International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(1): 569-572
www.biochemjournal.com
Received: 18-10-2023
Accepted: 23-11-2023

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Antimicrobial drug resistance profiling of *Pseudomonas aeruginosa* isolated from nosocomial infections

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1h.440

Abstract
As the second most important opportunistic pathogen, *P. aeruginosa* is thought to account for 10–11% of all nosocomial infections. It is associated with several nosocomial infections, such as burns and wounds, pneumonia, urinary tract infections, and cystic fibrosis. Out of 65 samples, ten *P. aeruginosa* isolates were recovered from different hospital-acquired infections in different species. After molecular-level conformation, the antimicrobial sensitivity of 23 drugs against all isolates was evaluated by performing a Cutler sensitivity test. Polymyxin-B, gentamicin, ceftazidime, meropenem, carbapenems, and ticarcillin were found to be 100% effective against all *P. aeruginosa* isolates. Aminoglycosides (i.e., amikacin and kanamycin) showed susceptibility to 80% of the isolates. Only half of the isolates exhibited sensitivity to chloramphenicol. Whereas cefuroxime, cefotaxime, amoxicillin, ampicillin, vancomycin, tetracycline, clindamycin, erythromycin, and trimethoprim exhibited 100% resistance. In conclusion, all isolates of *P. aeruginosa* recovered from nosocomial infections showed evidence of multidrug resistance. Out of 23 tested antibiotics, the *P. aeruginosa* isolates exhibited either a complete or partial drug resistance phenotype against 17 antimicrobal agents, which eventually can cause prolonged persistence infections and treatment failure.

Keywords: *P. aeruginosa*, antibiotic, antimicrobial, nosocomial infections

Introduction
Pseudomonas aeruginosa is a gram-negative bacteria that was initially identified by Carle Gessard, a chemist, in 1882 (Silhavy, 2010; Currans B *et al*., 2004) [17, 7]. This organism is found in a variety of habitats, such as soil, marine environments, and plants that can survive even without oxygen and withstand extreme temperatures up to 42 °C (Bjarnsholt and Givskov, 2007) [3]. As per the 2017 WHO report, Acinnetobacter, Pseudomonas, Enterobacteriaceae (carbapenem-resistant), and other ESBL-producing bacteria pose the biggest threat to human health. To combat these organisms, new medicines are critically needed. Among them, *P. aeruginosa* is regarded as the second most significant opportunistic pathogen, with a 10–11% share in all nosocomial infections. It is linked to pneumonia, urinary tract infections, problems from clinical burns and wounds, and cystic fibrosis (Branda *et al*., 2005; Mesaros *et al*., 2007) [4, 12].
Multidrug-resistant (MDR) *P. aeruginosa* infections were not easily treated which ultimately caused longer hospital stays and raised mortality rates. Pseudomonas exhibits multidrug resistance through a variety of mechanisms, including genetic mutations, the overexpression of multidrug efflux pumps, the formation of biofilms, the harboring of dormant persister cells, and the horizontal transfer of drug resistance genes (Levy, 1998b; Mazel and Davies, 1999; Poole *et al*., 1993) [8, 11, 13]. Therefore, in the current study, we investigated the antimicrobial drug resistance profile of *P. aeruginosa* isolates from nosocomial infections.

Materials and Methods
Isolation of *P. aeruginosa* and characterization
Based on cultural characteristics and biochemical profiling, *P. aeruginosa* was first isolated from hospitalized animals (Cowan and Steel, 1974; Quinn *et al*., 1994) [14]. Whereas the PCR amplification of 16S rRNA is also carried out for molecular-level conformation, as described by Clarridge *et al*. (2004) [5].

Antibiotic sensitivity test

The antibiogram of the isolates was ascertained using the Baur *et al*. (1966) [2] technique. Briefly, the isolates were inoculated for eighteen hours at 37 °C in 5 ml of sterile nutritional broth. After that, the opacity was adjusted using a normal saline solution to 0.5 McFarland opacity standards (Quinn *et al*., 1994) [14]. The Muller-Hinton agar surface was then covered with each inoculum using a sterile swab. After letting the plates sit at 37 °C for ten minutes to dry, the antibiotic discs were gently placed on the surface, leaving enough room for the antibiotics to diffuse. After incubating the plates for 24 hours at 37 °C, the organism's growth inhibition zone surrounding each disc was measured. The list of antibiotics and their concentrations used for the antibiogram study against *P. aeruginosa* are provided in Table 1.

Table 1: List of antibiotics used for antibiogram study against *P. aeruginosa*

Class of antibiotics	Antibiotics	Conc. (mcg)
2nd gen. Cephalosporin	Cefuroxime (CXM)	30
3rdgen.cephaloantipseudo	Ceftazidime (CAZ)	30
3rd gen. Cephalosporin	Cefotaxime (CTX)	30
4th gen. Cephalosporin	Cefipime (CPM)	30
Penicillinase stable	Oxacillin (OX)	1
Aminopenicillin	Ampicillin (AMP)	10
Carboxypenicillin	Ticarcillin (TI)	10
Polypeptide	Polymyxin-B (PB)	300
Monobactums	Aztreonam (AT)	30
Carbapenems	Meropenam (MRP)	10
Glycopeptides	Vancomycin (VA)	30
1stgen.aminoglycoside	Kanamycin (K)	30
2ndgen.aminoglycoside	Gentamicin (GEN)	10
3rdgen.aminoglycoside	Amikacin (Ak)	30
Natural tetracycline	Tetracycline (TE)	30
Lincosamides	Clindamycin (CD)	2
Macrolides (50-S)	Erythromycin (E)	15
Phenicoles (50-S)	Chloramphenicol (C)	30
2nd gen quinolone	Ciprofloxacin (CIP)	5
3rd gen quinolone	Levofloxacin (LE)	5
RNA synthesis inhibitor	Rifampicin (RIF)	5
Sulphonamide	Trimethoprim (TR)	5
Combination of sulpha + trimethoprim	Cotrimoxazole (COT)	25

Result and Discussion

From 65 clinical samples, only ten isolates were identified as *P. aeruginosa* since they produce pyocynin on cetrimide agar. The molecular characterization was carried out by PCR, as described previously by Clarridge *et al*. (2004) [5]. All isolates produce an identical amplicon of 16S rRNA in PCR.

The bacteria that recovered from nosocomial infections exhibited a higher propensity to withstand the toxicity of a wide range of disinfectants and antimicrobial agents. *P. aeruginosa* is regarded as the second most significant opportunistic pathogen, with a 10–11% share in all nosocomial infections. Therefore, in this study, we evaluated the sensitivity of our isolates against 23 antimicrobials and antibotics by using the Baur *et al*. (1966) [2] technique. According to manufacturer specifications, the organism's response was classified as sensitive, intermediate, and resistant (Himedi). The resistance profiles of all the isolates to particular antibiotics and percentages of drug resistance against antimicrobials are provided in Tables 2 and 3, respectively.

Fig 1: Antibiotic sensitivity test of *P. aeruginosa*

Table 2: Antibiotic sensitivity results for *P. aeruginosa* isolated from nosocomial infections

Antibiotics	RP-1	RP-2	RP-3	RP-4	RP-5	RP-6	RP-7	RP-8	RP-9	RP-10
Cefuroxime (CXM)	R	R	R	R	R	R	R	R	R	R
Ceftazidime (CAZ)	S	S	S	S	S	S	S	S	S	S
Cefotaxime (CTX)	R	R	R	R	R	R	R	R	R	R
Cefipime (CPM)	S	S	S	S	S	S	S	S	S	S
Oxacillin (OX)	R	R	R	R	R	R	R	R	R	R
Ampicillin (AMP)	R	R	R	R	R	R	R	R	R	R
Ticarcillin (TI)	S	S	S	S	S	S	S	S	S	R
Polymyxin- B(PB)	S	S	S	S	S	S	S	S	S	S
Azetreonam (AT)	S	S	S	I	I	R	R	R	R	R
Meropenam (MRP)	S	S	S	S	S	S	S	S	S	S
Vancomycin (VA)	R	R	R	R	R	R	R	R	R	R
Kanamycin (K)	S	S	S	S	S	S	S	S	R	R
Gentamicin (GEN)	S	S	S	S	S	S	S	S	S	S
Amikacin (AK)	S	S	S	S	S	S	S	S	R	R
Tetracycline (TE)	R	R	R	R	R	R	R	R	R	R
Clindamycin (CD)	R	R	R	R	R	R	R	R	R	R
Erythromycin (E)	R	R	R	R	R	R	R	R	R	R
Chloramphenicol (C)	S	S	S	S	S	R	R	R	R	R
Ciprofloxacin (CIP)	S	S	S	S	S	S	S	S	S	R
Levofloxacin (LE)	S	S	S	S	S	S	S	S	S	S
Rifampicin (RIF)	R	R	R	R	R	R	R	R	R	R
Trimethoprim (TR)	R	R	R	R	R	R	R	R	R	R
Co-trimoxazole (COT)	S	S	S	S	S	S	I	I	I	R

Table 3: Percentages of drug resistance against antimicrobials

Mechanism	Class of antibiotic	Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
Cell wall synthesis inhibitor	2nd gen. Cephalosporin	Cefuroxime (CXM)	0	0	100
	3rd gen. Cephalosporin	Ceftazidime (CAZ)	100	0	0
	3rd gen. Cephalosporin	Cefotaxime (CTX)	0	0	100
	4th gen. Cephalosporin	Cefipime (CPM)	100	0	0
	Penicillinase stable	Oxacillin (OX)	0	0	100
	Aminopenicillin	Ampicillin (AMP)	0	0	100
	Carboxypenicillin	Ticarcillin (TI)	90	0	10
	Polypeptide	Polymyxin-B(PB)	100	0	0
	Monobactums	Azetreonam (AT)	30	20	50
	Carbapenems	Meropenam (MRP)	100	0	0
	Glycopeptides	Vancomycin (VA)	0	0	100
Protein synthesis inhibitor (30-S)	1st gen. aminoglycoside	Kanamycin (K)	80	0	20
	2nd gen. aminoglycoside	Gentamicin (GEN)	100	0	0
	3rd gen. aminoglycoside	Amikacin (AK)	80	0	20
	Natural tetracycline	Tetracycline (TE)	0	0	100
Protein synthesis inhibitor (50-S)	Lincosamides	Clindamycin (CD)	0	0	100
	Macrolides (50-S)	Erythromycin (E)	0	0	100
	Phenicoles (50-S)	Chloramphenicol (C)	50	0	50
DNA synthesis inhibitor	2nd gen. quinolone	Ciprofloxacin (CIP)	90	0	10
	3rd gen. quinolone	Levofloxacin (LE)	100	0	0
RNA synthesis inhibitor	RNA synthesis inhibitor	Rifampicin (RIF)	0	0	100
Antimetabolite antibiotics	Sulphonamide	Trimethoprim (TR)	0	0	100
	Combination of sulpha + trimethoprim	Contrimoxazole (COT)	70	20	10

The highest level of sensitivity was noted for polymyxin-B (100%). Our findings are somewhat comparable to those of Sela *et al*. (2007) [16], who showed that polmyxin-B was sensitive against 100% of *P. aeruginosa* isolates recovered from mastitic. 90% of the *P. aeruginosa* isolates were found to be sensitive to ciprofloxacin, whereas 100% of the isolates were found to be susceptible to levofloxacin. Algun *et al*. (2004) [1] reported nearly identical ciprofloxacin sensitivity patterns for 87.50% of the isolates of *P. aeruginosa*. For this investigation, *P. aeruginosa* isolates displayed varying patterns of resistance towards Gentamicin, Amikacin, and Kanamycin. The most effective antibiotic against *P. aeruginosa* was found to be gentamicin, for which 100% of isolates were found to be sensitive. Kanamycin and amikacin were found to be 80% and 20% effective, respectively, which was similar to earlier reports (Magnet and Blanchard, 2005) [9].

Meropenam had 100% efficacy against *P. aeruginosa*, while ticarcillin showed 90% sensitivity. Our findings closely matched those of Martin *et al*. (2000) [10], who found that 86% of *P. aeruginosa* isolates were sensitive to ticarcillin. Among the various β-lactam antibiotics, 100% of *P. aeruginosa* isolates were found to be responsive to ceftazidime and cefepime, whereas 100% of isolates showed complete resistance to cefotaxime and cefuroxime. 100% resistance to oxacillin or ampicillin was found. Zetronam antibiotics exhibited 20% sensitivity and 30% resistance, while 50% of the isolates exhibited intermediate activity.

Every isolate exhibited 100% resistance to trimethoprim and chloramphenicol, respectively. The remaining antibiotics exhibited 100% resistance to rifampicin and tetracycline. 70% of the isolates exhibit co-trimoxazole sensitivity, 20% exhibit intermediate sensitivity, and 10% exhibit resistance. In contrast to the findings of Sela *et al*. (2007) [16] and Rashid *et al*. (2007) [15], this showed that 100% of *P. aeruginosa* isolates were resistant to co-trimoxazole.

Conclusion

In conclusion, all isolates of *P. aeruginosa* recovered from nosocomial infections showed evidence of multidrug resistance. Out of 23 tested antibiotics, the *P. aeruginosa* isolates exhibited either a complete or partial drug resistance phenotype against 17 antimicrobal agents, which eventually can cause prolonged persistence infections and treatment failure.

Acknowledgment

A part of this study belongs to MVSc thesis work of R. Panwar.

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