ORIGINAL ARTICLE

Detection of blaOXA-58-like and blaOXA-23-like Genes among Carbapenem Resistant Acinetobacter baumanii strains in Benha University Hospital

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ABSTRACT

Background: A. baumannii has now emerged as a leading cause of hospital and community-acquired infections. Multidrug resistant A. baumannii has been increasingly reported worldwide. Carbapenem Resistant A. baumannii (CRAB) is enlisted as number one in the critical priority of the “Global Priority Pathogen List” by WHO, 2017. A simple and useful molecular technique for identifying A. baumannii isolates is the identification of the blaOXA-like carbapenemase gene by Polymerase Chain Reaction (PCR) method. Objectives: The aim of this study was to detect the prevalence of blaOXA23 like and blaOXA58 like genes among 25 carbapenem resistant A. baumannii isolates at Benha University Hospital. Methodology: Different clinical samples were cultured on CHROMagarTM Acinetobacter medium and identified to the species level by Vitek2 automated system. All A. baumannii strains were screened for carbapenem resistance by culture on CHROMagarTM with MDR supplement and CRAB was confirmed by Vitek2 system. PCR was used for BlaOXA 23 gene and blaOXA58 gene detection in A. baumannii strains. Results: 25(20.8%) out of 120 different samples were positive for Acinetobacter by culture on CHROMagarTM Acinetobacter media. All Acinetobacter isolated strains (100%) were identified as A. baumannii by vitek2 identification cards. All strains of A. baumannii were carbapenem resistant (CRAB) as they were resistant to Imipenem and Meropenem. Also, all strains were Multi Drug Resistant (MDR). Four (16%) strains of A. baumannii were sensitive to each of Tigecyclin and Trimethoprim/sulfamethoxazole. in this study out of 25 resistant strains, 23 A. baumannii strains had carbapenem resistance genes (all had blaOXA23 and one of them had both blaOXA23 and blaOXA58) as diagnosed by PCR. Conclusion: PCR of the CRAB showed that blaOXA-23 gene had higher rate (92%) than blaOXA-58 gene (4%) in A. baumannii clinical isolates. Tigecycline can be considered a good therapeutic option due to the presence of some sensitive CRAB strains.

INTRODUCTION

A. baumannii is considered a serious pathogen being characterized by multidrug resistance (MDR); long-term survival on inanimate surfaces such as computer keyboards, pillows, curtains and other dry surfaces; and propensity for epidemic spread. For the past 30 years, strains of A. baumannii have acquired resistance to newly developed antimicrobial drugs; those strains are known as MDR A. baumannii. It became prevalent in many hospitals all over the world and has been recognized as a leading nosocomial pathogen. MDR Acinetobacter species can refer to being resistant to a minimum of three classes of antimicrobial drugs e.g. all penicillins and cephalosporins, fluoroquinolones, and aminoglycosides. Hospital strains of Acinetobacter species are usually multidrug resistant. The problem is complicated by increasing rates of resistance to broad-spectrum antibiotics including carbapenems. Carbapenems have been the drug of choice for the treatment of Acinetobacter species, however the number of isolates showing resistance to these antibiotics has increased.

Several mechanisms are responsible for conferring the resistance to β-lactam on Acinetobacter species, including the production of β-lactamases, changes in penicillin-binding proteins that prevent activities of β-lactam drugs, alterations in porin proteins that result in decreased permeability to antibiotics and the activity of efflux pumps that decreases the concentration of antibiotics within the bacteria. However, resistance to these antibiotics has emerged due to the production of carbapenem hydrolyzing β-lactamases among these pathogens. Two classes of molecular carbapenemases classes B & D have been identified, but those belonging to molecular class D

Key words: A. baumannii, blaOXA23, blaOXA58, CRAB, MDR

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OXA enzymes have emerged globally as the main mechanism responsible for carbapenem resistance\textsuperscript{5,6}. Four families of OXA carbapenemases (OXA-23-like, OXA-40-like, OXA-51-like and OXA-58-like) are limited to isolates of Acinetobacter species. The rapid detection of strains that produce these beta lactamases in clinical bacteriology laboratories allows appropriate therapy to be implemented promptly in order to reduce patient morbidity and mortality\textsuperscript{6}.

The aim of this study was to detect the prevalence of blaOXA23 like and blaOXA58 like genes among 25 carbapenem resistant \textit{A. baumannii} isolates at Benha University Hospital.

**METHODOLOGY**

This study was conducted in Medical Microbiology and Immunology Department, Faculty of Medicine, Benha university in the period from February 2020 to March 2021. The patients were included in the study after written informed consent was obtained from them. The study was approved by the Ethics Committee, Faculty of Medicine, Benha University.

**Subjects:**

The current study was conducted on 25 strains of carbapenem resistant \textit{A. baumannii} isolated from 120 clinical samples collected from 68 male and 52 female patients, their ages ranged from 11 years to 80 years. Those patients were admitted to Intensive Care Unit (ICU) at Benha University Hospitals. The collected samples were 45 sputum, 30 broncho alveolar lavage (BAL), 30 urine and 15 pus samples. All samples were immediately sent to the laboratory to be processed and examined within 2 hours.

**Culture and isolation of Acinetobacter spp.:**

All samples were cultured on CHROMagar\textsuperscript{TM} Acinetobacter medium. It was prepared according to manufacturer’s instructions.

**Identification of the isolated Acinetobacter spp.:**

All isolated Acinetobacter spp. strains were identified by:

\textbf{a.} Color of bacterial colonies on Chromagar TM:

- Acinetobacter sp. \rightarrow Red
- Other gram negative bacteria \rightarrow Mostly inhibited or blue
- Gram positive bacteria & yeasts \rightarrow Mostly inhibited

\textbf{b.} Microscopic examination of Gram stained film: Acinetobacter spp. appears as gram negative rod, cocobacillary, grouped in pairs or in chain.

\textbf{c.} Biochemical reactions

Oxidase test: Acinetobacter spp. are oxidase negative.

Catalase test: Acinetobacter spp. are catalase positive

**Identification of \textit{A. baumannii} subspecies using VITEK\textsuperscript{®} 2 Systems.**

**Phenotypic detection of carbapenem resistant Acinetobacter baumannii (CRAB):**

**Screening of CRAB:**

All strains identified as \textit{A. baumannii} by VITEK2 system identification cards were tested for carbapenem resistance by culture On CHROMagar\textsuperscript{TM} Acinetobacter with MDR Selective supplement.

**Confirmation of carbapenem resistance for \textit{A. baumannii} by VITEK 2 system.**

**Detection of blaOXA genes by PCR:**

All Carbapeneme-Resistant \textit{A. baumannii} (CRAB) isolates diagnosed by chromogenic media and confirmed phenotypically by (VITEK 2 system) were examined for the presence of blaOXA 23 and bla OXA 58 carbapenemases genes by PCR.

Bacterial DNA was extracted from \textit{A. baumannii} following the manufacture’s instructions G-spin\textsuperscript{™}

**Total DNA Extraction Kit Purification Protocol (iNtRON Biotechnology (iB), Korea).**

The blaOXA23 primer sequences included OXA23-F, 5'–GAT CGG ATT GGA GAA CCA GA-3' and OXA23-R, 5'-ATT TCT GAC CGC ATT TCC AT-3'. (Biosearch technologies, U.S.A) \textsuperscript{7}. The blaOXA58 primer sequences included OXA58-F, 5'- AAG TAT TAG GGC TTG TGC TG-3' and OXA58-R, 5'– CCC CTC TGC GCT CTA CAT AC-3'. (Biosearch technologies, U.S.A) \textsuperscript{7}.

In a PCR tube, a PCR amplification reaction of a total volume 50μl containing 5μl of the extracted DNA template, 25μl of 2x Dream Taq Green PCR Master Mix, (Fermentas, Life Science,USA), 1μl of the forward primer of both blaOXA 23 and blaOXA58 genes, 1μl of the reverse primer of both blaOXA 23 and blaOXA58 genes and 16μl of nuclease free water. The amplification reaction in the thermal cycler followed these steps: initial denaturation at 94°C for 5 mins, followed by 39 cycles of (94°C for 25 sec, 53°C for 40 sec, 72°C for 40 sec), and a final extension at 72°C for 7 mins. The expected PCR product for blaOXA23 gene was 501 base pairs and for blaOXA58 gene was 599 base pairs and was separated by electrophoresis on a 1.5% agarose gel using ethidium bromide and visualized by UV transillumination.

**RESULTS**

Twenty-five (20.8%) out of 120 different samples were positive for \textit{Acinetobacter.} all Acinetobacter isolated strains (100%) were identified as \textit{A. baumannii} by vitek2 identification cards.

Infections by \textit{A. baumannii} isolates were higher in males (56%) than females (44%), and higher in patients more than sixty years old (40%).
Table 1: Isolation rate of Acinetobacter by culture of samples on CHROMagarTM Acinetobacter media

<table>
<thead>
<tr>
<th>Acinetobacter</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>20.8</td>
</tr>
<tr>
<td>Negative</td>
<td>95</td>
<td>79.2</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 showed that sputum samples represented the major site for A. baumannii isolation (44%) followed by pus, urine and finally BAL samples (24%, 20%, 12%) respectively. Also, it showed that A. baumannii infection was higher in patients with hospital stay more than 5 days, patients on mechanical ventilation had higher rate of A. baumannii infection followed by those on antibiotic therapy, diabetic and hypertensive patients (84%, 76%, 36% and 32% respectively).

Table 2: Isolation rate of A. baumannii as regard to different variables

<table>
<thead>
<tr>
<th>Hospital stay</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5 days</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td>more than 5 days</td>
<td>18</td>
<td>72.0</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>11</td>
<td>44.0</td>
</tr>
<tr>
<td>Urine</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Pus</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>BAL*</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>17</td>
<td>68.0</td>
</tr>
<tr>
<td>+ve</td>
<td>8</td>
<td>32.0</td>
</tr>
<tr>
<td>DM*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>16</td>
<td>64.0</td>
</tr>
<tr>
<td>+ve</td>
<td>9</td>
<td>36.0</td>
</tr>
<tr>
<td>Mechanical Ventilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>+ve</td>
<td>21</td>
<td>84.0</td>
</tr>
<tr>
<td>Antibiotic therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>+ve</td>
<td>19</td>
<td>76.0</td>
</tr>
</tbody>
</table>

*BAL: Broncho Alveolar Lavage.
*DM: Diabetes Mellitus.

All strains of A. baumannii were carbapenem resistant (CRAB) as they were resistant to Imipenem and Meropenem. Also, all strains were Multi Drug Resistant (MDR).

Four (16%) strains of A. baumannii were sensitive to each of Tigecyclin and Trimethoprim/sulfamethoxazole.

Table (3) showed that 23 (92%) A. baumannii strains showed growth on CHROMagarTM with MDR supplement while all A. baumannii isolates (25) showed Multi Drug Resistance by Vitek2 system.

Table 3: Comparison between Vitek2 system and CHROMagarTM with MDR supplement for detection of MDR A. baumannii

<table>
<thead>
<tr>
<th>CHROMagarTM with MDR supplement</th>
<th>Vitek2 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 4 showed that 23 out of 25 (92%) CRAB isolates had blaOXA23 carbapenemase gene while one strain (4%) had both blaOXA23 and blaOXA58 carbapenemase genes.

Table 4: Results of PCR for detection of blaOX23 and blaOXA 58 genes among 25 Carbapeneme Resistant A. baumannii (CRAB):

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaOXA23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td>blaOXA 58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>96.0</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>Both genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

In this study, out of 25 resistant strains, 23 A. baumannii strains had carbapenem resistance genes (all had blaOXA23 and one of them had both blaOXA23 and blaOXA58) as diagnosed by PCR. All A. baumannii isolates (25) showed resistance to carbapenem as diagnosed by Vitek2 system as shown in table 5.

Table 5: Vitek2 system versus PCR in detecting carbapenem resistant strains

<table>
<thead>
<tr>
<th></th>
<th>Vitek2 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, out of the 120 different clinical samples cultured on chromagar TM, A. baumannii were isolated from 25 (20.8%) samples which coincides with the Egyptian study of See et al.8 who reported that out of 472 Hospital Acquired Infections identified in ICUs from 11 hospitals, The most common pathogens reported were Acinetobacter species (21.8%). Tolba et al.7 reported that A. baumannii infections represented 16.1% (n= 45/279) of their total gram-negative infections.

However, another study reported that out of 530 Clinical samples, 20 isolates were identified as A. baumannii (3.8%).9 So, that result was much lower than that reported in this study.

In the present study, A. baumannii infection was higher in males (56%) than females (44%) and the highest percentage of A. baumannii infection was reported among patients more than 60 years (40%). These findings agrees with that of Arafa et al.10 who reported that A. baumannii infection was higher in males.
Agodi et al. 14 in Italy, Falagas and Kopterides 12 in Greece and Baran et al. 13 in Turkey reported that longer duration of hospital ICU stay was a significant risk factor for Acinetobacter infections (p<0.05).

In the present study, the prolonged stay in hospital was associated with A. baumannii infection as 72% of patients infected with A. baumannii were staying in hospital more than 5 days which coincides with Arafa et al. 10 who reported that 73.3% of their patients with A. baumannii infection stayed more than 5 days in hospital.

In this study, patients on mechanical ventilation and those received antibiotic therapy had higher incidence of A. baumannii infection (84%, 76%) respectively. This is in agreement with Zakuan et al. 14, Hernández et al.15 and Ellis et al. 16 who reported that use of previous antibiotic treatment is one of the significant risk factors in Acinetobacter infection. Arafa et al. 10 found that 80% of their studied patients with A. baumannii infection were on mechanical ventilation and 73.3% were receiving antibiotics which also coincides with the present results.

In this study, it was found that sputum samples represented the major site for A. baumannii isolation (44%) followed by pus, urine and finally BAL samples (24%, 20%, 12%) respectively. Similar results were obtained by Tolba et al.7 who found that sputum samples represented the major site for A. baumannii isolation 41.2 % followed by wound swabs 17.63%, urine 16.91% and BAL 0.72%.

Also, in Japan, Endo et al. 17 reported that out of 305 Acinetobacter spp. isolates 44.6% and 18% were recovered from sputum and urine samples, respectively.

Zowawi et al.18 found that A. baumannii was mainly isolated from wound swabs (39%) followed by sputum (22%) and urine samples (6%) from patients in different hospitals in the Arabic Gulf area.

Also, Tawfik et al.19 reported that A. baumannii isolates were obtained in high percentage: 42.59% from wound swabs, 31.48% from urine and 5.55% from sputum samples.

These differences in rate of infections may be due to different communities, different hospital wards or variations in risk factors in ICUs such as: use of invasive devices [e.g. IV lines, Central Venous Catheter (CVC), urinary catheters], previous antibiotics, history of chronic diseases and malignancy or variation in application of aseptic precautions in ICUs.

The Vitek 2 compact system is very important in identifying Acinetobacter to the species level and detecting antimicrobial susceptibility of the isolated A. baumannii as reported by Tolba et al. 7.

In this study all strains of A. baumannii were resistant to at least three classes of antibiotics (MDR) as diagnosed by Vitek2 system. All of them were resistant to imipenem and meropenem (carbapenem resistant) (CRAB). As regards resistance to Tigecyclin, 48% and 36% of A. baumannii isolates showed resistance and intermediate resistance respectively. Also, 84% of A. baumannii isolates were resistant to Trimethoprim/sulfamethoxazole.

These results come in agreement with the study done by Josheghani et al.20 who found that all of A. baumannii isolates were MDR and all of the isolates from the ICU were resistant to imipenem and meropenem.

Tolba et al.7 reported that 88.9% of A. baumanntii isolates were carbapenem resistant, all of them100% were MDR.

Also, Arafa et al.10 showed that A. baumannti recorded high resistance rate to different antibiotics including Meropenem while the resistance to Tigecycline was only (33.3%).

Tawfik et al.21 reported that this could be due to the overuse and/or misuse and the routine use of these antimicrobials in hospitals. Tigecyclin showed lower resistance rate than other antibiotics so, it could be considered a potential alternative therapy for A. baumannii infections which are resistant to other classes of antibiotics.

In our study, all Carbapenem-Resistant A. baumannii (CRAB) isolates screened by chromogenic media and confirmed phenotypically by (VITEK 2 system) were examined for the presence of bla OXA 23 and bla OXA 58 carbapenemases genes by PCR. Twenty three out of 25 (92%) CRAB had blaOXA23 carbapenemase gene and one strain only (4%) had both blaOXA58 gene and blaOXA23 gene.

In agreement with the present study, Josheghani et al.20 found that 90% of CRAB were positive for the blaOXA-23 gene, and None were positive for blaOXA-58.

Also, a study done in Egypt by Tolba et al.7 revealed that blaOXA-23-like gene was detected in 90% of isolates while none of the isolates carried blaOXA-58-like gene.

Tawfique et al.19 found that blaOXA-23-like gene was existed in 90.74% of A. baumannii isolates and blaOXA58 gene was found in 2 (3.70%) of all A. baumannii isolates.

Upon the current results, the production of blaOXA-23-like gene is the dominant carbapenems resistance gene in A. baumannii isolates. This may be explained by the fact that blaOXA23 is located both on plasmid and chromosome and was detected in many species of bacteria other than Acinetobacter. So, the ability of the bacteria to acquire this gene is higher than other genes.22

Much lower results for blaOXA-23 gene were detected by Kuo et al. 23 who found that (52.6%) only had blaOXA-23-like and (1.1%) only had blaOXA-58-like. and Simo Tchuinte et al.24 who found that blaOXA-
23 was present in 53.3% only but blaOXA58 gene represented 6.7% of CRAB isolates.

The low prevalence of blaOXA-58-like gene among *A. baumannii* isolates may attributed to different geographic distribution of the gene as it is most frequently reported in Europe, South and North America, Asia and Australia. 25

The results of this study coincides with those of the Middle East and around the world which stated that the frequency of blaOXA58 gene was the lowest compared to other genes that responsible for the resistance against carbapenems.

Bonnin et al. 26 reported in their study performed in Saudi Arabia hospitals that no MDR *A. baumannii* isolates had a positive PCR result for blaOXA58 gene.

Other studies showed that carbapenemase OXA-58 had a very wide spread and may be the main cause of carbapenem resistance in *A. baumannii*, since it has been detected in *A. baumannii* isolates recovered from different countries like France, Argentina, Kuwait and United Kingdom. 27

**CONCLUSION**

Chromagar TM Acinetobacter medium is a good medium for isolation of Acinetobacter although it can’t detect the different species of Acinetobacter. Chromagar TM with MDR supplement can diagnose MDR Acinetobacter. Vitek2 system provides a rapid method for detection of different species of Acinetobacter and carbapenem resistant Acinetobacter species. It gives a profile of resistance to different antibiotics. PCR of the CRAB showed that blaOXA-23 gene had higher rate (92%) than blaOXA-58 gene (4%) in *A. baumannii* clinical isolates. Tigecycline can be considered a good therapeutic option due to the presence of some sensitive CRAB strains.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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