

Original Research Article

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Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* of Various Clinical Samples in Government Medical College, Aurangabad, India

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ABSTRACT

In this era spread of multidrug resistance is a global problem. *P. aeruginosa* is one of the most common nosocomial pathogen causing wide range of opportunistic infections. Rapid emergence of varying degrees of innate & acquired resistance mechanisms has put forward a major challenge in their detection as well as treatment options. We did a prospective study, from January 2016 to December 2016, in Department of Microbiology at Government Medical College, Aurangabad. Out of 3,340 culture positive samples, 676 (20.23%) were identified as *P. aeruginosa* by standard bacteriological identification methods, from various specimens. Antimicrobial sensitivity testing was done by Modified Kirby-Bauer disc diffusion method as per CLSI guidelines 2016. Results showed maximum antibiotic resistance to Nalidixic acid (100%), Ampicillin (83.3), Ceftriaxone (72.8%), Nitrofurantoin (72%), Cefepime (62.9%), Norfloxacin (65.2%), Cefotaxime (57.6%), Ceftazidime (56.1%) against Colistin (94.9%), Amikacin (93.9%), Piperacillin/Tazobactam (94.3%), Imipenem (82.2%), Cefoxitin (67.6%) Ceftazidime/Clavulanic acid (57.9%) which were identified as the most effective antibiotics. Prevalence of ESBL, AmpC and MBL producers was found to be 21.74%, 10.62% and 9.31% respectively. Conclusion: Emerging multi drug resistant *P. aeruginosa* needs inevitable screening and confirmation for β - lactamases production so that proper treatment can be initiated with appropriate antibiotics for commendable outcome, further their spread can be prevented by applying infection control policy and judicious use of antibiotics in the hospital.

Keywords

Pseudomonas aeruginosa (*P. aeruginosa*), ESBL, AmpC, MBL

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Introduction

P. aeruginosa is an opportunistic pathogen that causes severe invasive diseases in critically ill patients or immunocompromised patients. The frequency of infections caused by them is increasing and multidrug-resistant (MDR) strains, resistant to almost all available

antibiotics are emerging in hospitalized patients (Ozer *et al.*, 2009). Restricted permeability and efflux are common components of the resistance phenotype for β - lactams, aminoglycosides and quinolones which are essentially fundamental properties of the organism. *P. aeruginosa* innate antibiotic resistance results from the restricted

permeability of the cell wall. Resistance to β -lactams involves several mechanisms, the most prevalent mechanism is the production of β -lactamases, followed by permeability alterations, extrusion by efflux pumps, and to a lesser extent PBP alterations. Acquired resistance mechanism includes plasmid mediated AmpC β -lactamase, extended spectrum β -lactamase and metallo β -lactamase (MBL) enzymes (Manchanda and Singh, 2008).

A multidrug-resistant phenotype produces serious infections and often leaves us with only few reliable therapeutic options which have more side effects. As a result these types of infections are always associated with increased morbidity and mortality.

Emergence of multiple antibiotic resistance is a growing clinical concern, hence there is a need to conduct studies to profile resistance patterns in various hospitals so as to generate data that would guide clinicians to use antibiotics judiciously for treatment.

The main aim and objectives of this study to isolate and identify *P. aeruginosa* from various clinical samples. And to study the antibiotic profile of *P. aeruginosa*. Also to detect various type of β -lactamases producing *P. aeruginosa*.

Materials and Methods

We did a one year prospective study from January 2016 to December 2016 in Department of Microbiology at Government Medical College, Aurangabad. Various samples like blood, wound swabs/ pus, urine, sputum and tracheal aspirate were taken from hospitalized patients of different wards were evaluated and included in our study. In 2016, total number of culture positive isolates were 3,340; among that 676 (20.23%) were *P. aeruginosa*. Identification of bacterial isolates

was done by conventional bacteriological methods such as colony morphology, gram stain, catalase test, oxidase test, motility test, IMVIC tests and production of various pigments (pyocyanin, pyoverdin, pyomelanin and pyorubrum). Antibiotic susceptibility test was done by Modified Kirby-Bauer disk diffusion method on Muller Hinton Agar as per CLSI guidelines 2016 for various antibiotics, namely:

Colistin (10 μ gm), Ciprofloxacin (5 μ gm), Norfloxacin (10 μ gm), Nalidixic Acid (30 μ gm), Nitrofurantoin (300 μ gm), Ceftazidime/ Clavulanic acid (30/10 μ gm), Ceftazidime (30 μ gm), Cefepime (30 μ gm), Ceftriaxone (30 μ gm), Cefoxitin (30 μ g), Ampicillin (10 μ gm), Imipenem (10 μ gm), Amikacin (30 μ gm), Piperacillin+tazobactam (100/10 μ gm) (Clinical and Laboratory Standards Institute, 2016).

ESBL was detected by using Double disc synergy test in which difference of ≥ 5 mm between the zone diameters of ceftazidime and ceftazidime/clavulanate disks is seen, as per the CLSI 2016 guidelines. (Clinical and Laboratory Standards Institute, 2016) (As shown in Figure 1a).

AmpC β -lactamase production was tested by Disk Antagonism Test. Ceftriaxone (30 μ g) and cefoxitin (30 μ g) disks were placed 20 mm apart from centre to centre. Isolates showing blunting of the Ceftriaxone zone of inhibition adjacent to the cefoxitin disk were labelled AmpC β -lactamase producer (Upadhyay *et al.*, 2010) (Fig. 1b).

MBL production was detected by Disk Potentiation Test in which Imipenem (10 μ g) and Imipenem-EDTA (10/50 μ g) were placed on the plate and the inhibition zones were compared after 16 to 18 hours of incubation at 35 °C. If the increase in inhibition zone with Imipenem - EDTA disk

was ≥ 7 mm as compared to the Imipenem disk alone was considered to be the MBL producer (Yong *et al.*, 2002) (Fig. 1c).

Results and Discussion

Out of 676 *P. aeruginosa* in our study; maximum isolates 405 (59.91%) were isolated from wound swab; 81 (11.98%) from tracheal secretion; 67 (9.91%) from urine; 61 (9.02%) from sputum; 38 (5.62%) from pus and 24 (3.55%) from blood. Among infected patients, males infected were 58.43% and female 41.57%. *P. aeruginosa* infection was seen in all age groups but rate of isolation was seen more in age group of 41 – 50 years.

In our study *P. aeruginosa* showed maximum antibiotic resistance to Nalidixic acid (100%), Ampicillin (83.3), Ceftriaxone (72.8%), Nitrofurantoin (72%), Cefepime (62.9%), Norfloxacin (65.2%), Cefotaxime (57.6%), Ceftazidime (56.1%) against Colistin (94.9%), Amikacin (93.9%), Piperacillin/Tazobactam (94.3%), Imipenem (82.2%), Cefoxitin (67.6%) and Ceftazidime/Clavulanic acid (57.9%) which were identified as the most effective antibiotics (Table 1).

In our study we got 229 (33.87%) *P. aeruginosa* sensitive to all applied drugs, 72 (10.65%) monoresistant or bi-resistant *P. aeruginosa*, 198 (29.28%) MDR *P. aeruginosa* and 38 (5.6%) were resistant to all drugs.

As shown in the (Table 2), out of 676 isolates of *P. aeruginosa* detected from various clinical samples 21.74 % were ESBL producers, 10.65% were AmpC producer and 9.31% were MBL producer. Among all the clinical specimens wound swab showed highest percentage of ESBL, AmpC and MBL producer. Co-existence of resistance

mechanisms of *P. aeruginosa* were also studied. Only 0.44% isolates showed coexistence of ESBL, Amp C and MBL (Table 3).

P. aeruginosa has emerged as an important nosocomial pathogen as it is inherently resistance to many antimicrobial agents and antiseptics mainly due to synergy between multidrug efflux system and low outer membrane permeability (Manchanda and Singh, 2008). From the present study we observed that *P. aeruginosa* isolated from the burn patients was the most important cause of hospital acquired infection. *P. aeruginosa* isolates which were isolated in our institute showed remarkable resistance against penicillin and fourth generation cephalosporin. But the extended spectrum penicillin and the 3rd generation cephalosporin in combination with Tazobactam and clavulanic acid showed a significant decrease in resistance pattern as it expands the spectrum of activity, the dose as well as incidence of toxicity reduces subsequently. The increased prevalence of resistance to cefepime reflects the increased use, rather we can say misuse of this drug in recent years.

Carbapenems are β lactam antibiotics, presently considered as the most potent agents for the treatment of multidrug resistance pseudomonas infections as these agents are stable and have high rate of permeation through bacterial outer membranes. Carbapenem hydrolyzing MBL's have been reported in several countries and have emerged as the most important mechanisms of carbapenem resistance. Production of MBL by *P. aeruginosa* has tremendous consequences, since these organisms also carry other multi drug resistance genes and the only treatment option remains with us is potentially toxic colistin.

Table.1 Antibiogram of *P. aeruginosa*

Antibioticname	%S	%R
Colistin	94.9	5.1
Amikacin	93.9	6.1
Piperacillin/Tazobactam	94.3	5.7
Imipenem	82.2	17.8
Cefoxitin	67.9	32.1
Ceftazidime/Clavulanic acid	57.9	42.1
Ceftazidime	43.9	56.1
Cefotaxime	42.4	57.6
Ciprofloxacin	37.9	62.1
Norfloxacin	34.8	65.2
Cefepime	37.1	62.9
Nitrofurantoin	28	72
Ceftriaxone	27.2	72.8
Ampicillin	16.7	83.3
Nalidixic acid	0	100

Table.2 Number of isolation among various Beta-Lactamase producer

Total no. of isolates	ESBL	AmpC	MBL
676	147 (21.74%)	72 (10.65%)	63 (9.31%)

Table.3 Coexistence of ESBL, Amp C and MBL

	Multiple resistance	Number (%)
1.	ESBL + MBL	18 (2.66%)
2.	ESBL + AmpC	26 (3.8%)
3.	MBL + AmpC	13 (1.92%)
4.	ESBL + AmpC + MBL	3 (0.44%)

Fig.1 *P. aeruginosa* showing production of various type of β -lactamases



Fig 1(a): Positive Double disc synergy test

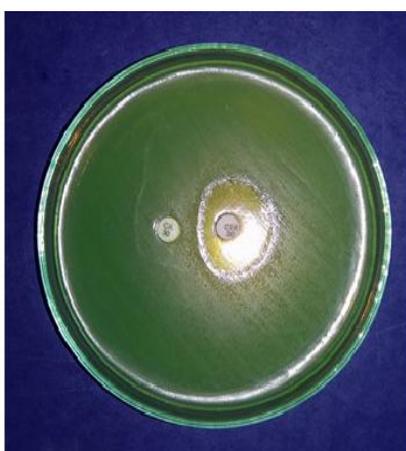


Fig 1(b): Positive Disc Antagonism Test



Fig 1 (c): Positive Disc Potentiation Test

In our study Piperacillin Tazobactam, Amikacin and colistin were highly effective against MBL producers. Better anti pseudomonal activity with colistin, piperacillintazobactam, amikacin and imipenem goes in accordance with various studies published (Chaudhari *et al.*, 2013; Amutha *et al.*, 2009; Joseph *et al.*, 2013; Senthamarai *et al.*, 2014).

In the present study 21.74% isolates were ESBL producers and 10.65% Amp C β lactamases producers similar to study done by Umadevi *et al.*, (19.4%) and (16.4%) but MBL in our study was found to be 9.31% which is very low as compared to Umadevi *et al.*, which reported 65.7% (Umadevi *et al.*, 2011). A study conducted at NIMS Hospital, Rajasthan by (Gagan Priya Pandey *et al.*, 2017) showed lower incidence of β lactamases production as compared to our study. They studied 50 isolates in which 8% showed ESBL production, 8% showed AmpC production and 6% showed MBL production (Gagan Priya Pandey *et al.*, 2017).

We have also studied the coexistence between ESBL – Amp C, Amp C – MBL, ESBL-MBL and ESBL- Amp C – MBL in isolates of *P. aeruginosa*. In our study coexistence between MBL + Amp C was found to be 1.92% which is similar to study conducted by (Gagan Priya Pandey *et al.*, 2017) showing 2%. Whereas coexistence between ESBL – Amp C, ESBL- MBL and ESBL- Amp C – MBL in isolates of *P. aeruginosa* were not reported in (Gagan Priya Pandey *et al.*, 2017) and we reported 2.6%, 3.8% and 0.44% respectively.

In case of pan drug resistance, colistin along with β lactams was advised in our institute to patients as per National treatment guidelines (National treatment Guidelines, 2016) which successfully treated the patients. In using combination therapy probability increases that

the infecting pathogen will be susceptible to at least one of the antibiotics; plus there is synergistic effect of the combination which prevents emergence of drug resistance.

Widespread use of antibiotics has altered etiological pattern of infections and their antibiotic susceptibility. Hence continuous monitoring of susceptibility pattern needs to be carried out in individual setting so as to detect the true burden of antibiotic resistance among organisms and prevent their further emergence by judicious use of drugs (Kaur *et al.*, 2008).

This study was done for the first time in our institute, as multi drug resistance producing organisms are emerging, it becomes our responsibility as microbiologist to screen and confirm for β - lactamases production of every sample so that proper treatment can be initiated with appropriate antibiotics for favourable outcome. It should be made mandatory for all clinician to send all the samples for microbiological analysis and their antibiogram before starting antibiotics so that emergence of drug resistance can be minimised. Control measures which include the judicious use of antibiotics, antibiotic cycling, and the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done to prevent the spread of *P. aeruginosa* infection.

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