Detection of Extended Spectrum Beta Lactamases Among Clinical Isolates of Pseudomonas aeruginosa

Shree Dhotre[1], Vilas Jahagirdar[2], Basavraj Nagoba[3]

[1]Assistant Professor Department of Microbiology, M.M. Patel Public Charitable Trust's, Ashwini Medical College & Hospital, Kumbhari-413006, Solapur, Maharashtra, India.

[2]Formerly Dean & Professor of Microbiology Govt. Medical College, Miraj, Maharashtra, India.

[3]Assistant Dean (R&D) & Professor of Microbiology Maharashtra Institute of Medical Sciences & Research, Latur-413 531, Maharashtra, India.

Eur J Basic Med Sci 2015;5(3): 45-50

Received: 19-01-2016

Accepted: 25-05-2016

Correspondence (Yazışma Adresi):
Dr. B. S. Nagoba
Assistant Dean, Research & Development, Maharashtra Institute of Medical Sciences & Research, Latur-413 531,M.S., India.
Email: dr_bsnagoba@yahoo.com, bsnagoba@gmail.com
Office Telephone: +912382227587
Mobile No.+919423075786/+917588237531

ABSTRACT

Infections caused by Pseudomonas aeruginosa are tough to treat as the majority of these isolates exhibit varying degrees of beta-lactamase mediated resistance. These enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. Resistance to broad-spectrum beta lactams, mediated by extended-spectrum beta lactamases (ESBLs), is an increasing problem worldwide. In addition, ESBL producing organism's exhibit coresistance to several other classes of antibiotics resulting in limitation of therapeutic option. This resistance poses problems for testing and reporting. Increased prevalence of ESBLs among clinical isolates creates a great need for laboratory testing methods that will accurately identify their presence. Thus the present study was designed to investigate the prevalence of extended-spectrum beta lactamases (ESBLs) in clinical isolates of P. aeruginosa. A total of 150 clinical isolates of P. aeruginosa were isolated from various clinical samples were tested for antimicrobial susceptibility by Kirby-Baeur disk diffusion method. 75 isolates showing a zone diameter less than 18 mm to Cefoxitin (screen positive) were tested for the presence of extended spectrum beta-lactamase (ESBL) by disk approximation method using cefoxitin inducer and cefotaxime indicator method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiogram of P. aeruginosa isolates revealed that, highest resistance was observed to gentamycin (73.67%), followed by ceftazidime (70.67%), amikacin (63.33%), ciprofloxacin (52.67%) in that order of frequencies. Maximum susceptibility was observed to carbenicillin (72%). Multiple drug resistance was common phenomenon observed, in more than 50% of strains. 78 (52%) isolates showed resistance to six or more antibiotics. 65.33% of P. aeruginosa strains showed extended spectrum beta-lactamase production in (70.73%) isolates from pus followed by urine (62.5%). The study emphasizes the high prevalence of multidrug resistant P. aeruginosa producing extended spectrum beta-lactamase using a simple disk approximation method. Thus proper antibiotic policy and measures to restrict the indiscriminative use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple beta-lactamase producing pathogens.

Key Words: *Extended spectrum beta-lactamase, P. aeruginosa, resistance.*

Abbreviations: *Amikacin (AMK), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftazidime (CAZ), Norfloxacin (NOR), Lomefloxacin (LOM), Gentamycin (GEN), Carbenicillin (CAR)*

Pseudomonas aeruginosa'nın Klinik İzolatları Arasında Geniş Spektrumlu Beta Laktamazların tespiti

ÖZET

Pseudomonas aeruginosa'nın neden olduğu enfeksiyonları, değişik derecelerde beta-laktamaz aracılı direnç gösteren bu izolatlara bağlı olarak çoğunlukla tedavi etmek zordur. Bu enzimler geniş spektrumlu sefalosporinler ve monobaktamları hidrolize edebilirler fakat sefamisinlere ve imipeneme karşı inaktiftirler. Geniş spektrumlu beta laktamazlar (ESBL) aracılığı ile geniş spektrumlu beta laktamlara direnç, tüm dünyada giderek artan bir sorundur. Buna ek olarak, antibiyotiklerin diğer birkaçına karşı birlikte direnç gösteren ESBL üreten organizmaların varlığı tedavi seçeneğinin sınırlı olması ile sonuçlanır. Bu direnç test ve raporlama için sorun teşkil etmektedir. Klinik izolatlar arasında ESBL sıklığının artışı, onların varlığını tam olarak tespit edecek laboratuvar test yöntemleri için büyük bir ihtiyaç doğurur. Bundan dolayı, mevcut çalışmada P. aeruginosa'nın klinik izolatlarında geniş spektrumlu beta laktamazların (ESBL) prevalansının araştırılması planlandı. Çeşitli klinik örneklerden izole edilen, P. aeruginosa'nın toplam 150 klinik izolatı Kirby-Bauer disk difüzyon yöntemi ile antimikrobiyal duyarlılık açısından test edildi. Klinik ve Laboratuar Standartları Enstitüsü (CLSI) kurallarına uygun olarak sefoksitin indükleyici ve sefotaksim gösterge yöntemini kullanarak disk yaklaşım metodu ile geniş spektrumlu beta-laktamaz (ESBL) varlığı açısından sefoksitin 18 mm'den daha az diameter zon gösteren (tarama pozitif) 75 izolat test edildi. P. aeruginosa izolatları'nın antibiyogramı, en yüksek direncin gentamisin (%73.67)'e, bunu takiben, sırasıyla seftazidim (%70.67), amikasin (%63.33), siprofloksasin (%52.67)'e olduğu gözlenmiştir. En fazla duyarlılık karbenisilin (%72)'e gözlenmiştir. Çoklu ilaç direnci, suşların %50'den daha fazlasında yaygın bir olgu olarak gözlenmiştir. 78 (%52) izolat, altı veya daha fazla antibiyotiğe karşı direnç göstermiştir. P. aeruginosa suşlarının %65.33'ü geniş spektrumlu ß laktamaz üretimini, cerahatttan (%70.73) bunu takiben idrardan (%62.5) elde edilen izolatlarda gösterdi. Bu çalışma basit bir disk yaklaşım yöntemi kullanılarak geniş spektrumlu beta-laktamaz üreten P. aeruginosa'nın çoklu ilaca direncinin yüksek prevalansını vurgulamaktadır. Bu yüzden sefalosporin ve karbapenemlerin gelişigüzel kullanımını kısıtlayacak uygun antibiyotik politikası belirlenmeli ve çoklu beta-laktamaz üreten patojenlerin ortaya çıkmasını en aza indirmek için önlemler alınmalıdır.

Anahtar Kelimeler: *Geniş spektrumlu beta-laktamazlar, P. aeruginosa, direnç.*

INTRODUCTION

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in community and hospital settings. The main mechanism of resistance to beta-lactam antibiotics among gram-negative isolates is beta-lactamase biosynthesis. Beta-Lactamases inactivate penicillins and cephalosporins by hydrolyzing the amide bond of the beta-lactam ring. The numerous beta-lactamase sequences allow them to be divided into four molecular classes according to their amino acid content, designated A to D (1). Resistance to extended-spectrum cephalosporins is usually observed in members of the family Enterobacteriaceae, with extended-spectrum variants from class A beta-lactamases TEM-1, TEM-2, and SHV-1 (2). These plasmid mediated extended-spectrum enzymes were first reported in *Klebsiella pneumoniae* and later in almost all other Enterobacteriaceae. These variants differ from their parent enzymes by only a few amino acid positions (3) within their catalytic sites (3) but can hydrolyze broad-spectrum beta-lactam antibiotics such as penicillins and cephalosporins, including oxyimino beta lactams (cefotaxime, ceftazidime, and aztreonam). However, they do not hydrolyze cephamycins (cefoxitin) or carbapenems (imipenem or meropenem) (3).

***Table 1**. Correlation of clinical history with source of isolation.*

Source	Diagnosis	No. of cases
Pus n=93 (62%)	Burn wound	54
	Acute otitis media	09
	Foot ulcer	17
	Chronic Osteomyelitis	01
	Cellulitis	04
	Abscess	06
	Empyema	02
Urine n=24 (16%)	UTI	24
Sputum n=12 (8%)	Pneumonia	04
	Bronchitis	08
Conjunctival Swab. n=12 (12%)	Immat/mat/Traumatic cataract	10
	Conjunctivitis	01
	Corneal abscess	01
Stool n=3 (2%)	PEM	02
	Intestinal Obstruction	01
Blood n=2 (1.33%)	Fever	02
CSF n=1 (0.66%)	Meningitis	01
Intra Costal drain n=1 (0.66%)	Hydropneumothorax	01
Total		150

***Table 2**. Antimicrobial susceptibility pattern of P.aeruginosa (n=150).*

Antibiotics	Disk Conc. µg	Symbols	Susceptibility Pattern Resistant	Susceptible
Gentamycin	10	GEN	118 (73.67%)	32 (21.33%)
Norfloxacin	10	NOR	71 (47.33%)	79 (52.67%)
Ciprofloxacin	5	CIP	79 (52.67%)	71 (47.33%)
Amikacin	30	AMK	95 (63.33%)	55 (36.67%)
Cephotaxime	30	CTX	48 (32%)	102 (68%)
Carbenicillin	100	CAR	42 (28%)	108 (72%)
Ceftazidime	30	CAZ	106 (70.67%)	44 (29.33%)
Lomefloxacin	10	LOM	73 (48.67%)	77 (51.33%)

Amikacin (AMK), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftazidime (CAZ), Norfloxacin (NOR), Lomefloxacin (LOM), Gentamycin (GEN), Carbenicillin (CAR)

Extended spectrum beta-lactams are routinely added in empirical antibiotic regimens for the treatment of Gram-negative infections. The evolution of extended-spectrum beta-lactamase (ESBL)-producing bacteria present a grave risk to the continued use of this family of antibiotics (4).

The current automated susceptibility test methods do not reliably detect ESBL production. Other techniques such as polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism and direct nucleotide sequencing, which demonstrate ESBL production, are of variable sensitivity and may be time consuming, expensive or technically difficult to perform. There is a need for an easy, rapid and reproducible method for the detection of ESBLs, suitable for use in the routine diagnostic laboratory. Additionally, if the method could utilize the same methodology as antimicrobial susceptibility testing, the use of extra one or two discs only would enable all clinical isolates to be screened during routine susceptibility testing (4).

Hence, the present study was designed to investigate the prevalence of extended spectrum beta-lactamase enzymes in clinical isolates of *P. aeruginosa* using a simple disk approximation method, which could be easily used in the routine diagnostic laboratory.

MATERIAL AND METHOD

This study is a prospective study, which was approved by the institutional ethical committee. The study included a total of 150 consecutive non-duplicate isolates of *P. aeruginosa* obtained from different clinical specimens from patients who were admitted in different wards as well as from those who attended the outpatient departments of our tertiary care hospital.

All the isolates of *P. aeruginosa* which showed susceptibility to cefoxitin were evaluated for ESBL production by using the phenotypic disk approximation confirmatory test (5,6). The disk approximation test was used for detection of ESBL in all the isolates of *P. aeruginosa*. A test isolate (with a turbidity equivalent to that of 0.5 McFarland standards) was spread over a Mueller Hinton agar (Hi-Media)

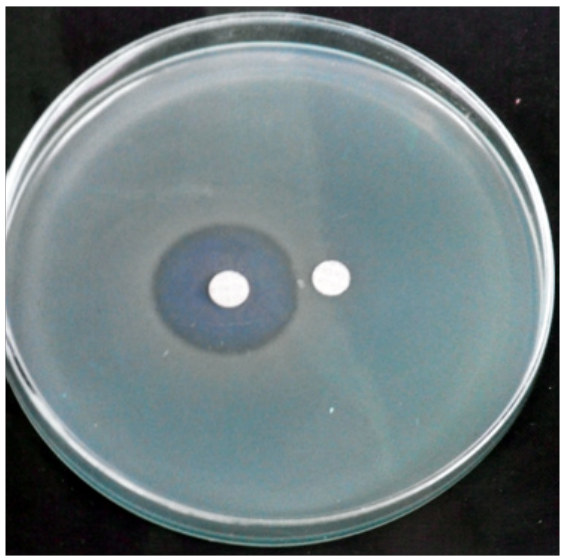

***Figure 1**. Positive ESBL test (Disk Approximation Method) showing no zone of inhibition around cefoxitin and flattering of inhibition zone around cefotaxime disk.*

***Table 3**. R-pattern observed among P.aeruginosa strains.*

No. of Strains	Resistance	R-pattern	Resistance (%)
P.aeruginosa (141)	08	GEN, NOR, CIP, AMK, CTX, CAR, CAZ, LOM	33 (22%)
	07	GEN, NOR, CIP, AMK, CAR, CAZ, LOM	20 (13.33%)
	07	GEN, NOR, CIP, AMK, CTX, CAZ, LOM	08 (5.33%)
	06	GEN, NOR, CIP, CTX, CAZ, LOM	17 (11.33%)
	05	GEN, NOR, CIP, CAZ, LOM	16 (10.67%)
	04	AMK, CTX, CAR, CAZ	04 (2.67%)
	04	GEN, NOR, CIP, CAZ	04 (2.67%)
	03	GEN, CTX, CAZ	07 (4.67%)
	03	GEN, CAZ, LOM	05 (3.33%)
	02	CTX, CAZ	06 (10.67%)
	02	GEN CAZ	05 (3.33%)
	02	GEN, CTX	04 (2.67%)
	02	GEN , LOM	04 (2.67%)
	01	GEN	04 (2.67%)
	01	CAZ	04 (2.67%)

Amikacin (AMK), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftazidime(CAZ), Norfloxacin (NOR), Lomefloxacin (LOM), Gentamycin (GEN), Carbenicillin (CAR)

plate. Cefotaxime (30µg) and cefoxitin (30µg) (Hi-Media Mumbai) disks were placed 20 mm apart from centre to centre. Isolates showing blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin disk were screened as positive for ESBL (5,6) (Figure 1).

Antimicrobial susceptibility was performed by the Kirby-Baeur disk diffusion method for various antibiotics, namely: Amikacin (30µg), Ciprofloxacin (5µg), Cefotaxime (30µg), Ceftazidime (30µg), Norfloxacin (10µg), Lomefloxacin (10µg), Gentamycin (10µg), Carbenicillin (100µg) (Hi-Media Mumbai). The results were interpreted according to the standard table provided along with the disk (6).

RESULTS

Table 1 shows the correlation of clinical history and source of isolation. In the present study majority of *P. aeruginosa* strains were isolated in pus samples 93 (62%) followed by urine 24 (16%), conjunctival swab and sputum were 12 (8%) each. The maximum numbers of isolates were from burn wounds (36%) and foot ulcer (11.3%).

The antibiogram of 150 *P. aeruginosa* isolates is shown in table 2. Among the antibiotics used resistance to GEN (73.67%), CAZ (70.67%) was most frequently observed, followed by AMK (63.33%) and CIP (52.67%). Maximum susceptibility was observed with CAR i.e. (72%).

Among the 150 *P.aeruginosa* isolates 141 were resistant to one or more antibiotics (Table 3). Most of *P.aeruginosa* strains 33(22%) were resistant to all 8 antibiotics, followed by 28 (18.67%) strains resistant to 7 antibiotics (GEN, NOR, CIP, AMK, CAR, CAZ, LOM) which was the most frequent resistance pattern. Majority of the *P. aeruginosa* isolates 94(62.67%) were resistant to 5 or more antibiotics. 9 strains were found to be sensitive to all the antibiotics tested.

Table 4 summarises the distribution of extended spectrum beta lactamase activity of *P. aeruginosa* isolates from different clinical source. 49 (65.33%) *P. aeruginosa* isolates

***Table 4**. ESBL positivity according to their clinical source.*

Clinical Source	Total	ESBL Positive	ESBL Negative
PUS	41	29 (70.73%)	12 (29.27%)
Urine	16	10 (62.5%)	06 (37.5%)
Conjunctival Swab	07	04 (57.14%)	03 (42.86%)
Sputum	06	04 (66.67%)	02 (33.33%)
Stool	02	---	02 (100%)
CSF	01	---	01 (100%)
Intra costal drain	01	1 (100%)	---
Blood	01	1 (100%)	---
Total	75	49 (65.33%)	26 (34.67%)

***Table 5**. R-pattern and ESBL activity.*

Strains	Resistance	R-pattern	No. of strains	ESBL Positive
75	07	GEN NOR, CIP, AMK, CAR, CAZ, LOM	20	+ (20)
P.aeruginosa	06	GEN NOR, CIP, CAR, CAZ, LOM	17	+ (16)
	05	GEN, NOR, CIP, CAZ, LOM	16	+ (13)
	04	GEN, CIP,CAZ, LOM	05	---
	03	GEN, CIP, CAZ	05	---
	02	GEN, LOM	04	---
	01	GEN	04	---
	01	CAZ	04	---
Total			75	49 (65.33%)

Amikacin (AMK), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftazidime (CAZ), Norfloxacin (NOR), Lomefloxacin (LOM), Gentamycin (GEN), Carbenicillin (CAR)

produced ESBL. Majority of ESBL producing *P. aeruginosa* strains 29 (70.73%) were isolated from pus, followed by sputum (66.67%).

Table 5 summarises the distribution of extended spectrum beta lactamase activity in *P. aeruginosa* strains. Of the 49 (65.33%) ESBL positive strains 20 (100%) were resistant to seven antibiotics followed by 16 (94.12%) strains resistant to 6 antibiotics. All the ESBL positive strains were resistant to 5 or more antibiotics.

DISCUSSION

Production of ESBLs by *P. aeruginosa* has tremendous therapeutic consequences and poses a significant clinical challenge if it remains undetected, early identification of the infections due to these organisms is necessary as the appropriate treatment might reduce the spread of these resistant strains as well as reduce the mortality in hospitalized patients. This emphasizes the need for the detection of isolates that produce these enzymes to avoid therapeutic failures and nosocomial outbreaks. Since there is no standard guideline for detection of most of these beta-lactamase enzymes in *P. aeruginosa*, the comparison between studies becomes difficult as the patient population in particular centres and the methods of study differ. In comparison to the earlier studies conducted at other centres, we found a very high prevalence of these beta-lactamase producing P. aeruginosa (65.33%) (6,7). The increase in ESBL producing isolates may be indicative of the ominous trend of more and more isolates acquiring resistance mechanisms rendering all the available antimicrobial ineffective. Our study reported very high incidence of ESBL among *P. aeruginosa*, which contrasts an earlier study which showed 3.3% and 22.22% of ESBL production (6,7). The only beta-lactam active against ESBL producers are carbapenems; however, recently resistance to carbapenems has been increasing, In our study carbenicillin was found to be the most effective drug, showing maximum susceptibility of 72%, which is in agreement with earlier studies (6,8,9).

Although molecular methods appear sensitive detection of ESBL-producing strains in the clinical laboratory (10, 11) they are expensive, time consuming, and require specialized equipment and expertise. In this study we could reliably screen out the extended spectrum beta lactamase producing *P. aeruginosa* by using the disc approximation method which is in accordance with earlier studies (6,7,10). The disk approximation method affords a reliable option for ESBL detection for all Gram-negative bacteria to be screened in the routine diagnostic laboratory.

The present study emphasizes the high prevalence of multidrug resistant *Pseudomonas aeruginosa* producing extended spectrum beta-lactamase enzyme prevailing in present clinical settings. To combat these problems, epidemiological studies should be undertaken in hospital settings to monitor the source of infection. Early detection of these extended spectrum beta-lactamase producing isolates in a routine laboratory could help to avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility

testing. Furthermore, strict antibiotic policies and measures to limit the indiscriminative use of cephalosporins and carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple beta-lactamase producing pathogen whose spread would leave no other option to treat Gram-negative nosocomial infections.

Conflict of Interest: None to Declare

Source of funding: No funding

REFERENCES

1. *Ambler, R. P. The structure of β-lactamases. Philos Trans R Soc Lond 1980; B 289:321-331.*
2. *Philippon, A., G. Arlet, and P. H. Lagrange. Origin and impact of plasmid-mediated extended-spectrum beta-lactamases. Eur J Clin Microbiol Infect Dis 1994;13(Suppl. 1):17-29.*
3. *Philippon LN, Naas T, Bouthors A-typhaine, Barakett V, Nordmann P, Be A. OXA-18, a Class D Clavulanic Acid-Inhibited Extended-Spectrum β-Lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 1997;41(10):2188-95.*
4. *Zali FHM, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. JAC Detection of extended-spectrum β-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. J Antimicrobial Chemotherapy 2000;45:881-5.*
5. *Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL) – An emerging threat to clinical therapeutics. Ind J Med Microbiol 2004;22(2):75-80.*
6. *Revathi G, Singh S, Simrita S. Detection of expanded spectrum cephalosporin resistance due to inducible lactamases in hospital isolates. Ind J Med Microbiol 1997;15:113-115.*
7. *Peshattiwar PD, Peerapur BV. ESBL and MBL Mediated Resistance in Pseudomonas aeruginosa : An Emerging Threat to Clinical Therapeutics. J Clin Diagn Res 2011; 5(8): 1552-4.*
8. *Aggarwal R, Chaudhary U, Bala K. Detection of extended spectrum beta lactamase in Pseudomonas aeruginosa. Ind J Pathol Microbiol 2008;51: 222-224.*
9. *Hemlatha V, Sekar U, Kamat V. Prevalence of metallo beta lactamase producing Pseudomonas aeruginosa in hospitalized patients. Ind J Med Res 2005;122: 148-152.*
10. *Giriyapur RS, Nandihal NW, Krishna BVS, PatilAB, Chandrasekhar M R. Comparison of Disc Diffusion Methods for the Detection of Extended-Spectrum Beta Lactamase-Producing Enterobacteriaceae. J Lab Physicians 2011;3(1): 33-36.*
11. *Polsfuss S, Bloemberg GV, Giger J, Meyer V, Hombach M. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β-lactamase production in clinical Enterobacteriaceae isolates. J Antimicrob Chemother 2012;67(1):159-66.*