Molecular Detection of bla_{OXA-51} in Carbapenem-resistant *Acinetobacterbaumannii* Isolated from Different Clinical Sources

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Abstract

Carbapenems are the drugs of choice against serious infections caused by Gram-negative bacteria Carbapenem-resistant. *Acinetobacterbaumannii* (CRAB) 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 Carbapenem-Resistant genes in *Acinetobacterbaumannii* isolated from Baghdad and Ramadi hospitals. Among 48*A. baumannii* isolates, 33 isolates (68.75%) were resistant to imipenem and meropenem. One gene for carbapenem resistance (bla_{OXA-51} like) were amplified by PCR. The presence of *bla*OXA-51-like genes in 100% of CRAB isolates indicated that the *bla*OXA-51-like genes are the predominant mechanism for imipenem resistance in our isolates.

Keywords: *Acinetobacter baumannii; bla_{OXA-51}; Health management; genes*

Introduction

Acinetobacterbaumannii is a non-fermentative, strictly aerobic, non-motile, non pigmented, catalase-positive and oxidase-negative Gram-negative coccobacillus[(1)]. A. *baumannii* became clinically important pathogen due to its capability for outbreaks and resistance to most antibiotics including carbapenems[(2)]. nosocomial infections has become an increasingly prevalent cause especially in immunocompromised and in Intensive Care Units (ICUs) patients in the last few years[(3)].

Carbapenems are the drugs of choice for the treatment of serious nosocomial infections caused by A. baumannii[(4)]. Carbapenem resistant A. baumannii strains have been now emerged around the world . This resistance is principally caused by the production of carbapenemases[(5)]. 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 inimipenem-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[(6)].

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Materials and Method

Isolation and processing of samples: 48, urine,wounds, burns, blood and sputum, were collected in sterilizedcontainers from patients attending hospitals in Baghdad and Ramadi city. These strains were isolated through a period extended from August 2019 to December 2019.

All bacterial isolates were diagnosed by conventional method such as morphological, microscopic,and biochemical tests[(7)]. Additionally,identification was confirmed by Vitek-2 system (Biomerieux; France).

Antibiotic susceptibility was done for two antibiotics available in themarket. Disc agar diffusion test was performed according to the Kirby–Bauer standardized antimicrobial susceptibility single disc method[(8)].

An isolate was interpreted as susceptible, intermediate, or resistantto a particular antibiotic by comparison with standards inhibitionzones or MIC break point according to Clinical Laboratories Standards Institute (CLSI,2018)

Extraction of genomic DNA: DNA was extracted from *A. baumannii* isolates using a commercial purification system (Genomic DNA purification Kit, promega, USA), then the DNA concentration and purity were determined

Traditional PCR assay: PCR was achieved to amplify carbapenem resistance genes (oxacillinases) including: *bla* OXA-51-Like genes (which is also adopted for the identification of isolates to species level). Primers used in this study (Alpha DNA,Canada) were provided, in lyophilized form then dissolved in sterile deionized distilled water (Table I).

Table I. The sequences of primers used in this study

Gene	Primers' Sequences (5'→3')	TM (°C)	Product size (bp)
OXA-51	F:CGGCCTTGTAATGCTTTGAT	59.4	353
	R:TGGATTGCACTTCATCTTGG	57.3	

For PCR method, the initial denaturation phase for each PCR assay with primers was established on 94°C for 5 min also denaturation was 94°C for 1 min. The annealing time was 1 min and temperature was 49°C for. The extensiontime was 35 sec in 72°C. The final extension was done at 72°C for 7 min.

The reaction of PCR consisted of 12 µl Master mix (Bioneer,USA), 1 µl of each forward and reverse primers, 4 µl of template DNA, and 7 µl PCR grade water to a final volume 25 µl. The products of PCR were electrophoresed for 1hr and visualized with the aid of Red Safe staining (iNtRON, Korea) and UV transilluminator documentation system .

Result and Discussion

During the period of August 2019 to December 2019, forty eight *Acinetobacterbaumannii*s were collected from Ramadi city and baghdad. The collected isolates were from different clinical specimens (urine, wound, sputum, and burns) of in patients in this hospital. All isolates were identified by using the automated Vitek-2 system (Bio-Merieux/France) according to the manufacturer's instructions with the using ID-GNB (Identification card - Gram Negative Bacteria).

Antibiotic susceptibility testing: The antibiotic susceptibility test revealed that (68.75%) carbapenem-resistant *A.* from 48 clinical strains were multidrug-resistant.

Molecular detection of bla $_{OXA-51}$ in *A. buammanii* by traditional PCR: *A . baumannii* has successfully become a significant nosocomial pathogen because of its e remarkable ability to acquire antibiotic resistance and to survive in nosocomial environments. The bla$_{OXA-51}$-like genes were reported to be highly specific for the identification of *A. baumannii* at the species level[9]. PCR product illustrate the presence of blaOXA-51-like genes in all 15 (100%) A. baumannii clinical isolate close to Nadeema et al[10]. figure 1.

Nevertheless, similar findings were reported in countries other than Iraq, such as Bulgaria,China, Brazil, Afghanistan, Korea[9], Singapore and Thailand[11]. A study carried out by Hujer et al. demonstrated that 97% of A. baumannii strains (isolated from military and civilian personnelinjured in the Iraq/Kuwait region during Operations in Iraq haveblaOXA-69-like gene (a member of blaOXA-51-like genes). The blaOXA-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.

In conclusion, this study identifies the gene responsible for the carbapenem resistance in Ramadi which is important to understand the carbapenem resistance and to suggest plans for treatment of patients in future. The highdistribution of bla OXA-51-encodinggenes, presents an emerging threatin our hospital. The diversity of resistancegenes is particularly worrisome due to the difficult choice of empirical antibiotic therapy inseriously ill patients and the possible contribution to increased hospital stay and associatedcosts.

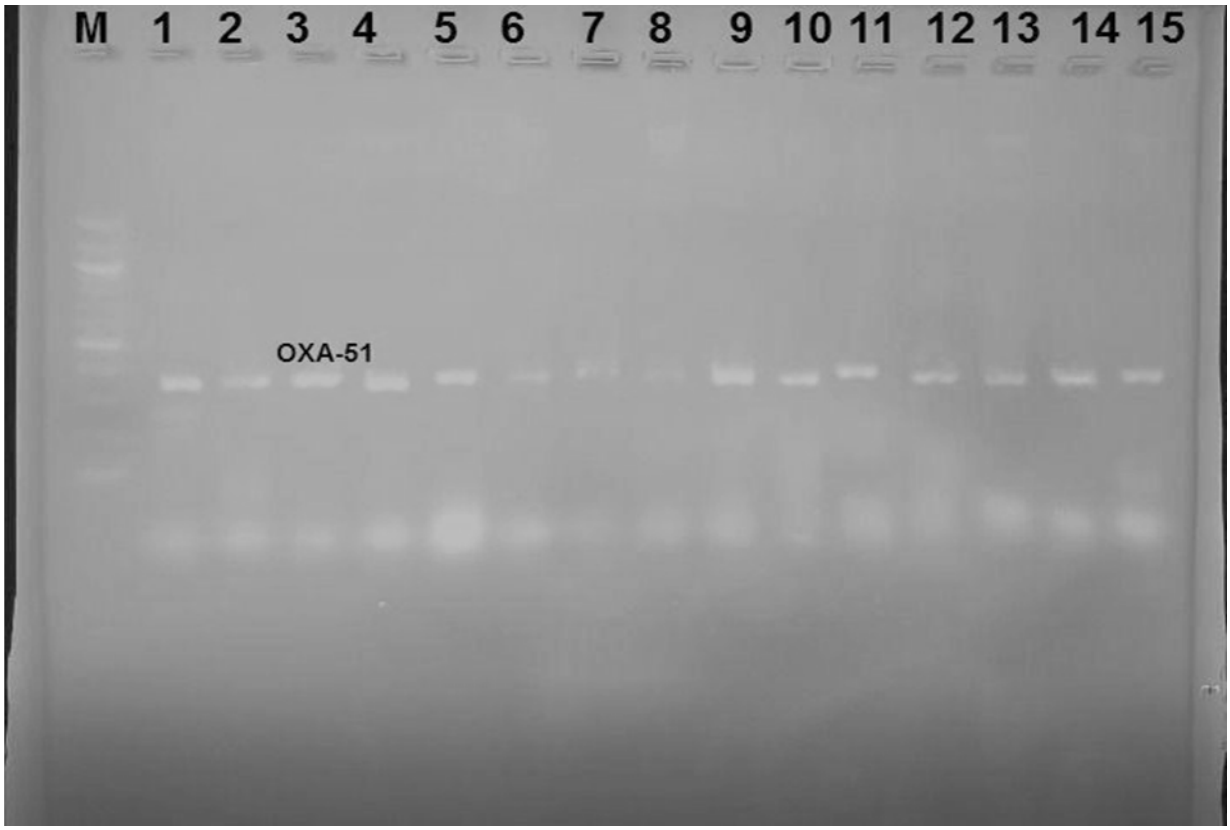

Figure 1 : PCR amplification fragments for the detection of bla OXA-51 gene (553 bp) carbapenem-resistant *Acinetobacterbauammanii* strains. Lanes 1 – 15: = *A. bauamanii* Lane M: 100-bp DNA ladder. Amplicons were electrophoresed on agarose gel (1%) at 70 V/cm for 1.5 h, stained with RedSafe(iNtRON, Korea), and visualized using an UV transilluminator documentation system.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

Funding: Self-funding

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