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Characterization of the Extended-Spectrum beta-Lactamase Producers among Non-Fermenting Gram-Negative Bacteria Isolated from Burnt Patients

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Background & Aims of the Study: Extended-spectrum beta-Lactamases (ESBLs) represent a major group of beta-lactamases which are responsible for resistance to oxyimino-cephalosporins and aztreonam and currently being identified in large numbers throughout the world. The objective of this study was to characterize ESBL producers among non-fermenter gram-negative bacteria isolated from burnt patients.

Materials & Methods: During April to July 2012, 75 non-fermenter gram-negative bacilli were isolated from 240 bacterial cultures collected from wounds of burnt patients admitted to the Burn Unit at Shahid Motahari Hospital (Tehran, Iran). Bacterial isolation and identification was done using standard methods. Antimicrobial susceptibility testing was performed by disk diffusion method for all strains against selected antibiotics and minimum inhibitory concentration was determined by microdilution test. The ability to produce ESBL was detected through double disk synergy test among candidate strains.

Results: Of 75 non-fermenter isolates, 47 *Pseudomonas aeruginosa* and 28 *Acinetobacter baumannii* were identified. The resistance of *P. aeruginosa* isolates to tested antibiotics in antibiogram test were 100% to cefpodoxime, 82.98% to ceftriaxone, 78.73% to imipenem, 75% to meropenem, 72.72% to gentamicin, 69.23% to ciprofloxacin and aztreonam, 67.57% to cefepime, 65.95% to ceftazidime, and 61.53% to piperacillin. The results for *Acinetobacter baumannii* were 100% to ceftazidime, cefepime, ciprofloxacin, imipenem, meropenem, cefpodoxime, and cefotaxim, 96.85% to gentamicin, 89.65% to ceftriaxone, 65.51% to aztreonam, and 40% to piperacillin. Double disk synergy test showed that 21 (28%) of non-fermenter isolates were ESBL producer.

Conclusions: None of the third or fourth generation of cephalosporins is suitable for treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from burnt patients.

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Background

Non-fermenting gram-negative bacteria are a group of bacteria which are unable to produce energy by fermenting sugars. Because of emerging multidrug resistant (MDR) strains, the members of this group have become a particular challenge in healthcare management. Most of the time, gram-negative non-fermenters are pathogens that cause opportunistic

infections in patients who are critically ill or immunocompromised (1).

Non-fermenting bacteria are distributed in nature, especially in soil and water. In the hospital environment, they may be isolated from humidifiers, ventilator machines, mattresses, and other equipment, as well as from the skin of healthcare workers (1). Therefore, recent clinical attention has focused on the increasing frequency of non-fermenting gram-negative pathogens, which account for hospital-acquired infections (2).

Among non-fermenting gram-negative rods, the most clinically important species are *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii*, which are frequently MDR (3). Infections with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are of particular concern for hospitalized patients, especially whom with MDR infection (4).

Multidrug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates are increasingly causing hospital-acquired infections. These clones are spreading into new geographic areas, and causing susceptible strains to acquire resistance genes. New extended-spectrum beta-lactamase and carbapenemase producers are emerging and leading to the evolution of pan-resistant strains.

Extended-spectrum beta-lactamases (ESBLs) represent a major group of beta-lactamases responsible for resistance to oxyimino-cephalosporins and aztreonam, and currently being identified in large numbers throughout the world (5). ESBLs are encoded by both chromosomal or plamid genes (4-7). Hence, acquired resistance to beta-lactams can result in therapeutic failure, particularly when it is associated with resistance to other classes of drugs, such as aminoglycosides and fluoroquinolones (8).

The risk of infection in burns is well-known, and in the recent decades, the frequency of antimicrobial resistance is increasing among isolated bacteria from burnt patients (9). Burn wound is an ideal environment for bacterial growth, and are potentially richer sources of infection than surgical wounds, predominantly due to the larger area involved and the longer duration of hospitalization (10).

Aims of the study: The objective of this study was to characterize extended-spectrum beta-lactamase (ESBL) producers among non-fermenter gram-negative bacteria isolated from burnt patients.

Materials & Methods

Bacterial isolates: During April to July 2012, 75 non-fermenter gram-negative bacilli were isolated from 240 bacterial cultures collected from wounds of burnt patients admitted to the Burn Unit at Shahid Motahari Hospital (Tehran, Iran). The wound exudates were collected by swabbing, and immediately transported in transport culture media under standard conditions to the laboratory of the Department of Microbiology of Shahid Beheshti University of Medical Sciences (Tehran, Iran).

Bacterial isolation and identification was done using standard methods such as "oxidase", "triple sugar iron", "oxidative-fermentative", and "motility" tests (11). Based on the oxidase test, the isolated bacteria were divided into two groups (oxidase-positive and oxidase-negative). Oxidase-positive strains were considered as members of *Pseudomonas* family, and further confirmation tests were performed, including growth on cetrimid agar, growth at 42°C, decarboxylation of lysine on lysine decarboxylase and arginine dihydrolase test. Oxidase-negative isolates were presumed as members of *Acinetobacter* family, and were confirmed by Microgen identification kit (Microgen™, UK).

Antibiotic susceptibility (antibiogram) test: Based on Clinical Laboratory Standards Institute (CLSI) 2012 protocol (12), antimicrobial susceptibility testing was

performed for all strains by disk diffusion method against following antibiotics:
Piperacillin (100 µg), meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefteriaxone (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), and gentamicin (10 µg), all purchased from MAST Co. (Mast Diagnostics, UK).

Minimum inhibitory concentration (MIC) test: For ceftazidime resistant strains in antibiogram test, MIC was determined by microdilution test based on Clinical Laboratory Standards Institute (CLSI) 2012 protocol (12).

ESBL screening: The ability to produce ESBL was detected by double disk synergy test (DDST) among candidate strains.

The ceftazidime resistant strains were candidates for ESBL detection by DDST method. In this test, colonies from overnight cultures on blood agar plates were suspended in Mueller-Hinton broth and their turbidity was adjusted to 0.5 McFarland standard. Then, the suspension was streaked onto Mueller-Hinton agar plates (Qlab, Montreal, Canada) (10). DDST test was performed by using plain ceftazidime disk (30 µg) and ceftazidime/clavulanic acid (30 µg/10 µg) and putting other third generation cephalosporins like cefotaxime, ceftriaxone, ceftazidime, and cefepime at 20 mm distance to ceftazidime/clavulanic acid disk, respectively. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used simultaneously as control strains.

Data analysis: The results were reported as percentages.

Results

Totally, 75 non-fermenter gram-negative bacilli were isolated from 240 bacterial cultures collected from wounds of burnt patients admitted to the Burn Unit at Shahid Motahari Hospital (Tehran, Iran).

Out of 75 non-fermenter isolates, 47 (62.67%) *Pseudomonas aeruginosa* and 28 (36.33%) *Acinetobacter baumannii* were identified. Antimicrobial resistance data are presented in table 1.

Using DDST, an increase in inhibition zone diameter of 5 mm for ceftazidime/clavulanic acid versus ceftazidime alone were detected among 21 (28%) non-fermenter isolates (table 2).

The MICs of ceftazidime for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are presented in table 3.

Table1) Antibacterial resistant among tested *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Resistance to: \ Antibiotics	imipenem	meropenem	ceftazidim	cefotaxime	ceftriaxone	cefpodoxim	cefepime	piperacilin	ciprofloxacin	aztereonam	gentamycin
Pseudomonas aeruginosa	78.73	75	65.95	82.97	82.98	100	67.57	61.53	69.23	69.23	72.72
Acinetobacter baumannii	100	100	100	100	89.65	100	100	40	100	65.51	96.85

Table 2) Frequency of extended-spectrum beta-lactamase producers among the *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates

Species	Number	extended-spectrum beta-lactamase -positive (%)
Pseudomonas aeruginosa	15	31.91
Acinetobacter baumannii	6	21.42

Table 3) Minimum inhibitory concentration (MIC) of ceftazidime among 75 non-fermenter isolates

Species	MIC50 (µg/mL)	MIC90 (µg/mL)	MIC range (µg/mL)
Pseudomonas aeruginosa	128	256	4>X≥256
Acinetobacter baumannii	256	>256	4>X>256

Discussion

In this study, existence of 100% resistance to 7 tested antibiotics, including imipenem, meropenem, ceftazidime, cefepime, cefpodoxime, cefotaxim, and ciprofloxacin among *Acinetobacter baumannii* shows that these groups of bacteria are more resistant to common antibiotics than *Pseudomonas aeruginosa* isolates. Therefore, based on the antibiotic resistance pattern, none of antibiotics tested in antibiogram test are effective against *Pseudomonas aeruginosa* or *Acinetobacter baumannii* (table 1).

Detection of ESBL producers among gram-negative bacilli is necessary for successful therapy, improving clinical outcomes, and limiting the spread of these MDR organisms (14). ESBL producing strains are usually found in hospitals where antibiotic usage is high and patients are in critical conditions (5). Importance of prolonged duration of staying in burn ward is for its serious threat of the spread of MDR bacterial pathogens, and consequently causing nosocomial infection (10).

31.91% of *Pseudomonas aeruginosa* isolates were ESBL-positive, which is in accordance with other studies conducted in Iran (28%), Bangladesh (37.8%), India (32.6%), and Turkey (31.5%) (5,15-17).

However, it is in contrast with two other studies in Iran that their results were lower (18%) and higher (42.8%) than our result. This discrepancy may be related to more usage of beta-lactam drugs and time of study (18,19).

In addition, 21.42% of *Acinetobacter baumannii* were identified as ESBL producer by phenotypic tests, which was similar to Owlia et al.'s study (21%) in Iran and another study in Poland (20%) (10,20). Despite the above findings, a study in Chile has reported a low rate (10%) of ESBL producer among *A. baumannii* strains, which were collected from pathological products of hospitals by the same DDST method (21).

Resistance to 3rd and 4th generation cephalosporins and carbapenems among *Pseudomonas aeruginosa* isolated in our study is comparable with a study by Rossolini et al. in Latin America, which reported the same rate of resistance to the following antibiotics: ceftazidime (65.95%), cefepime (67%), imipenem (78%) (23). Rate of resistance in our study were 66, 67, and 76 percent, respectively.

Similarity between the resistance rates, which were reported among *Acinetobacter baumannii* in the Owlia's study (98% to ceftazidime, 95% to cefotaxim, and 85% to imipenem) and in our study (100% for all) is another evidence that confirms our results (10).

Whereas, a kind of beta-lactams is inhibited by beta-lactamase inhibitors like clavulanic acid, the use of beta-lactam/beta-lactamase inhibitor combination (21%) therapy may be an alternative treatment, but the effect of this combination varies dependent on the subtype of ESBL present (5).

In conclusion, the antibiogram results in this study reveal that none of the third or fourth generation cephalosporins are suitable for treatment of *Acinetobacter baumannii*.

Footnotes

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Conflict of Interest:

The authors declare no conflict of interest.

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