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Prevalence and Antibiotic Resistant Pattern of *Pseudomonas aeruginosa* at a Tertiary Care Centre of North India

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A B S T R A C T

The aim of this study was to analyze the extended spectrum of β lactamase (ESBL), metallo β lactamase (MBL) and AmpC production in *Pseudomonas aeruginosa* in various clinical samples. A Total of 100 clinical isolates of *P. aeruginosa* were collected from different clinical specimen and confirmed by standard tests. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method. 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. Out of 100 clinical *P.aeruginosa* isolates, 33% were ESBL producer, 18 % MBL producer both ESB and MBL 9% and none were AmpC producer. Imipenem (81%), meropenem (82%), aminoglycosides (amikacin (72%), tobramycin (74%), netilmycin (71%) and Polymyxin B(100%) and colistin (100%) has got the better antipseudomonal activity. 28 (28%) *P.aeruginosa* was found to be Multi Drug Resistant (MDR). This study highlights the prevalence of ESBL, MBL and MDR *P.aeruginosa*. In our study Carbapenems and aminoglycosides are promising drugs with antipseudomonal activity while polymyxin b and colstin use as reserved drug.

Introduction

Pseudomonas aeruginosa belongs to a large group of aerobic, non-fermenting saprophytic, gram-negative bacilli widespread in nature, particularly in moist environment. (Govan, 2008; Du Bois *et al.*, 2001) However, its profound ability to survive on inert materials, minimal nutritional requirement, tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen. (Gales *et al.*, 2001) *Pseudomonas aeruginosa* has emerged as a major cause of infection in the last few decades. It is an increasingly prevalent opportunistic pathogen and is the fourth most frequently isolated nosocomial pathogen accounting for 10% of all hospital acquired infections. (Pathi *et al.*,) The organism has been incriminated in cases of meningitis, septicemia, pneumonia, ocular and burn

infection, osteomyelitis, cystic fibrosis related lung infection, malignant external otitis and urinary tract infections with colonized patients being an important reservoir (Hernandez *et al.*, 1997) *Pseudomonas aeruginosa* shows innate resistance to many disinfectants and antibiotics. (Syed Arshi *et al.*, 2007) Nosocomial infections mainly caused by ESBL, MBL, MDR and PDR *P.aeruginosa* strains creates enormous burden of morbidity, mortality and high health care cost.

The aims and objectives of this study is to determine the prevalence of (i) *Pseudomonas aeruginosa* strains from various clinical samples and their antibiotic resistance pattern. (ii) Prevalence of ESBL, MBL and AmpC production in *Pseudomonas aeruginosa* from various clinical samples in our tertiary care hospital PMCH Patna, Bihar, India.

Materials and Methods

The study was carried out in Department of Microbiology, Patna Medical College, Patna during the period from October 2017 till March 2018. All the samples were obtained from PMCH hospital, to Microbiology department were processed as per standard protocol. The *Pseudomonas aeruginosa* strains were isolated and identified from various clinical sample including urine, sputum, pus, wound swab, endo tracheal tube secretions (ETTsec.), blood and cerebrospinal fluid (CSF) etc. The specimens on receipt in the laboratory were inoculated on nutrient agar, blood agar and MacConkey agar. The plates were then incubated at 37°C for 24 hours, the growth on above media were then picked up and processed for further identification using standard procedures. *P.aeruginosa* was identified by colony character with peculiar diffusible pigment production, Gram staining, motility test and biochemical tests like- oxidase test, O/F test and growth at 42^0C. (Govan, 2006) The antibiotic susceptibility test of identified *P.aeruginosa* strains were performed by modified Kirby Bauer disk diffusion technique (Govan, 2006). The final bacterium inoculation concentration was approximately 10^8 cfu/ml that was equal to 0.5 McFarland prepared. Commercially available Muller Hinton Agar with HiMedia discs of using ceftazidime (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), cefepime (30mcg), gentamicin (10mcg), amikacin (30mcg), tobramycin (30 mcg), ciprofloxacin (5mcg), levofloxacin (Le, 5µg), piperacillin/ tazobactam (100/10mcg), imipenem (10mcg), meropenem (10mcg), polymyxinB (300 µg), colistin (10mcg), norfloxacin (10 mcg- for urinary isolates). According to CLSI guidelines on Muller Hinton agar plates. (Govan, 2006; Srinivas *et al.*, 2012)

Detection of various phenotypic resistance mechanisms

ESBL Screening (Clinical and Laboratory Standards Institute, 2016)

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) (Clinical and Laboratory Standards Institute, 2016)

A turbidity standard 0.5 McFarland suspension in peptone water was made from the colonies of *P.aeruginosa* isolate. By using this inoculum, lawn culture was made on Muller Hinton Agar plate. Discs of

ceftazidime and ceftazidime + clavulanic acid (30 mcg/10 mcg) and cephotaxime (30µg) and cephotaxime + clavulanic acid (30 mcg/10 mcg) were placed separately aseptically on the surface of MHA at a distance of 15 mm apart. Overnight incubation was done at 37°C. An increase of ≥ 5 mm in zone diameter of ceftazidime + clavulanic acid and cephotaxime + clavulanic acid in comparison to the zone diameter of ceftazidime and cephotaxime alone confirmed the ESBL production by the organisms.

Methods of Phenotypic Detection of MBL (Clinical and Laboratory Standards Institute, 2016)

Isolates resistant to Imipenem were tested for metallo β lactamase production by Imipenem EDTA double disc synergy test (DDST).

EDTA Double Disc Synergy Test (DDST) (Clinical and Laboratory Standards Institute, 2016)

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 37°C 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 (Clinical and Laboratory Standards Institute, 2016)

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 zone of inhibition of ceftazidime or cefotaxime adjacent to cefoxitin disc or showing reduced susceptibility to either of the above drugs and cefoxitin are considered as AmpC producer.

Results and Discussion

In our study, among the 1151 culture positive clinical samples, 100 isolates of *P.aeruginosa* were isolated (8.68%). The predominant sample of isolation was pus/wound swab (17.59%), followed by ETT Secretion (12.5%), Ear swab (9.79%), sputum (7.66%) urine (5.74%), Blood (1.96%) and CSF (1.02%) (Table 1).

In our study, among the used β lactam other than carbapenems, ceftazidime (61%), cefepime (53%) and fluroquinolones like cipofloxacin (63%) and levofloxacin(49%) showed highest resistant. Among the aminoglycosides, gentamicin (41%) showed highest resistant while tobramicin (26%) and amikacin (28%) exhibit less resistant.

Among the β-lactam combination (β-lactam combined with β latamase inhibitor) by Piperacillin/ tazobactam showed 42% resistance. The resistant pattern of Aztreonam is 51%. The urine isolates of *P.aeruginosa* shown 50% resistant to Norfloxacin. The carbapenems, Imipenem (18%), Meropenem (19%), and Doripenem (16%) showed less resistant. Most of isolates were found to be highly sensitive to Colistin (100%), Polymyxin B (100%),

Among 100 strains of *P.aeruginosa,* which were screened phenotypically for ESBL (33%), MBL (18%) and AMP C(0%), the prevalence of ESBL, MBL and Both ESBL and MBL is 33%, 18%, and 9% respectively. No strain was positive for AMP C (Table 2 and 3). Isolates from ETT. Sec (100%), Pus

(48.1%), Urine (75.0%) and wound swab (64.2%) showed maximum resistant to levofloxacin (Le). Among the combined drug Piperacillin/Tazobactam (25.0%) shown less resistant.

P.aeruginosa has emerged as a significant pathogen, due to its intrinsic ability to resist many classes of antibiotics as well as its ability to acquire resistance, its virulence, ability to resist killing by various antibiotics and disinfectant, it presents a serious therapeutic challenge for treatment of both community acquired and nosocomial infections. This affects mortility, morbidity 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.,* (1995) In other Indian study Pathi *et al.,* reported 8.43%. (Pathi *et al.,*) The prevalence in our study was found to be 8.68% which is comparable to above study.

Wound infection and respiratory tract infections were found to be commonly affected by *P.aerugiosa.* In this study the predominant sample of isolation was pus/wound swab (17.59%), followed by ETT secretion (12.5%), ear swab (9.79%), sputum (7.66%) urine (5.74%), Blood (1.96%) and CSF (1.02%). S. Senthamarai *et al.,* (47.11%) (Senthamarai *et al.,* 2014) and Vijaya Chaudhari *et al.,* (35.3%) also reported highest rate of isolation in pus. (Vijaya Chaudhari *et al.,* 2013)

In a study conducted in Punjab, India, Arora *et al.,* found highest recovery rates were from urine (36%), followed by wound discharge (20%), tracheal aspirate (8%), ear discharge (5%) and sputum (4%). (Arora *et al.,* 2011)

Another study by Javiya *et al.,* from Gujarat, India, reported higher isolation rates from urine, pus and sputum which accounts to 27% each, followed by ET secretion 14%. (Javiya *et al.,* 2008) This variation among these studies could be due to the difference in study period and sample size, geographical location and patient population.

Fig.1

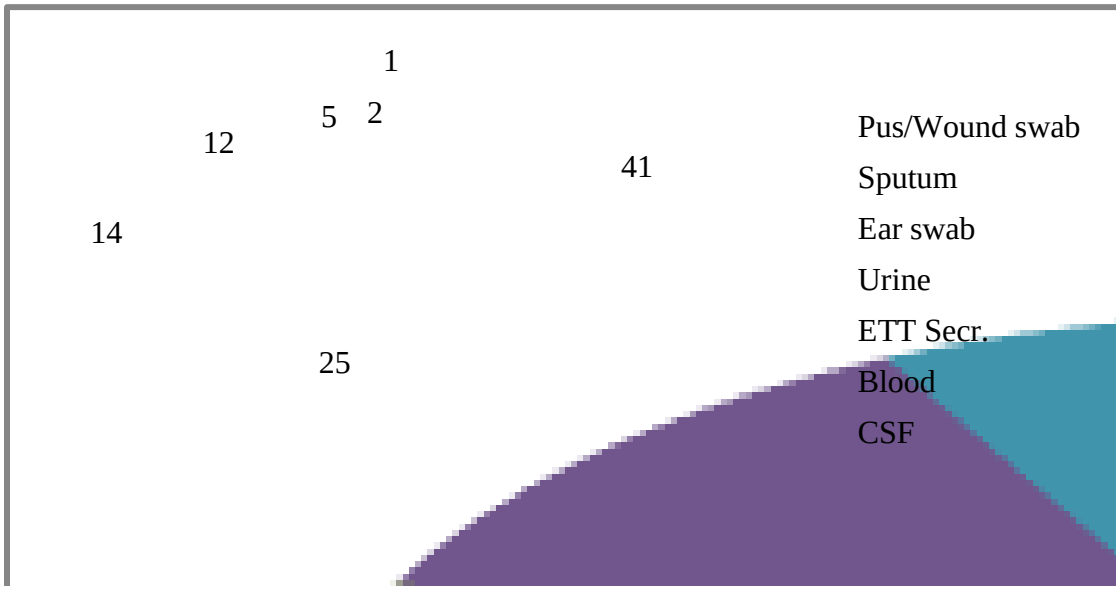

Table.1 Isolation rate of *P. aeruginosa* from different clinical Sample (N=1151)

Sample	N	No. (%)
Pus/ Wound swab	233	41 (17.59)
Sputum	326	25 (7.66)
Ear Swab	143	14 (9.79)
Urine	209	12 (5.74)
ET T Sec.	40	5 (12.5)
Blood	102	2 (1.96)
CSF	98	1 (1.02)
TOTAL	1151	100 (100)

Table.2 Antibiotic susceptibility pattern of *P. aeruginosa* in different clinical specimen

ANTIBIOTICS	SENSITIVE	RESISTANT
Ceftriaxone (30)	28	62
Ceftazidime (30)	39	61
Cefipime	47	53
Piperacillin-Tazobactam	58	42
Gentamicin	59	41
Amikacin	72	28
Tobramycin	74	26
Ciprofloxacin	37	63
levofloxacin	51	49
Imipenem	82	18
Meropenem	81	19
Colistin	100	00
Polymyxin b	100	00
Aztreonam	49	51
Norfloxacin	48	52

Table.3 Prevalence of ESBL, MBL, Amp c and from different clinical isolates (n=100)

N=100	No of isolates	Percentage
MDR	28	28
ESBL	33	33
MBL	18	18
BOTH ESBL AND MBL	09	9
AMP C	0	0

Most of isolates were found to be highly sensitive to colistin (100%), polymyxin B (100%), doripenem (89.0%) imipenem (84 %), amikacin (76.0%) and piperacillin + tazobactum (75%). As the bacterial strains that show resistance to three or more categories of antibiotics are defined as multidrug resistant (MDR) strains,

(Senthamarai *et al.,* 2014) MDR strains of *P.aeruginosa* isolated in this study were 28%.

In our study *P.aeruginosa* showed highest resistant to β-lactum antibiotics and fluroquinolones. Among the β lactam drugs, ceftazidime (61%) and cefepime (53%) showed the highest resistance in this present study. K.M Mohanasundaram *et al.,* (84.6%), (Mohanasundaram, 2011) Yapar *et al.,* (84%) (Ayse Yüce *et al.,* 2009) and Ibukun *et al.,* (79.4%), (Ibukun *et al.,* 2007) reported more resistance against ceftazidime in their study. Our study is in line with the reports of Diwivedi *et al.,* (63%) (Diwivedi *et al.,* 2009) & Arya *et al.,* (55.4%). (Arya *et al.,* 2005) The reason for high resistance of third and fourth generation cephalosporin may be due to indiscriminate use of third and fourth 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 in *Pseudomonas aeruginosa* are due to loss of outer membrane protein, production of class C AmpC β-lactamase and altered target sites.

Our study showed 33 (33%) isolates were ESBL producer. 42.30% ESBL producer were observed in the study of (Varun Goel *et al.,* 2013) Lower ESBL producer were seen in the studies by (Prashant *et al.,* 2011) and Agarwal *et al.,* which were 22.22% & 20.27% respectively (Aggarwal *et al.,* 2008)

The ESBL enzymes are inhibited by β-lactamase inhibitors, viz., clavulanic acid. 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. In our study β-lactamase inhibitor resistance was ranged from 42% to 57%. Similar resistance also observed by Senthamarai *et al.,* (37.5% to 56.73%) (Senthamarai *et al.,* 2014) and K.M Mohanasundaram *et al.,* (40.3%). (Mohanasundaram, 2011) In therapeutic part, increasing resistance to β lactam inhibitors is a major problem 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 (18%) which is comparable with the studies of Ibukun *et al.,* and Senthamarai *et al.,* (15.38%). (Senthamarai *et al.,* 2014; Ibukun *et al.,* 2007), (Prashant *et al.,* 2011; Agarwal *et al.,* 2008; Jayakumar and Appalraju, 2007; Navneeth *et al.,* 2002) and slightly raised level of carbapenem resistance were reported by Variya *et al.,* (25%). (Variya *et al.,* 2008) 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 higher resistance to many other classes of antibiotics, including fluoroquinolones (49% to 63%) and aminoglycosides (26% to 46%). 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 (41%). Minimal resistance was observed with other aminoglycoside such as tobramycin (26%) and amikacin (28%) which is shows promising effect in treatment. Ciprofloxacin showed (63%) 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.,* 2004).

It is evident from the study that nowadays *P.aeruginosa* is becoming resistant to cephalosporins, aminoglycosides and even beta lactam (BL) – beta lactamase inhibitor (BLI) combinations and Carbapenems. Furthermore, infections with such strains may result in poor or untoward clinical outcomes that may increase morbidity, mortality and economic burden. Proper use of antibiotics following a proper antibiotic policy is the best way to control spreading of this superbug. To prevent the spread of the resistant bacteria it is critically important to have strict antibiotic policies. To minimize the resistance to in use routine antibiotics, it is desirable that the antibiotic susceptibility pattern of bacterial pathogens like *P.aeruginosa* in clinical units should be continuously monitored. As there are few studies available 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 aeruginosa* infections.

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