

Metallo- β -lactamases among Multidrug Resistant (MDR) Gram Negative Bacteria Isolated from Clinical Specimens during 2009 in Sanandaj, Kurdistan Province

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Article information	Abstract
<p>Article history: Received: 30 Dec 2011 Accepted: 1 Feb 2012 Available online: 12 Mar 2012</p> <p>Keywords: Metallo-β-lactamase MDR (multi drug resistant) Hospital specimens</p> <p>*Corresponding author at: Department of Microbiology, Institute Pasteur of Iran, Tehran, Iran. E-mail: shahcheraghi@pasteur.ac.ir</p>	<p>Background: Today, there are numerous reports about emerging multi drug resistant gram negative bacteria all around the world, especially in ICUs. Rarely, Metallo-β-lactamase (MBL) enzymes are responsible for these cases. Study of MBLs for diagnosing and preventing distribution of the origin of infection are critical issues. In addition, we would like to compare the efficacy of Iranian and foreign-made antibiotic disks.</p> <p>Materials and Methods: During 2009 all entered clinical specimens to the laboratory tested for detecting gram negative bacteria. Isolated bacteria were tested by Kirby-Bauer method to antibiotic susceptibility test by Iranian and foreign (MAST) disks. For gram negative carbapenem resistant isolates, PCR technique used to detect VIM, GIM, and SIM variants of MBLs.</p> <p>Results: During one year, 17890 clinical specimens referred Besat laboratory. The most specimen was Urine (8172) followed by blood culture (5190) that in which 1110 gram negative and positives isolated. Out of which, 778 (70%) of isolates were gram negatives. MDR gram negatives were 157 (20.2%). Imipenem and meropenem were the most efficient antibiotics (all susceptible) and ceftriaxone was the least (19 % susceptible). E. coli was the most prevalent isolate. 79 Gram negative isolates (10.1%) were resistant to Iranian-made discs but all susceptible for foreign ones. All 79 isolates were tested by PCR for MBL genes, that, all were negative. Besides, Iranian imipenem and cefepime disks have had distinguishable difference in susceptibility of isolates.</p> <p>Conclusion: Fortunately, none of gram negative isolates were MBL producer, which revealed no colonization of MBL producing bacteria. Iranian-made disks appear efficient except for imipenem and cefepime.</p>

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Introduction

Recently, numerous articles have reported carbapenem resistant enteric, especially in ICU isolates [1-3]. One of the most important, but rarely, such resistant is due to Metallo- β -lactamase (MBL) enzymes that confer the ability of beta-lactam hydrolyzing activity. Genes that encode such enzymes not only are harbored by chromosomes but also by plasmids [3, 4]. Mobile genetic elements have the great impact to spread antibacterial resistance among bacteria. Of which, Integrons, rather, class 1 integrons play important role in gram negative bacteria [5]. MBL genes were discovered in early 1990 in *Pseudomonas aeruginosa* [6]. *P. aeruginosa* and *Acinetobacter spp.* are more reported species that produce MBLs [7, 8] but there are rare reports of enterobacteriaceae [9, 10]. Since enterobacteriaceae cause many bacterial infections and can easily uptake mobile genetic element, hence, this family is the most of concern. Because MBL inhibitor like those for beta-lactamases, e.g. clavulanic acid, is unknown, therefore strictly surveillance should be exerted to avoid mortality and pervading MBL producers. The

current study has designed to evaluate the distribution of carbapenem resistant isolates and molecular epidemiology of MBLs in Besat hospital of Sanandaj during a year.

Materials and Methods

In this cross-sectional study, during a year all of the referred clinical specimens were cultured.

Sampling: Entered specimens to Besat hospital laboratory cultured on 5% Sheep Blood Agar (Merck, Germany) and EMB (Merck, Germany). Sterile body fluid (e. g. cerebrospinal fluid) specimens at first were cultured in enrichment mediums, Thioglycollate broth (Merck, Germany) and Chocolate agar, further to prior mediums. MacConkey Agar and Hekton Enteric Agar (Merck, Germany) mediums were used for stool cultures. Isolated bacteria were characterized by biochemical reactions and oxidation/ fermentation activity. Age, sex, and previous hospitalization and ward of patients and type of specimens (blood, urine, tracheal aspirate, and so on) were recorded. Also, according to the monthly sampling

program from wards by infection control committee, samples related to the instruments associated to the patients were collected. Isolated Gram negative bacteria characterized as well as patients' isolates. All of the isolates were frozen at -20°C in LB broth (Merck, Germany) contained 18% glycerol (Merck, Germany) up to the end of study.

Susceptibility testing: Different antibiotic families were used for susceptibility testing including fluoroquinolones, cephalosporins, monobactams, aminoglycosides and carbapenems by Kirby-Bauer disk diffusion method according to CLSI recommendation standards [11]. Two brands (An Iranian brand and MAST from England) were chosen to compare to each other. Iranian brand antibiotics that were used by Besat hospital were ciprofloxacin (CP), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), gentamicin (GM), ceftriaxone (CRO), amikacin (AN), Nalidixic acid (NA), nitrofurantoin (FM), trimethoprim-sulphamethoxazole (SXT) and imipenem (IPM), whereas in our study selected antibiotics from MAST disks were ciprofloxacin (CIP), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), gentamicin (GM), ceftriaxone (CRO), amikacin (AK), aztreonam (ATM), imipenem (IMI) and meropenem (MEM). Isolates which were resistant to two or more families were considered as multi drug resistant (MDR). *E. coli* ATCC 25922 was used as negative control. **Polymerase chain reaction (PCR):** To ascertain whether there are MBL genes or not, PCR were done for four prevalence MBL genes including VIM, GIM, SIM and IMP. Primers used as previously described [12].

Results

During the year of our study 17890 clinical specimens referred to the Besat hospital laboratory (Table 1). 1110 causative organisms were isolated. Of which 778 (70%)

were gram negative bacteria. The most prevalent isolated gram negative were *E. coli* (67.7%) followed by *K. pneumoniae* (18.3%).

In susceptibility testing, as shown in table 2, imipenem was the most effective antibiotic such that there were no resistant (0%); also the most ineffective was ceftriaxone (81% resistant). Comparing Iranian brand with MAST for imipenem disks revealed that all of the isolates were susceptible to MAST imipenem disk but 79 (10.1%) of gram negative isolates were resistant to Iranian one. Also, striking differences is seen for cefepime when comparing disks from both brands. In total, 157 (20.2%) of gram negative isolates were MDR (Table 3). 19 gram negative bacteria were isolated from patients associated instruments, which no one were MDR.

PCR for all carbapenem resistant isolates were negative for VIM, GIM, SIM and IMP genes.

Discussion

MBL genes were not detected in this investigation that revealed no colonization of MBL producers. Comparing Iranian disks to MAST ones except for imipenem and cefepime, all of the rest of antibiotic disks were efficient. Urine cultures were the most specimens followed by blood culture referred to the Besat hospital. 1110 causative bacteria were isolated. Of which, 778 (70 %) were gram negatives that 526 were *E. coli*.

Tracheal aspirates are the most resistant specimen which MDR isolates to have (Table 3). Because, all of them were taken from ICU patients, it is expected. MDR isolates mainly *Acinetobacter spp.* and *K. pneumoniae*. They are normal flora of skin and intestine and easily conveyed patient to patient by hands of hospital staff and medical instruments.

Table 1. Clinical specimens and frequency of gram negative and positive isolates

	Urine culture	Blood culture	Stool culture	Cerebrospinal fluid	Tracheal aspirate	Wound & other body fluids	Patient associated instruments	Total
Number of specimens in 2009	8172	5190	2555	1164	77	252	480	17890
Grown	725(8.87%)	143(2.7%)	3(0.12%)	22(1.9%)	75(97%)	81(32%)	81(12.7%)	1110(6.2%)
Gram negative	656(90.5%)	28(19.4%)	3(100%)	7(31.8%)	41(54.7%)	24(29.6%)	19(31.1%)	778(70%)
Gram positive	69(9.5%)	116 (80.6%)	0	15(61.1%)	34(45.3%)	57(70.4%)	42(68.9%)	332(30%)

Table 2. Susceptibility testing and comparing Iranian with MAST disks for gram negative isolates (%)

Antibiotic disks	Percentage of resistant (MAST brand)	Percentage of resistant (Iranian brand)
Nalidixic acid (NA)	Not used	62
Ampicillin (AM)	Not used	99
Trimethoprim-Sulphamethoxazole (SXT)	Not used	81
Nitrofurantoin (FM)	Not used	39
Ceftriaxone (CRO)	81	87
Ceftazidime (CAZ)	80	78
Cefotaxime (CTX)	62	63
Amikacin (AN)	21	29
Ciprofloxacin (CP)	39	47
Cefepime (FEP)	23	48
Gentamicin (GM)	67	69
Imipenem (IPM)	67	11
Meropenem (MEM)	0	Not used
Aztreonam (ATM)	23	Not used

Table 3. Frequency of Gram negative and MDR isolates in clinical specimens

Specimen	Patient associated instruments	Wound & other body fluids	Tracheal aspirate	Cerebrospinal fluid	Stool culture	Blood culture	Urine culture	Total
Isolated Gram negatives	19(31.1)	24(29.6)	41(54.7)	7(31.8)	3(100)	28(19.4)	656(90.5)	778(70)
Number of MDR isolates per clinical specimens	0(0)	24 (100)	36(87.8)	0 (0)	3(100)	2(7.1)	92(14)	157(20.2)

The importance of these species is their LPS which makes ICU patient at risk especially in the case of septicemia. Therefore, hand hygiene should be the most of concern besides of monthly continues surveillance. A controversial issue about comparing antibiotic disc brands is the efficacy of Iranian imipenem and cefepime disk versus MAST ones (Table 2). This issue requires more considerations.

To prevent and control gram negative MBL producers lots of studies have assigned around the world. Indeed, periodical surveillance with respect to control bacterial resistance in many countries [5, 13]. In the United States, an investigation during 2002 to 2007 showed that 6644 out of 29723 gram negative isolates were MDR. Furthermore, *E. coli* was the most prevalence MDR and imipenem was the most efficient antibacterial. In Taiwan, a comprehensive study, that investigated frequently MBL genes by PCR and sequencing, showed that VIM2, 3, and 11 are the most frequent genes. SIM, GIM and SPM were not detected. Isolates that carry MBL genes were *A. baumannii* and *P. aeruginosa* [14]. In Iran, there are few reports for MBL genes, where, in Ahvaz [15], Isfahan [16], Tehran [17] and Kurdistan [18] VIM type has detected. In all of mentioned studies in Iran only *P. aeruginosa* were subjected as presumable MBL producer. As seen, not only there is no comprehensive surveillance

for all of Iran derived by Health Ministry, but also there were no individual studies for provinces.

Therefore, it should be of concern for the importance of issue. In conclusion, this study revealed that, fortunately, MBL producers do not colonize in Besat hospital, but, more precautions should be exerted to avoid them in future. Also, all antibiotic disks of the Iranian brand seem efficient except for imipenem and cefepime.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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