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Prevalence and Antibiogram of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) *Pseudomonas aeruginosa* Isolates in a Tertiary Care Hospital of West Bengal

Tapajyoti Mukherjee[1*], Aritra Bhattacharya[2], Binita Kangsabanik[3], Paulami Ghosh[4], Sohini Banerjee[5], Monalisa Majumdar[6]

[1]Assistant Professor, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India
[2]PGT, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India
[3]PGT, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India
[4]Senior Resident, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India
[5]PGT, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India
[6]Professor & Head, Department of Microbiology, Burdwan Medical College, Purba Bardhaman, West Bengal, 713104, India

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***Corresponding author:** Dr. Tapajyoti Mukherjee

Abstract **Original Research Article**

P. aeruginosa, particularly drug resistant phenotypes present a serious therapeutic challenge for treatment due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance. Considering the paucity of data on the prevalence of drug resistant *P.aeruginosa* isolates in West Bengal, the present study has been envisaged to assess its prevalence among hospitalized patients. This study was conducted to detect multidrug resistant (MDR) and extremely drug resistant (XDR) *Pseudomonas aeruginosa* isolates among patients in a tertiary care hospital in West Bengal. *Pseudomonas aeruginosa* was identified using standard methods from various clinical samples collected over a period seven months. Their antimicrobial susceptibility to 11 antimicrobial agents from 7 antimicrobial categories were determined by disk diffusion method and characterization of *P. aeruginosa* isolates as MDR and XDR was done according to standardized international terminology. MDR was defined as acquired non-susceptibility to at least one agent in ≥3 antimicrobial categories and XDR was defined as non-susceptibility to at least one agent in ≥6 antimicrobial categories. Out of total 91 *Pseudomonas aeruginosa* isolates, 25 (27.47%) of them were multidrug resistant and 1 (0.01%) was found to be extremely drug resistant. Most of them were located at ICU. Overall, the highest susceptibility was shown to polymyxins categories i.e. polymyxin B (96.8%) and colistin (91.7%) and the lowest to ceftazidime (21.2%) and gentamicin (49%). The high frequency of antimicrobial resistance in clinical isolates of *P. aeruginosa* is posing threat in health-care institutions. To minimize the emergence and spread of this organism, a regular surveillance of healthcare-associated infections with proper implementation of antimicrobial policy and infection control measures are need of the hour.

Keywords: *Pseudomonas aeruginosa*, antibiotic, prevalence, Multidrug Resistant, Extremely drug Resistant.

INTRODUCTION

Pseudomonas aeruginosa is not only opportunistic but also a common nosocomial pathogen causing pneumonia, bacteremia, wound infection and urinary tract infection [1]. This bacterium is notorious for its low antibiotic, susceptibility which is due to low permeability of the bacterial cellular envelopes and action of multidrug efflux pumps. This efflux pump is associated with elevated MICs with penicillin, cephalosporins, quinolones, tetracyclines, chloramphenicol, metallo-b-lactamases and later carbapenems [2-6]. Moreover, literature review showed that the resistance of *P. aeruginosa* to β-lactams, quinolones, aminoglycosides and carbapenems, especially imipenem has steadily increased [2, 5-7].

This ever-increasing problem of drug resistance is not only due to its intrinsic resistance but also *P. aeruginosa* can acquire resistance by mutation either in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants [8, 9]. It is observed that, rates of antibiotic resistance in *P. aeruginosa* are gradually increasing worldwide [8, 10]. Besides, some of isolates have shown nonsusceptibility to multiple antibiotics,

which could be mediated by several mechanisms including production of hydrolyzing enzyme, loss of outer membrane protein, efflux systems and target mutations [11].

Literature review shows that there is considerable controversy in defining multidrug resistant (MDR) and extremely drug resistant (XDR) *P.aeruginosa* isolates when they are resistant to multiple antibiotics [12, 13]. But standard definition of of these superbugs is essential for the sake of estimating the true prevalence [11] and comparison of data [14]. Therefore, international experts in this field, in 2011, proposed interim standard definitions for acquired resistance of these organisms which are followed in the majority of the published studies [14]. The proposed list of antimicrobial categories for characterization of MDR, XDR and PDR in *P. aeruginosa* is shown in Table 1. According to it, MDR, XDR and PDR were defined as "acquired non-susceptibility to at least one agent in three or more antimicrobial categories", "nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories" (i.e. bacterial isolates remain susceptible to only one or two categories) and "nonsusceptibility to all agents in all antimicrobial categories" respectively [14].

The present study was attempted to evaluate the prevalence of MDR and XDR *P.aeruginosa* isolates in this region as there is paucity of existing information regarding these special pseudomonal phenotypes.

MATERIAL & METHODS

The present study was conducted in Microbiology department, Burdwan Medical College, Purba Bardhaman, India, over 7 months from January 2019 to July 2019. After collection of various samples, first, direct smear was prepared and stained with gram stain and then the samples were cultured on various culture media, like Blood agar and MacConkey's agar. After 24hrs of incubation, colony morphology and pigmentation were noted and from the colony; gram stain, motility and oxidase test were performed. Then, only samples showing oxidase positive gram negative, motile bacilli were taken into account. After that biochemical parameters like oxidative carbohydrate utilization, ability to grow at 42 ˚C and on Cetrimide agar were considered to identify as *Pseudomonas* aeruginosa [15].

Antimicrobial susceptibility test

The antibiotic susceptibility pattern of all the *Pseudomonas aeruginosa* isolates were assessed by modified Kirby–Bauer disc diffusion method on Mueller–Hinton agar against the following antibiotics: amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), piperacillin-tazobactam (110µg), ceftazidime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), polymyxin B (300U) and colistin (10µg). After incubation of 24 h at 37°C, the zone diameters measured around each disc were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute (CLSI)[16].

Detection method for MDR and XDR isolates

P. aeruginosa isolates were defined as MDR and XDR according to new standardized international document [14] and they were estimated from the results of antimicrobial susceptibility test with all the antimicrobial agents listed in Table 1 except fosfomycin. Therefore, isolates of *P. aeruginosa*, which have shown non-susceptibility to at least one agent in ≥3 antimicrobial categories considered MDR, and isolates exhibit resistance to at least one agent in ≥6 antimicrobial categories known as XDR.

Table-1: Antimicrobial categories and agents proposed for characterization of MDR, XDR and PDR in *P. aeruginosa* [14]

Antimicrobial categories	Antimicrobial agents
Aminoglycosides	Amikacin
	Gentamicin
Fluoroquinolones	Ciprofloxacin
	Levofloxacin
Penicillins/β-lactamase inhibitors	Piperacillin-tazobactam
Cephalosporins	Ceftazidime
Monobactams	Aztreonam
Phosphonic acid	Fosfomycin
Carbapenems	Imipenem
	Meropenem
Polymyxins	Polymyxin B
	Colistin

RESULTS

In this present study, a total of 2439 samples were collected in the Microbiology department, Burdwan Medical College, over a period of seven months from January 2019 to July 2019. Of these, *Pseudomonas aeruginosa* was isolated in ninety-one (91) samples. Antimicrobial susceptibility of these 91 *P. aeruginosa* isolates against 11 antimicrobial agents from 7 antimicrobial categories was shown in Table 2.

Table-2: Antimicrobial susceptibility of 91 *P. aeruginosa* isolates against 11 agents from 7 antimicrobial categories

Antimicrobial categories	Antimicrobial agents	Number of isolates (%)	
		Resistant	Susceptible
Aminoglycosides	Amikacin	26(28.4)	65(71.6)
	Gentamicin	46(51)	45(49)
Fluoroquinolones	Ciprofloxacin	39(43.1)	52(56.9)
	Levofloxacin	37(41.2)	54(58.8)
Penicillins/β-lactamase inhibitors	Piperacillin-tazobactam	25(27.9)	66(72.1)
Cephalosporins	Ceftazidime	72(78.8)	19(21.2)
Monobactams	Aztreonam	43(46.9)	48(53.1)
Carbapenems	Imipenem	36(39.3)	55(60.7)
	Meropenem	42(46.5)	49(53.5)
Polymyxins	Polymyxin B	3(3.2)	88(96.8)
	Colistin	8(8.3)	83(91.7)

In this study, the highest susceptibility was shown to polymyxins categories i.e. polymyxin B (96.8%) and colistin (91.7%) and the lowest to ceftazidime (21.2%) and gentamicin (49%).

Table-3: Antimicrobial susceptibility patterns of 25 MDR and 1 XDR *P. aeruginosa* isolated from patients in Burdwan, West Bengal

Pattern no.	No. of classes nonsusceptible	No. of isolates	Drug resistance category	Resistance profile
1	6	1	XDR	A-C-PF--I-SY
2	5	5	MDR	AGC-PF-MI-
3	5		MDR	A-C-PF--I--
4	5		MDR	A---PF-M--S
5	5		MDR	A-C-PFM--
6	5		MDR	A--LPF-I-
7	4	8	MDR	A-C-PF------
8	4		MDR	A- -PF-M--
9	4		MDR	A-C- F—I- -
10	4		MDR	A-C- F--I- -
11	4		MDR	-G- PF-M-- -
12	4		MDR	-G-L F-M-- -
13	4		MDR	-G- PF-MI- -
14	4		MDR	---LPF-MI-
15	3	12	MDR	-C- F-M-- -
16	3		MDR	- -PF--I--
17	3		MDR	- -PF--I--
18	3		MDR	-C- F-M-- -
19	3		MDR	-C- F-M-- -
20	3		MDR	--L F-M-- -
21	3		MDR	-- PF--I---
22	3		MDR	A- - F--I---
23	3		MDR	A-C--F-----
24	3		MDR	A--L ---I---
25	3		MDR	A---PF------
26	3		MDR	AGC--F----

Profile: A= Amikacin, G= Gentamicin, C= Ciprofloxacin, L= Levofloxacin, P= Piperacillin+Tazobactam, F= Ceftazidime, M= Meropenem, I= Imipenem, Z=Aztreonam , S=Colistin, Y=Polymyxin-B.

We observed that, twenty five (27.47%) of these 91 isolates were multidrug resistant and only one (0.01%) was extremely drug resistant *Pseudomonas aeruginosa*. (Table 3) It was seen that the only XDR isolate was resistant to atleast one member of all of the 6 antimicrobial categories. Moreover, 5 isolates were nonsusceptible to 5 categories, 8 isolates to 4 categories and 12 isolates were resistant to 3 categories of antimicrobials. Thus, in total 25 isolates were nonsusceptible to ≥3 categories of antimicrobials and hence were MDR isolates. Furthermore, non-susceptibility to one and two categories were seen in 28 (30.77%) and 26 (28.57%) isolates, respectively. However, pandrug resistant *P. aeruginosa* was not detected, because non-susceptibility to all used agents was not seen in any isolates. Instead, 11 (12.09%) all

drug sensitive isolates of *P. aeruginosa* were detected in this study.

Table-4: Department wise distribution of MDR *P. aeruginosa* isolates. (N=25)

Department	MDR isolates n(%)
ICU	10(40)
OPD	6(24)
Surgery	3(12)
Medicine	3(12)
OBG	2(8)
ENT	1(4)

In our study, it is evident that ICU is truly the hub of superbugs, where we found 40% MDR *P.aeruginosa* isolates and the sole XDR *P. aeruginosa* isolate. The rest of the isolates were found in outdoor (24%), surgery (12%), medicine (12%), gynecology (8%) and ENT (4%) departments (Table 4).

DISCUSSION

In this present study, we have determined antimicrobial susceptibility of 91 *Pseudomonas aeruginosa* isolates against 11 agents from 7 antimicrobial categories. It was observed that these isolates demonstrated highest susceptibility to polymyxins categories i.e. (96.8%) and (91.7%) which is at par with the observation of Saderi *et al.* (polymyxin B and colistin 95.5% and 90.9% respectively) [17]. Moreover, many researchers also noticed that *P. aeruginosa* clinical isolates show resistance to all groups of antimicrobials except the polymyxins[9]. Colistin which was considered as last resort to treat these isolates but still there have been reports of *P. aeruginosa* resistance to colistin [5,18-21]. The emergence of colistin resistant *P. aeruginosa* has increased due to the use of colistin in the treatment of *P. aeruginosa* being on the rise [5, 20-22].

Among the aminoglycosides, amikacin was found to be superior than the gentamicin and susceptibility to amikacin (71.6%) and gentamicin (49%) were much better than the findings of Saderi *et al.* (amikacin and gentamicin 55% and 27.3% respectively) [17]. Fluoroquinolones, monobactams and carbapenems were fairly active against these isolates (56-59%, 53%, 53-60% respectively) which also mimics results obtained by Iranian researchers [17].

In our study, we noticed that piperacillin-tazobactam, in the penicillins/ß-lactamase inhibitors category, was relatively better in killing these isolates (72.1%) as compared to other groups and this observation was also comparable to that of Saderi *et al.* (63.6%) [17]. But susceptibility to ceftazidime in the cephalosporin category was worse in our study (21.2%) than Iranian group (63.6%) [17].

Our study revealed that, twenty five (27.47%) of these 91 isolates were multidrug resistant and only one (0.01%) was extremely drug resistant *Pseudomonas aeruginosa* but Saderi *et al.* found more MDR (54.5%) and XDR (33%) isolates [17]. However, prevalence of MDR *P. aeruginosa* in our study was in concordance with the observations of Salimi *et al.* (33.1%), De Francesco *et al.* (20%) [23, 24]. In the studies in other countries, further lower prevalence was usually reported; Morales *et al.* 5.46% and Tacconelli *et al.* 14% [25, 26]. On the contrary, much higher prevalence has been reported by Moazami-Goudarzi *et al.* and Ranjbar *et al.* (100%), Bayani *et al.* (60%), Nikokar *et al.* (45.3%) [27-30]. This difference was most probably due to factors like; geographic differences in antimicrobial resistance, population demographics, access to medical care and illicit drug use [11, 31].

However, one limitation of our study was the inability to perform fosfomycin susceptibility test for these isolates as because we used only disk diffusion test and interpretive criterion of fosfomycin for *P. aeruginosa* (recommended by CLSI and EUCAST) is not available yet [16, 32]. These possibly resulted less detection of MDR and XDR *P. aeruginosa* isolates.

Furthermore, we noticed 40% MDR *P. aeruginosa* isolates and the only XDR *P. aeruginosa* isolate were located in ICU which closely resembles the observation of Sharma *et al.* (42.8%) [33]. This finding emphasises that infection prevention control measures should be strictly followed as well as adherence to antimicrobial policy should be ensured to control the spread of these deadly superbugs.

CONCLUSION

Our study revealed that the percentage of MDR *P.aeruginosa* is fairly high and most of these isolates were inhabited in the ICU. Polymyxins, no doubt, are the most susceptible group of antimicrobial to these isolates. Above all, a regular surveillance of healthcare associated infection, monitoring of antibiotic sensitivity pattern of MDR *P. aeruginosa*, strict antibiotic policy with stringent implementation of antimicrobial stewardship programme with strict compliance to prevention and infection control strategies are mandatory to control the situation.

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