ORIGINAL ARTICLE

Assessment of Antibiotic Sensitivity of *Pseudomonas aeruginosa* Isolated from Different Clinical Samples

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ABSTRACT

Background: *One of the most significant threats to global public health and development is antimicrobial resistance (AMR). Pseudomonas aeruginosa (P. aeruginosa) is directly associated with the issue of AMR and is a major cause of both chronic lifelong diseases and life-threatening acute infections. In Iraq, the rising prevalence of carbapenem-resistant P. aeruginosa poses a substantial threat to public health.* ***Objectives:*** *This study aimed to compare and evaluate the results of drug sensitivity testing for P. aeruginosa isolates from different clinical samples.* ***Methodology:*** *Different samples were collected from clinical cases attending Al-Sadder Medical City Hospital, Iraq, these were wounds, otitis media, urinary tract infections (UTIs) and vaginitis. P. aeruginosa was isolated and identified by the routine bacteriological methods and validated by using the VITEK® 2 system. The antibiotic sensitivity of the isolates was determined by AST techniques.* ***Result:*** *One hundred P. aeruginosa strains were identified. The results indicated that the majority of P. aeruginosa showed high resistance to Amoxicillin + Clavulanic acid (AMC), In contrast, there was significantly less resistance to (IPM) Imipenem.* ***Conclusion****: Imipenem (IPM) and (MEM) Meropenem were the promising antimicrobial agents used to treat the P. aeruginosa infections.*

INTRODUCTION

Antimicrobial resistance (AMR) has been materialized as one of the most serious worldwide public health risks of the twenty-first century[1]. Antimicrobial drug resistance (AMR) arises from the evolution of microorganisms, including bacteria, fungi, viruses, and parasites, to medications that are often used to treat illnesses of this nature, such as antibiotics [2]. The major mechanisms of resistance include antimicrobial modification or destruction, reduced target access, and target change. These processes may be inherent in bacteria or acquired from other microbes [3]. Bacterial infections cause an estimated 7.7 million fatalities annually. Access to appropriate antibiotics when indicated increases life expectancy, decreases disability, lowers health-care costs and allows for access to other life-saving medical breakthroughs[4]. However, quick and on-site antibiotic susceptibility testing (AST) is critical for making optimal antibiotic selections to prevent AMR [5].

Model microorganisms for pathogenicity and social features of bacteria is *P. aeruginosa*, an opportunistic pathogen that is Gram-negative. It is the most commonly isolated pathogen in bronchiectasis and is associated with worse outcomes[6]. Antibiotic resistance mechanisms and the organism's tendency to build multicellular biofilms provide significant challenges to the treatment of infections in patients with immunosuppressive and chronic diseases, since they are a major cause of sickness and mortality[7]. *P. aeruginosa* continues to be a major source of infectious illness, mostly because of antibiotic resistance, despite developments in antimicrobial therapy and even the introduction of several potent vaccinations. Even while there are easily available treatments for it, they are not always effective, especially for specific patient groups and drug-resistant strains. The WHO and CDC both designate multi-drug resistant *P. aeruginosa* infections as important global problems[8]. So, Multi-Drug Resistant (MDR) *P. aeruginosa* is a major health problem in burn infection. Currently, carbapenems are used to treat the majority of pseudomonas infections. The broad-spectrum carbapenem antibiotic meropenem works well against bacteria that are Gram-positive and Gram-negative. It works by entering bacterial cells with ease and preventing the synthesis of necessary cell wall components, which kills the cells[9]. *Pseudomonas*

aeruginosa is an extremely troublesome drug-resistant bacterium in the world today. We are already encountering an increase of pan-drug-resistant *P. aeruginosa* clones within healthcare facilities.

Antimicrobial susceptibility testing is a critical step in identifying effective antimicrobial medicines to treat infectious illnesses. The procedures employed in diagnostic microbiology laboratories are constantly evolving. Disc diffusion and broth micro dilution are traditional phenotypic procedures that require a long turnaround time and are labor-intensive, yet they are still extensively used as the gold standard[10]. Conventional phenotypic AST techniques, such as Kirby-Bauer disc diffusion or broth micro dilution, measure bacterial growth to evaluate phenotypic susceptibility to antimicrobials. Phenotypic approaches have an edge over genotypic methods in terms of proving a pathogen's qualitative and quantitative antimicrobial susceptibility. However, most traditional manual procedures, such as disc diffusion, agar or broth dilution, and the concentration gradient approach, such as E-test, are time-consuming and need a culture inoculum as well as visual growth evaluation[11]. The objective of this work is to compare and evaluate the results of drug sensitivity testing for *P. aeruginosa* isolates from different clinical samples and determine the optimal treatment.

METHODOLOGY

Study Design and Specimens Collection

A total of 100 clinical samples have been collected between December 2023 to January 2024 from wounds, otitis media, urinary tract infections (UTIs) and high vaginal swabs from patients attending to AL sadder Hospital in AL_Najaf Province, Iraq. The specimens were stored in transport media and transported to laboratory for culture and identification. This study was conducted at Kufa University / College of Science / Department of Pathological Analyses/ Advanced Microbiology Laboratory. The age range of the participants was between 15 and ≥77 years. All participants were clinically diagnosed as positive for *P. aeruginosa* and were processed in accordance with the established standard operating procedures. To prevent contamination, sterile, disposable glass containers (50 mL) were used to collect clean-catch midstream urine specimens from the patients. Additionally, sterile cotton swabs were employed to collect wounds, ears and cervical samples.

Identification and Antimicrobial Susceptibility Test of *P. aeruginosa:*

P. aeruginosa isolates were originally identified based on their morphological features by inoculation on MacConkey and Blood agar media (Oxoid/ paris, France) and incubated at 37 °C for 24 hr. then colonies were sub cultured on Cetrimide agar medium (Biolife/Italy) as selective for *P. aeruginosa* later validated using the VITEK® 2 system (BioMerieux, France) following to the manufacturer's guidelines[12]. Antimicrobial susceptibility testing was conducted on Mueller-Hinton agar (Himedia, India) employing the disk diffusion (Kirby Bauer's) method in accordance with Clinical and Laboratory Standards Institute CLSI recommendations[13]. Patients of both genders were eligible if they were over the age of 14 years, had positive microbiological evidence of *P. aeruginosa* isolated from multiple samples, and agreed to participate in the study.

RESULTS

Table (1) illustrated that a total of 100 *P. aeruginosa* isolates, including 5 strains isolated from cases of Otitis media, 75 (75%) urine samples from patients suffering from urinary tract infections (UTI), and 10 strains (10%) from wounds and vagina, each.

Table 1: Frequency of *P. aeruginosa* isolates from different samples

Clinical condition	sample	No.	(%)
UTI	urine	75	75
Wounds	swap	10	10
Vaginitis	swap	10	10
Otitis media	swap	5	5

Demographic characteristics for 20 patients attending to Al-Sadder Medical City Hospital in AL-Najaf province revealed that male were 20 % and female were 80 %, as shown in figure (1).

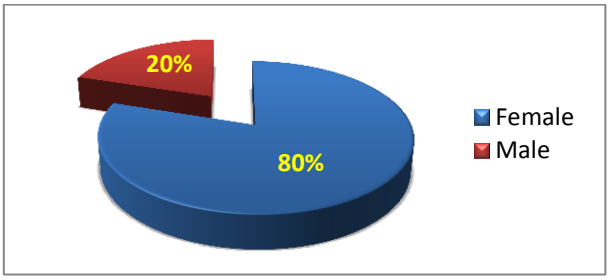

Fig. 1: Distribution of patients according to gender

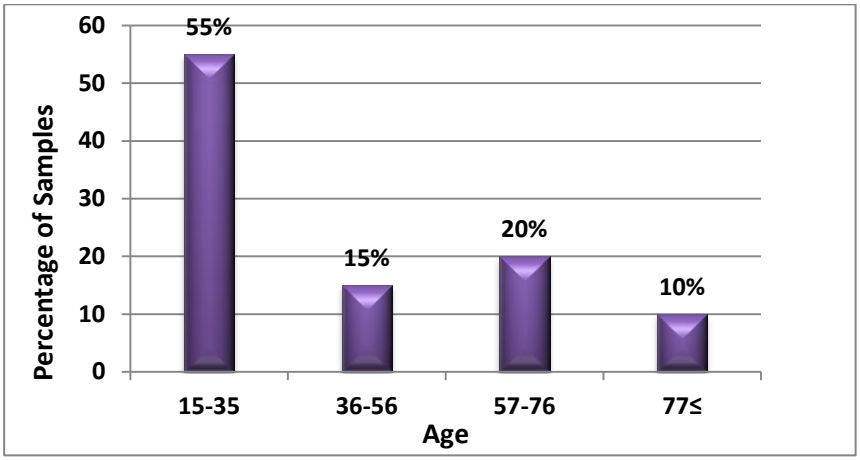

Fig. 2: Distribution of patients according to age group

From our study *P. aeruginosa*-infected individuals categorized based on their age ranges revealed 4 categories as shown in figure (2). The highest frequency of patients was in the age group 15-35 years followed by 57-76, 36-56 years' age groups and lowest frequency were ≥ 77 age group they were recorded (55 %, 20 %,15%, 10 %), respectively.

Following the guidelines provided by the Clinical and Laboratory Standard Institute, the antibiotic susceptibility on Mueller-Hinton agar was assessed using the Kirby-Bauer disc diffusion technique[13]. AMC Amoxicillin + Clavulanic Acid caused most *P. aeruginosa* bacterial isolates to exhibit strong resistance, according to the findings of the antibiotic sensitivity test, (PRL) Piperacillin and (CRO) Ceftriaxone with percentage 90%, 80%, 70%, respectively, while the bacterial isolates showed a varying resistance to each of (TMP)Trimethoprim, (AK) Amikacin 35 %, (LEV) Levofloxacin 25%, (NOR) Norfloxacine 25%, (CIP) Ciprofloxacin (20%), (MEM) Meropenem15%, and it was less resist to Imipenem (IPM) 10% as shown in table 2 and figure 3.

Table 2: Resistance patterns of *P. aerogenosa* isolates against different antibiotics

Antimicrobial Category	Antimicrobial Agent	Disk Content Potencies	Resistance Rate (%)		
			R	I	S
Aminoglycosides	AK	30 µg	35	15	50
Aminopenicillin	AMC	10 µg	95	5	0
Fluoroquinolones	CIP	5 µg	20	0	80
	LEV	5 µg	25	10	65
	NOR	5 µg	25	0	75
3rd generation Cephalosporin	CRO	30 µg	70	10	20
Carbapenems	IPM	30 µg	10	10	80
	MEM	10 µg	15	10	75
Penicillins	PRL	75 µg	80	0	20
Sulfonamides-Trimethoprim	TMP	5 µg	65	5	30

S: Sensitive, **I**: intermediate, **R**: resistant

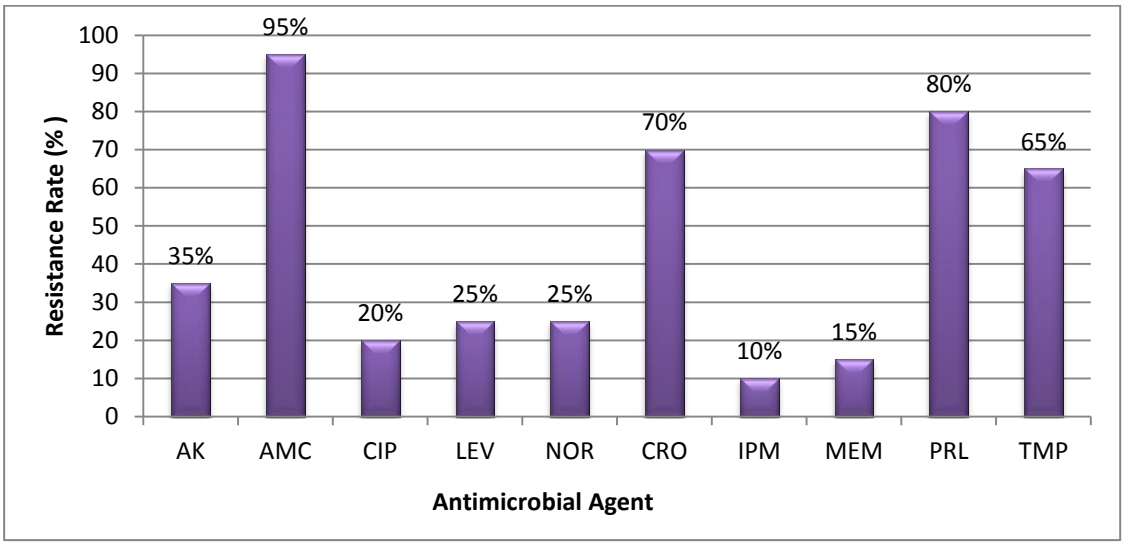

Fig. 3: Resistance rate percentage of *P. aeruginosa* (*n* = 20). AK: Amikacin, AMC: Amoxicillin + clavulanic acid, CIP: Ciprofloxacin, LEV: Levofloxacin, NOR: Norfloxacine, CRO: Ceftriaxone, IPM: Imipenem, MEM: Meropenem, PRL: Piperacillin, TMP: Trimethoprim

Statistical Analysis

The results obtained from the experiments were entered into a database and evaluated statistically using the statistical package for social science (SPSS) version 20 statistical software for Microsoft Windows and an abstract was presented using the descriptive statistics such as means and percentages.

DISCUSSION

In our study, a high prevalence of Pseudomonas infections was found in the female patients more than males similar to a study done in Kirkuk City, Iraq[14], This could be caused by a variety of host factors, anatomical predisposition, or urological mucosal adhesion to the mucopolysaccharide lining, but it disagrees with the result of Bindu and Saikumar, [15] who reported *P. aeruginosa* occurrence was predominant in males (54.05%).

Out of 100 isolates of *P. aeruginosa* 10% were isolated from wounds that relatively agree with the result of Jafar et al.[16] who recorded 11.4% from wound samples. While Olayinka et al.[17] in Zaria recorded 51.1% from urine. They calculated that. *P. aeruginosa* rates vary throughout researches, and these variations may be attributed to the kind of clinical specimens used, the hospitals used, the population under study, geographic areas, and variations in sanitary procedures.

Furthermore, our findings demonstrated that *P. aeruginosa* rates ranged from 10% in the senior age group to 55% in young individuals (ages 15 to 35) which agree with Hasan et al.[14], but these results disagree with studies in Ethiopia and in Al-Sulaimania city, Iraq[18]. Moreover, the results by Elsaid et al,[31] who reported no significant difference detected between isolates of *P. aeruginosa* in relation to gender or age.

The buildup of mixed biofilms on the artificial surface of the catheter or other implant was the primary cause of the recurring UTIs. Adherent microorganisms, their extracellular products, and host components deposited on the catheter form a biofilm on an indwelling urinary catheter. Antimicrobial treatment is not effective in treating chronic infections caused by biofilm on urinary catheters[19].

P. aeruginosa was present in healthcare-associated infection and recent urinary tract instrumentation and chronic indwelling urinary catheters[20]. In UTI *P. aeruginosa* is considered a key uropathogens with high prevalence in reported cases worldwide[21].

On the other hand, one of the most often identified bacteria in wounds is *P. aeruginosa*, a pathogen that is a normal component of human skin microflora [22]. Skin and soft tissue infections caused by *P. aeruginosa* range in severity from immediately fatal to benign (such as cellulitis and post-surgical infections). One of the most frequent pathogens seen in cellulitis, surgical site infections (SSI), post-trauma infections, and infections of chronic decubitus ulcers is *P. aeruginosa*[20]. Adeyemi et al.[23] reported 61 (25.7%) isolates of *P. aeruginosa* recovered from 237 wound samples, and a high isolation rate from surgical sites of caesarian sections (CS). The study was close to the results of Khan et al.[24] who reported that the isolation rate of *P. aeruginosa* was (26.6 %) from wounds.

P. aeruginosa mainly affects people with impaired immune systems and causes serious infections,

especially in healthcare settings[25]. It is naturally resistant to several antibiotics. It can acquire resistance to almost all antibiotics by mutating its chromosomal genes or through horizontal transfer. This has led to the worldwide dissemination of a few specific high-risk clones that are multidrug-resistant (MDR) or extensively drug-resistant (XDR) [26].

The current data are close to the result of Hassan et al.[27] who reported that *P. aeruginosa* had high resistance towards Ceftazidime with percentage 90 % and showed varying resistance to Ciprofloxacin (50%) Amikacin (40%) and Pipracillin (70%). On the other hand, Jubair et al.[28] recorded that Gram-positive and Gram-negative pathogens were sensitive to carbapenems [imipenem and/or meropenem]. Our data were near to the studies of Abass et al.[29] who recorded the antibiotic sensitivity test for *P. aeruginosa* isolates showing high resistance towards Ciprofloxacin and Piperacillin with percentage of 61.1%, 88.8 % respectively; while it was less resistant to Imipenem (IPM) 16.6 %.

When treating individuals who have contracted *P. aeruginosa*, cephalosporins are essential. Nevertheless, β-lactam resistance in several isolates of these bacteria makes infection treatment more difficult and results in inferior outcomes[30]. Current outcome demonstrated carbapenems, such as imipenem and meropenem, are thought to offer a therapeutic option for treating *P. aeruginosa* infections; nonetheless, an alarming rise in drug resistance has been seen, particularly when these medications are used carelessly in hospital settings. Furthermore, a rise in carbapenem-resistant microbes during the pandemic was seen, corroborating the rise in resistance observed in the past few years.

In our study, we found that, the resistance to third-generation cephalosporins was higher (70%) than in a prior research conducted in Iraq (41.2%) and other countries in the area including; (47.1%) Yemen, (66%) Libya and (68%) Egypt but met with a study from Tunisia (70%), and relatively comparable to those reported in Basra, Iraq[32,34].

Finally, the spread of antibiotic-resistant bacterial strains results in a decrease in the effectiveness of medications due to several factors such as inadequate knowledge, non-adherence by patients, indiscriminate use of antimicrobial antibiotics, and unsanitary conditions[35].

CONCLUSION

In the present study, *P. aeruginosa* isolated from urine was obtained at a high rate compared with others samples and females have a high incidence compared with male patients. On the other hand, *P. aeruginosa* isolates exhibited substantial high resistance towards (AMC) Amoxicillin + clavulanic acid, and it was less resistant to Imipenem (IPM) based on our study. Therefore, to address the rising issue of resistance, a combination of alternative antibiotics will be needed to be used.

Ethical Approval Declaration

The ethical approval was obtained from the Ministry of Health, Iraq. Agreement was also obtained from all patients with filling questioners from each patient.

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