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Evaluation of Number of Virulence Factors and Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* isolated from wound and burn infections in Samarra city

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ABSTRACT

Wound and burn infections is one of the serious problems that patients suffer from in hospitals, since the bacteria that cause wound and burn infections usually have resistance to most of the commonly used antibiotics.
The current study aimed to isolate and diagnose *Pseudomonas aeruginosa* from patients with burn and wound infections in Samarra City and to test the sensitivity of the isolates to commonly used antibiotics and the production of virulence factor by these isolates.
Swabs were taken from 110 patients attending Samarra General Hospital and some private medical clinics at Samarra who suffer from wound and burn infections. Twenty two isolates of *P. aeruginosa* were obtained with an isolation rate of 20%.
Production of some virulence factors by P. aeruginosa were detected, and the percentage was as follows:- The DNase at 0%, haemolysin at 81.8%, lecithinase & lipase at 100%, and urease at 81.8%.
A sensitivity test was conducted for (12) antibiotics, whereby *P. aeruginosa* gave complete resistance to Erythromycin, Trimethoprim - sulfamethoxazole, and Ceftriaxone, and the bacteria showed that it is susceptible to both Tobramycin antibiotics, and Ciprofloxacin, Levofloxacin, and Imepineme, also showed varying resistance to the other antibiotics.

Introduction

The human skin is the layer that separates everything inside the body from the internal organs, tissues, and body fluids from the external environment, in addition to its main function as a mechanical barrier in the immune system and its participation in the function of the nervous sense, as well as maintaining the water balance of the body. Any breach of the surface layers of the skin due to a wound or burn will lead to a disruption of the skin's protective mechanism and allow the passage of microorganisms into the internal tissues and blood, and thus will have a suitable environment that is moist and rich in proteins that help them grow [1].
A wound is defined as any cut or separation in the continuum of the skin and that damage may be due to mechanical, physical, biological or chemical, which allows the entry of pollutants into the tissues and blood [2].
Burns are defined as the area of tissue damage caused by the effects of heat. It may occur directly from the transfer of thermal energy or indirectly, when some other forms of energy are converted into heat forms, such as burning the skin resulting from electric shock (electrical energy) and others [3].
It was found through this study and other studies by other researchers that the most common bacterial causes of wounds and burns infections are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and other types of bacteria. *Pseudomonas aeruginosa* causes some diseases to humans, especially when the immune defenses are weak, and the virulence factors in the cause after colonization great damage to tissues

and may invade the bloodstream and reach other organs, causing systemic infections [4]., and it has a high capacity Sepsis can spread rapidly, damaged tissues, and cause sepsis with high rates of death, and acute infection can persist for weeks, months, and years in the face of intensive clinical treatment [5].

It is considered one of the types that threaten public health if it is characterized by being opportunistic bacteria that infect humans, especially weak immunity and the so-called immunosuppression, and those with wounds and deep burns, as well as leukemia [6]. Humans increase by 20% among hospitalized people, especially those who have and severe burns [7], as it affects wounds, burns, eyes, respiratory and urinary tracts, and middle ear, and may cause bacteremia [8].

One of the most important developments in medical sciences in the twentieth century was the introduction of antimicrobial drugs, and the use of these drugs at the beginning of their appearance resulted in a reduction in deaths resulting from bacterial infections and most of the infections that appeared at the time were controlled. [9]. The increase in infection rates was not only due to its resistance to antibiotics but also to its possession of many virulence factors that increase the severity of its pathologies, such as beta-lactamase, urease, lipase, lecithinase, haemolysin, as well as coagulation and hydrolytic enzymes[10].

Pseudomonas aeruginosa bacteria has a resistance to many antibiotics, sterilizers, and disinfectants [11], and what increases its danger is its possession of many virulence factors that help it when attacking host cells and spreading in them. *P. aeruginosa* has caused many skin infections, including local and some widespread, especially in those with wounds and burns, where it was found that (60%) of burn patients die due to infection with this germ, and this germ is one of the causes of acne [12], [13].

Materials and Methods

In this study, 110 samples were collected from patients hospitalized in Samarra General Hospital and some outpatient clinics in the city of Samarra, with infections of wounds and burns, of both sexes, and of different ages. The swabs taken included 80 swabs of wounds from patients whose ages ranged between 2 - 70 years, 39 of them were taken from females and 41 males, as well as 30 burn swabs for patients aged 5-71 years, 19 females and 11 males. *Pseudomonas aeruginosa* isolated from 22 cases at rate of 20% of the total swabs taken. Microscopic examination was done by observing the susceptibility of bacteria to staining with Gram stain. Screening for rapid initial diagnosis [14]. The tests for *Pseudomonas aeruginosa* were carried out as follows:

Pyocyanin Production Test:- *Pseudomonas aeruginosa* was cultured on nutrient agar media, and then the plates were incubated at 37°C for 24 hours. The positive test was indicated by the bluish-green coloration of the medium as a result of pyocyanin production, in addition to the appearance of bright color on the medium as a result of the spread of the fluorescent dye on the surface [15].

Growing at a temperature of 4°C and 42°C:-

In this test, a nutrient agar medium was used by inoculating the medium with bacterial colonies and incubating for 24 hours at a temperature of 4 and 42 degrees Celsius. The evidence of the positiveness of the test is the presence of growth on the medium [15].

Growth on cetrimide medium:-

This medium is used to diagnose *Pseudomonas aeruginosa* because it allows the growth of *Pseudomonas* bacteria without others [15].

Biochemical assay:-

Traditional (biochemical tests) were also conducted to diagnose these isolates. After that, the final diagnosis of *Pseudomonas aeruginosa* was confirmed using the API 20E system to confirm the final diagnosis of the bacteria and to determine the genus and species, as this system has the advantage that it diagnoses the bacteria within 24 hours and gives an accurate diagnosis of the pure isolation. , provided that it is not contaminated with other germs, as this system was used to ensure the purity of the isolated bacterial isolates under study.

Susceptibility test:-

Antibiotic susceptibility test was conducted for twelve antibiotics, and the diffusion method described by Kirby Bauer was used, using Muller-Hinton agar medium. The diameter of the inhibition zone were recorded using the (Interscience) Scan 4000 device, according to what was mentioned in [16].

Results and Discussion

It was diagnosed Twenty-two isolates of bacteria *Pseudomonas aeruginosa* from patients with wound and burn infection, and by isolating 20% of *P.aerougenosa* from the total swabs taken .this study agreed with other study reached by one of the researchers and by isolating (26.1%), [17]. These bacterial isolates were diagnosed by conventional method, and the isolates confirmed using the API 20E system, in order to ensure the final diagnosis of the bacteria and to determine the species. This system was used to diagnose the types of bacterial isolates after confirming them by the preliminary biochemical tests referred to previously. Table (1) shows the biochemical tests and figure (1) Shows the diagnosis of bacteria using the API 20E system.Table (1) shows the biochemical tests and figure (1) Shows the diagnosis of bacteria using the API 20E system.

Table 1: Results of biochemical tests for diagnosing isolates of Gram-negative *Pseudomonas aeruginosa* bacteria.

Lactose	Glucose	Motility	Hemolysin	Manitol	H2S	Urease	Oxidase	Catalase	Vogas proskauer	Citrate Utilization	Methyl Red	Indol test	Number of isolates	Bacteria name
-	+	+	+	–	–	V	+	+	–	+	–	–	22	***Pseudomonas aeruginosa***

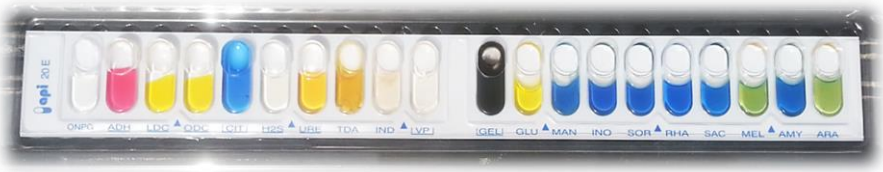

Fig. 1: Diagnosis of *Pseudomonas aeruginosa* using the *Api* 20E system.

Antibiotic sensitivity:

The resistance test was conducted for the bacterial isolates under the study of *Pseudomonas aeruginosa* to 12 antibiotics, and these bacteria could show resistance characteristics to various types of antibiotics, and the reason for the resistance may be that it is one of the most important types of opportunistic pathogenic bacteria that exploit the weakness of the immune system, especially in dormant patients. In the hospital and due to hospital infection as well as its high ability to produce the strongest bacterial virulence factors, *P. aeruginosa* gave complete resistance towards the antibiotics Erythromycin, Trimethoprim-sulfamethoxazole, and Ceftriaxone, and this came following the results of one Researchers [18], as the percentage reached 100%, and this result also agreed with what a researcher obtained in her study [19] regarding Erythromycin. It was close with a study [18] about Trimethoprim-sulfamethoxazole, the resistance reached 94%. It is noted that this percentage also converged with the results of researchers [20] and [21].

Bacteria have been shown to be non-resistant to both antibiotics Tobramycin, Ciprofloxacin, Levofloxacin, and Imepineme. As for the Amikacin in Figure (2), the resistance rate for it reached 4.5%, which approached that of the study One of the researchers [18], with a rate of 17%, and it differed with another researcher [20] where the percentage of resistance was 77.77%, and in the ciprofloxacin, it did not agree with a study by researchers [22] Where the percentage was 31.2% concerning the Ciprofloxacin, this result differed with what was reached by one of the researchers [23] in his study of some virulence factors of *Pseudomonas aeruginosa* bacteria, where the percentage of bacteria resistance to the Tobramycin was % 12, and the reason may be due to the frequent use of these antibiotics in recent times and the increase in resistant strains, as well as the different places and environments of isolation.

The bacteria also showed high resistance of about 86% to the antibiotics Azithromycin and Cefepim and 40.9% to the antibiotic Aztreonam, and the bacteria showed 18.1% resistance to the Gentamicin, and this result did not agree with a study conducted by him. One of the researchers [18] regarding the gentamicin because it was 70% and did not agree with another study, [24] where it was 93%, as the reasons for the high resistance are either due to the production of beta-lactamase enzyme or By possessing the permeability barrier represented by the outer membrane layer, as it is one of the most important types of resistance possessed by *P. aeruginosa* towards antibiotics and antiseptics [25]. Resistance may come by reducing the permeability of the outer membrane, which affects the rate of absorption of antibiotics by this bacterium, as this mechanism is found exclusively in Gram-negative bacteria, while Gram-positive bacteria lack it [26].

Table 2: The percentage of bacteria resistant to the antibiotics under study

Trimethoprim Sulphamethoxazole SXT-25	Levofloxacin LEV-5	Imepineme IPM-10	Ceftriaxone CRO-10	Ciprofloxacin CIP-10	Aztreonam ATM	Cefepim FEP-30	Gentamicin CN-10	Tobramycin TOB-10	Azithromycin AZM-15	Amikacin AK^{30}	Erythromycin E^{10}	Number of isolates	Bacteria name
%100	**4.5%**	**%0**	**%100**	**%0**	**40.9%**	**%72.7**	**18.1%**	**%0**	**86.3%**	**4.5%**	**%100**	**22**	*Pseudomonas aeruginosa*

It is noted from the foregoing that the bacteria *P. aeruginosa* isolated from the wounds, burns and surgical infections showed multiple resistance to many antibiotics, including those of recent generations, and this resistance may be caused by the characteristics of outer membrane proteins and chromosomal beta-lactamase enzymes, as well as the presence of multiple antibiotic resistance genes in these bacteria. In addition to the presence of the resistance plasmid R-plasmid, which gives these bacteria resistant to many antibiotics [27], as one of the researchers mentioned [28] that it is better to combine two antibiotics when treating infections caused by *P. aeruginosa* due to its high resistance to many antibiotics, as shown in Figure (2).

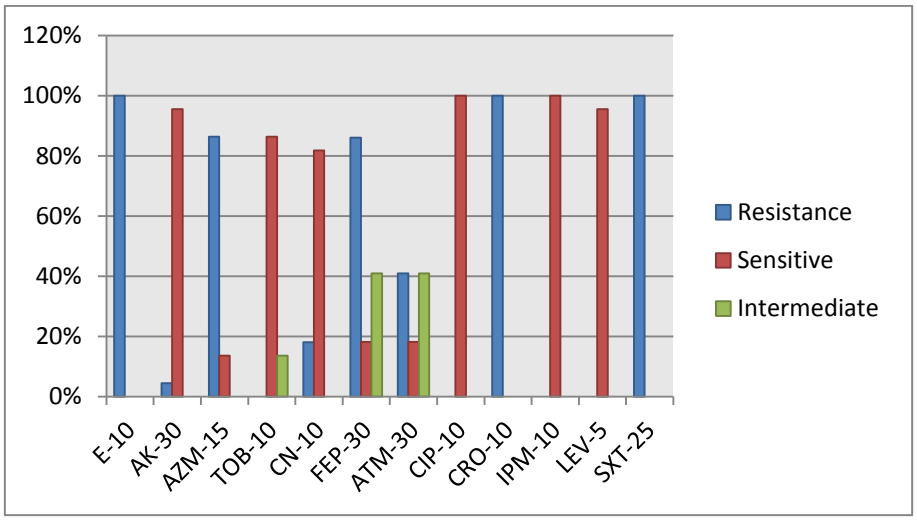

Fig. 2: Resistance and sensitivity of *Pseudomonas aeruginosa* to the studied antibiotics.

Investigate some virulence factors:

Some selective media were used to identify the ability of some bacterial isolates to produce some virulence factors and the ability to analyze some of the substances present in these media. The results of the formation of virulence factors for the bacterial isolates under study were as shown in Figure (3), which includes percentages For each of them:-

Hemolysin test:

It is considered one of the most important factors of virulence possessed by some bacteria and can destroy the cell membrane of red blood cells. The result appears in the presence of a transparent or green halo around the colony in the middle of the blood agar [29], and the ability to analyze blood Through this enzyme depends on many factors, including blood type and its ability to bind to the receptors of the glycoprotein, and it is transformed by polymerization into a complex compound that penetrates the lipid bilayer of the cell membrane, and the presence of cholesterol and serum in the used blood helps to inhibit the analysis process [30]. Where the rate of production of *Pseudomonas aeruginosa* by (81.8%) of the infections of wounds and burns was able to analyze the blood and produce hemolysin, where it agreed with a study by one of the researchers, [19] which was 76% and did not agree with what has reached another researcher, [20], where the percentage of analysis was 100% in isolates of *Pseudomonas aeruginosa*, and the results of the current study were similar with the results of [31] and [32], It was found that *P. areuginosa* isolates were hemolysin producers with a percentage of (84%) and (93%), respectively.

Hemolysin is the important virulence factors in *Pseudomonas aeruginosa* bacteria, as it works on the analysis of red blood cells, leading to anemia and weak host defenses, which provide large amounts of iron that the bacteria benefit from their metabolic activities. Red blood cells depend not only on the type of hemolysin but on the type of bacteria itself, the concentration of calcium ions in addition to the temperature, duration of incubation, and incubation conditions [33].

Production of the urease enzyme:

This enzyme is of great importance in the analysis of urea into ammonia and Co_2 gas, and the positive result of the examination is to change the colour of the medium from yellow to pink due to the formed ammonia, which in turn transforms the medium from acidic to basic after 4 hours from cultivation and at a temperature of 37 degrees . [34] The results of our study showed that *Pseudomonas aeruginosa* bacteria, the rate of its production of the enzyme was 81.8%, and this result agreed with what was reached by one of the researchers, [20] where 75% of the total isolates produced the enzyme, and this result agrees with another researcher [18]. by 80%, and differed in a study conducted by another researcher, [35] where she indicated that the percentage of isolates producing urease (42.7%).

DNase production:

This enzyme is considered to have a high ability to affect the synthesis of the DNA, and it is considered one of the most important characteristics of pathogenic bacteria, but the isolates under study did not produce this enzyme and this study agreed with one of the researchers [17].

Production of the enzyme lecithinase and lipase:

Lecithinase is one of the most important virulence enzymes, as it degrades lipids that are stable in cell membranes, and then decomposes and the tissue dies, separating the lipoprotein complexes in the medium containing the egg yolk. This reaction is called Leetho Vitellin [36]. As for lipase, it works on the analysis of fats in the cell and thus facilitates the process of penetration into the skin [37].

Where it was found that the bacteria *Pseudomonas aeruginosa*, through the study, that all its isolates are producers of Lecithinase, and this is concerning infections of wounds and burns, and this result agreed with one of the researchers, [23] as it was also found that all isolates of *Pseudomonas aruginosa*, which is specifically isolated from wound infections, is a producer of this enzyme, and it also agreed with another researcher [38].

The production of lecithinase is associated with the toxicity and virulence of *P. areuginosa* bacteria, and this enzyme causes phosphorous and choline to be released with the precipitation of fat, which is the source of the luster. Most organisms that produce lecithinase are gram-positive, and few gram-negative organisms possess this activity. The family of *Pseudomonasaceae* possesses the largest number of products for this enzyme. The difference between members of this family in their production of this enzyme depends on the colour of the enzyme. The pigment produced by each species [17].

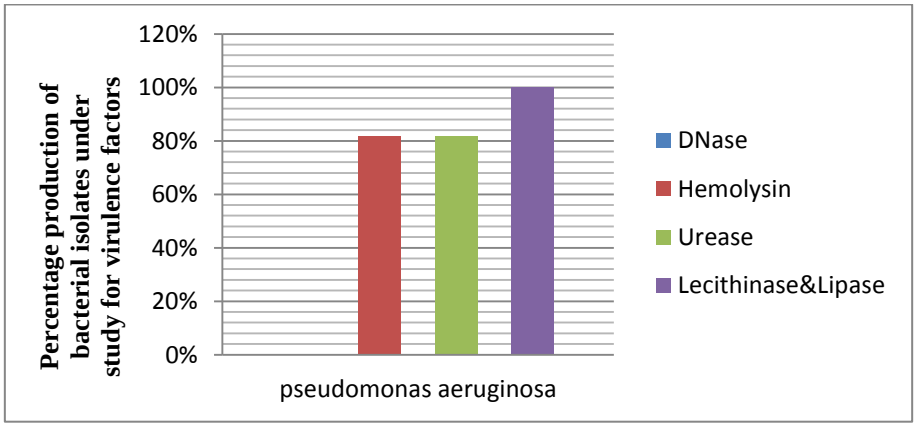

Fig. 3: The percentage of production of bacterial isolates of the virulence factors.

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تقييم عدد من عوامل الضراوة وأنماط مقاومة المضادات الحيوية في بكتيريا *Pseudomonas aeruginosa* المعزولة من التهابات الجرح والحروق في مدينة سامراء

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الملخص

تعد التهابات الجروح والحروق من المشاكل الخطيرة التي يعاني منها المرضى في المستشفيات ، حيث أن البكتيريا التي تسبب التهابات الجروح والحروق عادة ما تكون مقاومة لمعظم انواع المضادات الحيوية شائعة الاستخدام.

هدفت الدراسة الحالية إلى عزل وتشخيص بكتريا الزوائف الزنجارية من مرضى الحروق والجروح في مدينة سامراء واجراء اختبار حساسية العزلات للمضادات الحيوية شائعة الاستخدام والتحري عن إنتاج بعض عوامل الضراوة لهذه العزلات حيث تم أخذ العينات من المرضى في مستشفى سامراء العام وبعض العيادات الطبية الخاصة في سامراء من الذين يعانون من التهابات الجروح والحروق بمجموع 110 مسحة وتم الحصول على 22 عزلة من *P. aeruginosa* وبمعدل نسبة عزل 20٪. وتم الكشف عن قدرة هذه العزلات على إنتاج بعض عوامل الضراوة وكانت النتيجة كما يلي: – DNase بنسبة 0٪ ، الهيمولايسين بنسبة 81.8٪ ، الليسيثينيز واللاايبيز بنسبة 100٪ ، واليوريز بنسبة 81.8٪. وقد تم إجراء اختبار الحساسية لـ (12) مضاداً حيوياً حيث أبدت بكتريا الزوائف الزنجارية مقاومة كاملة لكل من Trimethoprim-sulfamethoxazole, Erythromycinو Ceftriaxone ، وقد أظهرت البكتيريا أنها غير مقاومة لكل من المضادات الحيوية Ciprofloxacin, Tobramycin، Levofloxacin و Imepineme وأيضاً كانت مقاومتها متفاوتة للمضادات الحيوية الأخرى.