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Original Article

Phenotypic Detection of Metallo-beta-lactamase Producing *Pseudomonas aeruginosa* Strains Isolated from Burned Patients

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ABSTRACT

Background and Objective: **Metallo-beta-lactamases (MBLS) mediated resistance is an emerging threat in hospital isolates of *Pseudomonas aeruginosa*. There is not enough information from Iran regarding the prevalence and the screening methods for such enzymes. The present study was undertaken to detect MBLS in strains of *P. aeruginosa* isolated from burned patient using phenotypic method.**

Materials and Methods: **For this purpose, 128 consecutive *P. aeruginosa* isolates obtained from hospitalized patients were subjected to susceptibility testing to antipseudomonal drugs by disc diffusion and minimal inhibitory concentration (MIC) for ceftazidime was determined. The production of MBL was detected by the zone size enhancement with EDTA impregnated ceftazidime disc.**

Results: **It was found out that 94 (73.44%) of the isolates were resistant to ceftazidime. These isolates screened as ESBLs producing strains and introduced for detection of MBL production. Out of the 94 *P. aeruginosa* that were resistant to ceftazidime, 50 (53.2%) isolates were MBL positive. This result indicated that 39.06% of all isolates were MBL positive.**

Conclusion: **MBL-mediated ceftazidime resistance in *P. aeruginosa* is a cause for concern in the therapy of critically ill patients. The MBL producing *P. aeruginosa* isolates were more resistant to various antimicrobial agents. This result suggests that MBL producing isolates in hospitals may cause serious infections that illustrated when these strains were responsible for a nosocomial outbreak.**

Key words: Ceftazidime, Metallo-beta-lactamase, *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa producing metallo-β-lactamases (MBLs) was first reported from Japan in 1991 (1) and since then has been described from various parts of the world including Asia (2,3), Europe (4-7), Australia (8), South America (9), and North America (10). The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by β-lactam resistant bacteria (11). However, resistance to extended spectrum beta-lactams has been frequently observed in non-fermenting bacilli such as *Pseudomonas aeruginosa* and Acinetobacter spp. The common form of resistance is mediated by lack of drug penetration (i.e. porin mutations and efflux pumps) and/or hydrolyzing β-lactamases. Based on molecular studies, carbapenem hydrolyzing enzymes are classified into four groups A, B, C and D. The MBLs belong to group B and are enzymes requiring divalent cations as cofactors for enzyme activity, being inhibited by the action of a metal ion chelator (12). The MBLs efficiently hydrolyze all β-lactams, except aztreonam in vitro (13).

Several phenotypic methods are available for the detection of MBL producing bacteria. All these methods are based on the ability of metal chelators such as EDTA and thiol-based compounds to inhibit the activity of MBLs. These tests include the double-disk synergy tests using EDTA with imipenem (IPM) or ceftazidime (CAZ) (14-16), 2-mercaptopropionic acid with CAZ or IPM (17), the Hodge test (14,15), a combined disk test using EDTA with CAZ or IPM (16-18), the MBL Etest (AB BioDisk company, Solna, Sweden) (19), and a micro dilution method using EDTA and 1,10-phenanthroline with IPM (20).

P. aeruginosa is a common Gram-negative bacillus associated with hospital infections and is often difficult to eradicate due to its resistant drug profile. Therefore, detection of MBL producing Gram-negative bacilli especially *P. aeruginosa* is crucial for the optimal treatment of patients particularly in critically ill and hospitalized patients and to control the spread of resistance (21). There is not much information available on MBL producing *P. aeruginosa* isolates from Iran. Therefore, we undertook this study to detect the MBL in *P. aeruginosa* isolates obtained from hospitalized burned patients.

Materials and Methods

Bacterial strains

In this study, 128 strains of *P. aeruginosa* were isolated from burned patients that hospitalized in Shahid Motahari hospital from March to October 2007. Bacteria were determined by biochemical tests and stored at -20 °C. Standard strain *P. aeruginosa* ATCC 27853 was used as control.

Antimicrobial susceptibility testing

The susceptibility to antipseudomonal drugs was done on Mueller Hinton agar using disc diffusion method in accordance with National Committee for Clinical Laboratory standards (NCCLS) incorporating standard strain of *P. aeruginosa* (ATCC 27853) (22). The antibiotics tested were gentamicin, amikacin, piperacillin, ciprofloxacin, ceftazidime, piperacillin, ceftriaxone and ceftizoxime (Hi-media Laboratories, Mumbai).

Screening of ESBL

Extended spectrum β-lactamase (ESBL) producing strains was screened by determination of MIC of CAZ. In this respect, MIC of CAZ for these isolates was done by agar dilution method in accordance with NCCLS standards (23). The pure form of the drug was obtained from Exir Pharmaceutical Company (Tehran, Iran). Isolates with MIC ≥16 μg/ml were screened as extended spectrum β- lactamase producing strains and used for confirmation of MBLs production.

Detection of MBLs

We used zone enhancement with EDTA impregnated ceftazidime discs (24) for phenotypic determination of MBLs. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the NCCLS (22). A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.2H2O in 1000 ml of distilled water and adjusting it to pH 8.0 using NaOH. The mixture was sterilized by autoclaving. EDTA solution was added to ceftazidime discs to obtain a desired concentration of 750 μg. The EDTA impregnated antibiotic discs were dried immediately in an incubator and stored at -20 °C in airtight vials. Then, 30 μg ceftazidime discs (with and without EDTA) were placed on the surface of an inoculated agar plate. The inhibition zones of ceftazidime and ceftazidime EDTA discs were compared after 16-18 h of incubation in air at 35 °C. Strains with enhancement zone in ceftazidime EDTA discs were recognized as MBL producing *P. aeruginosa*.

Results

Clinical bacterial strains

Out of the 128 isolates of *P. aeruginosa*, 12 (9.37%) were isolated from tissue culture, 1 (0.78%) from blood culture and 115 (89.85%) from wound (purulent) culture.

Screening of ESBLs

Out of the 128 clinical isolates of *P. aeruginosa*, 94 (73.44%) were resistant to 16 µg/ml of ceftazidime. These isolates screened as ESBLs producing strains and introduced for detection of MBLs production.

Detection of MBLs

Out of the 94 *P. aeruginosa* that were resistant to CAZ, 50 (53.2%) isolates were MBLs positive. This result indicated that 39.06% of all isolates were MBLs positive.

Antimicrobial susceptibilities of clinical strains

Out of the 128 *P. aeruginosa* clinical isolates included in this study, 95 (74.22%) were resistant to ceftazidime, 109 (85.16%) to ceftizoxime, 111 (86.72%) to ceftriaxone, 86 (67.19%) to piperacillin, 83 (64.85%) to amikacin, 49 (38.28%) to imipenem, 107 (83.6%) to gentamicin and 63 (49.22%) to ciprofloxacin (Table 1). A particularly important feature was that all the MBL producers were resistant to ceftazidime, ceftizoxime, and ceftriaxone, but resistance to gentamicin, piperacillin, amikacin, ciprofloxacin and imipenem were 96%, 88%, 80%, 52% and 46% respectively.

Table 1: Results of antibiotic susceptibility tests of isolated strains of *P. aeruginosa*

	Frequency (%) of:		
Antibiotics	**Resistant**	**Intermediate**	**Sensitive**
Ceftazidime	95 (74.22)	1 (0.78)	32 (25)
Ceftizoxime	109 (85.16)	17 (13.28)	2 (1.56)
Ceftriaxone	111 (86.72)	12 (9.38)	5 (3.9)
Piperacillin	86 (67.19)	14 (10.94)	28 (21.87)
Amikacin	83 (64.85)	3 (2.34)	42 (32.81)
Imipenem	49 (38.28)	14 (10.94)	65 (50.78)
Gentamicin	107 (83.6)	1 (0.78)	20 (15.62)
Ciprofloxacin	63 (49.22)	16 (12.5)	49 (38.28)

Discussion

In 1991, Japan reported the first plasmid-mediated metallo-beta-lactamase in *P. aeruginosa* (1). This was soon followed by another report of transferable metallo enzyme in B. fragilis (25). Apart from *P. aeruginosa*, other bacteria like *Serratia*, *Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, E. cloacae, Citrobacter freundii, Proteus vulgaris, P. putida, Acinetobacter and Alcaligenes xylosoxidans* were also shown to produce MBL (18). There are frequent reports of MBL production in *P. aeruginosa* from the Asian and the Pacific countries, namely Hong Kong, Taiwan and Japan (26). MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years, and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections (27-29). A case-controlled study from Japan showed that patients infected with MBL-producing *P. aeruginosa* were more likely to receive multiple antibiotics and, more importantly, that infection-related deaths due to IMP-producing *P. aeruginosa* were more frequent than deaths caused by MBL-negative *P. aeruginosa* (30). The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. The accurate identification and reporting of MBL-producing *P. aeruginosa* will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates (28-29).

In this study, we observed a resistance of 73.44% to ceftazidime among the *P. aeruginosa*. Meanwhile, 53.2% of screened bacteria were MBL-positive. Another study from south India reported 12% MBL-mediated imipenem resistance in *P. aeruginosa* (31). There is one report of MBL production in *P.*

aeruginosa from Ahwaz, Iran (32). They reported that 19.51% of imipenem resistant *P. aeruginosa* strains (isolated from burned patients) were MBL producer, but in our study, this was 53.2%.

Conclusion

This study clearly illustrated that MBL producing isolates of *P. aeruginosa* are important causes of ceftazidime resistance among this species isolated from the Shahid Motahari hospital. The MBL producing *P. aeruginosa* isolates were more resistant to various antimicrobial agents. This result suggests that MBL producing isolates in this hospital are responsible for serious infections, which was illustrated when these strains were responsible for a nosocomial outbreak.

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