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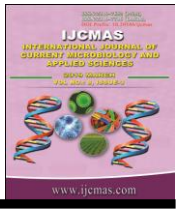

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Antibiotic Susceptibility Pattern in Clinical Isolates of *Pseudomonas aeruginosa* from a Tertiary Care Hospital of Tripura

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A B S T R A C T

Pseudomonas aeruginosa are established pathogens predominantly in various nosocomial infections which are often life threatening due to limited therapeutic options. The present study was undertaken to analyze the antibiotic susceptibility pattern in the clinical isolates of *Pseudomonas aeruginosa*, so as to establish the current therapeutic options available for treatment in this geographical area of North East India. The study was conducted on 150 strains of *Pseudomonas aeruginosa* isolated from various clinical samples. Isolation and confirmation of the organism in culture was performed using standard microbiological techniques. The antibiotic susceptibility testing was performed by Kirby Bauer Disc diffusion method. Out of all clinical samples 6.6% were identified to be *Pseudomonas aeruginosa* and 69.4% were elderly male patients. The highest numbers (62.7%) of isolates were from pulmonary samples like sputum, Endotracheal aspirate and Bronchoalveolar lavage, followed by urine (25.3%). 10.7% isolates were resistant to Imipenem, followed by Meropenem (12.7%), Piperacillin Tazobactum (14%) and Piperacillin (28.7%). Higher level of resistance was observed with Nitrofurantoin(71.1%), Gentamicin (60.7%), Amikacin (42%), Ciprofloxacin (46%) and Levofloxacin (42%). A total of 43(28.7%) isolates were Multi-Drug resistant. In our hospital, the antibiotic of choice for treatment of infections due to *Pseudomonas aeruginosa* could be Piperacillin Tazobactum. Emphasis on strict adherence to hospital infection control guidelines and antibiotic policy is also recommended as most of the isolates were recovered from hospitalized patients.

Introduction

Pseudomonas aeruginosa are gram negative, aerobic, non-fermentative bacilli, widely distributed in nature and hospital environment. It is responsible for 10 – 20% of nosocomial infections (Carmeli *et al.*, 1999). They are established pathogens in nosocomial Pneumonia, Urinary tract infections, skin and soft tissue infections, burns, injuries, septicemia and infections in immunocompromised conditions.

The infections caused by *Pseudomonas aeruginosa* are often life threatening due to limited therapeutic options, owing to the

constitutive low level of susceptibility to several antibiotics and multiple genetic mechanisms of resistance (Babay, 2007). Their resistance to antibiotics may be due to mutation in chromosomal genes which regulate the resistance genes and acquisition of additional genes from other organisms or environment via plasmids, transposons and bacteriophages. Increase in the prevalence of Multi-Drug Resistant (MDR) strains *of Pseudomonas aeruginosa* has been reported worldwide, complicating decisions on antibiotic policy and its relation to high morbidity and mortality (Babay, 2007; Ergin and Mutlu, 1999). The variations in antibiotic susceptibility exists in different geographical locations and periods, due to difference in pattern of prescribing habits, for which periodic analysis of antibiotic susceptibility pattern is essential to know the susceptible therapeutic options available for treatment. The objective of this study was to analyze the antibiotic susceptibility pattern in the clinical isolates *of Pseudomonas aeruginosa*, so as to establish the current therapeutic options available for treatment.

Materials and Methods

The prospective study was conducted in the Microbiology Department of Tripura Medical College & Dr. BR Ambedkar Memorial Hospital, a tertiary care centre of the North Eastern State of Tripura. The proposal for the study was approved by the Institutional Human Ethics Committee. The study was conducted on 150 strains *of Pseudomonas aeruginosa* isolated from various clinical samples during a period of one year six months from July 2014 to December 2015. The particulars and clinical data of the patients were recorded simultaneously.

In the laboratory all collected samples were cultured aerobically on Blood agar and MacConkey agar media plates at 37°C for 24 hours. Blood specimen were cultured in Brain Heart Infusion Broth and subsequently sub-cultured in Blood agar and MacConkey agar plates. Suspected Non-Lactose Fermenting colonies *of Pseudomonas aeruginosa* were identified using colony morphology, motility testing, Grams reaction and biochemical tests indicating positive oxidase test, alkaline slant in Triple Sugar Iron agar medium, negative Indole production test, positive citrate utilization test and positive nitrate reduction test. Definitive identification *of Pseudomonas aeruginosa* included identifying the production of the blue green pigment pyocyanin and its ability to grow at 42°C (Collee *et al.,* 2006). Antibiotic susceptibility testing was performed against Anti-Pseudomonal antibiotics by modified Kirby Bauer Disc diffusion method conforming to the CLSI guidelines. (CLSI, 2014) Anti-Pseudomonal antibiotics used for susceptibility testing were from the classes of Ureidopenicillins, Cephalosporins, Carbapenems, Aminoglycosides and Fluoroquinolones. For Quality control *Pseudomonas aeruginosa* ATCC 27853 strain was used. The Multi-Drug Resistant (MDR) strains *of Pseudomonas aeruginosa* were identified by the criteria that those which are resistant to three or more classes of Anti-Pseudomonal antibiotics (Magiorakos, 2011).

Results and Discussion

The data collected for a period of 18 months reveals that, out of 7368 samples, culture was positive in 2274 cases and 150(6.6%) isolates were identified to be *Pseudomonas aeruginosa*. The majority of the patients were males (56%) and of elderly age group of more than 60 years (48%) as shown in the Table 1 (Fig. 1).

Varied spectrum of the lower respiratory tract infections with Chronic obstructive pulmonary disease were the most common clinical cases

in which the organisms were isolated. The isolates *of Pseudomonas aeruginosa* were most commonly identified from lower respiratory tract secretions 94(62.7%) in specimen like sputum, Broncho-alveolar lavage and Endotracheal aspirates, followed by urine 38(25.3%) as mentioned in the Table 2 (Fig. 2).

Among the Beta-Lactams tested, the most effective agent was Imipenem 134(89.3%) followed by Meropenem 131(87.3%), Piperacillin 107(71.3%), Cefepime 98(65.3%), Ceftazidime 96(64%). The susceptibility results of combination of Beta-Lactams and Beta-Lactamase inhibitors tested were Piperacillin-tazobactum 129(86%) and Cefoperazone-sulbactum 103(68.7%). Among the Aminoglycosides, Netilmicin showed considerable sensitivity of 93(62%), followed by Amikacin 87(58%). Only 81(54%) isolates were sensitive to Ciprofloxacin and 87(58%) to Levofloxacin. Nitrofurantoin was additionally tested against the 38 urine isolates *of Pseudomonas aeruginosa*, in which only 11(28.9%) of the isolates were susceptible. The observation is depicted in Table 3 (Fig. 3). A total of 43(28.7%) isolates were Multi-Drug resistant, i.e. resistant to three or more antibiotic classes.

In the present study, *Pseudomonas aeruginosa* were isolated in 6.6% of the culture positive cases, which can be compared with reports from Odisha and Andhra Pradesh stating 8.43% and 9.3% prevalence rate respectively (Pathi *et al.,* 2013; Srinivas *et al.,* 2012). The prevalence rate may vary depending upon type of clinical specimen, status of healthcare centre, demographic profiles and geographical location (Dash *et al.,* 2014). A prevalence as low as 2.1% has been reported from Nigeria, whereas a high prevalence rate of 32.1% was reported from Gujarat (Okon *et al.,* 2010; Rajat *et al.,* 2012). In our observation, 56% of the patients were males and 48% of the patients belonged to age more than 60 years. Out of the total number of elderly patients, 69.4% were elderly male patients (Fig. 1). Other studies also reported similar observation, ranging from 62.5% to 71% of elderly male patients being infected with *Pseudomonas aeruginosa* (Javiya *et al.,* 2008; Mayank *et al.,* 2009)

In the present study, maximum number (62.7%) of isolates were from pulmonary samples like sputum, Endotracheal aspirate and Bronchoalveolar lavage, followed by urine (25.3%) and pus (7.3%) (Fig. 2). This observation is different from most of the reported studies, in which isolation *of Pseudomonas aeruginosa* from pus and urine samples predominates over sputum (Dash *et al.,* 2014; Okon *et al.,* 2010; Mohanasoundaram, 2011) A study from Uttar Pradesh reported that highest number of isolates (53.89%) *of Pseudomonas aeruginosa* were from pulmonary samples. (Prakash *et al.,* 2014) A study from Kathmandu reported that maximum number of isolates *of Pseudomonas aeruginosa* were from urine and sputum (36.3% each). (Shrestha *et al.,* 2016) The high rate of isolation *of Pseudomonas aeruginosa* from pulmonary samples during our study period may be due to a possible outbreak of infection in General Medicine ward and Intensive Care Unit, as most of the admitted patients were previously diagnosed as Chronic Obstructive Pulmonary Disease.

On analysis of Antibiotic susceptibility pattern, we observed that 10.7% isolates were resistant to Imipenem, followed by Meropenem (12.7%), Piperacillin Tazobactum (14%) and Piperacillin (28.7%). Higher level of resistance was observed with Gentamicin (60.7%), Amikacin (42%), Ciprofloxacin (46%), Levofloxacin (42%) and Nitrofurantoin in urine isolates (71.1%). The isolates did not reveal an acceptable level of sensitivity for therapeutic use to other tested

antibiotics like Cefepime, Ceftazidime, Netilmicin and Cefoperazone sulbactum (Fig. 3). In our centre, keeping the Carbapenems as "reserve drugs", the Ureidopenicillins preferably in combination with a Beta-Lactamase inhibitor like Piperacillin Tazobactum may be considered as drug of choice for treatment of patients suffering from infections due to *Pseudomonas aeruginosa*. Concurrent administration of a Beta-Lactamase inhibitor markedly expands the spectrum of activity of acid resistant Penicillins like Piperacillin and Ticarcillin. The dose and incidence of toxicity also gets subsequently reduced with Ureidopenicillins.

Similar observation was reported in a recent study from Odisha, stating Imipenem, Meropenem and Piperacillin Tazobactum to be the most effective drugs against infections due to *Pseudomonas aeruginosa*, exhibiting resistance rates as low as 6.4%, 8% and 11.3% respectively. However, in their observation the isolates were less resistant to Aminoglycosides and Fluoroquinolones, unlike our report (Dash *et al.*, 2014). This might be due to widespread administration of Aminoglycosides and Fluoroquinolones in hospital and community for treatment of infections and surgical prophylaxis in medical practice in this region.

In the present study, 28.7% isolates *of Pseudomonas aeruginosa* were Multi-Drug Resistant, which implies resistance to three or more antibiotic classes. Observations reported from Uttar Pradesh and Pakistan are quite similar as ours, stating 31.7% and 22.7% rate of isolation of Multi-Drug Resistant *Pseudomonas aeruginosa* respectively (Prakash *et al.*, 2014; Gill *et al.*, 2011). Multi-Drug Resistant Pseudomonas aeruginosa elaborates inactivating enzymes which can be chromosomally encoded or plasmid mediated, that make Beta-Lactams and Carbapenems ineffective, such as Extended Spectrum Beta Lactamases and Metallo Beta Lactamases (Vahdani *et al.*, 2012).

Table.1 Proportions of Age-Group of patients in relation to sex and isolates *of Pseudomonas aeruginosa*

Age – Group of patients	Number of Isolates N (%)	Males N (%)	Females N (%)
0 – 15 years	05 (3.3%)	02 (40%)	03 (60%)
16 – 30 years	11 (7.3%)	04 (36.4%)	07 (63.6%)
31 – 45 years	27 (18%)	10 (37%)	17 (63%)
46 – 60 years	35 (23.3%)	18 (51.4%)	17 (48.6%)
>60 years	72 (48%)	50 (69.4%)	22 (30.6%)
TOTAL	150	84 (56%)	66 (44%)

Table.2 Proportion of Isolates from various clinical specimen

Specimen	Isolates N = 150	Proportion (%)
Pulmonary samples	94	62.7 %
Urine	38	25.3%
Pus	11	7.3 %
Blood	07	4.6%

Table.3 In vitro susceptibility pattern *of Pseudomonas aeruginosa* isolates

Antibiotic (Disk concentration in µg)	Proportion of susceptible isolates [N(%)]	Proportion of resistant isolates [N(%)]
Imipenem (10)	134 (89.3%)	16 (10.7%)
Meropenem (10)	131 (87.3%)	19 (12.7%)
Piperacillin-tazobactum (100/10)	129 (86%)	21 (14%)
Piperacillin (100)	107 (71.3%)	43 (28.7%)
Cefoperazone – sulbactum (75/30)	103 (68.7%)	47 (31.3%)
Cefepime (30)	98 (65.3%)	52 (34.7%)
Ceftazidime (30)	96 (64%)	54 (36%)
Amikacin (30)	87 (58%)	63 (42%)
Gentamicin (10)	59 (39.3%)	91 (60.7%)
Netilmicin (30)	93 (62%)	57 (38%)
Levofloxacin (5)	87 (58%)	63 (42%)
Ciprofloxacin (5)	81 (54%)	69 (46%)
Nitrofurantoin (300) (For Urine isolates (n=38))	11(28.9%)	27 (71.1%)

Fig.1 Proportion of age group of patients in relation to sex and isolates *of Pseudomonas aeruginosa*

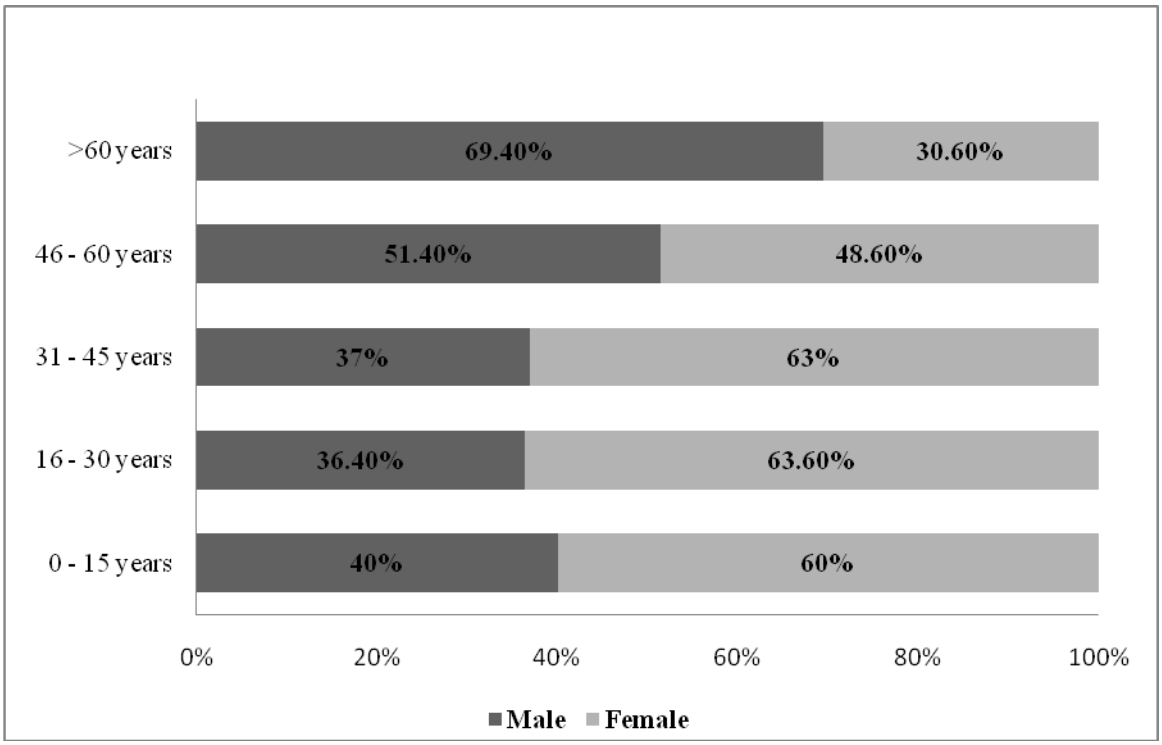


Fig.2 Proportion of isolates from various clinical samples

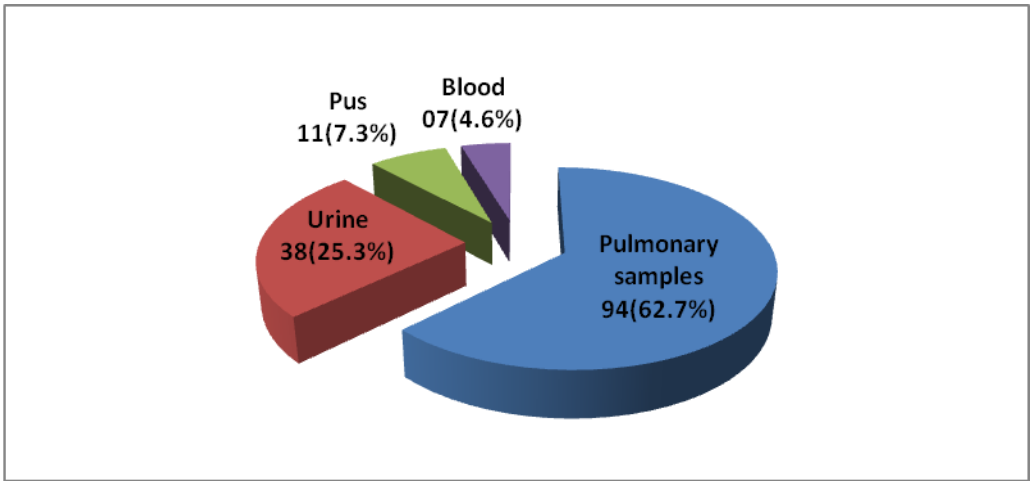
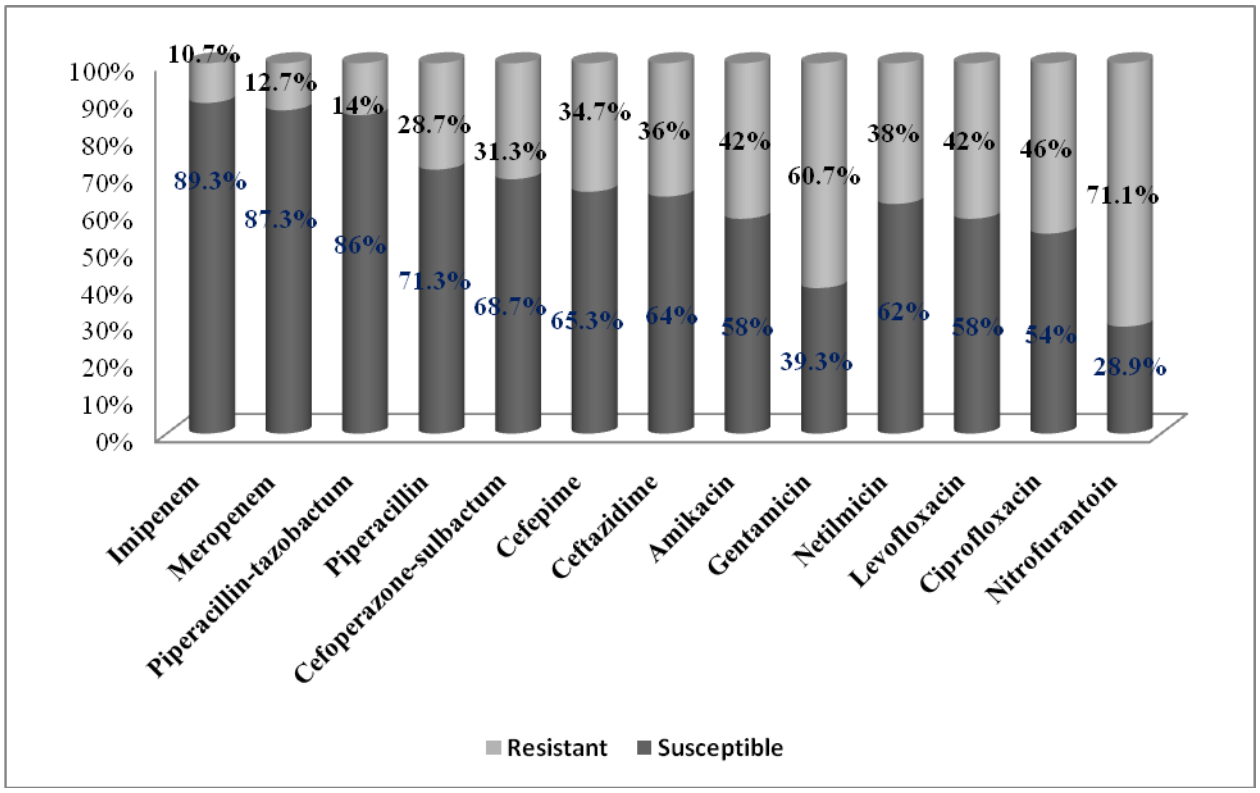


Fig.3 In vitro antibiotic susceptibility pattern of the isolated *Pseudomonas aeruginosa*



The increasing trend of drug resistance exhibited by *Pseudomonas aeruginosa* is an established fact, which can be due to irrational use of broad spectrum antibiotics and its unique feature to acquire resistance due to low permeability of the cell wall, production of inducible Cephalosporinases, an active efflux with a poor affinity to target sites. (Al-Tawfiq, 2007).

In conclusion, in our healthcare setting, Piperacillin Tazobactum can be considered as

the appropriate antibiotic for treating infections due to *Pseudomonas aeruginosa*. As, majority of the isolates were recovered from hospitalized patients, we need to emphasize upon strict adherence to hospital infection control guidelines and antibiotic policy. Periodic antimicrobial surveillance is also recommended to keep a track on the development and spread of Multi-Drug Resistant *Pseudomonas aeruginosa*.

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