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OCCURRENCE AND DETECTION OF EXTENDED SPECTRUM β-LACTAMASE AND AmpC β-LACTAMASE IN CLINICAL ISOLATES OF *Pseudomonas aeruginosa* AND *Acinetobacter baumanii* BY INHIBITOR BASED METHOD

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Abstract-

Background & Objective: *Pseudomonsa aeruginosa* and *Acinetobacter baumanii* are common non-fermenters which have emerged as the most common opportunistic pathogens in recent years. Persistent exposure of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* to β-lactam antibiotics leads to acquired resistance through mutation and over production of various enzymes which also include AmpC or class C β-lactamases and extended spectrum β-lactamase (ESBL). For clinical microbiologists, detection of ESBL and AmpC-mediated resistance together poses a problem because the phenotypic tests may be misleading; resulting in misreporting and treatment failures.

Methods: A total number of 94 consecutive, non-repetitive, imipenem sensitve clinical isolates of *Pseudomonas aeruginosa* (n=64) and *Acinetobacter baumanii* (n=30) obtained over a period of 6 months, were screened for β-lactamase production by nitrocefin disc and production of ESBL and AmpC β-lactamase is detected by Inhibitor based test.

Results: A total of 50 out of 94 isolates were positive for β-lactamase production; of which 17 (15.98%) and 22(20.68%) were ESBL and AmpC β-lactamase producers respectively.

Conclusion: The inhibitor based method is useful for detection of ESBL and AmpC β-lactamase and helpful to differentiate ESBL from AmpC producers. As high incidence of ESBL and AmpC β-lactamase production in gram negative isolates is alarming and urgent actions needs to be taken for therapeutic and infection control measure. This is only possible if correct detection of ESBL and AmpC β-lactamase is done in clinical laboratory.

Keywords- *Acinetobacter baumanii*, AmpC β-lactamase, Boronic acid- Inhibitor based method, Extended spectrum β-lactamase, *Pseudomonas aeruginosa*

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Introduction

Pseudomonsa aeruginosa and *Acinetobacter baumanii* are common non-fermenters which have emerged as the most common opportunistic pathogens in recent years. *P. aeruginosa* is physiologically versatile and flourishes as a saprophyte in multiple environments, including sinks, drains, respirators, humidifiers and disinfectant solutions. Infections due to P. aeruginosa are seldom encountered in healthy adults, but in the last two decades the organism has become increasingly recognised as the aetiological agent in a variety of serious infections in hospitalized patients, especially those with impaired immune defences [1]. Now a days, *P. aeruginosa* is one of the most common pathogen causing nosocomial infection [2].

Also, the *Acinetobacter* spp. especially *Acinetobacter baumanii* has emerged as some of the most important opportunistic pathogens within the hospital environment, being able to colonize and produce infections in most immunocompromised patients, especially from intensive care units and/or in the context of serious underlying disease. Antimicrobial treatment of such severe infections is complicated by a widespread multidrug resistance pattern [3].

P. aeruginosa is a pathogen with innate resistance to many antibiotics and disinfectants. P. aeruginosa shows acquired resistance due to plasmids, in addition to its innate resistance. Plasmid-mediated resistance involving modifying enzymes is particularly associated with topical antibiotic use and with sites where high levels of antibiotics are achieved [1]. ESBL and AmpC β-lactamase enzymes are one of them. Persistent exposure of *P. aeruginosa* to β-lactam antibiotics leads to mutation and over production of AmpC or class C β-lactamases [2,4].

Acquired resistance in *Acinetobacter baumani* is reported by different mechanisms such as production of plasmid mediated AmpC β-lactamase, extended spectrum β-lactamase and metallo β-lactamase (MBL) enzymes, penicillin binding protein alterations and reduced penetration across the outer membrane [3]. Nevertheless, β-lactamase production is one of the main mechanisms of resistance to β-lactams in *Acinetobacter baumanii.* Even AmpC β-lactamase was sequenced in clinically isolates of *Acinetobacter baumanni* in Spain [3,5].

For clinical microbiologists, detection of AmpC-mediated resistance in Gram-negative organisms poses a problem because the phenotypic tests may be misleading especially when extended spectrum β-lactamse (ESBL) co-exit; resulting in misreporting and failures in clinical treatment of patients. There is no recommended guideline for detection of this resistance mechanism and clinical laboratories need to address this issue since both may co-exist and mask each other [6,7]. Screening with cefoxitin disc is recommended for initial detection of AmpC β-lactamase. However, it does not reliably indicate Amp C production. There are some phenotypic tests which include the three-dimensional test [8], AmpC disc test [9] and modified disc diffusion test [10]. None of these tests are standardized and can be time consuming when screening large numbers of isolates, while detecting ESBL and AmpC β-lactamase, both.

At present Clinical and Laboratory Standards Institute (CLSI) guidelines do not describe any method for detection of ESBL and AmpC β-lactamase enzymes production in *P. aeruginosa* and *Acinetobacter baumanii.*

We therefore undertook this study to detect the presence of ESBL and AmpC β-lactamases in *P. aeruginosa* and *Acinetobacter* spp. by inhibitor based method using boronic acid (BA) as inhibitor of AmpC β-lactamase [6,11].

Materials and Method

The study was conducted for a period of 6 months (January – June 2009). A total number of 94 consecutive, non-repetitive, imipenem sensitive clinical isolates of *P. aeruginosa* (n= 64) and *Acinetobacter baumanni* (n=30) were isolated from different clinical specimens such as urine, pus, sputum, blood, endotracheal tube secretions and others, which were received for Culture and Sensitivity test at Department. of Microbiology from various OPD, hospital wards and Intensive Care Unit patients at a tertiary care hospital. These organisms were confirmed using standard biochemical identification tests [12,13].

Antibiotic susceptibility testing is performed by modified Kirby Bauer method on Muller Hinton Agar according to CLSI protocols [14,15]. The drugs tested were Ampicillin-sulbactam, Cefuroxime, Ceftazidime, Ceftriaxone, Cefoxitin, Cefepime, Ciprofloxacin, Levofloxacin, Gentamicin, Amikacin and Cefoperazone-Sulbactam.

The presence of β-lactamase in all isolates was checked with Nitrocefin (chromogenic cephalosporin) test (Cefinase, B-D microbiology systems) [13,16].

Detection of ESBL and AmpC β-lactamase

Inhibitor based method: A disk containing 30 µg of cefoxitin and another containing 30 µg of cefoxitin with 400 µg of boronic acid were placed on the agar. Similarly, discs of ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) were placed on the medium at a distance of 20 mm. Inoculated plates were incubated overnight at 35°C [6,17,18]. An organism demonstrating 5 mm or greater zone size increase around the ceftazidime -clavulanic acid disk compared to the ceftazidime disk was considered indicative of ESBL production. Likewise, an organism exhibiting a zone diameter around the disk containing cefoxitin and boronic acid 5 mm or greater zone diameter around the disk containing cefoxitin alone was considered an AmpC β-lactamase producer [6,11].

Result and Discussion

The each and every 94 isolates of *Acinetobacter baumanni* and *P. aeruginosa* were tested for antibiotic susceptibility pattern against a panel of antibiotics, which was described earlier. [Table-1] shows number of isolates which shows resistance to a particular antibiotic.

Table 1- Antibiogram (showing resistance to a particular antibiotic)

Antibiotics	Isolates	
	Pseudomonas aeruginosa (n=64)	*Acinetobacter baumanii* (n=30)
Ampicillin+ Sulbactam	32	10
Cefuroxime	34	22
Cefoxitin	32	18
Ceftriaxone	32	18
Ceftazidime	28	18
Cefepime	10	4
Cefoperazone+ Sulbactam	0	2
Gentamicin	52	20
Amikacin	30	16
Ciprofloxacin	28	20
Levofloxacin	26	6

Using Nitrocefin disc test a total of 50 out of 94 isolates; out of which 32 (50%) of *P. aeruginosa* and 18 (60%) of *Acinetobacter baumanii* were positive for β-lactamase production. ESBL and AmpC β-lactamase production are shown in [Table-2].

Table 2- Showing ESBL and AmpC β-lactamase production

Isolates	Frequency			
	ESBL		AmpC β-lactamase	
	(n)	(%)	(n)	(%)
A. baumannii	3	10	6	20
P. aeruginosa	14	21.88	18	28.13

Antibiogram of isolates showed resistance to majority of commonly used antibiotics. When they were tested by Inhibitor based method for ESBL and AmpC β-lactamase production, production of AmpC β-lactamase is much higher compared to ESBL production in both *Acinetobacter baumanii* and *P. aeruginosa*. Also AmpC β-lactamase production is much higher in *P. aeruginosa* compared to *Acinetobacter baumannii*. Two Indian studies reported 20.27% and 19.3% of ESBL and AmpC β-lactamase production in *P. aerugino-*

sa [2,19]. ESBL and AmpC β-lactamase production in *Acinetobacter baumanii* is already reported from other countries [3,20]. As ESBL and AmpC β-lactamase production may mask detection of each other, detection by various other phenotypic methods mentioned earlier, may miss their detection in routine clinical laboratories, which ultimately lead to in-vivo treatment failure. But by using inhibitor based method, we can detect both ESBL and AmpC β-lactamase. In our study, though we didn't encounter any single organism which has shown both ESBL and AmpC β-lactamase, this should not be ignored.

In *P. aeruginosa* and *Acinetobacter baumanii*, various mechanisms of the drug resistance are seen. Till now most worrisome resistance mechanism is Metallo-β-lactamase production [21]. *P. aeruginosa* is also known for chromosomally mediated AmpC β-lactamase production since 1980, when β-lactam antibiotics like cephalosporins, carbapenems and monobactem which have greater β-lactamase stability were introduced [22,23]. But because of long term exposure to multiple antibiotics in hospital environment, there is always a chance to get encounter with multiple drug resistance strains of these organisms with involvement of multiple plasmid mediated mechanisms.

Therefore, detection of AmpC β-lactamase is very important in clinical laboratories along with detection of other commonly occurring enzyme production as chromosomally encoded enzyme can mediate resistance to many cepholosporins like cepholothin, cephazolin, cefoxitin, most penicillins, and also β-lactam/ β-lactam inhibitor combinations. AmpC encoded by both plasmid and chromosomal mediated genes are also evolving to hydrolyze broad-spectrum cephalosporins more efficiently. Carbapenems can usually be used to treat infections due to AmpC-producing bacteria, but carbapenem resistance can arise in some organisms by mutations that reduce influx (outer membrane porin loss) or enhance efflux (efflux pump activation) or MBL production [21,24]. This ultimately leaves us with no antibiotics available for treatment of infections.

Conclusion

Inhibitor based method is very useful in routine clinical laboratories for detection of both ESBL and AmpC β-lactamase simultaneously in a single organism. This saves the time and also cost-effective compared to other more confirmative genotypic methods. With the increase in occurrence and types of these multiple β-lactamase enzymes, early detection is very important and decisive, the benefits of which include implementation of proper antibiotic therapy and infection control policy.

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