

Identification of Imipenem-Resistant Genes in *Acinetobacter baumannii* Isolated from Baghdad Hospitals

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Abstract

Imipenem-resistant *Acinetobacter baumannii* (IRAB) represents one of the important causing agents of nosocomial infections especially in immunocompromised and Intensive Care Units (ICUs) patients. The aim of this work was to identify the Imipenem-Resistant genes in *Acinetobacter baumannii* isolated from Baghdad hospitals. Among 128 *A. baumannii* isolates, 67 isolates (58.26%) were resistant to imipenem and meropenem. Four genes for imipenem resistance (*bla*OXA-23 like, *bla*OXA-24 like, *bla*OXA-51like and *bla*OXA-58 like) were amplified and sequenced. The presence of *bla*OXA-23-like genes in 91.03% of IRAB isolates indicated that the *bla*OXA-23-like genes are the predominant mechanism for imipenem resistance in our isolates. Sequencing of PCR products showed the presence of new OXA-genes in local *A. baumannii* isolates including: OXA-207, OXA-239 and OXA-229 among the genes of OXA-24-like, OXA-23-like and OXA-58-like genes, respectively. In conclusion, this study identifies the genes responsible for the imipenem resistance in Baghdad which is important to understand the imipenem resistance and to suggest plans for treatment of patients in future.

Keywords: *Acinetobacter baumannii*; Beta lactamases; Rep-pcr; IRAB; Imipenem

Introduction

During the last two decades *A. baumannii* became clinically important pathogen due to its capability for outbreaks and resistance to most antibiotics including carbapenems [1]. The outbreak of *A. baumannii* associated with United States military operations in Iraq generated special interest in this organism [2]. The features of *A. baumannii* for resistance to most available antibiotics and environmental stress enable the bacteria to persist in hospitals and epidemic spread [3]. Several studies reported that carbapenem-resistant *A. baumannii* strains have been now emerged around the world [4-6]. This resistance is principally caused by the production of carbapenemases [7]. Carbapenem-hydrolysing class D β -lactamases (CHDLs) are the most often reported mechanisms of carbapenem resistance in *A. baumannii* and four groups of CHDLs have been identified in imipenem-resistant *A. baumannii*, including intrinsic and chromosomally located OXA-51-like β -lactamases and acquired OXA-23-like, OXA-24-like and OXA-58-like β -lactamases [8,9].

Till now, no study has been performed in Iraq identification of Imipenem-Resistant *A. baumannii* genes among Iraqi patients. Therefore, this study aimed to investigate the Imipenem-Resistant bla-OXA β -lactamases genes in *A. baumannii* among patients in four hospitals in Baghdad, Iraq.

Materials and Methods

Isolation and processing of samples

One thousand and nine hundreds specimens comprising, urine, wounds, burns, blood and sputum, were collected in sterilized containers from patients attending four hospitals in Baghdad-Medical city including: Baghdad Teaching Hospital, the Martyr Gazi Al-Hariry Hospital, Welfare Teaching Hospital and the Burn Specialist Hospital.

All bacterial isolates were identified by routine morphological, biochemical carried out according to Forbes et al. [10]. Additionally, identification was confirmed by api20E (bioMerieux, France).

Furthermore, species identification of *Acinetobacter baumannii* isolates was performed by Polymerase chain reaction (PCR) to detect blaOXA-51-Like genes [11,12]. The results of bacterial identification were published separately [13].

Antibiotic susceptibility was done for 20 antibiotics available in the market. Disc agar diffusion test was performed according to the Kirby-Bauer standardized antimicrobial susceptibility single disc method [14]. Serial dilution agar method was applied for the determination of minimal inhibitory concentrations (MICs) to Imipenem and Meropenem [15]. *Escherichia coli* (*E. coli* ATCC 25922) was used as a quality control in susceptibility determination.

An isolate was interpreted as susceptible, intermediate, or resistant to a particular antibiotic by comparison with standards inhibition zones or MIC break point according to Clinical Laboratories Standards Institute (CLSI) [16].

Extraction of genomic DNA

DNA was extracted from *A. baumannii* isolates using a commercial purification system (Genomic DNA Mini Kit, Geneaid, Thailand), then the DNA concentration and purity were determined [17].

Multiplex PCR assay

Multiplex PCR was achieved to amplify four carbapenem resistance genes (oxacillinases) including: *bla* OXA-51-Like genes (which is also

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adopted for the identification of isolates to species level) [11,12], *bla* OXA-23-Like genes, *bla* OXA-24-Like genes and *bla* OXA-58-Like genes using the listed primers in Table 1 (primers synthesized by Alpha DNA, Canada). Multiplex PCR was optimized using the PCR premix (Accupower, bioneer-Korea).

Sequencing of PCR products

Multiplex PCR products for *bla*OXA-23 like, *bla*OXA-24like, *bla*OXA-51 like and *bla*OXA-58 like genes were detected by agarose gel electrophoresis and purified by Gel/ PCR DNA Fragment Extraction Kit (Geneaid, Thailand), and then the sequencing was carried out using the ABI capillary system (Macrogen, Korea). PCR products were subjected to direct sequencing. DNA sequences were analysed and similarity searches were carried out with the Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>).

Results and Discussion

Antibiotic susceptibility

Data published previously [14] indicated a high level resistance of *A. baumannii* clinical isolates to most of the antibiotics under test. The MIC values for imipenem and meropenem resistant isolates ranged from 16 µg/ml to 512 µg/ml.

Detection of carbapenem resistance genes (oxacillinases) by multiplex polymerase chain reaction (PCR)

The results of resistance genes amplification presented in Figure 1

OXA-genes	Primer name	Sequence	Product size
<i>bla</i> OXA-51-Like	OXA-51-Like-F	5'-TAATGCTTTGATCGGCCTTG-3'	353 bp
	OXA-51-Like-R	5'-TGGATTGCACCTTCATCTTGG-3'	
<i>bla</i> OXA-23-Like	OXA-23-Like-F	5'-GATCGGATTGGAGAACCAGA-3'	501 bp
	OXA-23-like-R	5'-ATTTCTGACCGCATTTCAT-3'	
<i>bla</i> OXA-24-Like	OXA-24-Like-F	5'-GGTTAGTTGGCCCCCTTAAA-3'	246 bp
	OXA-24-Like-R	5'-AGTTGAGCGAAAAGGGGATT-3'	
<i>bla</i> OXA-58-Like	OXA-58-Like-F	5'-AAGTATTGGGGCTTGCTGCTG-3'	599 bp
	OXA-58-Like-R	5'-CCCCTCTGCGCTCTACATAC-3'	

Table 1: Primers used for multiplex PCR amplification of *A. baumannii* oxacillinases genes.

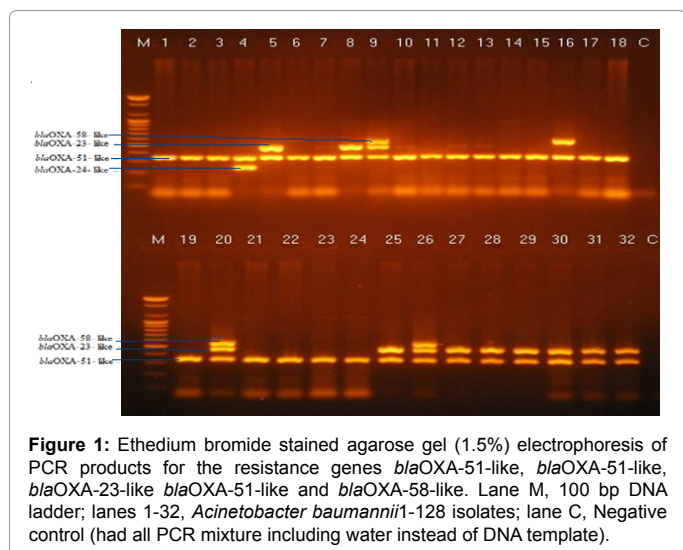


Figure 1: Ethidium bromide stained agarose gel (1.5%) electrophoresis of PCR products for the resistance genes *bla*OXA-51-like, *bla*OXA-23-like, *bla*OXA-24-like and *bla*OXA-58-like. Lane M, 100 bp DNA ladder; lanes 1-32, *Acinetobacter baumannii* 1-128 isolates; lane C, Negative control (had all PCR mixture including water instead of DNA template).

<i>bla</i> OXA type	Imipenem-resistant <i>A. baumannii</i> (n=78)	Imipenem-sensitive <i>A. baumannii</i> (n=50)
<i>bla</i> OXA-51-like alone	2	48
<i>bla</i> OXA-51-like plus <i>bla</i> OXA-23-like	61	ND
<i>bla</i> OXA-51-like plus <i>bla</i> OXA-23-like and <i>bla</i> OXA-58-like	10	ND
<i>bla</i> OXA-51-like plus <i>bla</i> OXA-24-like	5	ND
<i>bla</i> OXA-51-like plus <i>bla</i> OXA-58-like	ND	2
Total	78	50

ND=Not Detected; P<0.05

Table 2: Distribution of *bla*OXA genes among *A. baumannii* isolates.

and Table 2 illustrate the presence of *bla*OXA-51-like genes in all 128 (100%) *A. baumannii* clinical and environmental isolates (regardless to imipenem susceptibility). The *bla*OXA-51-like genes were reported to be highly specific for the identification of *A. baumannii* at the species level [18,19]. The *bla*OXA-23-like genes present in 91.03% of IRAB which indicated its responsibility for the dominant carbapenem resistance gene in the local *A. baumannii* isolates. Nevertheless, similar findings were reported in countries other than Iraq, such as Bulgaria, China, Brazil, Afghanistan [20], Korea, Singapore and Thailand [21].

A study carried out by Hujer et al. [22] demonstrated that 97% of *A. baumannii* strains (isolated from military and civilian personnel injured in the Iraq/Kuwait region during Operations in Iraq) have *bla*OXA-69-like gene (a member of *bla*OXA-51-like genes). *bla*OXA-23-like genes detected in 11% of those strains and the *bla*OXA-58-like genes were detected in 12% of the isolates; whereas *bla*OXA-24-like genes were not detected at all. This may specify the non-relatedness between Hujer's isolates and those isolated in the present study.

It is markedly noticed that two (4%) out of fifty imipenem sensitive *A. baumannii* environmental isolates harboured *bla*OXA-58-like genes in addition to *bla*OXA-51-like genes. Although these two isolates have *bla*OXA-58-like genes, no resistance was developed phenotypically. Such finding could be assigned to the absence of the insertion sequence *ISAbal* upstream *bla*OXA-58-like genes. Corvec et al. [23] and Zhong et al. [24] stated that in *A. baumannii* the OXA genes are expressed when the insertion sequence *ISAbal* inserts upstream of the gene where it provides a promoter for gene expression.

Sequencing of multiplex PCR products

Results revealed that GenBank accession numbers for the nucleotide sequences of the *bla*OXA-51-like, *bla*OXA-58-like, *bla*OXA-23-like and *bla*OXA-24-like genes fragments are JX305928, JQ409994, JQ343841 and JX310716, respectively. In OXA-51-like genes sequencing we found some differences in the nucleotides of our query and the subject in position 5, 11, 21, 35, 40, 111, 247, 268, 289 and 316 of the subject. In the case of OXA-58-like gene the differences are 315, 323, 324, 342, 355, 519, 522, 531, 532 and 537 of the subject. In regard to OXA-23-like gene the differences are 127, 131, 145, 148, 158, 164, 167, 186, 207, 241, 250 and 256 of the subject. Regarding OXA-24-like gene the differences are 195, 198, 204, 207, 210 and 215 of the subject.

Interestingly, OXA-207 gene was found among the OXA-24-like genes in local *A. baumannii* isolates; this gene (OXA-207) has not been previously reported in *A. baumannii* strains. Nevertheless, it was mentioned to be found in other species of the genus *Acinetobacter*.

In this study we identify the sequences of Imipenem-resistance genes in our local *A. baumannii* isolates; this will improve the management and control of this pathogen in Baghdad hospitals.

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