



ANTIBIOTIC SUSCEPTIBILITY PATTERN OF PSEUDOMONAS SPECIES AT A TERTIARY CARE HOSPITAL IN CENTRAL INDIA.

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ABSTRACT

Introduction: Pseudomonas is widely distributed in nature. Intrinsic resistance to numerous antibiotics contribute to their virulence. Aims & **Objectives:** To isolate the Pseudomonas species from different clinical specimens and to study their antibiotic susceptibility pattern.

Material & Methods: Study was conducted in Department of

Microbiology, Indira Gandhi Govt. Medical College, Nagpur from August 2011 - August 2013. Pseudomonas isolates were processed to species level by standard procedures and antibiotic susceptibility was done according to CLSI guidelines 2011. **Observations & Results:** A total of 210 Pseudomonas strains were isolated and maximum 44% were from pus. Amongst them, 95% were *P. aeruginos*, 2.4% were *P. putida*, 1.4% were *P. stutzeri* and 0.95% were *P. fluorescens*. Amongst the 200 *P. aeruginosa*, least resistance was found to colistin (2.5%) and imipenem (21%) followed by piperacillin-tazobactam (25%) and amikacin (27%). Resistance to cephalosporines, aminoglycosides, tobramycin and netillin was in the range of 30 – 35%. Ciprofloxacin was 52% and norfloxacin was 67% resistant. Metallo-beta lactamase (MBL) production was observed in 12% isolates. The Pseudomonas isolates other than *P. aeruginosa*, were more sensitive as compare to *P. aeruginosa*. They were 100% sensitive to colistin and imipenem followed by 80% sensitivity to piperacillin - tazobactam, 70% to amikacin & gentamycin, 60% to tobramycin & netillin and 50% to cephalosporines, quinolones and aztreonam.

KEYWORDS: Pseudomonas species, antibiotic resistance, metallo-beta lactamase.

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] Pseudomonas species that are opportunistic pathogens in patients, immunologically compromised by diseases or treatment, *P. aeruginosa* is pre-eminent followed by *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. alcaligenes* and *P. pseudoalcaligenes*.^[2]

Pseudomonas exhibit reduced susceptibility to beta lactam (β -lactam) antibiotics by number of mechanisms. By far the most common mechanism is enzymatic inactivation of β - lactam by β -lactamases.^[3] The occurrence of an MBL positive *P. aeruginosa* isolate in a hospital environment poses not only a therapeutic problem, but is also a serious concern for infection control management.^[4]

Very less data is available about Pseudomonas species from our area. The present study was undertaken to know the pattern of antibiotic sensitivity of Pseudomonas isolates.

MATERIAL AND METHODS

The Prospective study was conducted from August 2011 to July 2013 in Department of Microbiology in a tertiary care hospital at Nagpur, the Central part of India.

Case history data of the patients was recorded as per the proforma. Various clinical samples like sputum, pus, pleural fluid, urine, ET aspirate, bronchoalveolar lavage, ear swabs and wound swabs were collected and processed. Pseudomonas species were identified by standard procedures.^[5]

Antibiotic sensitivity was done by Kirby Bauer disc diffusion method as per CLSI guidelines 2011.^[6] Metallo-beta lactamase production was detected by disc potentiation test.^[7] All the isolate showing resistance to imipenem were evaluated for metallo-beta lactamase (MBL) production. The isolate was inoculated onto a Mueller-Hinton Agar (MHA) plate. Disc of 10 μ g imipenem and disc of imipenem with EDTA (10/750 μ g) were placed on the plate at 15 mm distance. After overnight incubation, if zone of imipenem with EDTA is >7 mm than that of imipenem disc alone, test strain was considered to be MBL positive.

RESULTS

Table – 1: Pseudomonas isolates from various clinical specimens

Specimens (n = 210)	Total (%)
Pus (pus, wound swab ,ear swab)	92 (43.8)
Respiratory specimen (sputum, ET Aspirate, pleural fluid)	59 (28.1)
Urine	31 (14.7)
Blood	16 (07.6)
Others (corneal scrapping, catheter tip, drain fluids)	12 (05.7)
Total	210

Table - 2: Various species of Pseudomonas isolated from different clinical specimens

Specimens	<i>P.aeruginosa</i> (%)	<i>P. putida</i> (%)	<i>P. stutzeri</i> (%)	<i>P. fluorescens</i> (%)
Pus	89 (44.5)	02 (40)	00	01 (50)
Respiratory specimens	56 (28)	01 (20)	01 (33.3)	01 (50)
Urine	29 (14.5)	01 (20)	01 (33.3)	00
Blood	15 (07.5)	00	01 (33.3)	00
Other	11 (05.5)	01 (20)	00	00
Total	200 (95.2)	05 (2.4)	03(1.4)	02 (0.95)

Table - 3: Antimicrobial susceptibility of *Pseudomonas aeruginosa* (n = 200)

Antimicrobial agents	Sensitive (%)	Resistant		
		Intermediate	Resistant	Total Resistant (%)
Piperacillin	114 (57.0)	08	78	86 (43.0)
Piperacillin – tazobactam	151 (75.5)	12	37	49 (24.9)
Ceftazidime	134 (67.0)	18	48	66 (33.0)
Cefepime	140 (70.0)	16	44	60 (30.0)
Aztreonam	102 (51.0)	06	92	98 (49.0)
Imipenen	158 (79.0)	07	35	42 (21.0)
Colistin	195 (97.5)	00	05	05 (02.5)
Gentamycin	138 (69.0)	08	54	62 (31.0)
Tobramycin	132 (66.0)	12	56	68 (34.0)
Amikacin	147 (73.5)	15	38	53 (26.5)
Netillin	130 (65.0)	13	57	70 (35.0)
Ciprofloxacin	96 (48.0)	23	81	104 (52.0)
Norfloxacin (n = 29)	10 (37.5)	05	14	19 (65.5)

Table – 4: Antimicrobial susceptibility of *Pseudomonas* species other than *P. aeruginosa* (n = 10)

Antimicrobial agents	<i>P. putida</i> (n = 5)	<i>P. stutzeri</i> (n = 3)	<i>P. fluorescens</i> (n = 2)	Total (n = 10)
	S (%)	S (%)	S (%)	S (%)
Piperacillin	3 (60)	2 (66.7)	1 (50)	06 (60)
Piperacillin - tazobactam	4 (80)	3 (100)	1 (50)	08 (80)
Ceftazidime	2 (40)	2 (66.7)	1 (50)	05 (50)
Cefepime	3 (60)	2 (66.7)	0 (0)	05 (50)
Aztreonam	2 (40)	2 (66.7)	1 (50)	05 (50)
Imipenem	5 (100)	3 (100)	2 (100)	10 (100)
Colistin	5 (100)	3 (100)	2 (100)	10 (100)
Gentamycin	4 (80)	2 (66.7)	1 (50)	07 (70)
Tobramycin	3 (60)	2 (66.7)	1 (50)	06 (60)
Amikacin	5 (100)	2 (66.7)	0 (0)	07 (70)
Netillin	3 (60)	1 (33.3)	1 (50)	05 (50)
Ciprofloxacin	3 (60)	1 (33.3)	1 (50)	05 (50)
Norfloxacin (n=2)	1 (50)	1 (50)	---	02 (20)

Table -5: Antibiotic sensitivity pattern in MBL and nonMBL producing *P. aeruginosa* isolates

Antimicrobial Agents	MBL producer (n = 24)		Non MBL producer (n = 176)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Piperacillin	01 (04.1)	23 (95.9)	113 (64.2)	63 (35.8)
Piperacillin – tazobactam	11 (45.8)	13 (54.1)	140 (79.5)	36 (20.5)
Ceftazidime	06 (25.0)	18 (75.0)	128 (72.7)	48 (27.3)
Cefepime	07 (29.1)	17 (70.9)	133 (75.6)	43 (24.4)
Aztreonam	12 (50.0)	12 (50.0)	095 (53.9)	81 (46.1)
Polymixin	24 (100)	00 (00.0)	-	-
Colistin	20 (83.3)	4 (16.7)	175 (99.4)	1 (0.6)
Gentamycin	09 (37.5)	15 (62.5)	129 (73.2)	47 (26.8)
Tobramycin	08 (33.3)	16 (66.7)	124 (70.4)	52 (29.6)
Amikacin	09 (37.5)	15 (54.2)	135 (76.7)	41 (23.3)
Netillin	08 (33.3)	16 (66.7)	122 (69.3)	54 (30.7)
Ciprofloxacin	06 (25.0)	18 (75.0)	090 (51.1)	86 (48.90)
Norfloxacin (n = 29)	00 (0%)	02(100)	10(37)	17(63)

In the present study, 210 *Pseudomonas* were isolated and different species were identified. Amongst these, 110 (52%) were from males and 100 (49%) were from females. The maximum 58 (28%) isolates were observed in the age group of 41-50 yr followed by 41(20%) in the age group of 51-60 yr.

Amongst the total 210 isolates, *P. aeruginosa* isolates were 200 (95%) and other species contribute 10 (5%) isolates. Amongst these 10 isolates, *P. putida* were 5 (2%), *P. stutzeri* 3 (1%) and *P. fluorescens* were 2 (0.95%).

Amongst the total 200 *P. aeruginosa* isolates, Colistin was found to be least resistant (2.5%), followed by imipenem (21%), piperacillin – tazobactam (24.5%) and amikacin (26.5%). Resistance to cefepime was found to be 30 %, 31% to gentamycin, 33% to ceftazidime, 34% to tobramycin and 35% to netillin. Most of the resistance was found to piperacillin (43%), aztreonam (49%), ciprofloxacin (52%), and norfloxacin (65.5 %). Out of these 200 isolates, 42 were resistant to imipenem and 24 (12%) were found to be MBL producers. We found that 24 (12%) MBL strains of *P. aeruginosa* were 100% sensitive to polymixin and 83.3% to colistin while 100% resistant was observed to norfloxacin. Polymixin B was not tested in non MBL producers while sensitivity to colistin was 99%. Non-MBL strains were sensitive to most of the antibiotics.

Amongst the 10 *Pseudomonas* isolates other than *P. aeruginosa*, all were 100% sensitive to colistin and imipenem, 80% to piperacillin - tazobactam, 70% to amikacin and gentamycin, 60% to tobramycin and netillin and 50% to cephalosporins, quinolones and aztreonam.

DISCUSSION

Infections due to *P. aeruginosa* are seldom encountered in healthy adults; but in the last two decades, the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients, especially those with impaired immune defenses. In addition to its innate resistance, acquired additional resistance due to plasmids is also a problem in *P. aeruginosa*. Plasmid-mediated resistance involving modifying enzymes is particularly associated with indiscriminate antibiotic use and with sites where high levels of antibiotics are achieved.^[8]

In the present study, 210 *Pseudomonas* isolates were more common in males (52%) as compare to females (49%). This finding is comparable with Bashir et al.^[9] Other studies had also reported higher isolation rates (>60%) in males.^[10,11] *Pseudomonas* infections were more common in the age group of 41-50 yr followed by age group of 51-60 yr which was comparable with Rajat et al^[12] while other studies have observed different findings.^[10,13] This age group in present study was might be due to more admission reported in 40-50 age group .

Pseudomonas were most commonly isolated from pus (44%) followed by respiratory specimens (28%). Similar pattern was observed by other studies^[14, 15] but holds a deviation from Ergin et al^[16] who found more (33%) isolates from respiratory specimens.

P. aeruginosa is the Pseudomonad most frequently recovered from clinical specimens.^[17] In the present study, the most common isolate from all clinical specimens was *P. aeruginosa*. It's isolation from pus was 44.5% which was comparable with Agrawal et al.^[18]

P. putida was the agent of catheter related bacteremia isolated from sputum, joints, peritoneal fluid and urine related infections.^[17] In present study, *P. putida* was isolated from pus, respiratory specimens, urine and other specimens. The isolation rate was comparable with Ergin et al^[16] while Osterhout et al^[19] had reported higher percentage.

P. stutzeri is an unusual cause of human infection and reported as a pathogen in 1895. It was isolated from urine, blood, wound and respiratory tract of intubated patient.^[20] In the present study, 3 (1%) *P. stutzeri* were isolated from sputum and urine which is similar with the finding of Gad et al.^[21] It's isolation rate was comparable with Ergin et al^[16] while higher percentage was reported by Osterhout et al.^[19]

P. fluorescens was recovered from urinary tract infection and wound infection.^[17] In present study, 2 (0.95%) isolates of *P. fluorescens* were from pus, sputum and urine which was in agreement with Juyal et al.^[15] The isolation rate was comparable with Sidhu et al^[22] and Noble et al^[20] while other studies have reported higher percentage.^[15,19]

The sensitivity of *P. aeruginosa* often varies between communities, hospitals in the same community and among different patient population in the same hospital. Hence it is important to remain updated with prevalence and antimicrobial susceptibility pattern of the circulating pathogen to decide the empiric therapy.

In the present study, amongst the 200 isolates of *P. aeruginosa*, colistin was found as least resistant (2.5%) drug followed by imipenem (21%) and piperacillin-tazobactam (24.5%), cephalosporins (30-33%) and aminoglycosides (25 - 35%), which is similar with the findings by Juyal et al.^[15]

Our findings differ from Rajat et al^[12] who had found piperacillin – tazobactam (4%) and imipenem (14%) as least resistant drugs and Peshettiwar et al^[11] who had found ceftazidime

(5%) followed by imipenem (13%) and piperacillin – tazobactam (21%) as least resistant drugs.

All the drugs were sensitive in study by Raja *et al*^[23] while Behara *et al*^[24] have found all the drugs more resistant.

Norfloxacin (65.5%) and ciprofloxacin (52%) followed by aztreonam (49%) were found as most resistant drugs in our study. The findings are similar with those of Juyal *et al*.^[15]

In the present study, resistance to ceftazidime was found in 33% and 30% to cefepime. Higher resistance to ceftazidime was reported by other studies.^[11,12,15,25] Many studies have not studied the resistance to cefepime. Malini *et al*^[14] have observed similar finding while Juyal *et al*^[15] and Javiya *et al*^[25] have reported it in higher percentage.

In the present study, resistance to colistin was found in 5 (2.5%) isolates. Amongst them, 4 were MBL producer and 1 was having other mechanism of resistance. Very few studies had observed colistin resistance. Varaiya *et al*^[26] had found 42.5% and 33% in MBL and non-MBL strains respectively. Manoharan *et al*^[27] had observed 100% sensitivity in MBL producers.

There are very few reports on antibiotic sensitivity pattern of *Pseudomonas* species other than *P. aeruginosa*.

In present study, these 10 *pseudomonas* isolates were 100% sensitive to colistin and imipenem followed by 80% sensitivity to piperacillin - tazobactam, 70% sensitivity to amikacin and gentamycin, 60% sensitivity to tobramycin and netillin , 50% sensitivity to cephalosporins, quinolones and aztreonam.

Sidhu *et al*^[22] had reported 25-75% resistance by *P. stutzeri* and *P. putida* showed 50% resistance while Malini *et al*^[14] had reported 5-91% resistance by *P. fluorescens* while Juyal *et al*^[15] had reported it in the range of 21-79%.

Although different phenotypic methods have been described for a long time, there was no standard guideline for screening of carbapenemases. CLSI has recommended modified Hodge test for detection of carbapenemase activity in enterobacteriaceae but not in nonfermenters.

In present study, amongst the 200 isolates of *P. aeruginosa*, 42 isolates were resistant to Imipenem and 24(12%) were found to be MBL producers by disc approximation method. This is comparable with Peshettiwar et al.^[11]

Renu G^[28] had reported the existence of MBL in carbapenem susceptible organism. Such imipenem susceptible organism carrying hidden MBL genes may spread unnoticed that might be the reason for lower percentage of MBL in present study than most of the studies.

Resistance of MBL producers to various antibiotics ranged between 17-96 % while that of non-MBL producers was 1 to 49%. Non MBL strains were sensitive to most of the antibiotics. Higher resistance to various antibiotics by MBL producers as compared to non producers has also been reported by Bashir et al.^[9]

In many cases, the MBL genes may be located on plasmid encoding other antibiotic resistant determinants. Such MBL positive strains are usually resistant to betalactams, aminoglycosides and fluoroquinolones.^[9] In our study, 100% resistance was shown by MBL producers to norfloxacin, 96% resistance to piperacillin and more than 50% resistance to most of the antibiotics. MBL positives are usually susceptible to polymyxin, aztreonam and colistin.^[29] Our study also observed 100% sensitivity to polymixin B and 83% to colistin but only 50% to aztreonam. Varaiya et al^[26] and Juyal^[15] et al have reported low sensitivity to aztreonam while higher percentage is observed by Rajat et al^[12]. Sensitivity of 58% to colistin have been reported by Varaiya et al^[26] and Rajat et al^[12] have observed 55% sensitivity to polymixin B.

CONCLUSION

P. aeruginosa are becoming less sensitive to piperacillin – tazobactam, gentamycin, amikacin, cefepime, tobramycin and netillin . Early laboratory identification of the infections due to these multiresistant organisms is necessary to avoid therapeutic failures resulting in reduction in mortality in hospitalized patients and helps in surveillance and infection control measures to avoid the nosocomial outbreaks.

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