبة 25.7% لبكتيريا S. epidermidis (Staph)، و4 عزلات بنسبة 11.4% لبكتيريا *Strept pyogenes*، *Strept agalactiae Viridans Strept*). وأظهرت النتائج وجود فرق معنوي (P > 0.01) بين الذكور المصابين بنسبة 68.7% مقارنة بالإناث بنسبة 31.2%، كما وجد أن معدل الإصابة أعلى في الفئة العمرية 5-20 سنة للمرضى الذكور بنسبة 50.9% والإناث بنسبة 36%، مع وجود فرق معنوي (P > 0.05) مقارنة بالفئات العمرية الأخرى من نفس الجنس. وأظهرت غالبية عزلات المكورات العنقودية الذهبية المعزولة من التهابات الجلد حساسية للمضادات الحيوية فانكومايسين، أوفلوكساسين، جنتاميسين / نورفلوكساسين، إيميبينيم، وميروبينيم، في حين أظهرت العزلات مقاومة للمضادات الحيوية تريميثوبريم، سيفترياكسون، وبنسلين ج. ومن ناحية أخرى، أظهرت عزلات الزائفة الزنجارية حساسية للمضادات الحيوية أوفلوكسين وإيميبينيم، في حين كانت العزلات مقاومة للمضادات الحيوية أزتريونام، تريميثوبريم، سيفترياكسون، وأمبيسلين.