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Detection and Pathogenicity Features of *Pseudomonas Aeruginosa* in Patients with Skin Infection

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***Abstract*— *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen often concerning studies in bacterial resistance and pathogenicity. Nosocomial infections from burns and surgical site infections are often caused by destruction of the natural defenses of the skin, exposed matrix proteins and inflammatory factors being compromised making it easier *for P. aeruginosa* to colonize the area leading to infection. For this experiment, 113 wound and burn samples collected from hospitalized immunocompromised patients. 26 isolates tested positive for *P. aeruginosa* with associated virulence factors. Identification of bacteria concluded biochemical assays, antibiotics susceptibility, and the ability of biofilm formation and the presence of *P.aeruginosa* in different skin samples. The study showed 66.53% of the isolates were resistant to antibiotics including (AK, GN, IPM, LEV, CIP, ATM, PRL and TZP), while prevalence of sensitivity was to Meropenem (65.38%) and Cefepime (19.23%).**

Biofilm formation assay showed all *P.aeruginosa* isolates formed biofilms at different levels (65.3%) weak (27%) moderate and (7.7%) strong biofilm formation. Following the study's conclusion, consider specific strategies for managing and shielding future generations against P.aeruginosa bacterial infections.

***Keywords*— Nosocomial infection, Virulence factor, Antimicrobial resistance, Susceptibility, Biofilm**

I. INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, opportunistic bacterium well known for its virulence and social characteristics. It is rod-shaped, 1-2 µm length, 0.5-1.0 µm wide, heterotrophic, with flagellum. A facultative aerobe that survives on nitrate and arginine-rich aerobic respiration and has minimal or no fermentative activities [1]. It usually consists of a single carbon and energy source, yet it may also thrive on a growth medium that has minimal salt and relatively limited minerals. Although *P. aeruginosa* can survive in temperatures as high as 4–42 °C, 37 °C is ideal for its growth. *P. aeruginosa* is commonly responsible for bloodstream infections, nosocomial infections, pneumonia, and surgical site infections [2].

The risk of *P.aeruginosa* infection increased by proximity to hospitals and other healthcare facilities. It possesses an amazing capacity to build resistance to widely used antibiotics, including aminoglycosides, carbapenems, acquired and adaptive resistance mechanisms, many of which are expressed concurrently [3]. It is naturally resistant to antibiotics sold commercially for infections in suitable areas, such as cystic fibrosis-affected lungs or burned skin. In skin related infections the natural defenses of the skin is destroyed by burn and wound injuries, which makes it easier for *P. aeruginosa* to colonize and cause infection because of exposed matrix proteins and inflammatory factors [4].

The most characterized resistance mechanisms of *P. aeruginosa* include modifications to the outer membrane's permeability, porins, efflux pumps, enzymes that render drugs inactive, and alterations to target binding sites [5]. Often, a *P. aeruginosa* infection causes the simultaneous expression of many resistance systems in a specific patient [5].

In burn patients, the extent of the burn determines the damage received by patients. The more extensive the burn area, the higher the patient's risk of morbidity as well as mortality [6].

Among burn patients, *P. aeruginosa* is still one of the most dangerous bacterial infections. The skin's capacity to resist bacterial infection when its barrier function is compromised physically [7]. Staphylococcus aureus and Streptococcus pyogenic are two examples of Gram-positive bacteria that colonize wounds before *P. aeruginosa* infection. Along with other bacteria and yeasts, *P. aeruginosa* eventually colonizes wounds from the local flora of the upper respiratory tract and/or gastrointestinal system in patients. [7].

The degree of damage of a burn depends on several factors, including its location, temperature, and duration of patient contact [8]. *P.aeruginosa* and *S. aureus* are the most common bacteria that cause significant infections in burn wound patients. This could potentially lower the effectiveness of burn wound therapy. After five to seven days, if the patient is not given antibiotics, the skin will start to colonize with bacteria, both gram-positive and gram-negative, and microorganisms from the hospital environment [9].

Additionally, *P. aeruginosa* can result in infections of the skin, bones, and joints. The spinal column, the pelvis, and

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the sternoclavicular joint are the most often involved sites. Penetrating injuries, surgical procedures, or diseases of the soft tissues above can all cause an infection to spread either simultaneously. Pseudomonas spp has special role in severe tissue damage caused by poor blood circulation in the foot leading to diabetic foot infection [10]. This pathogen is well known to grow on moist skin, but it cannot develop on dry skin [11].

This study aimed to identify and characterize *P.aeruginosa* strains isolated from burn and wound samples of immunocompromised patients in intensive care units in hospitals of Thi-Qar province.

II. METHODS:

A. Sample collecting

A total of 113 samples were collected from patients with burns, accident wounds, and surgical wounds from Al-Turkey Hospital and private clinics in Thi-Qar province. Samples on different culture media including MacConkey agar, were collected for microbiological examination. All samples were transported immediately on transport media swabs to the Microbiology Laboratory at the University of Thi-Qar College of Science, for cultivation and identification.

B. Isolation and Identification of P. aeruginosa

The samples were cultivated aerobically for 24 hours at 37 °C after the swab media was spread out on MacConkey agar. The pale pink colonies shown on MacConkey indicated the non-lactose fermentative ability, then subcultering the bacteria on blood agar and cetrimide agar by streaked a loopful of bacterial colony, the petri dishes were then incubated for 24 hours at 37°C in an aerobic condition. Cetrimide is a selective medium well known for identifying *P.aeruginosa* by presenting a green fluorescent color on the agar, whereas blood agar is used to demonstrate the beta hemolysis properties of *P. aeruginosa*.

Gram's stain was also used to identify the recovered isolates; this method was introduced by Christian Gram to distinguish between two species of bacteria based on variations in the structure of their cell walls. The thick layer of peptidoglycan in the cell walls of gram-positive bacteria allows them to retain the crystal violet color. While a very thin coating of peptidoglycan degrades in response to the presence of alcohol in gram-negative bacteria. Results in this study reveal gram-negative bacteria in the form of pink non-spore forming rods. Characteristics of the culture, motility, generation of pigments (fluorescent pigments), and biochemical reactions utilizing the tests that Mac Fadden previously described: oxidase, indole, catalase, urease, methyl red, citrate utilization, mannitol fermentation, and Voges-Proskauer tests.

C. Antimicrobial Activity

Antibiotics susceptibility was investigated by using Muller Hinton agar and disc diffusion method. Bacterial isolates were sub cultured on MacConkey agar and incubated at 37 C for 24 hrs. One colony was suspended in normal saline 0.085% and adjusted to 0.5 McFarland. A total of ten antimicrobial discs including (Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Piperacillin, Imipenem, Cefepime, Aztronem, Meropenem and Pipercillin/Tazobactam combination) were placed in Muller Hinton agar plates and incubated at 37 °C for 24 hours. Guidelines established by CLSI, 2023 were adhered for interpreting the test findings and specific inhibition zone of each antibiotic revealing resistance and sensitivity. *P. aeruginosa* strains being evaluated demonstrated XDR and MDR in accordance with [12].

D. Biofilm Formation Test

The biofilm growth of the *P.aeruginosa* isolates was measured using a 96-well micro titer plate assay that was adapted from. After diluting Brain Heart Infusion Broth (BHI) 1:100, the overnight cultures were grown apart.

1. Using a micropipette, add 100 µL of the overnight suspension to a new BHI tube. Shake well with a vortex. Next, add 200 µL to each well in a 96-well plate. As a negative control, three wells with just BHI solution are utilized. In quantitative investigations, we typically use three replicate wells for each bacterial sample.
2. For 4–24 hours, incubation of the microtiter plate at 37°C. The micro titer plate wells were emptied and allowed to air dry in the proper order following three washings in double-distilled water (DDW). The biofilm layer solidified, then micro titer plate wells were emptied and left to air dry. For 20 minutes at room temperature, the fixed biofilm layer was dyed with 0.1 percent (w/v) crystal violet.
3. Rinsing of the plate three to four times with (DDW) water to get rid of all the leftover cells and dye. Then, shaking it off and blotting firmly on a stack of paper towels.
4. 200 µL of a 99% ethanol (or acetone) solution was added to each well in order to measure the optical density (OD). After 15 minutes, they were placed in an Enzyme-Linked Immunosorbent Assay (ELISA) reader. There were three runs of each test; ODc of the negative control was contrasted with the standard deviation and average OD of every test.

The outcomes were obtained in compliance with the ODC guidelines for the negative control. (Table 1).

TABLE I. CALCULATION OF CUTOFF VALUE OR BIOFILM FORMATION.

Mean OD value	***Biofilm Formation***
OD ⩽ Odc	No biofilm
OD < OD ⩽ 2 * Odc	Weak Biofilm
2 * ODc <OD ⩽ 4* Odc	Moderate biofilm
4 * ODc < OD	Strong biofilm

E. Statistical Analysis

Using a descriptive, non-parametric Chi-Square at p. value < 0.05, the data from the current study were statistically analyzed using SPSS (Statistical Package of Social Science, version 26).

III. RESULTS

A. *Distribution of Pseudomonas aeruginosa isolates*

113 samples were collected from September 2023, to January 2024 from hospitals and private clinics in Thi-Qar Provence. Skin samples from patients with serious skin infections from wounds or burns were obtained using a sterile swab containing transfer media. The bacterial isolates were identified morphologically, as small, spherical, colorless or pale pink colonies Fig. 1(A). Indicating, it is incapable of breaking down lactose. Furthermore, MacConkey agar is the primary source of *P.aeruginosa's* distinctive, grape-like odor, used to identify the bacteria most frequently. On the other hand, colonies of P. aeruginosa on blood agar exhibited adhesive textures, white to gray coloration, of colorless area that suggested a specific type of beta hemolysis. In terms of specificity, Fig. 1(B) *P. aeruginosa* can be cultivated specifically on Cetramide agar, which can also be employed to inhibit the growth of other Pseudomonas species. *P. aeruginosa* colonies appear smooth, elevated center, with flat edges, and are vivid green or yellow-green in color due to the pigments pyocyanin and pyoverdine seen clearly on Cetrimide agar. Fig. 1(C).

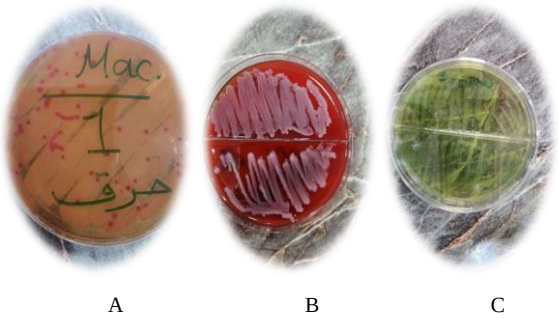


Fig 1: *Pseudomonas aeruginosa* strains on MacConkey agar (A), Blood agar (B) and Cetrimide agar (C)

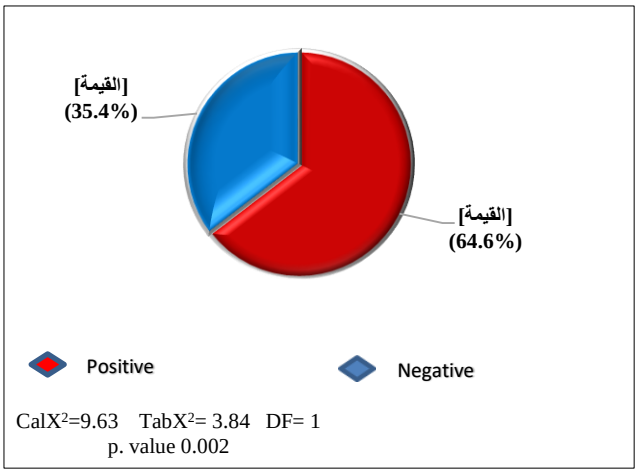


Fig 2: Types of bacterial isolates in skin infections

B. *Microscopic identification*

Under a light microscope, *P. aeruginosa* cells show a negative Gram stain and appear as single or double, pink non-spore forming rods with a single flagellum.Fig.3.

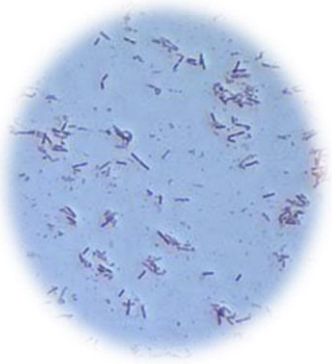


Fig 3: *Pseudomonas aeruginosa* under light microscope

C. *Biochemical identification*

Biochemical outcomes displayed in (Table 2).

TABLE II. RESULTS OF DIFFERENT BIOCHEMICAL TESTS FOR IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA*

Test	*Results*	*Appearance*
Indole Test	-	No Change
Methyl Red	-	No change (yellow)
Vogues-Proskauer	-	No change (yellow)
Catalase Test	+	Bubbles
Oxidase Test	-	Dark Purple
Triple sugar Iron (TSI)	+	Slant/Butt k\k No change /No H2S

Vitec 2 compact system was used for further identification, revealing prevalence of 26 positive isolates of P. aeruginosa .9 isolates (34.6%) incidents in patients with burns, and 17 isolates (65.4%) in those with wounds. (Fig: 4)

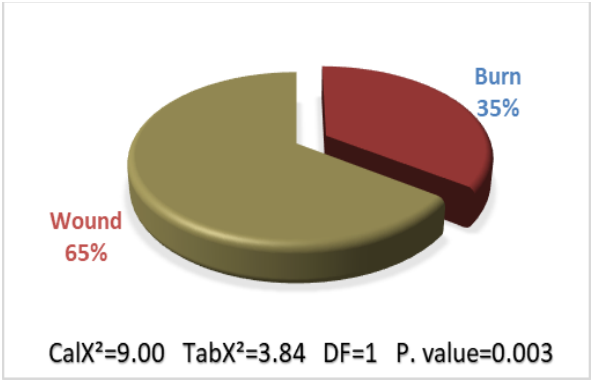
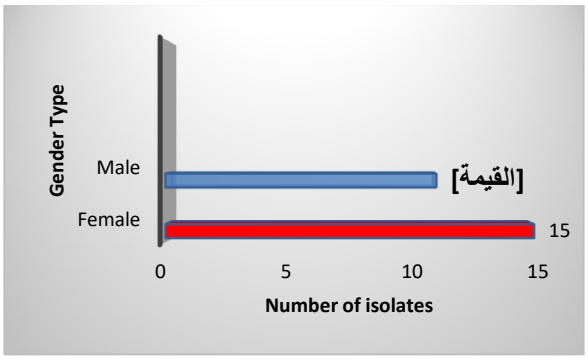


Fig 4: Percentage of Pseudomonal infections in burn and wound patients.

Outcomes relating to ex revealed that male patients (11 isolates) had lower outcomes (42.3%) compared to female patients (15 isolates), with greater results (57.7%). (Fig 4)



CalX²=2.56 TabX²=3.84 DF=1 P. value=0.110

Fig 5: Comparison of Pseudomonas aeruginosa skin infections according to Sex

D. *Antibiotic susceptibility of P. aeruginosa in skin infection*

Ten different antibiotic tests, each with a different mechanism of action, were performed on the recovered bacteria. Based on the studied results, 10.38% of the *P. aeruginosa* isolates were susceptible to antibiotic activity, 23.07% of the bacteria showed an intermediate response to antibiotic action, and 66.53% of the bacteria were resistant to antibiotics.

TABLE I. ANTIBIOTICS SUSCEPTIBILITY OF PSEUDOMONAS AERUGINOSA IN PATIENTS WITH SKIN INFECTIONS.

Antibiotics	*No. of isolates (S)*		*No. of isolates (I)*		*No .of isolates (R)*	
	No	*%*	*No*	*%*	*No*	*%*
Amikacin(AK)	2	7.7	10	38.46	14	53.85
Gentamicin(GN)	1	3.8	2	7.69	23	88.46
Imipenem (IPM)	0	0	1	3.85	25	96.15
Meropenem (MEM)	17	65.38	2	7.69	7	26.92
Cefepime(FEP)	5	19.23	6	23.08	15	57.69
Levofloxacin (LEV)	0	0	11	42.31	16	61.54
Ciprofloxacin(CIP)	0	0	15	57.69	10	38.46
Aztreonam(ATM)	2	7.69	11	42.31	13	50
Piperacillin (PRL)	0	0	0	0	26	100
Piperacillin+Tazobactam (TZP)	0	0	2	7.69	24	92.31
Susceptibility%		10.38		23.07		66.53
P. value<0.001	Calx²=159.3		Calx²=159.3		DF= 18	

(S)-Sensitive (I)-Intermediate (R) Resistance.

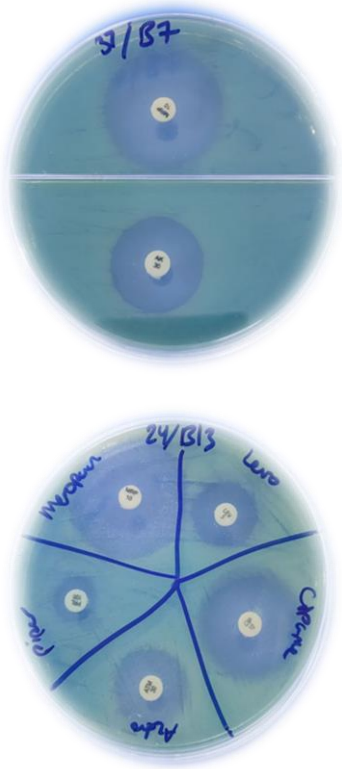


Fig 6: Antibiotic susceptibility test

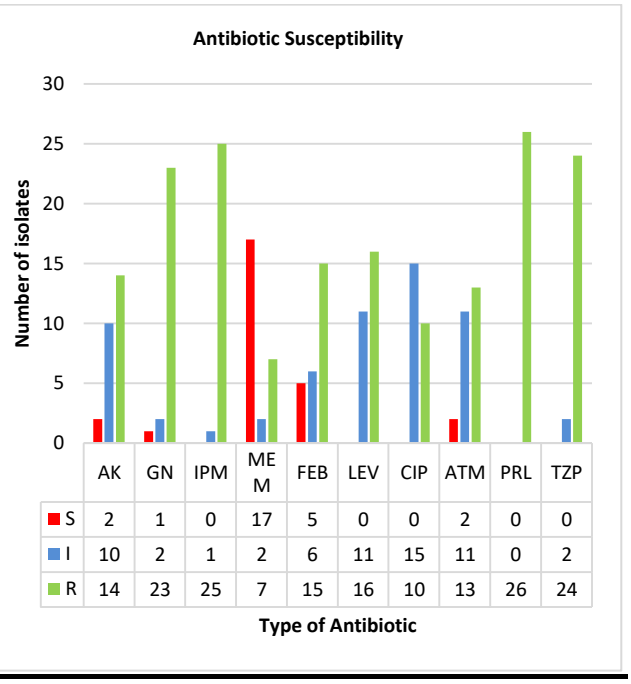


Fig 7: Antibiotics susceptibility of *Pseudomonas aeruginosa* in patients with skin infections

E. *Additional bacteria discovered in patients with skin conditions*

Throughout the current experiment, numerous bacterial colonies have been seen to develop on MacConkey agar medium to distinguish gram-negative bacteria from gram-positive bacteria. As shown in (Fig. 8)

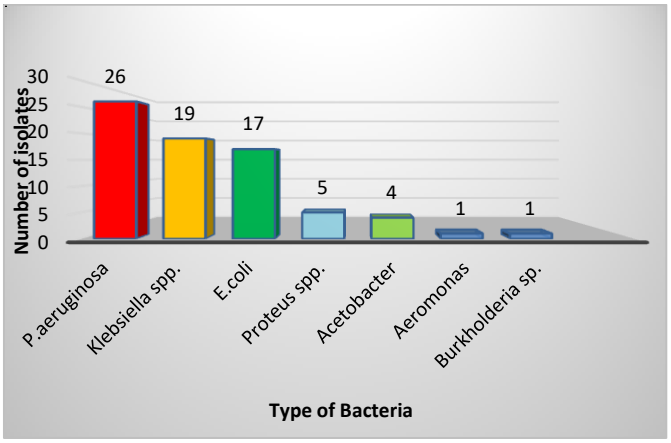


Fig 8: Type of bacteria isolates from skin.

F. Biofilm Formation of P.aeruginosa isolates

According to the study, *P.aeruginosa* forms biofilms at different rates. (Fig. 9) illustrates that 27% of the samples formed a moderate biofilm, 7.7% a strong biofilm, and 65.3% weak biofilm.

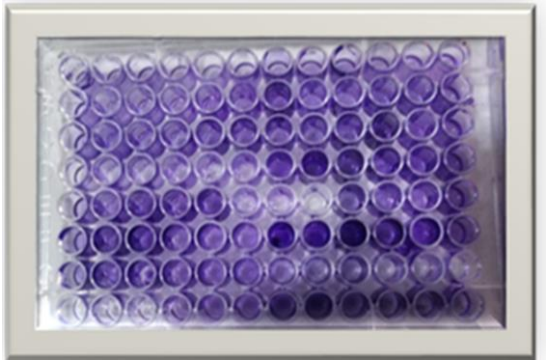


Fig 9: *Pseudomonas aeruginosa* Biofilm Formation on Micro titer plate.

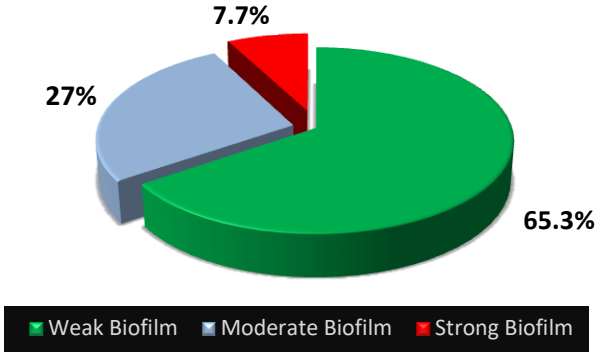


Fig 10: Types of Biofilm Formation of *Pseudomonas aeruginosa*

IV. DISCUSSION

Monitoring the distribution of *P. aeruginosa* in clinical and hospital settings from Thi-Qar Province is one of the goals of the current investigation. Based on different data mainly conducted by observing immunocompromised patients with varied medical problems, to concur the occurrence of pseudomonas infections. Additional observations show that male and female patients differ in terms of infection rates, types of injuries, and burn stages. Over the past ten years, *P. aeruginosa* has become a significantly commonly occurring nosocomial infection in Iraqi patients, primarily in hospitalized patients [11]. The most concerning feature is the remarkably high fatality rate linked to Pseudomonal infections received by hospitalized patients. The most common risk factor is a breakdown of the integument.

Thirty-five percent of the isolated samples that tested positive for *P.aeruginosa* in this experiment were from burn victims. These patients spent one to three weeks in the hospital with burns that ranged from minor to severe. Most of the isolates had burns categorized as third degree burns. In cases with burn skin this severe, there is a 70% chance of developing *P.aeruginosa* skin infections due to prolonged hospital stays and contact with infected items such as utensils or bed linens [13]. Taking into account the poor sterilization practices and unsanitary environments found in many hospitals. The burn site exhibits a green coloration in the area being treated.

P.aeruginosa's infection spread is influenced by the distinction between acute and chronic wounds. Regardless of their severity, they represent a major global public health issue [11]. It takes four to six weeks for an acute wound to heal physiologically. On the other hand, the wound can aggravate or become chronic if the healing process takes longer than six weeks to finish. A wound is nutrient-rich, warm, and moist, making the ideal environment for hazardous bacteria to colonize, grow, and infect [11].

The findings corroborated previous discoveries; wounds that have been drained are more likely to experience surgical site infections than those that have not. In order to make a wound infection diagnosis, one of the following needs to be satisfied: the wound needed to be clear of pus, serous or non-purulent, and devoid signs of inflammation (edema, redness, warmth, elevated local temperature, 38°C fever, tenderness, and induration) [14]. In addition, the surgeon needed to carefully open the wound due to a localized collection. *P. aeruginosa* was found in 65.4% of the isolates from burns and 34 % from wounds in this study in contrast to previous research [14].

Gender-related clinical isolates reveal that *P. aeruginosa* infections are more common in females (60%) than in males (40%), confirmed by an Iraqi investigation [15]. On the other hand, some research revealed an opposing pattern, with men making up 61.8% and women 38.2% [16]. This can be due to the sample chosen from different areas of the body. The results according to age (18-40 years) observed that female patients account for 57.7% of the isolates tested. These samples were from patients who underwent three to seven days in the hospital after a C-section. Additional samples obtained from women who had burns to their hands and neck, mainly a result of hot cooking oil and kitchenware. Signs suggested that inappropriate administration of specific medications, and improper washing and disinfection of the burnt areas were the cause of these severe burn infections when questioned about it. This mostly seen in the rural districts of Thi-Qar province,

where burn patients receive care in inadequate or nonexistent hospitals. In contrast, 42.3% of the patients were male, mostly with injuries from sports and auto accidents. The underuse of antibiotics and other therapies led to infections these wounds caused. Patients seem to utilize painkillers more frequently than prescribed antibacterial medication. The previous studies underline the importance of establishing sanitation and prevention in medical facilities in order to manage the spread of P. aeruginosa as it is a major contributing factor to nosocomial infections globally, including in Iraq, especially in burn victims [17].

The susceptibility patterns of *P. aeruginosa* differ in terms of antibiotic resistance. Beta-lactam antagonists are a class of drugs that comprises cephalosporin's (Ceftazidime, Cefepime), carbapenems (Imipenem, Meropenem), penicillins (Piperacillin, Tazobactam\Piperacilli, Ticarcillin, Ticarcillin/Clavulanic acid), and the monobactam group (Aztronam). By a number of means, these antibiotics either stop *P.aeruginosa* from growing or destroy it. The pathogen is prevented from building their cell walls by their adhesion to Penicillin Binding Proteins (PBPs). This prevents Transpeptidas, the enzyme responsible for creating peptide bridges to the peptidoglycan layer, from functioning [18-19]. The data indicated that, out of the 10 medications used, Meropenem had the highest range of sensitivity (65.38%) against *P.aeruginosa* bacterial strains. According to the results of antibiotic susceptibility tests in previous studies, *P.aeruginosa* is resistant to the majority of antibiotics, including ciprofloxacin, amikacin and imipenem although found to be Cefotaxim-sensitive [20]. .Numerous risk factors related to the severity of the infection are linked to the development of resistance, and resistance itself is linked to higher fatality rates. Sensitivity was determined by measuring the antibiotic's inhibition zone, which has a diameter greater than 19 mm. commercially, dermatologists and burn victims alike are well known for using this antibiotic to treat a range of skin infections. Meropenem belongs to the carbapenem group and interacts with a variety of penicillin-binding proteins [21-22] stated that this medication works well against *P. aeruginosa*.

The second antibiotic with increased sensitivity was Cefepime (19.23%).This cephalosporin is a most commonly administered medication, even though hospital standards indicate that the resistance rate was higher due to the prevalence of resistant strains. Conversely, *P.aeruginosa* exhibited the highest level of antibiotic resistance to Imipenem and Piperacillin (96–100%), with an inhibitory zone that was less than 15–17 millimeters in diameter.

The use of an ELISA reader and 96- well micro titer plate, the biofilm-forming capacity of 26 *P. aeruginosa* isolates was evaluated. The outcomes showed that all isolates produced biofilms. These medical isolates developed three different kinds of biofilm: moderate producer (27%) weak producer (65.3%), and strong producer (7.7%). This was carefully compared to a study conducted in 2019, which found that *P. aeruginosa* isolates used in clinical settings have a high capacity to produce biofilms (90.74%) [23]. A significant and notable influence could also come from the initial number of cells that connected as well as variations in the quantity and caliber of auto inducers (quorum sensing signaling molecules) generated from each isolate [24].

P. aeruginosa contributes to the synthesis of several virulence factors that maintain the pathogen's capacity to cause death. The organism's resistance to several antimicrobial treatments is most apparent when it is seen developing in a biofilm. According to a recent study by [25] most of the *P. aeruginosa* cells were killed by mild dosages of antibiotics, while *P. aeruginosa* biofilms were found to be resistant to death even at larger antibiotic concentrations

V. Conclusion

Pseudomonas aeruginosa was demonstrated to be the most often isolated bacterium in burn and wound patients. When appropriate treatment is not received, the burn wound serves as an excellent culture medium for the colonization and growth of several endogenous and foreign microorganisms. According to antibiotic susceptibility testing, Meropenem and Cefapime were the most effective antibiotics against the majority of *P.aeruginosa* isolates. However, Penicillin and Impenim caused the greatest resistance. On the other hand, all *P. aeruginosa* isolates developed biofilms with varying rates.

VI. Acknowledgment

We would like to express our deepest appreciation to everyone who helped complete this study.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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