

Detection of ESBLs in *Pseudomonas aeruginosa* isolated from different body fluids.

ABSTRACT

Background: The present study was carried out in order to detect the production of ESBLs in *Pseudomonas aeruginosa* and their susceptibility pattern against different antibiotics.

Material and methods: A total of 100 isolates of *P. aeruginosa* was analyzed for the production of ESBLs by using phenotypic confirmatory test and the results were determined. The antibiotic susceptibility against various antibiotics was performed by Kirby-Bauer Disc diffusion method on Mueller-Hinton Agar.

Results: Out of the 100 isolates tested 20 isolates were positive for the production of ESBLs, with a high prevalence of 36.3% found in the pus samples followed by the sputum (26%), blood (22.2%) and urine (5.4%), respectively. Imipenem was found to be the most effective antibiotic with cent percent?? susceptibility followed by ofloxacin. A drug resistance of 57% for ceftazidime was noted against *P. aeruginosa* followed by cefotaxime. A high prevalence rate of *P. aeruginosa* was detected in males.

Conclusion: This study showed the rising tendency of multidrug-resistant strains of enterobacteriaceae family, especially *P. aeruginosa* in the hospital settings, which is a great concern for clinicians and patients to treat these resistant strains.

Keywords: *P. aeruginosa*, Drug resistance, ESBLs, β -lactams.

1. INTRODUCTION

One of the central mechanisms of resistance in Gram-negative bacteria is the manufacturing of β -lactamases, which hinder protein transpeptidases participating in creation of bacterial cell wall [1, 2]. Presently there are several such enzymes known but ESBLs and AmpC β -lactamases are of particular clinical and epidemiological significance that is capable of inactivating the broad spectrum cephalosporins and penicillins. *Pseudomonas aeruginosa* is a primary cause of

nosocomial infections, including pneumonia, urinary tract infections, and bacteremia. The infections can be predominantly brutal in patients with impaired immune systems, such as in neutropenic or cancer patients [3]. Extended-spectrum β -lactamases are enzymes that arbitrate resistance to extended spectrum cephalosporins (ESCs), such as cefotaxime, ceftriaxone, and ceftazidime, and the monobactam aztreonam [4]. Although modern advances in sanitation amenities and the introduction of a wide range of antimicrobial agents with antipseudomonal activities, life threatening infections caused by this agent continue to cause devastation in the hospitals. The resistance in *P. aeruginosa* is primarily mediated by β -lactamases [5]. Although, the most important ones are metallo beta lactamases, but several studies indicated the presence of (ESBLs) in *Pseudomonas* as well [6, 7].

2. MATERIALS AND METHODS

A total of 225 different specimens was processed for the isolation of *P. aeruginosa* out of which 100 isolates of *P. aeruginosa* were recovered by using various conventional methods. The identification was based on the various biochemical tests and colony morphology of the organism [8]. The antibiotic susceptibility was performed against different antibiotics including, cefotaxime (30 μ g), ceftazidime (30 μ g), ofloxacin (5 μ g), gentamicin (10 μ g), amikacin (30 μ g), tobramycin (10 μ g), piperacillin (100 μ g), piperacillin/tazobactam (100 μ g/10 μ g), meropenem (10 μ g) and imipenem (10 μ g) using Kirby disc diffusion method [9]. Results were interpreted according to CLSI guidelines [10]. All the isolates of *P. aeruginosa* were tested for the production of ESBLs by using phenotypic confirmatory test [11]. A 0.5 McFarland's suspension of every isolate was spread on a Mueller-Hinton agar (MHA) plate (Hi-Media) and a 30 μ g disc of ceftazidime and ceftazidime/clavulanic acid 30 μ g/10 μ g discs were positioned aseptically on the agar plate. The two discs were kept apart at a distance of about 15mm and the plates were incubated at 37°C overnight. The results were interpreted and a distance of \geq 5mm increase in the zone diameter of the antimicrobial agent that was tested in combination with clavulanic acid, against its zone diameter when tested alone, confirmed the organism as ESBL producer [Figure 1].

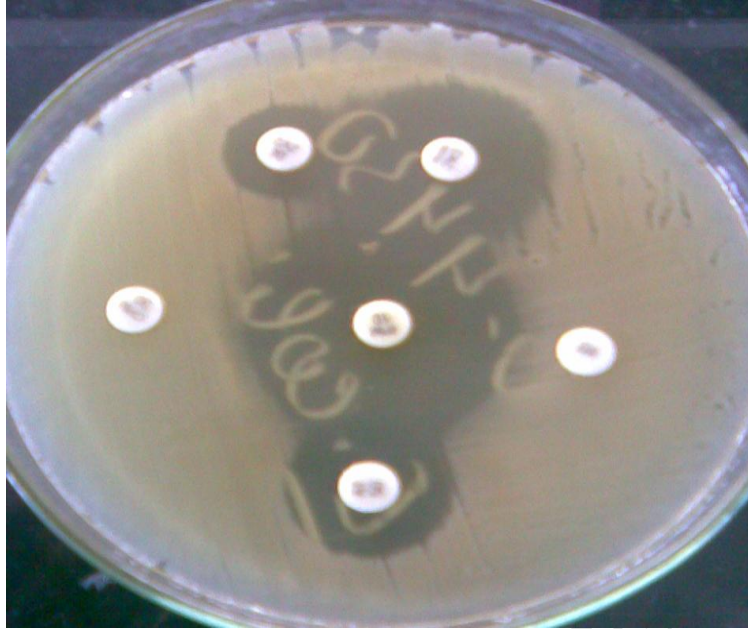


Figure 1: Detection of ESBL production in *P. aeruginosa*

3. RESULTS

Out of 100 *P. aeruginosa* isolates 20 were found to produce ESBLs. The maximum prevalence of ESBLs was set up in the isolates detected from the pus specimens (36.3%) while as low 5.4% was detected in urine [Table 1]. All the ESBL producing strains showed drug resistance to maximum number of antibiotics. Imipenem was found to be the drug of choice with 100% sensitivity followed by ofloxacin, while as highest resistance of 57% was noted against the ceftazidime followed by cefotaxime 51% [Table 2]. The majority of the isolates (76%) was obtained from male patients.

Table 1: Distribution of *P. aeruginosa* (ESBL) producing strains in different specimens

Sample	No. of isolates	No of ESBL strains	ESBL %age
Sputum	23	6	26%
Pus	22	8	36.3%
Blood	18	4	22.2%
Urine	37	2	5.4%
Total	100	20	

Table 2: Antibiotic resistance of *P. aeruginosa* strains

Antibiotic	Resistance
cefotaxime (30µg)	51%
ceftazidime (30µg),	57%
ofloxacin (5µg),	8%
gentamicin (10µg),	37.5%
amikacin (30µg),)	32.3%
tobramycin (10µg),	29%
piperacillin (100µg),	40%
piperacillin/tazobactam (100µg/10µg)	21.2%
meropenem (10µg)	13.7%
imipenem (10µg)	0

4. DISCUSSION

Extended-spectrum β -lactamases (ESBLs) are rapidly developing β -lactamases which are talented of conferring bacterial resistance to penicillins, first, second, third generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics [12]. Clavulanic acid can be used as β -lactamase inhibitor to screen for ESBL production. Because of possibility of inoculum effect, their use for treatment of ESBL-producing bacteria is not encouraged [13]. In the present study the prevalence of ESBLs-producing *P. aeruginosa* was found to be 20% which is quite similar to the studies conducted previously [14, 15], which have reported 20.27% and 22.22%, respectively, while another study had reported a 64% incidence of ESBL producing *P. aeruginosa* [16]. Yet another study noted a low ESBL production of 7.7% [17]. During the ongoing study, imipenem was found to be the drug of choice with 100% sensitivity which is similar to the studies reported [18, 19]. The current investigation has recorded a prevalence rate of 76% of *P. aeruginosa* in male patients which is comparable to the findings of Garg *et al.* [20], who reported 78% isolates from the male patients in her study. However, [21] reported predominance of males as (68%). In our study ceftazidime showed a resistance rate of 57% which is related to the conclusion of [22] who showed a

resistance rate of 55.4% while as another finding have recorded 53.17% [15], whereas as one more study has noted 63% ceftazidime resistance [23]. The emergence of ESBLs and their wide-ranging spectrums and unrivalled drug resistance is creating a therapeutic challenge for clinicians and microbiologists throughout the world. Therefore, we put forward that the detection of ESBL in *P. aeruginosa* must be a regular practice. We suggest a routine inspection on antibiotic resistance in the hospitals to curb the growing resistance.

5. CONCLUSION

The current study highlights that *P. aeruginosa* relics a significant cause of nosocomial wound infections worldwide. The incidence of β -lactamase-producing *P. aeruginosa* is on the rise. Although this study underlines the exceptional problem of ESBL-mediated resistance, which has produced a therapeutic confront? for the clinicians and microbiologists. To conquer the problem of appearance? and the stretch? of multidrug resistant *P. aeruginosa*, a joint communication and collaboration among the microbiologists, clinicians and the infection control team is desired. We suggest the regular surveillance of antibiotic resistance inside and outside the hospitals.

6. ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s). This should have benn indicated under Materials and Methods

REFERENCES:

1. Bradford PA . Extended-spectrum-beta-lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. *Clinical Microbiology Reviews*. 2001; 14: 933–951.
2. Paterson DL, Bonomo RA . Extended-spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews*. 2005; 18: 657–686.
3. Pagani L, Mantengoli E, Migliavacca R, Nucleo E, Pollini S, Spalla M, Daturi R, Romero E, and Rossolini GM. Multifocal detection of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extendedspectrum -lactamase in northern Italy. *J. Clin. Microbiol.* 2004; 39:1865–1870.

4. Livermore DM, and Brown DFJ. Detection of β -lactamase mediated resistance. J. Antimicrob.Chemotherapy. 2001; 35: 281–294.
5. Bonomo RA, Szabo D. Mechanism of multi drug resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa*. Clin. Infect. Dis. 2006; 43: 49-56.
6. Clinical Laboratory Standard Institute (CLSI) for antimicrobial disc susceptibility tests. Performance standards for antimicrobial susceptibility testing 15th information supplement (M100 –S15). Clinical Laboratory Standard Institute **2005**.
7. Jiang X, Zhang Z, Li M, Zhou D, Ruan F, Lu Y. Detection of Extended-Spectrum β -Lactamases in Clinical Isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2006; 50(9): 2990-5.
8. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney practical medical microbiology. 14th ed. Edinburgh : Churchill Livingstone. 1996; 151-79.
9. Bauer AW, Kirby WMM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol 1966; 45: 493.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk tests; Approved Standards, 9th ed. CLSI Document M2-A9, Vol. 26 No.1 Wayne PA 2006.
11. Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing-Ninth information supplement: M100-S10. NCCLS. Wayne, PA, USA 2000.
12. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β -lactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995; 39: 1211-33.
13. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Win WC. Antimicrobial Susceptibility Testing In: Color atlas and textbook of diagnostic microbiology. 6th ed. JB Lippincott Co: Philadelphia. 2006; 945-1021.
14. Agarwal P, Ghosh AN, Kumar S, Basu B, Kapila K . Prevalence of extended spectrum beta lactamases among *Escherichia coli* & *Klebsiella pneumoniae* isolates in a tertiary care hospital. Indian J Pathol Microbiol. 2008; 51:139-42.

15. Prashant Durwas et al. ESBL and MBL Mediated Resistance in *Pseudomonas Aeruginosa*. Journal of Clinical and Diagnostic Research. 2011; 5(8): 1552-1554.
16. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gain R and et al. Evaluation of methods for Amp C beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol. 2005; 23: 120-4.
17. Jacobson KL et al. The relationship between antecedent antibiotic use and resistance to extended spectrum cephalosporins in group-I producing beta-lactamase producing organisms. Clin Infect Dis. 1995; 21: 1107-13.
18. Thomson KS et al. Detection of extended spectrum β -lactamases in family enterobacteriaceae. Comparison of the double disk and three dimensional tests. Antimicrobial agents chemotherapy. 1992; 36: 1877-82.
19. Ritu Agarwal, Uma choudary, Kiran bala. Detection of extended spectrum β -lactamase producing *P. aeruginosa*. Indian Journal of Microbiology and Pathology. 2010; 51(2): 222-224.
20. Garg R; GuptaV; Aggarwal A. Resistance pattern of *Pseudomonas aeruginosa* isolates from Surgical Wounds. Journal of Clinical and Diagnostic Research. 2008; 2: 802-803.
21. Arfaj K AL-AL, Twum –Danso, Hegaz MG. Burn wound infection . Indian J Surg. 1990; 52 (6): 271-75.
22. Arya M , AryaP , Biswas , Prasad D R. The antimicrobial susceptibility pattern of the bacterial isolates from post operative wound infections .Indian J Pathol Microbiol. 2005; 48(2): 266-69.
23. Diwivedi M, Mishra A, Singh RK, Azim A, Baronia AK, Prasad KN. The nosocomial cross - transmission of *Pseudomonas aeruginosa* between patients in a tertiary intensive care unit. Indian J Pathol Microbiol. 2009; 52(4): 509-13.