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Examining the biofilm patterns and antibiotic sensitivity of P. aeruginosa isolated from burn and wound patients in Iraq

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Abstract: ***Background:*** Pseudomonas aeruginosa is an opportunistic bacterium that causes infections via a variety of mechanisms, including as biofilm development and medication resistance. Aim: Examining the biofilm patterns and antibiotic sensitivity of P. aeruginosa isolated from burn and wound patients was the aim of the current study. ***Methods:*** At Samarra Hospital in Samarra, Iraq, 163 clinical specimens from burn and wound patients have been collected. The isolates were identified using both traditional techniques and the VITEK 2 technology. The microtiter plate (MTP) method was used to detect biofilm development, and the isolates underwent the antibiotic susceptibility test (AST).***Results:*** Forty P. aeruginosa isolates were obtained from 163 clinical specimens, of which eighteen (45%) were from burns and twenty-two (55%) were from wound swabs. The results revealed high rates of resistance to Colistin (98%), Piperacillin-tazobactam (90%), Ceftazidime (88%), and Meropenem (80%), as well as moderate levels of resistance to Tigecycline (73%) and Aztreonam (63%). Resistance rates to gentamicin (8%), amikacin (10%), and ciprofloxacin (34%) were low. The biofilm test revealed that 57.5% of the isolates formed strong biofilms, 20% formed intermediate biofilms, and 7.70% formed weak biofilms. ***Conclusion:*** Antibiotic resistance has been observed to positively correlate with the highly proportional potential for biofilm formation displayed by clinical isolates of P. aeruginosa.

Keywords: P.aeruginosa, biofilm, antibiotic, resistance, MTP, AST, Iraq .

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterial pathogen linked to many illnesses, including chronic wound infections, surgical infections, and lung disorders linked to cystic fibrosis (CF). To start and sustain infections, P. aeruginosa employs a variety of tactics, including as biofilm development, multidrug resistance, and antibiotic tolerance [1]. Antimicrobial resistance (AMR) poses a growing threat to public health, particularly in developing countries. Numerous factors, including improper prescribing practices, inadequate supply chain management, insufficient treatment courses, and unchecked over-the-counter antibiotic access, contribute to its expansion [2]. The extracellular matrix that the bacteria make on their own covers the highly ordered bacterial cell neighborhood known as the biofilm. Biofilms that may include one or more microorganisms include surface-attached biofilms, free-floating aggregates, surface-linked aggregates, flocs, and mats [3].

The components of P. aeruginosa's biofilm consist of at least three different exopolysaccharides: Psl, Pel, and alginate [4]. The purpose of this study was to examine the biofilm patterns and antibiotic susceptibility of P. aeruginosa isolated from burns and wounds..

MATERIAL AND METHODS

Specimens' collection

Specimens (163) of wound and burn infection were sampled during hospital visits. All specimens from male and female patients of Samarra Hospital, were collected under aseptic conditions, over a period of four months, from February 2023, until May 2023 with ages ranging from (22 to 64) years old.

Bacterial isolation and identification

Bergey's handbook of systematic bacteriology, second edition [5], served as the basis for the morphological characteristics and biochemical tests used to distinguish P. aeruginosa isolates. The identification was verified using molecular detection and the Vitek 2 compact system..

Antibiotic Susceptibility Test

Nine antibiotics (AK, ATM, CT, CAZ, CIP, GN, TI, MER, and TPZ) were investigated using the Kirby-Bauer disc diffusion method, which was created by [6] based on the Clinical and Laboratory Standards Institute CLSI 2022..

Assay for P. aeruginosa isolates' biofilm development

The micro-titer plate test was used to evaluate biofilm development in accordance with [7].

Statistical analysis

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RESULTS AND OBSERVATIONS:

The isolation and characterization of Pseudomonas aeruginosa.

This research includes 40 (24.53%) P. aeruginosa strains from 163 samples collected from patients of both sexes at Samarra Hospital in Samarra. Of the P. aeruginosa isolates, 55% came from wound infections and 45% from skin burns.

Gram staining and a compound microscope with a 100x objective lens were used to analyze Pseudomonas aeruginosa strains.

According to the cultural description, every P. aeruginosa isolate flourished on blood agar.

Pseudomonas Cetrimide agar on MacConkey agar.

Pseudomonas aeruginosa was identified using a number of biochemical methods. The findings are listed in Table 1. All of the isolates had positive results for oxidase, catalase, and citric acid consumption even though they were Gram-negative.

Table 1. Biochemical analyses of the isolates of P. aeruginosa**.**

Biochemical examination	Result
Test for catalase	Positive (+)
Test for citrate	Positive (+)
Test for oxidase	Positive (+)

The isolates that were cultivated on blood agar, pseudo-cetrimide agar, and MacConkey agar and produced positive biochemical test results were verified using the Vitek 2 compact system. According to the investigation's findings, P. aeruginosa was found in 40 isolates out of 163 samples (Table 2).

Table 2. identifying P. aeruginosa isolates using the vitek-2 method.

The origin of the isolates	The quantity of isolates	Percentages of isolates
Injury	22	55%
Burns	18	45%
Total	40	100%

The susceptibility of Pseudomonas aeruginosa to antibiotics is evaluated.

The susceptibility of P. aeruginosa to nine different types of antibiotics was evaluated using the disk diffusion method. Figure 1 shows how susceptibility patterns vary considerably across several drugs. In P. aeruginosa, 90% of the isolates displayed a range of piperacillin-tazobactam resistance patterns. Colistin (98%) was moderately resistant to tigecycline (73%), aztreonam (63%), and ciprofloxacin (34%), followed by ceftazidime (88%) and meropenem (80%). Based on the data, ciprofloxacin (CIP), often referred to as gentamicin, was shown to be more effective than the other antibiotics. But amikacin and gentamicin were the most beneficial drugs. Additionally, multiple-drug resistance was shown by all 40 P. aeruginosa isolates, suggesting that different isolates may have unique defense mechanisms against these drugs.

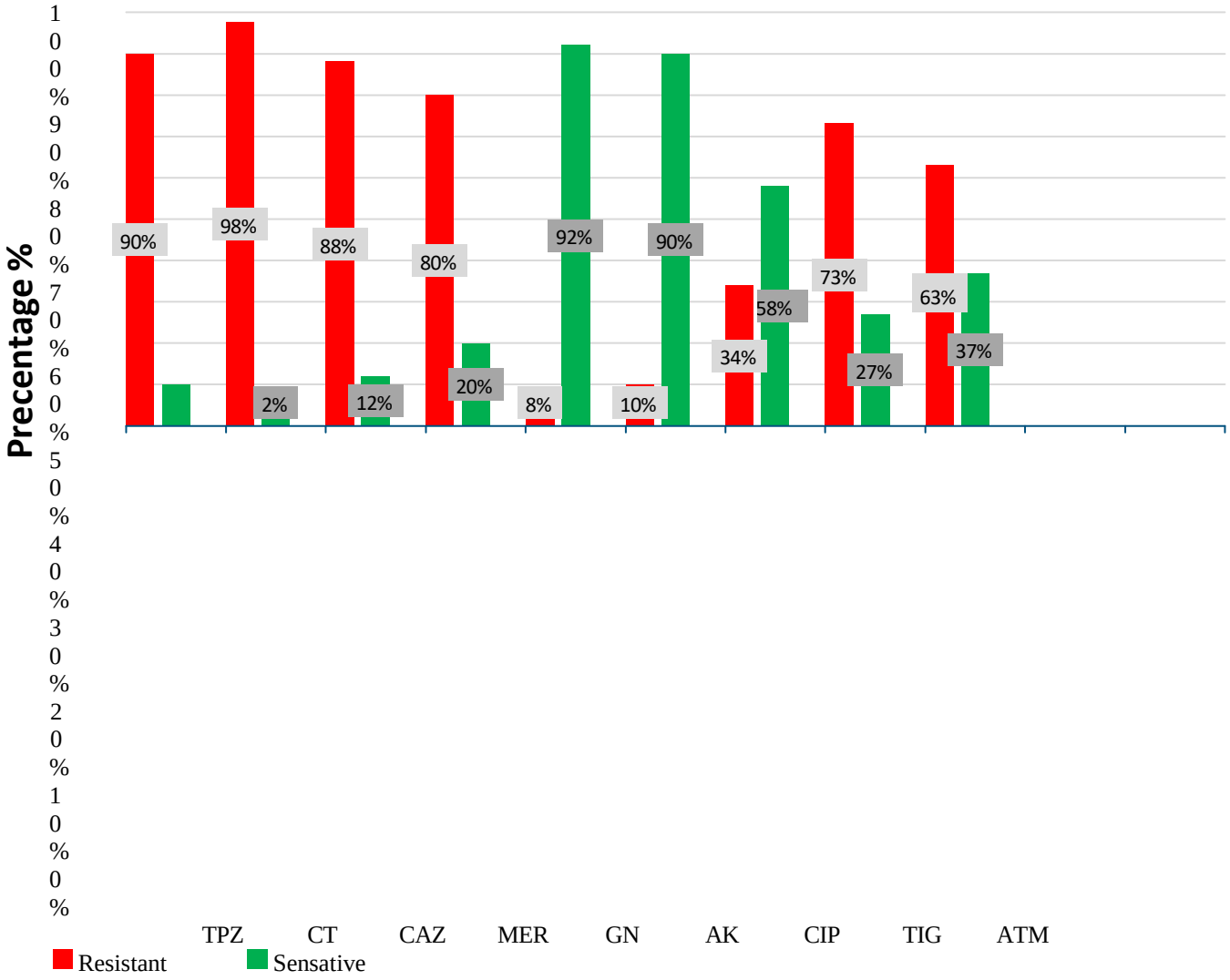

Figure 1. *P. aeruginosa* isolates susceptibility towards antibiotics.

Pipracillin tazobactam TPZ, Colistin CT, Ceftazidim CAZ, Meropenem MER, Gentamicin GN, Amikacin AK, Ciprofloxacin CIP, Tigecycline TIG, Aztreonam ATM

Biofilm formation assay for *P. aeruginosa* isolates

The ability of Pseudomonas aeruginosa strains that had previously been isolated to form biofilms was evaluated. Four categories were created from the results: no biofilm-forming capability, strong biofilm-forming capacity, moderate biofilm-forming capacity, and weak biofilm-forming capacity.

Only 57.5% of the P. aeruginosa isolates were able to form biofilms, according to Table 3, with the remaining isolates having a poor to moderate capacity to do so. These percentages are 20 and 22.5% for moderate and robust biofilm formation, respectively..

Table 3. The ability of P. aeruginosa isolates to produce biofilms**.**

kind of biofilm	The percentage %	The quantity of isolates
Powerful	57.5%	23
Average	20%	8
Inadequately	22.5%	9
non-producer of biofilm	0 %	0
	100%	40

DISCUSSION

Pseudomonas aeruginosa is a crucial bacteria that causes a variety of acute and chronic disorders. Although it seldom infects healthy hosts, it is an effective opportunistic pathogen that may cause serious infections in patients on mechanical ventilation, immunocompromised people, HIV patients, and cancer patients [8]. One of the main causes of eye infections in Iraq is P. aeruginosa [9].

In the current study, 40 isolates from 163 samples were identified as P. aeruginosa. Of these isolates, 22 (55%) had wound infections and 18 (45%) had burn infections. The isolates were identified using a variety of techniques, such as microscopic, cultural, biochemical, and Vitek 2 compact system testing. Gram staining revealed bacteria without spores, single bacteria connected to other bacteria, and smaller rods. These findings align with those reported by [10] and [11].

The cultural description revealed that all of the P. aeruginosa isolates developed large, flat colonies and produced a β-hemolytic ring with a grape-like flavor, in contrast to the majority of Pseudomonas isolates that displayed β-hemolysis on blood agar [12]. Pseudomonas agar has been used to identify P. aeruginosa since all colonies on this medium produce a blue-green or brown pigment that is exclusive to this bacterium. P. aeruginosa colonies are thought to be distinguished from other bacteria by their green color and the transition from a colorless to a light green medium [13,14]. MacConkey's agar is small, spherical, raised, rough, light or fruity in color, and lactose-free. It also smells good. These findings align with [15]. The bacteria seem to favor Pseudomonas cetrimonium bromide agar, a selective medium for Pseudomonas species. Furthermore, several strains produced light-emitting pigments like "pyocyanin."

Pseudomonas aeruginosa was identified using a number of biochemical methods. The findings are shown in Table 3-1. All of the isolates had positive results for oxidase, catalase, and citric acid consumption even though they were Gram-negative.

The oxygen-producing technique yielded significant results in two to three seconds. The bacteria possessed cytochrome c oxidase, which may utilize oxygen in the electron transport chain to produce H2O or $H2O_2$ for energy, as shown by the creation of oxidase. This was connected to the positive results. These findings align with [16]. All of the isolates passed the catalase test, which gauges the amount of the enzyme that converts hydrogen peroxide into oxygen and water and produces gaseous bubbles.

The susceptibility patterns of P. aeruginosa isolates in our study varied greatly across several drugs, as seen in Figures 3-4.

A research carried out in Iraq [17] found that the most common ampicillin-resistant bacterium, accounting for 81.1% of the cases, was pathogenic P. aeruginosa. Ceftriaxone and amoxicillin-clavulanic acid were also often resistant (78.4% and 75.6%, respectively). Other ampicillin-resistant bacteria included imipenem (45.9%), ceftazidime (51.4%), and ciprofloxacin (56.7%). At 40.5%, amikacin has the lowest rate of antibiotic resistance.

However, a study by [18] revealed that P. aeruginosa had a gentamicin and imipenem resistance rate of 0.0%. Additionally, gentamicin and imipenem resistance was found in 41.9% and 55.4% of additional P. aeruginosa isolates, respectively [19]. Azithromycin (66%) and aztreonam (74%), trimethoprim/sulfamethoxazole (64%), tigecycline (62%), meropenem, amikacin, tobramycin, and amoxicillin/clavulanic acid (58%), imipenem (56%), and ciprofloxacin and cefepime (44%) are all extremely resistant to P. aeruginosa isolates. A recent study by [20] confirmed this.

Numerous antibiotics, including aminoglycosides, quinolones, and β-lactams, have been shown to cause resistance in P. aeruginosa [21]. The development of several P. aeruginosa bacteriocides has limited the treatment of severe infections. Prompt and effective infection control strategies are necessary to reduce the occurrence of these disorders [22]. Because of its enhanced resistance to antibiotics, which may be partly due to the overuse of broad-spectrum antibiotics, P. aeruginosa has a selective survival advantage [23]. Although P. aeruginosa may form massive biofilms, another study found that the bacterium is resistant to treatment. This capacity to form biofilms not only forms a physical barrier but also prevents antibiotics from entering the biofilm [24].

The ability to form biofilms is closely linked to antibiotic resistance in P. aeruginosa strains that have been discovered from clinical specimens, and these bacteria are more likely to exhibit multiple resistant traits. Biofilms are difficult to remove because they are resistant to both medications and the host's defenses. P. aeruginosa's virulence is negatively impacted by biofilms, which are often the source of chronic infections [9,25].
Many techniques have been used or modified to understand the physiology, composition, and structure of biofilms. It is believed that a bacterium's capacity to produce biofilms is essential to its pathogenicity [26].

According to Chua's team [27], biofilm formation is a developmental process that involves adhesion and migration to surfaces, microcolonies, maturity, and ultimately dispersion. The formation of ESP, antibiotic resistance, flagellar rotation, type IV pili retraction, surface adhesive expression, secondary metabolite

production, and biofilm dissemination all have an impact on cyclic diguanylate's ability to regulate biofilm development [28]. This approach is believed to be the most effective, sensitive, and repeatable technique to identify biofilm formation by clinical strains of P. aeruginosa and has the advantage of being a quantitative comparison tool for the adherence of different strains [29].

[30] discovered a relationship between biofilm production and multidrug resistance, with 21/51 (41.17%) of the samples being non-MDR and 30/51 (58.83%) of the samples being MDR. Antibiotic resistance associated with biofilms is mostly caused by the biofilm matrix, which is made up of extracellular polysaccharides, DNA, proteins, lipids, and multidrug efflux pumps [31].

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