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PREVALENCE AND ROLE OF ISABA1 ASSOCIATED WITH BLAOXA-51 AND BLAOXA-23 GENES IN CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII*

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ABSTRACT

The global spread carbapenem-resistant *Acinetobacter baumannii* has become a serious problem especially in burn units. The purpose of this study was to detect the association of blaOXA-51 and blaOXA-23 carbapenmase genes with ISAba1 and their effect on carbapenem resistance. To detect insertion sequences ISAba1/blaOXA-23 and ISAba1/blaOXA-51, mapping by PCR using combinations of forward primers ISAba1 and reverse primers of blaOXA-23 and blaOXA-51 were used. A total of 96 isolates of *A. baumannii* were obtained from burns and wounds infections from Baghdad hospitals, Iraq. Antibiotic sensitivity patterns showed that 70.8% (n=68) of the isolates were resistant to Imipenem and Meropenem, while 13 isolates were susceptible to both antibiotics (13.5%). Of 68 isolates resistant to Imipenem and Meropenem, ISAba1 was detected in 61 isolates (89.7%). 50 isolates carried blaOXA-23 gene, all contained ISAba1 upstream of blaOXA-23 gene, and 41 contained ISAba1 upstream of blaOXA-51 gene. ISAba1 was found upstream of blaOXA-23 only in isolates resistant to carbapenems. Both susceptible and resistant isolates had ISAba1 upstream of blaOXA-51. The ISAba1-activated blaOXA-23 genes were prevalent among the carbapenem-resistant *A. baumannii* isolates in our hospitals and carbapenems resistance may be due mainly to ISAba1 upstream of blaOXA-23 gene. The present study revealed that the presence of ISAba1/blaOXA-51 in *A.baumannii* isolates was not contributing in the carbapenems resistance.

KEYWORDS: *Acinetobacter baumannii*, ISAba1, carbapenems resistance.

INTRODUCTION

Acinetobacter baumannii, an opportunistic pathogen, is often involved in nosocomial outbreaks and many reports indicated to be resistant to carbapenems.[1] Carbapenem-hydrolysing class D β-lactamases (CHDLs) are responsible for carbapenems resistance in *A. baumannii.* OXA-carbapenemases classified into eight subgroups, four of the OXA-carbapenemases have been identified in *A. baumannii*, namely, blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58.[2] The blaOXA-23 was first identified in *A. baumannii* in 1985 from Scotland, which was the main cause of Imipenem resistance in this species. The blaOXA-23 gene is mainly found on plasmids and has been associated with the ISAba1 element.[3] The movement of Insertion Sequence (IS) can have functional consequences for bacterial genomes, IS have been implicated in large changes to genome structure.[4] The ISAba1 element has been found in association with carbapenem-resistance genes blaOXA-51, blaOXA-23, blaOXA-58 and AmpC cephalosporinase.[5] The ISAba1 element of upstream of blaOXA-23 genes provide promoter sequences that enhance their expression.[3] Tn2006 transposon consist of blaOXA-23 with two copies of ISAba1 that surround the beta-lactamase gene in order to regulate blaOXA-23 mobility.[6]

The present study was performed to analyze the prevalence of ISAba1 associated with the blaOXA-51 and blaOXA-23 genes in clinical isolates of *A. baumannii* isolated from burns and wounds infections in Baghdad hospitals, Iraq, also investigation its role in carbapenems resistance.

MATERIALS AND METHODS

Bacterial isolates

A total of 96 *A. baumannii* clinical isolates were collected from 476 clinical specimens represented burns and wounds infections from patients in Baghdad hospitals, Iraq. The clinical isolates were identified by using standard biochemical tests, API20 E and Vitek2 system (bioMerieux, France). Species identification was performed by presence of blaOXA-51 gene.[7]

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed using the minimal inhibitory concentrations (MICs) by using microdilution method in Mueller-Hinton broth based on the results reported by the Clinical and Laboratory Standards Institute (CLSI) guidelines.[8] Imipenem and Meropenem were used in this test. *Pseudomonas aeruginosa* ATCC- 27853 was used for quality control in antimicrobial susceptibility testing.[9]

Ampification of the blaOXA genes by PCR method
All isolates were subjected to the multiplex PCR to detect blaOXA-51 and blaOXA-23, described previously by Woodford *et al.* (2006).[10] All primers used in this study and the PCR conditions was described previously in our study.[9]

Detection of ISAba1 by PCR
A. baumannii strains were assayed for ISAba1sequence by PCR with primers ISAba1F and ISAba1R (Table 1) giving rise to a 549 bp fragment. The amplification conditions were following: initial denaturation at 95°C for 5 min, 30 cycles of 95°C for 45 s, 55°C for 45 s, 72°C for 3 min and final elongation at 72°C for 5 min.[11]

To confirm the presence of IS element to the upstream of blaOXA genes; PCR mapping experiments were used.[12], the positive isolates of ISAba1 gene were performed for PCR reaction using two sets of primers. The forward primer of ISAba1 gene, the reverse primer of blaOXA-51 gene and the forward primer of ISAba1 gene and reverse primer of blaOXA-23 gene with 1.2 kb and 1.4 kb of the amplified product respectively (Table 1). ISAba1 upstream of blaOXA-51 and blaOXA-23 were amplified using the following conditions: initial denaturation at 95°C for 2 min, 30 cycles of 95°C for 10 s, 53°C for 40 s and 72°C for 3min and a final elongation at 72°C for 60 s.

Table 1: Primers used for detection ISAba1 gene.

Primer	Sequence	Product size
ISAba1F ISAba1R	5' CAC GAA TGC AGA AGT TG 3' 5’ CGA CGA ATA CTA TGA CAC3’	549 bp
ISAba1F BlaOXA-23R	5' CAC GAA TGC AGA AGT TG 3' 5' ATT TCT GAC CGC ATT TCC AT 3'	1.4 kb
ISAba1F BlaOXA-51R	5' CAC GAA TGC AGA AGT TG 3' 5' TGG ATT GCA CTT CAT CTT GG 3'	1.2 kb

PCR products detection
Amplicons were visualized under UV fluorescence and following electrophoresis at 2% agarose gel prepared in TBE buffer and stained with ethidium bromide. Gels were photographed digital camera (Canon, Japan). The size of PCR products was compared with 100bp DNA ladder.

RESULTS
Our study concerned a collection of 96 *Acinetobacter baumannii* isolates recovered from patients with burns and wounds infections in Baghdad hospitals, Iraq.[7]

MIC for carbapenems antibiotics were determined using the microdilution method in Mueller-Hinton broth. It was found that 68 isolates (70.8%) of all isolates were resisted to carbapenems antibiotics (Imipenem and Meropenem) used in this study, while 13 isolates (13.5%) were sensitive to both these antibiotics. For both Imipenem and Meropenem, the numbers of isolates that gave MIC values equal to or more than break point (≥ 16 µg/ml) were 68 (70.8%) of clinical isolates and these values assure resistance of studied *A. baumannii* isolates for these antibiotics; while 28 (29.2%) *A. baumannii* clinical isolates gave MIC values equal to or less than 8 µg/ml and these values assure sensitivity of studied *A. baumannii* isolates for these antibiotics. The number of isolates which had the highest MIC values (≥ 265 µg/ml) for both Imipenem and Meropenem were 13 isolates.

Detection of the OXA carbapenemases genes including (blaOXA-51 and blaOXA-23) in carbapenem-resistant isolates was carried out using a multiplex PCR assay. The results revealed the presence of blaOXA-51 gene in all isolates, while blaOXA-23 gene was detected in 50 from 68 carbapenems resistant isolates.[9]

The prevalence of ISAba1 elements and association ISAba1 upstream of the blaOXA-23 and blaOXA-51 genes were detected in 68 of the carbapenem-resistant *A. baumannii* isolates and in 13 of carbapenem-susceptible *A. baumannii* isolates by using PCR and PCR mapping (Table 2).

Table 2. Distribution of blaOXA genes and ISAba1 elements among carbapenems resistant and susceptible *A.baumannii* clinical isolates.

	Number of positive isolates (%)	
Type of gene and element	**Carbapenems resistant isolates (n=68)**	**Carbapenems sensitive isolates (n=13)**
ISAba1	**61(89.7%)**	**7(53.8%)**
BlaOXA-51	**68 (100%)**	**13(100%)**
BlaOXA-51/ISAba1	**41(60.3%)**	**5(38.5%)**
BlaOXA-23	**50 (73.5%)**	**3 (23.1%)**
BlaOXA-23/ISAba1	**50 (73.5%)**	**0**

The current study revealed that ISAba1 was detected in 61(89.7%) of resistant isolates and in 7 (53.8%) of sensitive isolates. In carbapenem-resistant isolates, the results indicated to presence blaOXA-51/ISAba1 in 41 isolates while blaOXA-23/ISAba1 was found in 50 isolates (73.5%), in other word, all resistant isolates which contained blaOXA23 gene carried ISAba1 upstream of this gene. In susceptible isolates, it was found that blaOXA-51/ISAba1 present in 5/13 isolates (38.5%) while blaOXA-23/ISAba1 was not present in any susceptible isolate in spite of the existence 3 blaOXA23 genes among these isolates. The current study demonstrated that blaOXA-51/ISAba1 was found in both carbapenem-resistant and susceptible *A.baumannii* local isolates.

Amplification of purified DNA yielded a PCR product with specific primers for ISAba1 element (Figure 1). The complete sequence of the fragment 549 bp was 99% identical to that described for the ISAba1 gene using the alignment tools of NCBI (Accession numbers LC136852 and LC136853) (Figure 1).

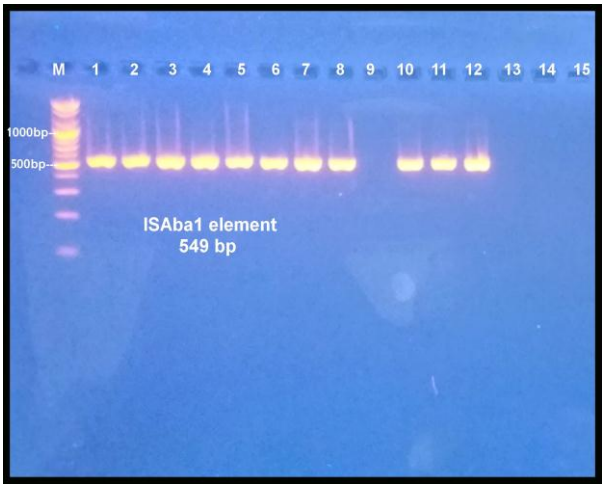

Figure 1. Ethidium bromide stained agarose gel (2%) electrophoresis for PCR products of ISAba1 elements for *A.baumannii* isolates 1-14, only lanes 9, 13 and 14 gave negative results. M is a 100 bp size marker ladder. Lane 15 is a negative control.

PCR mapping showed ISAba1 to be located in the promoter region of the blaOXA-23 gene in 50 carbapenem-resistant isolates; these amplicons were 1.4 kb in size. The sequence obtained for the ISAba1-blaOXA-23 amplicon was 99% identical to that described for the insertion sequence ISAba1 transposase gene and the blaOXA-23 gene using the alignment tools of NCBI (Accession numbers LC130573 and LC130574) (Figure 2). 41 of resistant isolates and 7 of susceptible isolates also had ISAba1 located upstream of the blaOXA-51 gene and these amplicons were 1.2 kb in size (Figure 3).

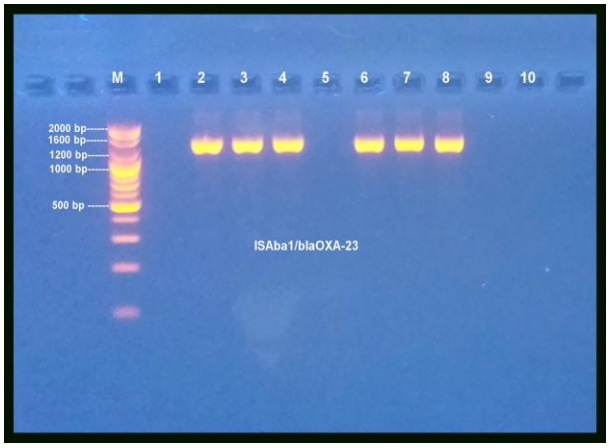

Figure 2. Ethidium bromide stained agarose gel (2%) electrophoresis for PCR products carried out using the ISAba1 forward primer and blaOXA-23 reverse primer for *A.baumannii* isolates 1-9, Imipenem and Meropenem susceptible isolates with blaOXA-23 failed to give a band in the PCR (lanes 1, 5 and 9) while resistant isolates gave a band (lanes 2, 3, 4, 6, 7 and 8). M is a 100 bp size marker ladder. Lane 10 is a negative control.

Figure 3. Ethidium bromide stained agarose gel (2%) electrophoresis for PCR products carried out using the ISAba1 forward primer and blaOXA-51 reverse primer for *A.baumannii* isolates 1-9, Imipenem and Meropenem susceptible isolates (lanes 1, 5 and 9), resistant isolates (lanes 2, 3, 4, 6, 7 and 8). M is a 100 bp size marker ladder. Lane 10 is a negative control.

DISCUSSION

A.baumannii isolates included in this study were isolated in February to July 2015, from patients with burns and wounds infections hospitalized in a Baghdad hospitals in Iraq. The MIC of Imipenem and Meropenem was determined. The most of isolates were highly resistant to carbapenems. *A. baumannii* strains became one of the most important pathogens associated with nosocomial infections. Carbapenems were regarded as the most powerful antibiotics because of its low toxicity and extremely effective antibacterial activity, but the emergence of carbapenem resistance in *A. baumannii* has

become a global concern recently.[13] Of the 68 carbapenem-resistant *A. baumannii* isolates included in our study, 50 contained the carbapenemase blaOXA-23 responsible for their carbapenems resistance. Outbreaks of blaOXA-23 producing *A. baumannii* have been reported from various regions of the world. It was believed that blaOXA-23 is responsible for carbapenem antibiotic resistance.[14] In the present study, the minimum inhibitory concentration for Imipenem and Meropenem was equal or more than 64 μg/ml for 40 isolates positive to both blaOXA-51 and blaOXA-23. Our findings referred that ISAba1 was represented 89.7% in our local isolates. The study of Nowak *et al*.(2012)[15] revealed that ISAba1 was found in all carbapenem-resistant *A. baumannii* isolates obtained from patients hospitalized in Krakow hospital Poland. Our results of ISAba1 prevalence did not agree with Nasrolahei *et al*. (2014)[16] who reported to the presence of ISAba1 in 32.2% of 61 *Acinetobacter baumannii* resistant to aminoglycosides and carbapenems. The current study observed that ISAba1 was detected in most of the strains which was positive for the carbapenemase gene blaOXA-23. ISAbal which is upstream blaOXA genes, plays a major role in the carbapenems resistance in *A.baumannii* strains.[17] In our isolates containing blaOXA-23, these were resistant to Imipenem and Meropenem if they possessed ISAba1 upstream of blaOXA-23 in comparison with the susceptible isolates, blaOXA-23 was not associated with ISAba1 (Table 2). 50 isolates showed the presence of 1.4kbp band which is positive for ISAba1-blaOXA-23 (Figure 2). Hence it is confirmed that IS element was acting as the promoter region for blaOXA gene expression**.** The correlation between the presence of ISAba1/blaOXA-23 gene and carbapenems resistance in *A.baumannii* isolated from ICU and burn units has been reported in many previous studies and worldwide.[18,19] One of the studies conducted on *A.baumannii* isolates from a tertiary medical centre in Malaysia referred to sufficient of ISAba1-blaOXA-23 to confer carbapenems resistance in clinical *A.baumannii* isolates even without the assistance of other blaOXA genes and the resistance characteristic is due to the presence of ISAba1-blaOXA-23 in these isolates.[20]

It was demonstrated both carbapenems resistant and susceptible isolates of local *A.baumannii* contained ISAba1 element in association with blaOXA-51, and ISAba1/blaOXA-51 association was found in 60.3% of carbapenems resistant *A.baumannii* isolates. These findings were concordant with a study published by Bratu *et al*. (2008)[21] in which *A. baumannii* Imipenem-susceptible isolates showed existence the ISAba1/blaOXA-51 association. Pagno *et al*., (2012)[22] established that ISAba1 element was not correlated with carbapenems resistance in *A.baumannii* when found upstream blaOXA-51. Our findings revealed that some isolates were positive for both blaOXA-23 and blaOXA-51 genes, ISAba1 was only associated with blaOXA-23-like genes. This indicates that the expression of these two genes might be associated with different promoters.[5] The development of carbapenem resistance in *A.baumannii* may be due to another mechanisms such as multidrug efflux pump and loss of membrane porins.

CONCLUSION

This study revealed that the presence of insertion sequence (ISAba1) upstream of blaOXA23 gene, among Imipenem and Meropenem-resistant clinical isolates of *A. baumannii* isolated from burns and wounds infections, might be responsible for carbapenems resistance. Our results highlight the need to monitor the mechanisms of resistance caused by insertion sequences among *A. baumannii* strains.

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