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Original Research Article

Antibiotic Resistance Profile & β-Lactamase Production in *Pseudomonas Aeruginosa*

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ABSTRACT

Background & objectives: In the recent years, antibiotic resistance in *Pseudomonas aeruginosa* is on the rise. It can develop resistance by mutation, by acquiring resistance genes or by producing newer β-lactamases like ESBLs, AmpC β-lactamases and MBLs. Hence, the study was aimed to detect the antibiotic resistance profile in *P. aeruginosa* and the incidence of newer β-lactamases producing strains.
Methods: The present cross-sectional study included 50 clinical isolates of *P. aeruginosa.* Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method and ESBLs, AmpC β-lactamases and MBLs were detected by phenotypic methods.
Results: Highest antibiotic resistance was observed with Cefoxitin (98% strains). 45 (90%) strains produced ESBLs, AmpC β-lactamases and MBLs either alone or in combination.
Interpretation & Conclusion: Phenotypic detection of newer β-lactamases should be done in Clinical Microbiology Laboratory.

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance, newer β-lactamases.

INTRODUCTION

Antimicrobial resistance (AMR) is a major threat to patient care and Infection Control Programme in any Health-care set up worldwide. Antimicrobial resistance leads to increased morbidity and mortality and increased cost of health care. In 2011, WHO declared 'Combat drug resistance- No action today, No cure tomorrow'. [(1)] In the recent years, antibiotic resistance in *Pseudomonas aeruginosa* is on the rise.

Pseudomonas aeruginosa is one of the most important causes of healthcare associated infections especially in patients of Intensive Care Units (ICUs), Postoperative wards, Burn units, Trauma units, Oncology units etc. Though major scientific advances have been achieved by Medical fraternity in patient care and management and invention of wide variety of antimicrobial agents with antipseudomonal activities, life threatening infection continue to occur resulting in high mortality and morbidity because of development of antibiotic resistant strains. [(2)] Multiple factors contribute to development of antibiotic resistance in *Pseudomonas aeruginosa* [(3)] e.g. it is intrinsically resistant to antibiotics due to low permeability of cell wall, or develop resistance by mutation or by acquiring resistance genes from other bacteria through plasmids, transposons etc. Production of newer β-lactamases like Extended Spectrum β-Lactamases (ESBLs), AmpC β-lactamases and Metallo-β-Lactamases (MBLs) have compounded the problem. In most of the ESBL and AmpC β-lactamase producing *Pseudomonas aeruginosa*, Carbapenems

are the last antibiotics for treating the infected patients. Carbapenem resistance commonly occurs in *Pseudomonas aeruginosa* due to increased efflux system and production of Carbapenem hydrolyzing enzymes i.e. Carbapenemases. Most commonly produced Carbapenemases are Metallo beta lactamases (MBL). [(4)]

Hence, the present study was undertaken to detect the antibiotic resistance profile in *P. aeruginosa* and to detect phenotypically ESBL, AmpC β-lactamases and MBL producing strains.

MATERIALS & METHODS

This short term cross sectional study was conducted in the department of Microbiology, as ICMR short term studentship from 1st April to 31st May 2015. A total number of 50 *Pseudomonas aeruginosa* strains isolated from different clinical samples only and characterized by conventional tests [(5)] e.g. Gram staining, motility, pigment production and biochemical tests were included in the study.

Pseudomonas aeruginosa strains were isolated from different clinical samples like- urine, blood, pus and wound swab, sputum, tracheal secretions, high vaginal swabs, medical devices etc. The clinical samples were received from different indoor patient departments (IPD) and Intensive Care Units (ICUs) like Medicine ICU (MICU), Neonatal ICU (NICU), Operation Theater ICU (OT ICU), Neuro surgery ICU etc. of our hospital, which is a tertiary care hospital in a rural set up.

Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method [(6)] as per CLSI guidelines 2014. [(7)] Detection of Extended Spectrum β-lactamases (ESBLs) was done by combine disc method using Ceftazidime (CAZ-30µg) and Ceftazidime plus Clavulanic acid (CAC-30/10µg) discs. If zone of inhibition with CAC was ≥5 mm than CAZ, the strain was reported as ESBL positive. [(7)]

Detection of AmpC β-lactamases: was done by disc potentiation test using Ceftazidime and Ceftazidime plus 3-Aminophenyl boronic acid (3-APB) discs. [(8)] If zone of inhibition with CAZ+3-APB was ≥5 mm than CAZ, the strain was interpreted as AmpC β-lactamase positive.

Carbapenemase production was detected by Classical Hodge test (CHT) [(9)] by putting lawn culture of indicator strain as Escherichia coli ATCC25922 on Mueller Hinton agar plate and then lawn culture of test strain was done on same agar plate and an Imipenem disc was put in the center. After overnight incubation at 37^0C, the presence of a distorted zone around Imipenem disc was interpreted as CHT positive.

MBL was detected by Disc Potentiation test using Imipenem (IPM) and Imipenem plus EDTA disc. [(10)] If zone of inhibition with IPM+EDTA was ≥7 mm than IPM, the strain was interpreted as MBL positive.

Detection of Klebsiella pneumoniae carbapenemases and OXA D carbapenemases were not included in the study.

OBSERVATION & RESULTS

A total number of 50 *Pseudomonas aeruginosa* strains isolated from different clinical samples and characterized by conventional methods were included in study.

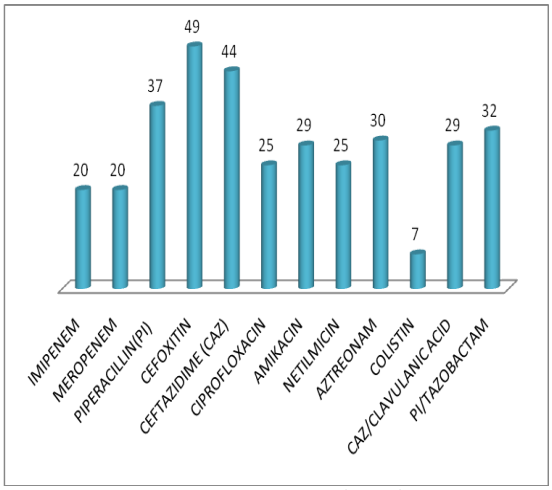

Figure 1: Antibiotic resistance profile of clinical isolates of P*seudomonas aeruginosa* strains studied (n= 50)

In figure:1 the highest resistance was observed with Cefoxitin 49 (98%) strains while lowest resistance was detected with Colistin 7 (14%) strains. For 7 Colistin resistant strains, Colistin disc test was performed thrice and Colistin resistance was further confirmed by E-Test (BioMerieux) and in all 7 strains Colistin MIC was ≥ 8µg/ml 44 (88%) *P. aeruginosa* strains were resistant to Ceftazidime, 25 (50%) strains were resistant to Ciprofloxacin and Netilmicin respectively. Carbapenems such as Imipenem and Meropenem resistance were observed in 20 (40%) strains.

TABLE 1: Detection of ESBL, AmpC β-lactamases and MBL producing Pseudomonas aeruginosa strains (n=50)

ESBL (combined disc method)		AmpC β-lactamases		MBL
CAZ/CAC	PI/PIT	D zone +ve IPM/CAZ	DP test CAZ/CAZ+APB	DP test IPM/IPM+EDTA
30 (60%)	30(60%)	30(60%)	36 (72%)	20 (40%)

Table 1 shows if the newer β-lactamases are considered individually 30 (60%), 36 (72%) and 20 (40%) strains were ESBL, AmpC β-lactamases and MBL producers respectively. All 50 strains were screened for carbapenem hydrolysis by classical Hodge test (CHT) and for MBL production by disc potentiation (DP) test. Out of 20 Carbapenem resistant strains 13 (26%) were Classical Hodge test positive and 17 (34%) were positive for MBL by DP test, Among 30 Carbapenem sensitive strains, 2 (4%) were CHT positive and 3 (6%) were positive by DP test. Hence, out of total 50 P. aeruginosa strains studied, only 15 (30%) were CHT positive (Photograph-1) and 20 (40%) strains were positive for MBL by DP test (Photograph-2.)

Photo1- Classical Hodge Test (CHT) +ve

Photo 2- Disc Potentiation (DP) Test +ve

Table 2: Isolation of newer β-lactamases producing *Pseudomonas aeruginosa* strains

P.aeruginosa strains	ESBL only	AmpC only	MBL only	ESBL + AmpC	ESBL + MBL	AmpC+ MBL	ESBL + AmpC+ MBL
Total (n=50)	4	9	1	12	4	5	10
Wards (n=39)	3	8	1	10	2	5	6
ICUs (n=11)	1	1	0	2	2	0	4

Table 2 shows the isolation of newer β-lactamases producing *P. aeruginosa* strains. 45 (90%) strains produced newer β-lactamases either singly or in combinations from different wards and ICUs. Out of 50 *P. aeruginosa* strains studied, 39 were isolated from different wards, such as Medicine (4), Surgery (9), Obsterics & Gynecology (12), Pulmonary Medicine (5), Pediatrics (4), ENT (3), Orthopedics (2) and Dermatology (1). Out of these 39 *P. aeruginosa* strains isolated from wards, 35 (72%), strains produced newer β-lactamases i.e. ESBL, AmpC and MBL either alone or in

combinations. Among 11 strains isolated from different ICUs, 4 each were from Medicine ICU (MICU) and Neonatal ICU (NICU), 2 were from Operation Theatre ICU (OTICU) and 1 was from Neurosurgery ICU. From ICUs, 10 (90%) strains produced newer β-lactamases i.e. ESBL, AmpC and MBL either alone or in combinations. All 3 types of β-lactamases in combinations i.e. ESBL plus AmpC plus MBL were produced by 4 (4/11 i.e. 36.4%) strains isolated from ICUs, compared to 6 (6/39 i.e. 15.4%) strains isolated from different wards.

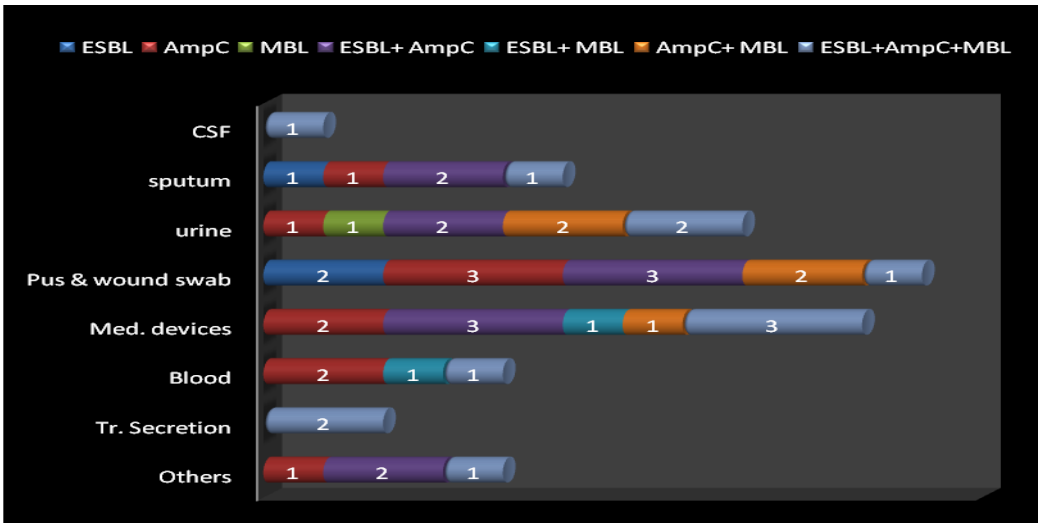

Figure 2: Isolation of newer β-lactamases producing *Pseudomonas aeruginosa* strains from different clinical specimens (n=50)
Tr. Secretion –Tracheal secretion; Med. Devices-Medical devices

Figure 2 shows the incidence of newer β-lactamases producing *Pseudomonas aeruginosa* strains from different clinical specimens. P.aeruginosa strains were isolated from pus & wound swab (13), urine (8), blood (4), sputum (5), medical devices (12), tracheal secretion (2), CSF (1) and others (5). The medical devices (12) included Foley's catheter tip (5) and neocan & intracath tip (7). Others included ear swab (2), nasal pack (1) and high vaginal swab (2). All 3 types of newer β-lactamases i.e. ESBL plus AmpC plus MBL producing *Pseudomonas aeruginosa* strains were isolated from tracheal secretions of patients from OTICU (2) and from medical devices (3).

DISCUSSION

The antibiotic resistance among bacteria especially *P. aeruginosa*, Acinetobacter sp and Burkholderia sp. is emerging as a global health problem. Presently, the major mechanism of antimicrobial resistance in *P. aeruginosa* is due to production of newer β-lactamases such as ESBL, AmpC and MBL etc. As there is no specific CLSI guidelines for detection of these enzymes for *P. aeruginosa*, many clinical laboratories have difficulties in detecting these enzymes which has led to their uncontrolled spread and therapeutic failure. ESBL producing strains may give ion false sensitive zone in routine disc diffusion test. [(11)] Carbapenem resistant, especially MBL producing *P. aeruginosa* are difficult to treat as therapeutic use of inhibitors have not been developed till now, which leads to their rapid dissemination to other different Gram negative bacilli. This emphasizes the urgent need for accurate detection of these newer β-lactamases producing strains.

In the present study, *P. aeruginosa* strains showed a very high degree of resistance to Cefoxitin (98%) which is similar to the reports of Upadhyay et al in 2010 as 97%. [(12)] Quite high resistance to Ceftazidime (88%) was observed in our study, but similarly 70% and 78% strains resistant to Ceftazidime have been reported by Behare et al. and Kumar et al.

respectively. [13,14] 40% of *P.aeruginosa* strains studied were resistant to Imipenem which correlated well with reports of other workers who have reported 37.9% of their strains resistant. [15] Though most of the workers have found that Polymyxin -B was the most effective antibiotic and had 0% resistance, [16] there are other studies that reported 8% of their *Pseudomonas aeruginosa* strains were Polymyxin B resistant. [17,18] But in this study it was found that 7 (14%) strains were resistant to Colistin (Polymyxin E). It is important to note that, Polymyxin B was used clinically in 1970s and because of its toxicity such as nephrotoxicity, ototoxicity and neuromuscular blockade, their use have been discontinued. [19]

The incidence of ESBL producing Pseudomonas aeruginosa strains in our study correlated well with other studies. [20] There is paucity of data regarding inducible Amp C β-lactamases producing *Pseudomonas aeruginosa* not only in India but worldwide also. By using DP test, 72% strains were detected as AmpC β-lactamases producers whereas other studies reported 59.4% [12] and 85.8% [21] respectively. The variations in incidence may be because of different methods employed by different workers, and many predisposing factors present such as indiscriminate use of 3rd generation Cephalosporins, prolong hospital stay etc.

In recent years the dictum of detection of MBL only in Carbapenem resistant strains has been changed as MBL gene can be carried by Carbapenem susceptible strains also according to CLSI guidelines. [7] Hence, detection of MBL was done in both Carbapenem resistant and Carbapenem sensitive strains.

The incidence of MBL producers in the present study was 40% which correlated well with other studies as 33.3%, 36%, 41% respectively. [16,17,22] There are few studies that reported quite high incidence of MBL producing *Pseudomonas aeruginosa* strains as 61.5% [13] and 69.5%. [15]

In the present study the coexistence of newer β-lactamases i.e. ESBL, Amp C and MBLs were studied and it was found that all types of possible coexistence such as ESBL plus Amp C (24%), ESBL plus MBL (8%), Amp C plus MBL (10%) and all 3 types i.e. ESBL plus Amp C plus MBL (20%) have been detected phenotypically. Very few studies have reported coexistence of newer β-lactamases. In one of the study, ESBL plus Amp C production was reported to be 3.3% and Amp C plus MBL as 46.6% of *Pseudomonas aeruginosa* strains [12] and another study reported Amp C plus MBL in 86% *Pseudomonas aeruginosa* strains. [23] In the present study 4 (8%) strains produced ESBL only, 9(18%) strains produced Amp C only and 1 (2%) strains produced MBL only. Out of 20 MBL producing strains, 19 (95%) strains produced newer β-lactamases in combination and 10 /20 (50 %) strains produced all 3 types of newer β-lactamases i.e. ESBL plus Amp C plus MBL.

It is a matter of great concern that out of 12 *Pseudomonas aeruginosa* strains isolated from medical devices 3 (25%) strains produced all 3 types of newer β-lactamases i.e. ESBL plus Amp C plus MBL. Out of 20 MBL producing strains 19 (95%) strains produced newer β-lactamases in combinations and 10 (10/20i.e. 50%) strains produced all 3 types i.e. ESBL plus Amp C plus MBL.

Several workers have reported that Class D enzymes i.e. OXA- 48 types are the most difficult carbapenemase producers to be identified phenotypically [24] Detection of *Klebsiella pneumoniae* carbapenemases (KPCs) and OXA-D carbapenemases were not included in the study. So, phenotypic detection of Class D carbapenemases were not included in the study. If any carbapenemases other than Class B (MBL) were produced by the *Pseudomonas aeruginosa* strains in the present study that could have been detected by classical Hodge test. But no strain was found to be Classical Hodge test positive and negative by confirmatory disk potentiation (DP) test for MBL.

Though Polymerase Chain Reaction (PCR) is considered as gold standard for detection of newer β-lactamases but it is very costly and requires expertise and are beyond the scope of routine Clinical Microbiology Laboratories in India. Considering the existence of so many types of newer β-lactamases, the main disadvantage of PCR is that it requires tailor-made DNA primers, cannot differentiate between variants and may not detect new variants. Similarly, E tests for ESBL, Amp C and MBL can be used but they are also very costly and cannot be used routinely.

Hence, to conclude for detection of ESBL combined disc method using Ceftazidime/Ceftazidime plus Clavulanic acid (CAZ/CAC) discs, for detection of Amp C β-lactamases disc potentiation test using Ceftazidime/ Ceftazidime plus 3-Aminophenyl boronic acid (CAZ/CAZ+3-APB) discs, for detection of MBLs disc potentiation test using Imipenem/ Imipenem plus EDTA (IPM/IPM+EDTA) discs should be used in Clinical Microbiology Laboratory to prevent the dissemination of newer β-lactamases producing *Pseudomonas aeruginosa* in Health care set up and also for a good therapeutic outcome

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Conflict of interest: None declared.

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