Burden of different beta-lactamase classes among clinical isolates of AmpC-producing *Pseudomonas aeruginosa* in burn patients: A prospective study

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**Abstract**

**Background:** *Pseudomonas aeruginosa* is one of the most common pathogens causing infections in burns, and shows increasing resistance to β-lactam antibiotics by producing different classes of beta-lactamases. It is also not unusual to find a single isolate that expresses multiple β-lactamase enzymes, further complicating the treatment options. Thus, in this study, we aimed to determine the coexistence of different beta-lactamase enzymes in clinical isolates of *P. aeruginosa* in the burn ward. **Materials and Methods:** A total of 101 clinical isolates of *P. aeruginosa* from the burn ward were identified and tested for the presence of different beta-lactamase enzymes (extended spectrum beta lactamase (ESBL), Amp C and metallo β-lactamases (MBL) from October 2006 to May 2009. **In vitro** susceptibility pattern of antipseudomonal antibiotics was done by the Kirby Bauer disc diffusion method. **Results:** A total of 33 (32.7%) isolates were confirmed to be positive for AmpC beta-lactamase. Co-production of AmpC along with ESBL and MBL was reported in 24.5% and 45.5% isolates, respectively. A total of 12 (11.9%) isolates were resistant to three or more antibiotic classes (multidrug resistance). Imipenem and piperacillin/tazobactam showed high sensitivity, with 86.1% and 82.2%, respectively. **Conclusion:** This study reveals the high prevalence of multidrug-resistant *P. aeruginosa* producing beta-lactamase enzymes of different mechanisms in this region from burn patients. The emerging antimicrobial resistance in burn wound pathogens poses serious therapeutic challenge. Thus proper antibiotic policy and measures to restrict the indiscriminate use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple beta-lactamase producing pathogen.

**Keywords:** AmpC, extended-spectrum beta lactamase, metallo β-lactamases, *Pseudomonas aeruginosa*

**Introduction**

Burns are one of the most common and devastating forms of trauma. The breached skin barrier is the hallmark of burn injury. Microorganisms colonizing the burn wound originate from the patient’s endogenous skin, gastrointestinal and respiratory flora.[1-4] Microorganisms may also be transferred to a patient’s skin surface via contact with contaminated external environmental surfaces, water, fomites, air and the soiled hands of health care workers.[5-6] *Pseudomonas aeruginosa* is a known opportunistic pathogen frequently causing infections in burned patients.[7] About 45% of mortality in burn patients is due to infections.[8] The nosocomially acquired resistant *P. aeruginosa* in burn patients results in higher mortality rate, antibiotic costs, hospital stay and surgical procedures.[9,10] Infections caused by *P. aeruginosa* are difficult to treat as the majority of isolates exhibit varying degrees of innate resistance. In *P. aeruginosa*, resistance to various antimicrobials may be due to outer membrane impermeability, target site modification and multidrug
efflux pumps.\textsuperscript{[11,12]} Acquired resistance is also reported by the production of beta lactamase enzymes like extended-spectrum beta lactamase (ESBL), AmpC and metallo beta-lactamases (MBL).\textsuperscript{[13]} ESBLs are typically inhibitor-susceptible beta-lactamases that hydrolyze penicillins, cephalosporins and aztreonam and are encoded by mobile genes. AmpC beta-lactamases preferentially hydrolyze cephalosporins and cephams and resist inhibition by clavulanate, sulbactam and tazobactam. MBLs hydrolyze carbapenems and other beta-lactams. Resistance to carbapenems is of great concern as these are considered to be antibiotics of last resort to combat infections by multidrug-resistant bacteria, especially in intensive care units and burn wards.

Genes for all these three enzymes are often carried on plasmids, facilitating rapid spread between microorganisms.\textsuperscript{[14]} The presence of ESBLs and AmpC beta-lactamases in a single isolate reduces the effectiveness of the beta-lactam/beta-lactamase inhibitor combinations, while MBLs and AmpC beta-lactamases confer resistance to carbapenems. Often, these enzymes are co-expressed in the same isolate. With the increase in occurrence and types of these multiple beta-lactamase enzymes, early detection is crucial, the benefits of which include formulation of a policy of empirical therapy and infection control policy in high-risk units where infections due to resistant organisms are much higher.

In view of the paucity of information on different beta-lactamase producing \textit{P. aeruginosa} infections in burn patients, we aimed to determine the frequency and coexistence of ESBL-, Amp C- and MBL-producing \textit{P. aeruginosa} in burn patients admitted to a tertiary care hospital.

Materials and Methods

A total of 101 consecutive non-repetitive (i.e., one per patient) isolates of \textit{P. aeruginosa} were collected from patients admitted to the burn wards of tertiary care hospital and confirmed at the Department of Microbiology. All the confirmed \textit{P. aeruginosa} isolates were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.\textsuperscript{[15]} The antibiotics used were imipenem, piperacillin/tazobactam, cefoperazone/sulbactam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, amikacin, gentamicin, tobramycin, netilmicin and carbenicillin.

The initial screening and phenotypic confirmatory tests recommended by CLSI were carried out for AmpC beta-lactamases detection. In the initial screening test, a disc of cefoxitin (FOX-30 µg) was placed in a Mueller Hinton agar plate already inoculated with the test organism. Zones of inhibition around the cefoxitin disc were observed after overnight incubation. Isolates that yielded a zone diameter less than 18 mm were labeled as AmpC beta-lactamases positive.

Disc antagonism test\textsuperscript{[16]} was performed for detection of inducible AmpC beta-lactamases. A test isolate (with a turbidity equivalent to that of 0.5 McFarland standards) was spread over a Mueller Hinton agar plate. Cefotaxime (CTX-30 µg) and Cefoxitin (FOX-30 µg) disks were placed 20 mm apart from center to center. Isolates showing blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin disk were screened as positive for AmpC beta-lactamase [Figure 1].

Confirmation of AmpC beta-lactamases production was done by a modified three-dimensional test.\textsuperscript{[13]} Fresh overnight growth from Mueller Hinton agar was

\textbf{Figure 1: Disc antagonism test (FOX, cefoxitin; CTX, cefotaxime)}

\textbf{Figure 2: Modified three-dimensional test (clear distortion - 1, minimal distortion - 2 and 3, no distortion in negative control - 4)}
transferred to a preweighed sterile microcentrifuge tube. The tube was weighed again to determine the bacterial mass and to obtain 10–15 mg of bacterial wet weight. The bacterial mass was suspended in peptone water and pelleted by centrifugation at 3000 rpm for 15 min. The crude enzyme extract was prepared by repeated freeze-thawing (10 cycles) of the bacterial pellet. The surface of the Mueller Hinton plate was inoculated with *E. coli* ATCC 25922. Cefoxitin (FOX-30 µg) was placed at the center of the inoculated plate. With a sterile scalpel blade, a slit beginning 5 mm from the edge of the disc was cut within the agar in an outward radial direction. By using a pipette, 50µL of enzyme preparation was dispensed into the slit, beginning near the slit and moving outward. It was incubated overnight at 35°C. Any distortion of the zone of the cefoxitin disc toward the slit confirmed AmpC β-lactamase production [Figure 2].

A combined disc diffusion test was performed for ESBL detection. In this test, the test organisms were grown on Mueller Hinton agar and discs of cefotaxime (CTX-30 µg) and ceftazidime (CAZ-30 µg) separately and each of these in combination with clavulanic acid (CA-10 µg) were placed on the surface of the lawn of bacteria. A difference of ≥5 mm between the zone of inhibition of a single disc and in combination with clavulanic acid was considered as an ESBL-positive isolate [Figure 3].

All the isolates were subjected to the Imipenem-EDTA disc method for the detection of MBL producers. Isolates were identified as MBL positive if the increase in the inhibition zone with the imipenem and the EDTA disc was ≥7mm than the imipenem disc alone [Figure 4].

### Results

A total of 101 clinical isolates of *P. aeruginosa* from burn patients were identified and tested for antimicrobial sensitivity to different antibiotics and presence of different beta-lactamase enzymes (ESBL, Amp C and MBL). Among the beta-lactams tested, the most effective agent was imipenem (86.1%), followed by piperacillin (69.3%), cefepime (68.3%), ceftazidime (66.3%), ceftriaxone (48.5%), carbenicillin (35.6%) and netilimicin (34.6%). Susceptibility results of combination of beta-lactams and beta-lactamase inhibitors tested were: piperacillin + tazobactam (82.2%) and, cefoperazone + sulbactam (70.3%). Among aminoglycosides, amikacin showed good activity (63.4%), followed by gentamicin (55.5%). Only 58.4% of the isolates were susceptible to ciprofloxacin [Table 1]. A total of 12 (11.9%) *P. aeruginosa* isolates were multidrug resistant, i.e. resistance to three or more antibiotic classes.

In this study, out of 101 *P. aeruginosa* isolates tested, cefoxitin resistance was seen in 79 (78.2%) isolates while 33 (32.7%) isolates were confirmed to be AmpC β-lactamase producers. Among the test isolates, 28 (27.7%) were detected as inducible AmpC producers while five (4.95%) of the isolates were confirmed to be noninducible [Table 2]. The co-existence of AmpC and ESBL was reported in eight (24.5%) isolates, whereas AmpC and co-production of MBL was shown by 16 (48.5%) of the isolates [Table 3]. Among these 16 MBL producers, 14 isolates were found to show resistance towards Imipenem.

### Discussion

Results of antimicrobial susceptibility reveal that resistance in *P. aeruginosa* is increasing to the commonly used antibiotics, i.e. penicillins, cephalosporins, and Aminoglycosides, etc. Worldwide, resistance
In our study, imipenem was found to be the most effective drug, showing a maximum susceptibility of 86.1%, which is in agreement with earlier studies. 

Multiple beta-lactamase producing *P. aeruginosa* can cause major therapeutic failure, and poses a significant clinical challenge if they remain undetected. Therefore, 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. Of 101 *P. aeruginosa* strains, 33 (32.7%) were AmpC β-lactamase producer. Other studies from different parts of India showed 17.3-59.4% of AmpC production. Co-production of AmpC along with ESBL was seen in 24.5% of the isolates, which contrasts an earlier study that showed 3.33%. This increase in percentage may be due to the rising trend of acquiring resistance mechanism and thus making the antimicrobials ineffective. Carbapenems are the only β-lactam antibiotic that are active against co-AmpC and ESBL producers; however, resistance to carbapenems has been increasing, which is mostly due to the production of MBL. Our findings showed a high percentage of MBL-producing *P. aeruginosa* (48.5%) among AmpC producing isolates; however, earlier studies in this country showed a low (7.5%) to moderate (20.8%) prevalence of MBL.

In conclusion, our study emphasizes the high burden of coexisting different beta-lactamase enzymes (i.e., AmpC -32.7%, AmpC + ESBL - 24.5% and AmpC + MBL - 48.5%) in clinical isolates of *P. aeruginosa*, which represent a serious therapeutic challenge for clinicians caring for burn patients. Strict infection control practices (i.e., physical isolation in a private room, use of gowns and gloves during patient contact and hand washing before and after each patient visit), appropriate empirical antimicrobial therapy and early detection of these β-lactamase-producing isolates could help to reduce the burden of infections. Thus, management of beta-lactamase-producing *P. aeruginosa* from burn patients urges for liaison between plastic surgeons, infectious disease physicians, and clinical microbiologists to facilitate the development of burn unit-specific empirical treatment algorithms based on an updated yearly antibiogram data and outcome analyses.

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### References


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