Resistance Pattern of *Pseudomonas aeruginosa* in a Tertiary Care Hospital of Kanchipuram, Tamilnadu, India

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ABSTRACT

Purpose: This study was undertaken to analyze the extended spectrum of β lactamase (ESBL), metallo β lactamase (MBL) & AmpC production in *Pseudomonas aeruginosa* in various clinical samples.

Materials & Methods: One hundred four non repetitive clinical specimens were inoculated onto nutrient agar, blood agar and incubated at 37ºC overnight. The colonies were tested for oxidase test and other biochemical tests and antibiogram. ESBL screening was done using 3rd generation cephalosporins and confirmatory combined double disc test, imipenem-EDTA double disc synergy test for MBL enzyme and AmpC test using Cefoxitin disc.

Results & Analysis: Out of 104 *P.aeruginosa* isolates, 42.30% were ESBL producer, 15.38 % MBL producer and none were AmpC producer. Imipenem, Ofloxacin, and aminoglycosides (amikacin (29.8%) tobramycin (29.8%) and netilmycin (13.46%) has got the better antipseudomonal activity in this study. 43 (41.35%) *P.aeruginosa* was found to be Multi Drug Resistant (MDR).

Conclusion: This study highlights the prevalence of ESBL, MBL and MDR *P.aeruginosa*. Carbapenems and aminoglycosides are promising drugs with antipseudomonal activity in our study.

Keywords: *P.aeruginosa,* 3rd generation cephalosporins, β lactam

INTRODUCTION

Pseudomonads are diverse group of established and emerging pathogen and are major agents of nosocomial and community acquired infections, widely distributed in the hospital environment where they are particularly difficult to eradicate [1].

P.aeruginosa is notorious for being intrinsically resistant to many structurally unrelated antimicrobial agents by exhibiting low permeability of its outer membrane, the constitutive expression of various efflux pumps and the naturally occurring chromosomal AmpC β lactamase, and it can acquire additional resistant gene form other organisms via plasmids, transposons, bacteriophages, and also by biofilm production [2,3]. Despite advances in medical and surgical care and wide variety of anti pseudomonal agents, life threatening infections caused by *P.aeruginosa* is still considered as most challenging pathogen. Emergence of infections caused by ESBL, MBL, MDR and PDR *P.aeruginosa* strains is alarming which creates serious health problem resulting in an enormous burden of morbidity, mortality and high health care cost.

AIM OF THE STUDY

This study was aimed to determine the prevalence, antibiotic resistance pattern and various mechanisms of resistance such as ESBL, MBL and AmpC production in *Pseudomonas aeruginosa* from various clinical samples in our tertiary care hospital at Kanchipuram, Tamil nadu, India.

MATERIALS AND METHODS

The study was carried out in Microbiology department in Meenakshi Medical College Hospital & Research Institute (MMCH&RI) at Kanchipuram during period of February 2012 to January 2013. Total 104 non repetitive clinical isolates of *P.aeruginosa* collected were urine, sputum, blood fluids, pus and wound swab. Ethical committee clearance was obtained from the Institute and informed consent was obtained from all the patients. All the samples were inoculated onto nutrient agar, blood agar and incubated at 37ºC overnight. The colonies were tested for oxidase test and other biochemical tests for *P.aeruginosa.*

The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available discs (Hi-Media) on Muller Hinton Agar using Gentamycin (10mcg), Amikacin (30mcg), Tobramycin (30 mcg), Netilmicin (30mcg), Ciprofloxacin (5mcg), Ofloxacin (5mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefotaxime (30mcg), Piperacillin (10mcg), Piperacillin Tazobactam (100/10mcg), Amoxyclav 20/10 (30mcg), Ticarcillin-Clavulanic-acid (75/10mcg), Cefoperazone-Sulbactam (75/15mcg), Imipenum (10mcg), Nitrofurantoin (300mcg- for urinary isolates). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

DETECTION OF VARIOUS PHENOTYPIC RESISTANCE MECHANISMS

ESBL Screening [4]

Screening of *P.aeruginosa* for ESBLs production was performed according to the procedures as recommended by the CLSI, using indicator cephalosporins, ceftriaxone (30μg), ceftazidime (30μg), and cefotaxime (30μg). Isolates exhibiting zone size ≤ 25 mm with ceftriaxone ≤ 22 mm for ceftazidime and ≤ 27mm with cefotaxime were considered as ESBLs producer.

Phenotypic Confirmatory Test for ESBL: (Combined Disc Diffusion Method) [4]

0.5 McFarland turbidity standard suspension was made from the colonies of *P.aeruginosa* isolate. Using this inoculum, lawn culture was made on Muller Hinton Agar plate. Discs of Ceftazidime and Ceftazidime+Clavulanic acid (30 mcg/10 mcg) were placed aseptically on the surface of MHA. The distance of 15 mm was kept between the disc and overnight incubation was done at 37ºC. An increase of ≥ 5 mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organisms.

Methods of Phenotypic Detection of MBL [4]

Isolate with resistance to Imipenem were tested for metallo β lactamase production by Imipenum EDTA double disc synergy test (DDST).

Imipenem EDTA Double Disc Synergy Test (DDST) [4]

Lawn culture of the test organism was made onto MHA plates and Imipenum disc (10 µg) was placed 10 mm edge to edge from a blank disc contained 10 µl of 0.5 M EDTA (750 µg). Plates were incubated at 37ºC overnight. Enhancement of zone of inhibition in the area between Imipenem and EDTA disc in comparison with the zone of inhibition on the far side (other side) of the drug is interpreted as a Positive test.

AmpC β lactamase detection methods [4]

Organisms showing resistance to Cefoxitin (zone size <18mm) should be considered as probable AmpC producer and should be confirmed by other methods. Ceftazidime (30µg), Cefotaxime (30 µg) were placed at a distance of 20 mm from Cefoxitin (30µg) on a MHA plate inoculated with test organism. Isolates showing blunting of Ceftazidime or Cefotaxime zone of inhibition adjacent to Cefoxitin disc or showing reduced susceptibility to either of the above drugs and Cefoxitin are considered as AmpC producer.

RESULTS

Among the 3760 total clinical samples, 104 isolates of *P.aeruginosa* were isolated (2.76%). Pus (47.11%) was the predominant sample of isolation, which was followed by sputum (36.53%), urine (12.5%) and blood (3.84%). Males (55.76%) were commonly affected and maximum number of cases were seen between age group 21-40 years [Table/Fig-1].

S.No.	Age Groups (Yrs)	Male (%) (N=58)	Female (%) (N=46)	Total (%)
1	0-20	4	8	12(11.54%)
2	21-40	21	20	41(39.42%)
3	41-60	22	9	31(29.81%)
4	>60	11	9	20(19.23%)
Total		58(55.76%)	46 (44.23%)	104(100%)

[Table/Fig-1]: Age and sex distribution

Antimicrobial Agent (mcg)	Resistance (%)	
Ceftazidime (30)	68	65.38
Ciprofloxacin (5)	64	61.53
Piperacillin (100)	62	59.61
Ticarcillin/Clavulanic Acid (75/10)	59	56.73
Ceftriaxone (30)	58	55.76
Cefotaxime (30)	54	51.92
Gentamycin (10)	54	51.92
Amoxyclav (30)	45	43.26
Piperacillin-Tazobactam	41	39.42
Cefoperazone-Sulbactam (75/10)	39	37.5
Amikacin (30)	31	29.8
Tobramycin(10)	31	29.8
Ofloxacin (5)	24	23.07
Imipenem (10)	20	19.23
Netilmicin (30)	14	13.46
Nitrofurantoin (300) (n=13)	0	0

[Table/Fig-2]: Antimicrobial susceptibility testing by disc diffusion method

Highest resistance were observed for Ceftazidime (65.38%), Ciprofloxacin (61.53%), Piperacillin (59.61%), Ticarcillin/Clavulanic Acid (56.73%), Ceftriaxone (55.76%), Cefotaxime (51.92%), & Gentamycin (51.92%) [Table/Fig-2]. Those strains showed resistance to Ceftazidime, Ceftriaxone & Cefotaxime were subjected to ESBL detection tests.

MDR

Among 104, 43 (41.35%) *P.aeruginosa* was found to be Multi drug resistant (MDR). Resistance was seen in three or more different classes of antibiotics.

N=104	No of isolates	Percentage
ESBL	47	45.19 %
MBL	16	15.38 %
AmpC	0	0
BOTH ESBL & MBL	8	7.69%

[Table/Fig-3]: Various phenotypic resistance mechanisms

Among 104 strains of *P.aeruginosa*, which were screened phenotypically for various mechanisms of resistance, 47 (45.19%) showed ESBL production and 16 (15.38 %) showed MBL production. None of the isolate showed AmpC production [Table/Fig-3].

DISCUSSION

P.aeruginosa has been emerged as a significant pathogen and is the most common dreadful gram negative bacilli found in various health care associated infections all over the world due to its virulence, well known ability to resist killing by various antibiotics and disinfectants. The bacterial resistance has been increasing and this has both clinical and financial implication in therapy of infected patients.

In India, prevalence rate of *P.aeruginosa* infection varies from 10.5% to 30%. It ranged from 3 to 16%, in a multicentric study conducted by Ling JM et al., [5]. The prevalence in our study was found to be 2.76% which is comparable to above study.

P.aeruginosa were predominantly isolated from pus (47.11%), followed by sputum sample (36.53%). The same has been reported with Okon et al., (39.2%) [6], & Vijaya Chaudhari et al., (35.3%) [7]. Wound infection and respiratory tract infections were found to be commonly affected by *P.aeruginosa*.

Male preponderance (55.76%) was noted in this study. Similar observations were made by, Anupurba et al., (60%) [8] & Siti Nur et al., (57%) [9]. Outdoor activity, personal habits, nature of work and exposure to soil, water and other areas which are inhabited by organism could be the reason for male preponderance. More no of cases 41(39.42%) cases, were seen between 21-40 years. This is in accordance with other studies reported by Okon K.O et al., (24.6%) [6] and Anupurba S et al., [8] (45.88%), the common age group was between 21–40 in these studies too.

Among the β lactam drugs, Ceftazidime (65.38%), Piperacillin (59.61%), Ceftriaxone (55.76%) and Cefotaxime (51.92%) showed the highest resistance in this present study. K.M Mohanasundaram et al., (84.6%) [3], Yapar et al., (84%) [10] and Ibukun et al., (79.4%) [11], reported more resistance against ceftazidime in their study. Our study is in line with the reports of Diwivedi et al., (63%) [12] & Arya et al., (55.4%) [13]. Indiscriminate use of 3rd generation cephalosporin as broad spectrum empirical therapy and the secretion of ESBL enzymes mediate the resistance by hydrolysis of β-lactam ring of β-lactam antibiotics. Other mechanisms of drug resistance to β-lactam group of antibiotics are loss of outer membrane protein, production of class C AmpC β-lactamase and altered target sites.

Our study showed 47 (45.19%) isolates were ESBL producer. 42.30% ESBL producer were observed in the study of VarunGoel et al., [14]. Lower ESBL producer were seen in the studies by Prashant et al., [15] and Agarwal et al., [16] which were 22.22% & 20.27% respectively. Whereas, Uma et al., observed high percentage of isolates (77.3%) to be ESBL producer [17].

The ESBL enzymes are inhibited by β-lactamase inhibitors, viz., clavulanic acid and sulbactam. Hence the use of β-lactam/β-lactamase inhibitor combination may be an alternative to 3rd generation cephalosporin, but the effect of this combination varies depending on the subtype of ESBL present. β-lactamase inhibitor resistance was ranged from 37.5% to 56.73% in our study. Similar resistance also observed by K.M Mohanasundaram et al., (40.3%) [3]. High resistance (96.66%) was seen to Ticarcillin/Clavulanate and 63.33% of resistance was observed to Ampicillin /Sulbactam by Agarwal et al., [16]. Increasing resistance to β lactam inhibitors

is a problem in therapeutic part which makes them less reliable for therapeutic purposes.

Though imipenem was found unaffected by the action of the enzymes in many studies, MBL production in our study was 15.38% which is comparable with the studies of Ibukun et al., [11] Prashant et al., [15] Agarwal et al., [16] Jayakumar et al., [18] Navneeth et al., [19] and, slightly raised level of carbapenem resistance were reported by Variya et al., (25%) [20]. The percentage variation in the resistance mechanism could be due to the study environment where the study was done. These carbapenem agents may be of benefit in the treatment of ESBL infection; however, indiscriminate use of these agents may promote increased resistance to carbapenems. None of our isolates showed AmpC β lactamase.

P.aeruginosa showed resistance to many other classes of antibiotics, including aminoglycosides and fluoroquinolones. This is due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL. This fact has also been observed in our study. Among the aminoglycoside group, Gentamycin showed highest resistance (51.92%). Minimal resistance was observed with other aminoglycoside such as amikacin (29.8%) tobramycin (29.8%) and netilmycin (13.46%) which shows promising effect in treatment.

Ciprofloxacin showed 61.53% resistance to *P.aeruginosa* in our study. In various reports on ciprofloxacin resistance to *P.aeruginosa* was ranged between 0-89% (Algun et al.,) [21]. Compared to this, ofloxacin (23.07%) found to be useful to keep as reserve drug or as combination therapy.

Multi Drug Resistant *P.aeruginosa* (MDR PA) is defined as isolates resistant to at least 3 classes of drugs in anti-pseudomonal cephalosporins, carbapenem, aminoglycosides and fluoroquinolones. MDR is pervasive and growing clinical problem, which is recognized as a threat to public health in causing significant on morbidity and mortality and increased economic burden which stems from the misuse of antibiotics particularly excessive use. The percentage of MDR PA in India ranges from 11.36% reported by Siti Nur Atiquah Idris et al., [9] to 91.6% reported by S.Panranjothi et al., [22]. In our study, 41.35% *P.aeruginosa* were found to be Multi drug resistant (MDR) which is comparable with above studies.

LIMITATIONS OF THE STUDY

1. Limited research works are available about prevalence of *P.aeruginosa* resistance pattern in our area.

2. To formulate the antibiotic policy, and to reduce the emergence of resistance a large scale molecular study has to be conducted to analyze the resistant gene prevalent in our area.

CONCLUSION

Due to the availability of few studies in our locality, studies like this would help to formulate the antibiotic guidelines to the physician in treatment part which in turn has a great impact in preventing the mortality and morbidity associated with *Pseudomonas* infections.

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