Frequency of IMP-1 and VIM Genes among Metallo-beta-Lactamase Producing Acinetobacter spp. Isolated from Health Care Associated Infections in Northeast of Iran

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ABSTRACT

Background: The emergence and rapid spread of metallo-beta-lactamase (MBL) producing Acinetobacter spp. are of great concern worldwide due to limited treatment options. Epidemiologic studies of the causing genes are important for prevention.

Methods: In this study, 70 imipenem-resistant Acinetobacter strains were isolated from health care associated infections. These isolates were screened for detection of metallo-beta-lactamase (MBL) using inhibitor potentiated disk diffusion tests with ethylenediaminetetraacetic acid (EDTA). PCR was designed for detection of bla_vim and bla_imp-1 using specific primers.

Results: Among these 70 strains, 50 strains appeared to produce metallo-beta-lactamase. Three isolates were detected by PCR to carry metallo-beta-lactamase gene bla_vim but bla_imp-1 gene was not detected.

Conclusion: These findings suggest that in our area other genetic elements are responsible for resistance against metallo-beta-lactams.


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**Introduction**

Acinetobacter is a Gram-negative, opportunistic pathogen which causes various infections specially nosocomial infections (1-3). The emergence and rapid spread of multidrug-resistant (MDR) isolates of *Acinetobacter* spp. are of great concern worldwide (4, 5). In recent years, carbapenems has been proposed as a last resort for treating serious infections attributable to multidrug-resistant isolates of Acinetobacter. The most common mechanism of carbapenem resistance among *Acinetobacter* spp. is the acquisition of carbapenemases, including certain class B metallo-beta-lactamases (MBLs) (6). Metallo-beta-lactamases can hydrolyze beta lactams from all classes except the monobactams. The genes encoding metallo-beta-lactamases are often found on mobile genetic units, such as transposons and plasmids so can rapidly spread (7, 8).

The IMP and VIM-type metallo-beta-lactamase producing *Acinetobacter* spp. have been increasingly isolated from clinical specimens in many part of the world (9). Higher mortality has been reported in patients infected with the IMP-1- producing strains. Therefore reliable epidemiological data about the prevalence and the contributing genes is essential for the optimal control of the spread of resistance (9).

Although several studies have reported the frequency of MBL-producing *Acinetobacter* spp. and the contributing genes but there is not any report about the metallo-beta-lactamase producing *Acinetobacter* spp. in northeast of Iran (10, 11). The purpose of this study was to investigate the frequency of IMP-1 and VIM genes among metallo-beta-lactamase producing *Acinetobacter* spp. isolated from health care associated infections in Northeast of Iran, Mashhad.

**Materials and Methods**

Clinical isolates of *Acinetobacter* spp. were collected during a period of 6 months from patients admitted to the Ghaem and Emamreza university hospital of Mashhad. *Acinetobacter* spp. was isolated from different clinical specimens, including wound (41 specimens) and respiratory secretions (29 specimens). The isolates were identified using standard laboratory methods. Antimicrobial susceptibility of isolates was determined using the standard Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) (12). Colistin, Ceftizoxime, Cefepime, Imipenem, Ceftriaxox, Ciprofloxacin, Sulfamethoxazol, Gentamycin, Piperacilin, Meropenem were the antibiotics evaluated in this study.

The imipenem / imipenem + EDTA disk method was performed for detection of metallo-beta-lactamase. The isolates with an increase in inhibition zone diameter produced by imipenem + EDTA compared with imipenem were considered positive for metallo-beta-lactamase production. All isolates positive for metallo-beta-lactamase production were screened by PCR for bla VIM and bla IMP-1 genes with primers specific for these genes (*Table 1*).
Table 1. PCR Primers for the detection of genes encoding metallo-beta-lactamases (bla VIM and blaIMP-1)  

<table>
<thead>
<tr>
<th>Genes Identified</th>
<th>Nucleotide Sequence</th>
<th>Amplicon Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bla VIM</strong></td>
<td>TTTGGTCGCATATCGCAACGCATTACAGCCAGATCGGCATT</td>
<td>500 bp</td>
</tr>
<tr>
<td><strong>bla IMP-1</strong></td>
<td>CTACCGCAGCGAGTCTTTGACCCAGTTTTGGCCCTTACC</td>
<td>587 bp</td>
</tr>
</tbody>
</table>

DNA from all imipenem resistant isolates were extracted by boiling and used as template in PCR assay to amplify blaVIM, blaIMP-1 genes. The amplification condition was: initial denaturation at 95°C for 5 min, followed by 35 cycle (1min at 94°C, 1min at 62°C and 1 min at 72°C with a final extension for 10 min at 72°C for bla IMP-1 gene; and initial denaturation for 5 min at 95°C, followed by 35 cycle (1min at 94°C, 1min at 56°C and 1 min at 72°C and 10 min at 72°C for blaVIM gene. PCR products were electrophoresed and then purified by AccuPrep PCR Purification Kit (Bioneer). DNA Sequencing was performed based on Sanger Dideoxynucleotides by Bioneer sequencing company. The sequence data were studied using chromas and MEGA softwares.

**Results**

_Acinetobacter_ spp. were screened for metallo-beta-lactamases production among hospitalized patients. Imipenem resistant clinical isolates were isolated from 36 male patients and 34 females during 6 months from Mashhad. Antimicrobial susceptibility profiles of these 70 isolates were shown in table 2.

Among 70 imipenem resistant isolates, 50 appeared to produce metallo-beta-lactamases inhibitor potentiated disk diffusion tests with ethylenediaminetetraacetic acid (EDTA). Of these 50 metallo-beta-lactamases positive strains, 3 were found to harbored blaVIM gene, but no blaIMP-1 gene was identified among our strains by PCR. The sequence data showed close similarity among these three blaVIM genes.

**Discussion**

The aim of this study was to assess the prevalence of blaIMP-1 and blaVIM metallo-beta-lactamases genes. Among these 70 imipenem nonsusceptible strains, 50 strains appeared to produce metallo-beta-lactamases. Of these 50 strains, 3 were found to carry blaVIM gene, but no blaIMP-1 gene was identified among our strains by PCR. Such a high prevalence of metallo-beta-lactamase producing Acinetobacter in hospitals indicates the urgent need for action to prevent further spread of these bacteria. Previous experiences with Methicillin-Resistant _Staphylococcus aureus_, vancomycin-resistant Enterococcus and...
Extended-spectrum β-lactamase-producing bacteria indicate that once resistant bacteria can become widespread they cannot be controlled (13). So it is strongly recommended that hospitals must promote infection control practices.

Our results showed that these two genes are not the major source for resistance against imipenem. And it can be assumed that in northeast of Iran other metallo-beta-lactamases genes such as SPM-1, GIM-1, SIM-1 or NDM-1 may contribute in resistance against metallo-beta-lactams.

Since the first report of blaIMP-1 and blaVIM metallo-beta-lactamase in Japan and Italy respectively, metallo-beta-lactamases producing Gram-negative bacilli have been increasingly reported in many countries (14). In a study during 2002 to 2004 in France, 46% of imipenem nonsusceptible P. aeruginosa isolates were metallo-beta-lactamases positive using phenotypic methods while 97.2% of them were PCR positive for metallo-beta-lactamases genes (15). In another study which conducted in India during 2011 by Amudhan et al., among 179 imipenem nonsusceptible isolates, 144 isolates (%80.4) were metallo-beta-lactamases producing. In this study PCR detected the metallo-beta-lactamases genes blaVIM/blaIMP in 51.4% of cases (16). And in Japan during 2012 among 54 Acinetobacter isolates 48 isolates were positive for MBL genes. The blaIMP-1 genes were detected in four isolates (17). In lee’s study during 2003 in Korean, 11 isolates (28.9%) of 38 metallo-beta-lactamases positive isolates of Acinetobacter spp. carry blaIMP (18). In another study in southern Taiwan, among 123 strains of gram negative bacilli, 116 strains (94.3%) carry the intI1 gene and 21 strains contain integron-associated blaVIM-3, blaVIM-11 and blaIMP-8 genes. This finding suggests that clinical spread of this blaVIM-11 gene is a matter of great concern for carbapenem resistance (19).

The results of these studies differ from the results of ours. Although in all above mentioned study blaVIM was much more common than blaIMP which is somehow similar to our study but in our study the frequency of these genes were much lower. This aspect of our results is different from above mentioned results which confirmed different source and level of resistance in northeast of Iran in contrast to other parts of the world.

In a study in Iran during 2012 by Dr Shahcheraghi et al., 13 metallo-beta-lactamases resistant Acinetobacter strains were isolated which carry blaVIM-1 but similarly in that study also no blaIMP genes were detected among the strains (20). Another study in Iran was performed by peymani during 2010 in tabriz among 63 carbapenem non-susceptible isolates. In his study 31 (49%) isolates were found to be metallo-beta-lactamases producers. Among these 31 metallo-beta-lactamases producing isolates, 19 (61%) isolates carried the blaIMP gene and 9 (29%) isolates carried the blaVIM gene (21).

Conclusion

Our finding are different in some aspect from the results of the other studies and this difference makes our study important because it reveals that in the North East of Iran these genetic elements are not the main
mechanism for metallo-beta-lactamases production. So, further studies are needed to investigate the genetic source of metallo-beta-lactamases production.

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Conflict of interest

None declared conflicts of interest.

References


