



PREVALENCE OF METALLO-B-LACTAMASE GENES IN SOME GRAM NEGATIVE BACTERIAL ISOLATES

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ABSTRACT

Introduction: the emergence of antibiotic resistance pathogens is an important health risk. Usually Gram negative bacteria acquire resistance to beta-lactam antibiotics by beta-lactamase production. The objective of this study was to assess the prevalence of *Metallo-β-lactamase* genes (*MBLs*) in some Gram Negative bacterial isolates. **Methods:** Genomic DNA was extracted using boiling method with further precipitation by ethanol. *VIM-1*, *SIM*, and *GIM-1* *MBLs* genes were investigated in clinical G-ve bacterial isolates by PCR based methods using gene specific primers. **Results:** Genomic DNA extraction resulted in high DNA yields for *Escherichia coli* (218 ng/ml), *Klebsiella pneumoniae* (224 ng/ml), and *Pseudomonas aeruginosa* (786 ng/ml). To evaluate the prevalence of *MBLs* genes (*VIM-1*, *SPM*, and *GIM-1*) in these isolates, genomic DNA was amplified using specific primers, results of amplification showed that these genes were detected in the three isolates after electrophoresis the amplified products on agarose gel with amplicon size of 477 bp for *VIM-1*, 261 bp for *SPM*, and 229 bp for *GIM-1*. It was also found that these isolate carrying at least one of those *MBLs* genes. **Conclusion:** This study showed that *MBLs* genes is prevalent in the studied G-ve bacteria which refer to the horizontal transfer of these genes among these isolates during nosocomial infections.

KEYWORDS: *Metallo-β-lactamase* Genes, Gram Negative Bacteria, β-lactam Antibiotics.

INTRODUCTION

Beta-lactamase genes are one of the most important groups of antimicrobial resistance genes in human and animal health. Therefore, continuous surveillance of this group of resistance genes is needed for a better understanding of the local epidemiology within a country and global dissemination.^[1] *Beta-lactamase* genes are prevalent in Gram-negative bacteria and are a major mechanism of resistance to beta-lactam antibiotics. These genes encode enzymes that break down beta-lactam rings in antibiotics, rendering them ineffective. The prevalence of specific *beta-lactamase* genes varies among different bacterial species and geographic locations. The spread of carbapenemase-producing gram-negative pathogens, especially those producing *MBLs*, has become a major health concern. *MBLs* are molecularly the most diverse carbapenemases, produced by a wide spectrum of gram-negative organisms.^[2] These carbapenemases include Ambler class A and class D serine-β-lactamases (SBLs), and class B *MBLs*.^[3-5] Among these, *MBLs* are the most diverse carbapenemases, molecularly, and are capable of hydrolyzing most β-lactams using metal ion cofactors in their active sites.^[5,6] *MBLs* are divided into three subclasses: B1, B2, and B3, mainly dependent on the

differences in the amino acid sequence and zinc ion dependence. The subclass B1 has emerged as the most clinically relevant, and includes KHM (Kyorin Hospital *MBL*), *GIM* (German imipenemase), *SIM* (Seoul imipenemase), *DIM* (Dutch imipenemase), *SPM* (Sao Paulo *MBL*), *TMB* (Tripoli *MBL*), *VIM* (Verona integron-encoded *MBL*), *IMP* (imipenemase), and *NDM* (New Delhi *MBL*) *MBLs* among others.^[7,8] Among these, the most prevalent *MBLs* are *NDM*, *IMP*, and *VIM*.^[9,10] The resistance to the carbapenem group antibiotics is due to the presence of *MBL* genes, which can be easily transferred to other strains.^[10] Analysis of the genetic background of the *IMP* and *VIM* *MBL* genes has revealed that these genes mostly occur as cassettes along with other resistance genes within integrons and are integrated on chromosomes or plasmids.^[7,8,12] The *NDM* genes are associated with mobile genetic elements such as plasmids belonging to different replicon or *Inc* types (*IncFII*, *IncHI2*, *IncN*, and *IncX3*), insertion sequences (*ISAb125*, *ISCR1*), and transposons (*TnJ25*).^[13] The B2 and B3 *MBLs* are usually chromosomally encoded and generally not transmissible, though ubiquitously present in their host species.^[12] In light of this, the current study was undertaken to investigate the prevalence and

coexistence of metallo-beta-lactamase genes in some clinical Gram-negative bacterial isolates.

MATERIALS AND METHODS

Bacterial isolates: Three clinical Gram-negative bacterial isolates *P. aeruginosa*, *E. coli* and *K. pneumoniae* were obtained from the Department of Molecular and Medical Biotechnology /University of Al-Nahrain, and they were activated by culturing in BHI broth at 37 °C for 24 hours.

Maintenance of bacterial isolate: Bacterial isolates were maintained on nutrient agar slants in screw-capped tubes and incubated at 37 for 24hours, and then kept at 4 °C for few weeks for routine work.

Extraction of genomic DNA: Genomic DNA was extracted from *P. aeruginosa*, *E.coli* and *K. pnemmoniae* isolates by using conventional boiling method followed by alcohol precipitation according to Moore *et al.*^[14] Then, Nano-drop spectrophotometer was used to measure the concentration and purity of DNA solution depending on the ratio of sample absorbance at wave lengths of 260 and 280 nm.

Amplification of metallo β -Lactamase genes: Conventional PCR was used for detection of *MBLs* genes (*VIM-1*, *SPM*, and *GIM-1*) in bacterial isolates. Table 1 showed the sequences of oligonucleotide primers used for amplification.

Table (1): Oligonucleotide primers used for amplification of β -Lactamase genes.

Primer	Sequence (5'---3')	Tm (°C)	Product size (bp)	Reference
<i>GIM-1</i>	F: TCGACACACCTTGGTCTG	55	477	(15)
	R: AACTTCCAACCTTGCCATG			
<i>SPM</i>	F: GGGTACGCAAACGCTTATGG	64	229	
	R: CCTTCGCTTCAGATCCTCGT			
<i>VIM-1</i>	F: AGTGGTGAGTATCCGACAG	55	261	
	R: ATGAAAGTGCGTGGAGAC			

Each reaction had a volume of 25 μ L, including 10 μ L master mix (Promega, USA), 1 μ L of each primer, 4 μ L DNA template, and 10 μ L RNase free distilled water. PCR reaction was initiated with a pre-denaturation step at 94 °C for 5 min, followed by 35 amplification cycles; denaturing at 94 °C (30s), annealing at 55 °C for *GIM-1* and *VIM-1*, and 64 °C for *SPM* (30 sec.), and extension at 72 °C (1 min), then finally extension at 72 °C for 10 min. PCR products were analyzed by 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Extraction of genomic DNA by boiling method

In this study, boiling method was used for rapid extraction and pure bacterial genomic DNA isolation

from each bacterial isolate for PCR analysis. Alcohol precipitation was mediated by the addition of ethanol commonly used for concentrating, desalting, and recovering nucleic acids. The extracted genomic DNA purity and concentration was indicated in table (2). The boiling method, also known as thermal lysis, is a rapid and simple technique for DNA extraction, particularly effective for bacterial DNA isolation. It involves heating a cell suspension to high temperatures (95°C - 100°C) to break open cells and release their DNA. This method is often used for PCR-based DNA analysis due to its speed and cost-effectiveness. These results are similar to those obtained by Lesiani *et al.*^[16] who mentioned that DNA extraction using the boiling approach can still give good results for DNA analysis.

Table (2): Concentration and purity of genomic DNA extracted from bacterial isolates.

Bacterial isolate	Genomic DNA	
	Concentration (ng/ μ l)	Purity
<i>E. coli</i>	218	1.36
<i>K. pneumoniae</i>	224	1.44
<i>P. aeruginosa</i>	786	1.39

The quality and integrity of DNA solutions were checked by electrophoresis on 1% agarose gel. Results of electrophoresis illustrated in figure (1) showed clear and sharp DNA bands visualized under UV ray.

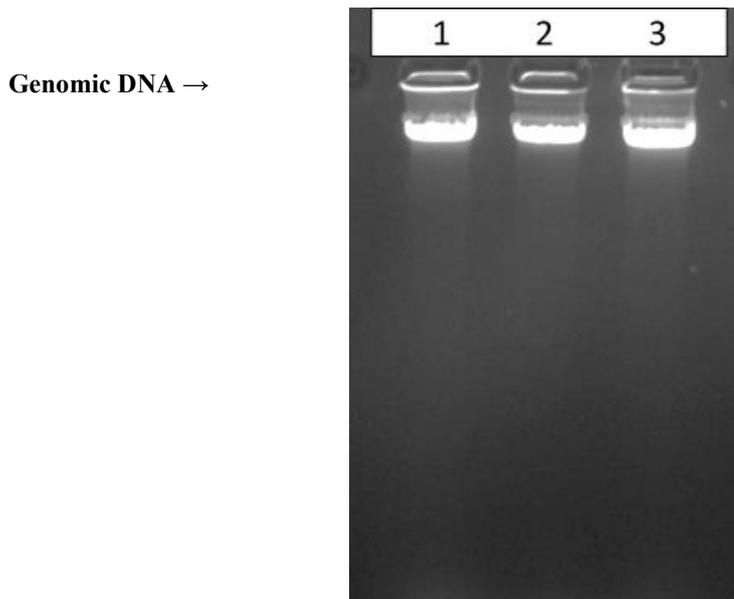


Figure (1): Genomic DNA extracted from bacterial isolates after Electrophoresis on agarose gel (1%) at 5 V/cm for 60 min. Lane (1): *Escherichia coli*; Lane (2): *Klebsiella pneumoniae*; Lane (3): *Pseudomonas aeruginosa*.

Boiling method proved to be the most efficient for the isolation of bacterial DNA, it is a simple, economical, fast method without the need for specialized reagents and is an appropriate alternative for carrying out molecular studies, compared to the commercial Kits. Since, boiling method seems to be an efficient way to extract bacterial DNA and suitable for other molecular techniques.^[17]

Detection of Metallo β -lactamase genes in bacterial isolates

Pseudomonas aeruginosa, *E. coli*, and *K. pneumoniae* isolates were subjected to polymerase chain reaction

(PCR) for the detection of *GIM-1*, *SPM*, and *VIM-1 MBLs*. Results illustrated in figure (2) showed *GIM-1* was detected in *P. aeruginosa* and *E. coli* after gene amplification and electrophoresis on agarose gel (2%), with an amplicon size of 477 bp, while this gene was not detected in *K. pneumoniae* isolate. On the other hand, *SPM Metallo β -lactamase* was detected in only *K. pneumoniae* isolate with an amplicon size of 229 bp, but not detected in the other two isolates. Furthermore, *VIM-1 Metallo β -lactamase* was detected in *K. pneumoniae* and *E. coli* with an amplicon size of 261 bp, while this gene was not detected in *P. aeruginosa*.

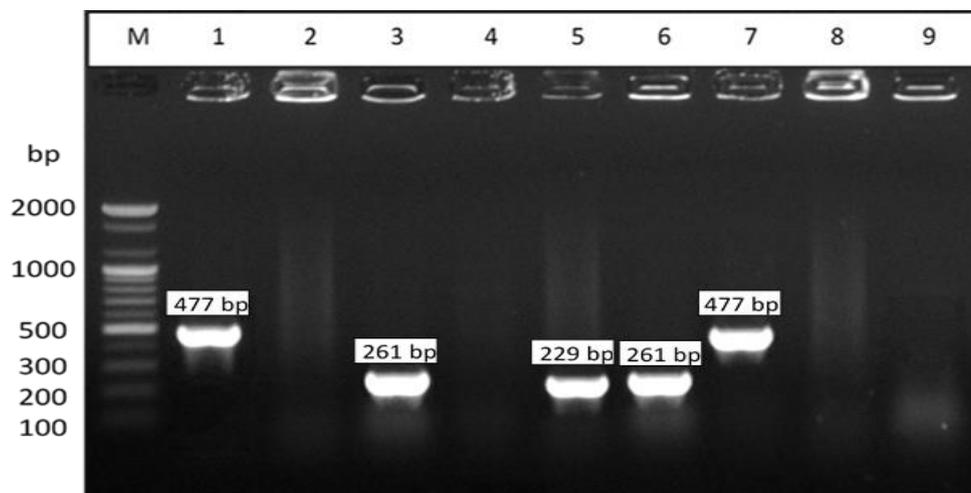


Figure (2): Electrophoresis of PCR products for the amplified product of metallo β -lactamase genes in bacterial isolates after electrophoresis on agarose gel (1%) at 5 V/cm for 60 min. *GIM-1* (477 bp); *SPM* (229 bp); *VIM-1* (261 bp); M: DNA ladder marker; Lanes (1-3): *Escherichia coli*; Lanes (4-6): *Klebsiella pneumoniae*; Lanes (7-9): *Pseudomonas aeruginosa*.

The increasing trend of β -lactam resistance among Enterobacteriaceae is a worldwide threat. β -lactamase genes in Enterobacteriaceae are a significant concern due

to their role in antibiotic resistance, particularly to beta-lactam antibiotics. The distribution of β -lactamase genes in Enterobacteriaceae can be linked to several factors,

such as hospital-acquired infections, community transmission, frequent travels, and most significantly the negative impact of overuse and misuse of antimicrobials, along with poor healthcare practices.^[18]

Prevalence of *MBLs* genes among the studied isolates showed that each isolate carrying at least one of those genes, and not more two as indicated in table (3-2).

Table (3-2): Prevalence of metallo β -lactamase genes among bacterial isolates.

Bacterial isolate	β -lactamase gene		
	<i>GIM-1</i>	<i>SPM</i>	<i>VIM-1</i>
<i>P. aeruginosa</i>	+	-	+
<i>E. coli</i>	+	-	-
<i>K. pneumoniae</i>	-	+	+

MBL infections are emerging as a global public health threat with increase in prevalence and spread in all the regions, including non-endemic areas. The spread of carbapenemase-producing gram-negative pathogens, especially those producing metallo- β -lactamases (MBLs), has become a major health concern.^[19]

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