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Detection of resistance bacteria isolated from skin infections to many antibiotics in several hospitals Mosul city

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ABSTRACT

Objectives: Bacterial infecting burns and wounds is an important problem that sometimes leads to death, so the best method for diagnosing bacterial isolates from burns and wounds was chosen in this study based on their phenotypic characteristics and biochemical tests. Isolated bacteria were diagnosed in several ways to confirm them before using antibiotics, including API20E and APISTAPH test. The VITIK test was also used, which is easier, more accurate and faster in bacterial diagnosis than the traditional methods approved for diagnosis, then using antibiotics and studying the extent of their impact to choose the best antibiotics to avoid problems. As a result of excessive and indiscriminate use of antibiotics by patients

Methodology and results

The study was conducted in Mosul hospitals to investigate the pathogenic bacteria of burn injuries, wounds, and abscesses, which is considered one of the most important medical problems in the world, which cause an increase in the number of deaths in particular. Among the patients of specialized hospitals for the period from (May 2021 to October 2021). They are grown on various media, including MocConkey agar, solid blood agar, methylene blue medium, mannitolheart and brain infusion broth, and other differential media; bacterial cells are Gram-negative and Gram-positive. *Staphylococcus aureus* isolates from burns and wounds constituted (28%) Of the total isolates that gave a growth on MocConkey agar with the highest wound infection, which means that they.56 isolated The isolated bacterial isolates predominated, followed by the gram-negative *Pseudomonas aeruginosa* isolates with (44) inpercentageby (22%). After incubation temperature (37%) to obtain pure isolates, the mentioned bacterial isolates showed high sensitivity towards Trimethoprim and Ipenem and were the most resistant to vancomycin and tetracycline

The study, its importance and impact

Wounds are a moist, warm and nourishing environment that helps microbes to colonize and reproduce. It should be noted that several factors influence the abundance and diversity of microbes in wounds, including the type, depth, location and quality of the wound. Hospital-acquired infections have been reported for more than a century as a serious problem that affects the quality of health care patients receive in hospitals. (20%) of these injuries are avoidable [1] Wound contamination arises from three sources, the first source being the microbes of the external environment present in the external environment [2] The most common bacteria in the external environment that can cause wound infections are antibiotic-resistant *Staphylococcus aureus* [3]

The skin is the second source of wound contamination, including many normal skin flora, such as *staphylococcus*, *micrococci* and *dermatophytes*. The third source of wound contamination is from internal sources, including the mucous membranes lining the gastrointestinal and urogenital tracts On the other hand, the emergence of bacterial strains that are resistant to antibiotics and antiseptics has contributed to the increased risk and spread of infection and the difficulty of infection control, as has been reported

Key word: *Staphylococcus aureus*, *Psuedomonas aeruginosa*, *Tetracyclin*, *Impenem*

Interdiction

The reason for the dominance of *Staphylococcus aureus* over the dominant bacterial species in the research mentioned above is due to the presence of different enzymes, such as lipase and hyaluronidase, that help diffuse connective material, causing an

imbalance in the skin tissue.[6] Does this study agree with [3]

In addition, some wounds are infected; – by the presence of purulent exudate or some signs that indicate a host response to tissue damage caused by

pathogenic microorganisms [7] Most strains of *Staphylococcus aureus* are resistant to many antibiotic [8] Studies have indicated that most *S. aureus* isolates are sensitive to the antibiotics, Ciprofloxacin and Imipenem [9] while they resist the antibiotics Cefazidime, Tetracycline [10]

Resistance of bacterial isolates to antibiotics is one of the most important problems faced by hospitals worldwide. There has been an increase in the rate of resistance among strains of these bacteria in many parts of the world. The main reason for this may be excessive or irregular use of antibiotics without consulting a doctor, as well as the bacterial acquisition of genetic factors that carry antibiotic resistance [11]

Materials and methods

Isolation and identification of bacterial isolates from burns and wounds. 200 swabs were collected from patients with wounds and burns, including burn infections and surgeries, for all ages of both sexes. Bacteria are isolated on blood agar medium as a general isolation medium and MacConkey medium as an optional medium for Gram-negative Enterobacteriaceae. The isolates were confirmed using API20E. API STAPH It is shown in the picture below 1,2.

Laboratory Diagnostics

Bacterial isolates were diagnosed on a Berge classifier using methods from [12] and [13]. Including Microscopic tests performed using chromium stain, and biochemical examination tests according to [14] and [15] Catalase, Oxidase, and Coagulase tests, Glucose fermentation, growth test on lipid, and Triglyceride iron (TSI) tests. Motility and indole are by API 20E and API Staph assay, and the sensitivity of isolates to antibiotics was also used. When microscopic examination of bacterial *P.aeruginosa* smears dyed with the gram-negative result of the dye in the form of single bacilli or chains. Biochemical tests were conducted for the isolates positive for the oxidase test, which is one of the important tests as it activates the oxidase enzyme that works to transfer the electron between each of the donor bacteria and the dye that reduces violet color. The appearance of violet during (10-5) the appearance of oxidase factor on the ability of bacteria to oxidase enzyme shown in the picture and adjusted for the Catalase test and As well as its growth on TSI medium without any change occurring (Citrate utilization and non-producing gas[16] H₂S, API20E also tested. In this regard, culture tests were conducted for *P. aeruginosa* bacteria grown on MacConkey agar. They appeared as smooth, round, pale-pale colonies because they did not ferment lactose and produced a grape-like smell called amino acid final. When planted on Nutrient agar medium, it appeared Bluish green

Results and discussion

The study of the growth, microscopic and biochemical properties of isolates from wounds and

burns swabs showed that the total number of bacterial isolates under study are (172) isolates distributed between (2) Gram-positive (32.5%) and (6) Gram-negative (67.5%) isolates (Table 1). Of the total number of isolates, *S.aureus* bacteria gave the ability to grow on menthol solid saline medium. And swabs of Gram-negative bacteria on MacConkey agar being lactose-fermenting, including *P. aeruginosa*.

The study showed that *Staphylococcus aureus* was the dominant and most isolated of the bacterial isolates under study, followed by *P.aeruginosa* in the second place of the total isolates, where the number of clinical isolates reached (172) swabs taken from wounds and burns. This difference in percentages may be due to the locations and number of swabs taken, or to the level of cleanliness of the hospital environment and the instruments used shown in Table (1).

Table 1: The numbers and percentages of bacterial isolates isolated from patients with wound and burns infection.

bacterial isolated	The number of injurie	Percentage
Gram positive bacteria	2	32%
Gram negative bacteria	6	68%
Total number	8	100

The percentage of isolation of *Staphylococcus aureus* from wound swabs

S. aureus has been classified as globular, Gram-positive, non-motile, non-sporogenic, and oxidase-negative, its Positive coagulation enzyme and catalase test. (AL-Ameen, 2017). It grows on a blood agar medium that contains 5% of human blood and gives its dissolution. It gives positive fermentation on the mannitol medium. It can ferment glucose anaerobically.

The research results found that the percentage of *Staphylococcus aureus* isolates is (56.%) of the total isolates, and *P.aeruginosa* (39%) of the total isolates (Table 2).

The result is somewhat consistent with the results of several studies. Research has indicated that *S. aureus* is prevalent among the isolated bacterial isolates and the percentage of *P.aeruginosa* bacteria ranged from (28%) and (22%) of the total isolates, respectively. There are many isolated aerobic bacterial species, including *Klebsiella*, *A. bummani*, and *Enterobacter cloacae* [18] and this does not represent a comprehensive rule, but rather is an illustration of the aerobic bacterial species that can be present in wounds. These results agree to some extent with the results of the current study.

As for anaerobic bacteria, – [3] indicated that the percentage of anaerobic bacteria isolated from cases of burns, wounds, and the outer ear. It gave bacterial growth of *Strep. pyogenic*

The difference in the rates of isolation of these bacterial species may be due to the different places where swabs are taken by *Staphylococcus aureus*.

There may be other reasons that made *Staphylococcus aureus* the most common type of bacteria in the current study, including its widespread in the environment, poor hygiene operating theaters, and overcrowding that significantly increase the spread of germs

Table 2: percentages of isolating bacterial species from wound swabs

insulation ratio	number of isolate	microbial species
28%	56	<i>S. aureus</i>
22%	44	<i>P.aeruginosa</i>

The antibiotic sensitivity of *S. aureus* and *P. aeruginosa* is the most common Gram-negative bacteria and the most dominant Gram-positive bacteria that are sensitive to some antibiotics and resistant to others. The increase in the resistance of bacterial species to antibiotics is due to several factors, Excessive use of treatments and antibiotics for various skin infections will increase bacterial resistance to these antibiotics in addition to the side effects and toxicity they cause. To overcome these problems, develop treatments and make them safe, effective, and low-cost [11]

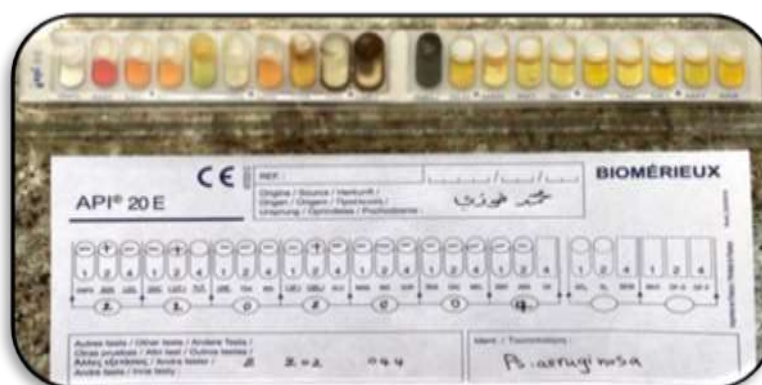
The highest resistance rate we obtained for *S. aureus* isolates was for Trimethoprim, where the percentage was (100%), which is close to what the researcher found [19] While γ was sensitive to the antibiotic. Imipenem, by (100%), and this agrees with what was reached by [20] Positive bacteria are effective against many diseases of the skin and soft tissues because they destroy the enzyme beta-lactamase– *S. aureus* isolates also showed resistance towards Penicillin, as it was indicated that the resistance ratio among their isolates was high. Approximately (96%) towards the antagonist amoxicillin clavulanic acid. *S. aureus* resistance to penicillin is due to its secretion of the enzyme penicillinase or the encoded beta-lactamase. The gene (blaZ) this enzyme when staphylococci are exposed to beta-lactam antigens.

This enzyme breaks the beta-lactam ring and converts it to the inactive form ,There are other causes of resistance, for example, the occurrence of spontaneous mutation in deoxynucleic acid (DNA) as a result of insertion, deletion, or substitution of one or more nucleotides within the genome of the micro-organism, or decreased permeability of the antibiotic through the cell membrane Penicillin-binding proteins (PBPs) which are enzymes involved in building the bacterial cell wall in addition to maintaining its appearance, so here the necessity of using clavulanic acid, which acts as a beta-lactamase inhibitor As this acid binds to these enzymes, turning them into the inactive form, it protects the antibiotics, which are the basis for these enzymes[21]

The resistance of tetracycline reached to (87%). It was in agreement with what was reached [22] which indicated that its isolates of *S. aureus* showed resistance to tetracycline, which amounted to about (86.6%), and this is close to what we found, as the percentage of resistant isolates was the reason for this. It is due to the bacteria possessing refractor which reflects their inability to synthesize this antagonist factor. The sensitivity of aminoglycoside depends on the presence of a drug transport system that requires oxygen.

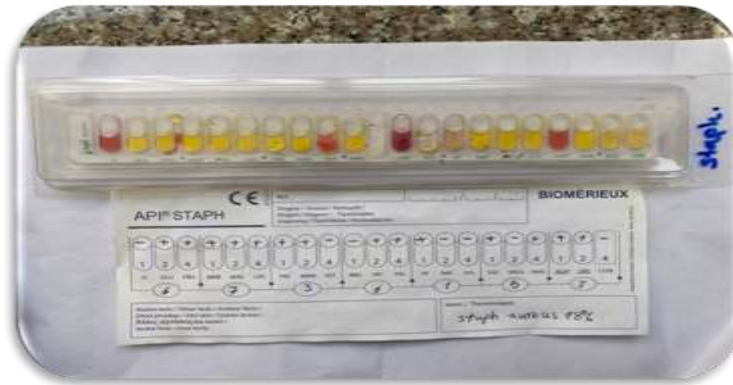
Table 3: Percentage of resistance and sensitivity of *S. aureus* to antibiotic

Anti biotic	Sensitivity rate	resistance ratio
Imipenem	100%	0%
Tetracyclin	22%	87%
Cefotaxim	20%	80%
Ceftazidime	2 %	98%
Clindamycin	40%	60%
Ciprofloxacin	100%	0%
Amixacin clavunicacid	4 %	96%
Trimethoprim	100%	0%
Gentemycin	60%	40%
Vancomycin	0%	100%



API STAPH

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API20E

DR. RACHWAN ALJAMAAH- LAB		Printed Jan 24, 2020 16:36 CST	
BioMérieux Customer:		Microbiology Chart Report	
Patient Name: salem		Patient ID: 111	
Location: Lab ID: 306		Physician: Isolate Number: 1	
Organism Quantity: Selected Organism: <i>Pseudomonas aeruginosa</i>			
Source: swab		Collected:	
Comments:			
Identification Information		Analysis Time: 4.88 hours Status: Final	
Selected Organism: *		97% Probability: <i>Pseudomonas aeruginosa</i>	
ID Analysis Messages		BioNumber: 0043483043500250	
Biochemical Details			
2 APPA -	3 ADD -	4 PyrA -	5 IARL -
10 H2S -	11 GNAG -	12 AGLTp -	13 dGLU -
17 BGLU -	18 dMAL -	19 dMAN -	20 dMNE -
23 ProA -	26 LIP -	27 PLE -	29 TyrA -
33 SAC -	34 dTAG -	35 dTRE -	36 CIT -
40 LATs -	41 AGLU -	42 SUCT -	43 NAGA -
46 GlyA -	47 ODC -	48 LDC -	53 HGSa -
56 O129R -	59 GGAA -	61 MLTs -	62 ELLM -
7 dCEL -	9 BGAL -	14 GGT -	15 OFF -
21 BXYL -	22 dAlap -	31 URE -	32 dSOR -
37 MNT -	39 SKG -	44 AGAL -	45 PHOS -
57 BGLR -			

VITIK2 *P.aeruginosa*

Al Mervar Laboratory		Patient ID: 481	
BioMérieux Customer:		Microbiology Chart Report	
Patient Name: 4, 4		Physician: Isolate Number: 3	
Location: Lab ID: 481			
Organism Quantity: Selected Organism: <i>Staphylococcus aureus</i>			
Source:		Collected:	
Comments:			
Identification Information		Analysis Time: 4.82 hours Status: Final	
Selected Organism: *		96% Probability: <i>Staphylococcus haemolyticus</i>	
ID Analysis Messages		BioNumber: 010003151330231	
Biochemical Details			
2 AMY -	4 PFPLC -	5 BXYL -	8 ADH1 -
13 APPA -	14 CDEX -	15 AspA -	16 BGAR -
25 LeuA -	23 ProA -	24 BGLR -	25 AGAL -
29 AlaA -	29 TyrA -	30 dSOR -	31 URE -
36 dRIB -	39 LATs -	42 LAC -	44 NAG -
47 NOVO -	50 NCE.S -	52 dMAN -	53 dMNE -
57 dRAF -	58 O129R -	59 SAL -	60 SAC -
84 OPTO -			
9 BGAL -	11 AGLU -	17 AMAN -	18 PHOS -
26 PyrA -	27 BGLR -	32 dPOLYB -	37 dGAL -
45 dMAL -	46 BACI -	54 NBDG -	56 PUL -
82 dTRE -	83 ACH2s -		

VITIK 2 *S. auerus*

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**الكشف عن البكتيريا المقاومة من الالتهابات الجلدية للعديد من المضادات الحيوية المعزولة في عدد من
مستشفيات مدينة الموصل**

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الملخص

تعد البكتيريا التي تصيب الحروق والجروح مشكلة مهمة تؤدي أحياناً إلى الوفاة ، لذلك تم اختيار أفضل طريقة لتشخيص العزلات البكتيرية من الحروق والجروح في هذه الدراسة بناءً على خصائصها المظهرية والاختبارات البيوكيميائية. اذتم تشخيص البكتيريا المعزولة بعدة طرق لتأكيدا قبل استخدام المضادات الحيوية ومن بين هذه الفحوصات API20E و APISTAPH test كما وتم استخدام اختبار VITIK الذي يعد اسهل وادق واسرع في التشخيص البكتيري من الطرق التقليدية المعتمدة في التشخيص ثم استخدام المضادات الحيوية ودراسة مدى تأثيرها لاختيار افضل المضادات الحيوية لتلافي المشاكل نتيجة الاستخدام المفرط والعشوائي للمضادات الحيوية من قبل المرضى المصابين