PSEUDOMONAS AERUGINOSA DRUG-RESISTANT ISOLATES IN ICU PATIENTS WITH A SPECIAL PROFILE RELATED TO B-LACTAMASE

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ABSTRACT

Objective: In addition to assessing the phenotypic development of biofilms and beta-lactamases, in the current study, *Pseudomonas aeruginosa* isolates from intensive care units (ICU) patients with prevalent drug resistance are being evaluated.

Methods: Standard CLSI recommended protocols were utilized to detect beta-lactamases such as extended-spectrum β-lactamases (ESBL), metallo beta-lactamase (MBL), and AmpC, and a standard tissue culture plate method was used to detect the development of biofilms in the study.

Results: 51 (36% of 142) Most *P. aeruginosa* isolates with multidrug resistance (MDR) phenotypes were discovered in endotracheal aspirates, followed by BAL fluid, pus, and blood, and the least number were found in urine samples. Of the 142 isolates of *P. aeruginosa*, 132 (93%) produced biofilms, while the remaining 10 (7%) did not. Our study reported that of the 51 MDR isolates, 14 (27%) produced ESBLs, 9 (18%) MBLs, and 5 (10%) AmpCs. Of the 51 MDR phenotypes, 48 (94%) of the isolates produced biofilm, and 3 (6%) did not. There were 142 clinical isolates of *P. aeruginosa*, 14 (27%) of which came from female patients, and 37 (73%) from male patients. Imipenem (10.4%) and meropenem (12.5%) showed the lowest rates of resistance, whereas gentamicin (60.4%) and amikacin (66.6%) had the greatest rates. There was no resistance to colistin or polymyxin B.

Conclusion: For the best antibiotic choice and management of acute disease in ICU patients, all *P. aeruginosa* bacterial isolates should undergo routine beta-lactamase profiling and biofilm formation.

Keywords: Multidrug-resistant, Biofilm, Beta-lactamases, Intensive care unit.

INTRODUCTION

Infections are more likely to happen in intensive care units (ICU) because the people there are so vulnerable (lower host defenses deregulating the immune system). In addition, numerous clinical procedures and the use of intrusive technologies that deform patients' anatomical integrity-protective barriers are the root causes of these illnesses. In addition, a variety of medications (tranquilizer, muscle relaxant drugs, etc.) might impair one's capacity to cough and swallow, making one more susceptible to infections such as pneumonia, or alter their body's normal balance of non-pathogenic microorganisms [1].

Public health is at risk due to the possibility of antibiotic resistance [2]. Among resistant infections, *Pseudomonas aeruginosa* is of special concern due to its frequent ability to cause a significant rate of death in patients undergoing intensive care units. *P. aeruginosa* is not only invulnerable to a number of antimicrobials, but it also has a high rate of rapid development of resistance to newly developed antimicrobials. The first bacteria to show multidrug resistance (MDR) characteristics is *P. aeruginosa* [3]. MDR isolates are those that have three or more antimicrobial class resistances [4]. One prominent source of nosocomial infections is *P. aeruginosa*, and it accounts for between 10% and 20% of all severe infections among patients undergoing treatment in intensive care units [5].

Beta-lactams and carbapenems are rendered ineffective by MDR phenotypes that can produce different types of beta-lactamases [6]. Midway through the 1980s, *P. aeruginosa* strains that produced extended-spectrum β-lactamases (ESBLs) were discovered in Europe, while metallo beta-lactamase (MBL)-producing strains were first discovered in Japan in 1991. They then quickly spread to various parts of the world [7].

P. aeruginosa is able to manufacture enzymes and is inherently resistant to antibiotics. The widespread occurrence of beta-lactamase resistance is due to beta-lactamases. These beta-lactamases break down the distinctive beta-lactam ring's amide bond which manifests the antibiotic as ineffective [8].

As MBL-producing *P. aeruginosa* emerges in ICUs, there is strong selection pressure because of the high utilization of wide-ranging antibiotics in ICUs. The number of MBL-producing *P. aeruginosa* that is circulating in intensive care units is rapidly increasing. As a result, the competing flora is eliminated, which leads to the selection of strains that are multidrug-resistant [9].

Biofilms are sessile communities created by bacteria and are defined by their tightly bound cells to a substrate or to one another. They are incorporated in an extracellular polymeric material matrix and have altered gene transcription, and growth rates (EPS) [10]. Many pathogenic microorganisms are infected by biofilms, which can cause long-term infections. They typically cause nosocomial infections and are linked to a variety of medical conditions, such as dental, urogenital tract, upper respiratory tract infections, and indwelling medical devices [11,12].

Aim
In addition to assessing the phenotypic development of biofilms and beta-lactamases, in the current study, *P. aeruginosa* isolates from ICU patients with prevalent drug resistance are being evaluated.

METHODS

The study was conducted in the Microbiology Department from March 2021 to February 2022. Blood, urine, sputum, wound swabs, catheter tips, tracheal aspirate, pus, and other body fluids collected from patients

at the Venkateshwara Institute of Medical Sciences Hospital in Gajraula, Uttar Pradesh, for routine diagnostic workup were processed according to the normal protocol. Before beginning the study, permission from the Institutional Ethics Committee was obtained (Reference No. SVU/VIMS/). A documented informed consent form was signed by each participant in the study. To identify and describe the bacterium, conventional microbiological techniques were employed. The study utilized 142 *P. aeruginosa* clinical isolates from diverse sources, which was carried out over the course of a year. *P. aeruginosa* non-repetitive isolates that were isolated from patients were chosen for further analysis.

Antibiotics susceptibility testing

All isolates underwent AST using the conventional agar diffusion assay on Mueller–Hinton agar. All clinical isolates were treated with the following antibiotics: Ceftazidime (30 µg), cefepime (30 µg), ceftazidime and clavulanic acid, piperacillin-tazobactam (100 µg)/10 µg, imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and levofloxacin. By measuring the widths of the generated zones and comparing them to CLSI recommendations, the results of the antibiotic susceptibility tests were interpreted.

ESBL detection procedure

The development of ESBLs was evaluated in isolates that were not susceptible to any third-generation cephalosporins, at least.

Metallo Beta-lactamase (MBL) detection method

The imipenem-EDTA double disk synergy test, which was described by Lee *et al.* was used to confirm clinical isolates with imipenem resistance [13]. CLSI claims that the test organism was spread using grass culture on MHA plates. The blank disc containing 10 µL of EDTA was positioned 10 mm from the edge of the imipenem 10 µg disc for incubation at 37°C over night. The amplification zone surrounding the IMP+EDTA disk influenced whether the results were favorable (7 mm).

AmpC detection method

Double disc synergy test

On an MHA plate, a lawn culture of bacteria was created. Two antibiotic disks were positioned on MHA plates with a center-to-center spacing of 24 mm, and incubation was at overnight 37°C. One antibiotic disk contained cefoxitin (30 ug), while the other contained cloxacillin (230 ug). A zone difference of ≥4 mm between cefoxitin and cefoxitin+cloxacillin disk was considered positive AmpC test results [14].

Biofim detection method

Tissue culture plate method

The standard tissue culture plate method followed the guidelines established by Christensen *et al.* At 570 wavelength optical density, ELISA readers were used to read [15] optical densities. A three-OD average was calculated following triplicate testing. According to Table 1, each well's OD values were interpreted.

RESULTS

The microbiology lab isolated, identified, and tested the AST patterns on each sample it received. *P. aeruginosa* isolates of 142 different types were found overall in clinical samples obtained over the course of a year. 51 (36% of 142) The majority of *P. aeruginosa* isolates with MDR phenotypes were found in endotracheal aspirates, then in BAL fluid, pus, and blood, and the least number was discovered in urine samples. As demonstrated in Table 2, the bulk of the positive isolates from these clinical samples came from ET aspirates and pus samples. In our investigation, a quantitative tissue culture plate technique was used. Of the 142 isolates of *P. aeruginosa*, 132 (93%) produced biofilms, while the remaining 10 (7%) did not, as shown in Fig. 1. In accordance with Fig. 2, of the 51 MDR isolates, 14 (27%) produced ESBLs, 9 (18%) MBLs, and 5 (10%) AmpCs. Of the 51 MDR phenotypes, 48 (94%) of the isolates produced biofilm and 3 (6%) did not. *P. aeruginosa* clinical

isolates totaled 142; 14 (27%) of them came from female patients, whereas 37 (73%) came from male patients. As shown in Table 3, gentamicin (60.4%) and amikacin (66.6%) had the highest rates of resistance, followed by ciprofloxacin (56.2%), piperacillin (54.1%), and aztreonam (50%) with imipenem (10.4%) and meropenem (12.5%) having the lowest rates of resistance. Bacterial isolates did not exhibit any polymyxin B or colistin resistance.
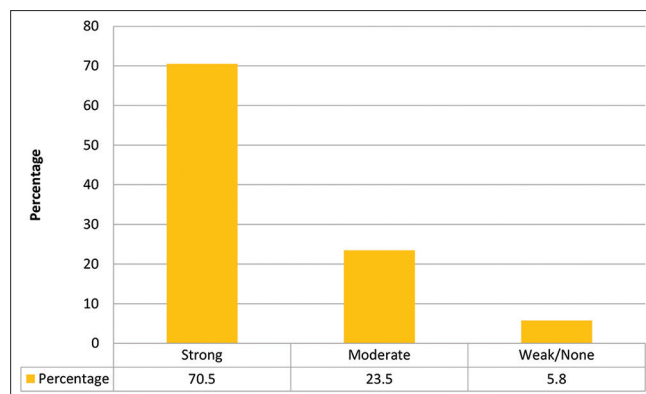

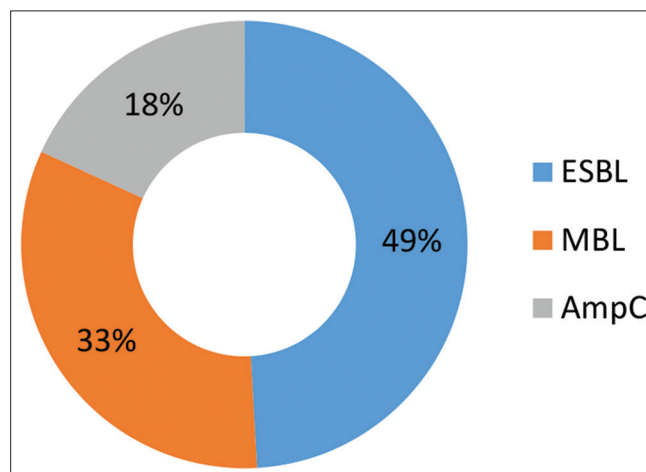**Fig. 1: Prevalence of biofilm production in MDR *Pseudomonas aeruginosa***

Fig. 2: Prevalence of different beta-lactamases in MDR *Pseudomonas aeruginosa*

Table 1: Bacterial adhesion classification using the tissue culture plate method

Mean OD value	Adherence	Biofilm formation
<0.120	Weak	Weak
0.120–0.240	Moderate	Moderate
≥0.240	Strong	High

Table 2: On the basis of clinical samples, the distribution of MDR *P. aeruginosa* isolates

Clinical sample types	Multi Drug resistant *P. aeruginosa* n (%)
ET aspirate	18 (35.2)
Pus	10 (19.6)
Urine	03 (5.88)
Blood	08 (15.68)
BAL fluid	12 (23.5)
Total	51

P. aeruginosa: Pseudomonas aeruginosa

Table 3: *Pseudomonas aeruginosa* drug resistance pattern pertaining to the production of biofilms

Antibiotics (Potency in mcg)	Producers of biofilm n (%)
Colistin (10 mcg)	Nil
Amikacin (30 mcg)	32 (66.6)
Pipracillin Tazobactam (100 mcg/10 mcg)	17 (35.4)
Pipracillin (100 mcg)	26 (54.1)
Gentamicin (10 mcg)	29 (60.4)
Meropenem (10 mcg)	6 (12.5)
Imipenem (10 mcg)	5 (10.4)
Ciprofloxacin (5 mcg)	27 (56.2)
Ticarcillin/Calvulinic acid (75 mcg/10 mcg)	20 (41.6)
Azetronam (30 mcg)	24 (50)
Cefepime (30 mcg)	15 (31.2)
Ceftazidime (30 mcg)	19 (39.5)
Polymyxin B (300 Units)	Nil

DISCUSSION

Treatment of these resistant versions of the common pathogen MDR *P. aeruginosa* has proven to be a very challenging task for clinicians in recent years. These phenotypes make use of a variety of molecular mechanisms that result in resistance to these antibacterial medications. Several factors, such as the bla genes insertion into integrons, the creation of various beta-lactamases, and the failure of the porin genes or modify target sites, contribute to drug resistance [16]. Our research discovered a 36% prevalence rate for MDR *P. aeruginosa*, which is lower than Gill *et al.*, 50% prevalence rate from their study [17]. MDR *P. aeruginosa* was found to be prevalent in Iran at a rate of 54.5%; Saderi and Owlia claim that the prevalence rate is 16.5%, despite the fact that Mirzaei *et al.* in Tehran discovered the opposite [18,19]. Multidrug-resistant *P. aeruginosa* morphologies were most frequently obtained from endotracheal aspirates in the current investigation, followed by BAL fluid, pus, and blood samples. Gupta R. *et al.* reported similar outcomes as well [20]. Urine and wound samples were, however, taken into consideration for the bulk of the positive isolates, according to Gill *et al.* In the current investigation, out of 142 *P. aeruginosa* isolates, 132 (93%) produced biofilms and 10 (7%) did not. These outcomes matched those of Gupta *et al.* Bankole *et al.* reported a greater percentage of biofilm producers (85.72%) [21]. Saha *et al.* with Kulkarni *et al.* reported lower results, respectively, of 29.1% and (26.83%) [22,23]. According to Shrivastava *et al.*, our research showed that ESBL, MBL, and AmpC were all present in 14, 27, and 18% of people, respectively [24]. However, according to Sarkar *et al.*, the prevalence of ESBL, MBL, and AmpC was 36.8%, 12.9%, and 12.4%, respectively [25]. *P. aeruginosa's* production of numerous beta-lactamases poses a therapeutic challenge, and immediate legislative action is required to stop the spread of these kinds of resistant bacteria. Globally, there is a problem with bacterial drug resistance to routinely used antibiotics, but drug resistance is particularly problematic in underdeveloped nations because of how easily accessible over-the-counter antibiotics are there.

CONCLUSION

To stop the establishment of drug-resistant *P. aeruginosa*, strict antimicrobial policies and regular antimicrobial resistance surveillance programs should be implemented. The spread of such resistant microorganisms must be stopped by strict regulations. Anti-multidrug-resistant *P. aeruginosa* variations nevertheless show good sensitivity to colistin and polymyxin B. All *P. aeruginosa* bacterial isolates should undergo routine beta-lactamase profiling and biofilm creation for optimal antibiotic choice and management of serious illness in ICU patients.

AUTHOR' CONTRIBUTIONS

Dr. Sanjeev H. Bhat and Dr. Deepali Gupta established the conceptual framework, designed the draft, and conducted the data analysis. Dr. Deepali Gupta also participated in the gathering and analysis of the data, while Dr. Rajesh Kumar wrote the manuscript and oversaw the final editing.

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