

## ORIGINAL ARTICLE

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

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## ABSTRACT

**Key words:**

*A. baumannii*, blaOXA23, blaOXA58, CRAB, MDR

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**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 CHROMagar™ *Acinetobacter* medium and identified to the species level by Vitek2 automated system. All *A. baumannii* strains were screened for carbapenem resistance by culture on CHROMagar™ 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 CHROMagar™ *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<sup>1</sup>.

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<sup>2</sup>.

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<sup>3,4</sup>.

Several mechanisms are responsible for conferring the resistance to  $\beta$ -lactam on *Acinetobacter* species, including the production of  $\beta$ -lactamases, changes in penicillin-binding proteins that prevent activities of  $\beta$ -lactam drugs, alterations of porin proteins that result in decreased permeability to antibiotics and the activity of efflux pumps that decreases the concentration of antibiotics within the bacteria<sup>5</sup>.

However, resistance to these antibiotics has emerged due to the production of carbapenem hydrolyzing  $\beta$ -lactamases among these pathogens. Two classes of molecular carbapenemases classes B & D have been identified, but those belonging to molecular class D

OXA enzymes have emerged globally as the main mechanism responsible for carbapenems resistance<sup>3,6</sup>.

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<sup>6</sup>.

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

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 *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™ *Acinetobacter* medium. It was prepared according to manufacturer's instructions.

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

All isolated *Acinetobacter* spp. strains were identified by:

- a. Color of bacterial colonies on Chromagar TM:
  - *Acinetobacter* sp. → Red
  - Other gram negative bacteria → Mostly inhibited or blue
  - Gram positive bacteria & yeasts → Mostly inhibited
- b. Microscopic examination of Gram stained film: *Acinetobacter* spp. appears as gram negative rod, cocobacillary, grouped in pairs or in chain.
- c. Biochemical reactions
 

Oxidase test: *Acinetobacter* spp. are oxidase negative.

Catalase test: *Acinetobacter* spp. are catalase positive

Identification of *A. baumannii* subspecies using VITEK® 2 Systems.

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

#### Screening of CRAB:

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

Confirmation of carbapenem resistance for *A. baumannii* by VITEK 2 system.

#### Detection of blaOXA genes by PCR:

All Carbapenem-Resistant *A. baumannii* (CRAB) isolates diagnosed 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.

Bacterial DNA was extracted from *A. baumannii* following the manufacture's instructions **G-spin™ 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**)<sup>7</sup>. The blaOXA58 primer sequences included OXA58-F, 5'- AAG TAT TGG GGC TTG TGC TG-3' and OXA58-R, 5'- CCC CTC TGC GCT CTA CAT AC-3'. (**Biosearch technologies, U.S.A**)<sup>7</sup>.

In a PCR tube, a PCR amplification reaction of a total volume 50µl containing 5µl of the extracted DNA template, 25µl of 2× 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 *Acinetobacter*. all *Acinetobacter* isolated strains (100%) were identified as *A. baumannii* by vitek2 identification cards.

infections by *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 CHROMagar™ *Acinetobacter* media**

		No.	%
<b>Acinetobacter</b>	<b>Positive</b>	25	20.8
	<b>Negative</b>	95	79.2
<b>Total</b>		120	100

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**

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

\*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 CHROMagar™ with MDR supplement while all *A.baumannii* isolates (25) showed Multi Drug Resistance by Vitek2 system.

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

		<b>Vitek2 Positive</b>	
		No.	%
<b>CHROMagar™ with MDR supplement</b>	<b>Positive</b>	23	92
	<b>Negative</b>	2	8
<b>Total</b>		25	100

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 blaOXA23 and blaOXA 58 genes among 25 Carbapenem Resistant *A. baumannii* (CRAB):**

		No.	%
<b>blaOXA23</b>	<b>Negative</b>	2	8.0
	<b>Positive</b>	23	92.0
<b>blaOXA 58</b>	<b>Negative</b>	24	96.0
	<b>Positive</b>	1	4.0
<b>Both genes</b>	<b>Positive</b>	1	4.0

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**

		<b>Vitek2 Positive</b>	
		No.	%
<b>PCR</b>	<b>Positive</b>	23	92
	<b>Negative</b>	2	8
<b>Total</b>		25	100

## DISCUSSION

In the present study, out of the 120 different clinical samples cultured on chromagar™, *A. baumannii* were isolated from 25 (20.8%) samples which coincides with the Egyptian study of *See et al.*<sup>8</sup> 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.*<sup>7</sup> 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%).<sup>9</sup> 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.*<sup>10</sup> who reported that *A. baumannii* infection was higher in males

(60%) than females (40%) and among patients more than 60 years (40%).

Agodi et al.<sup>11</sup> in Italy, Falagas and Kopterides<sup>12</sup> in Greece and Baran et al.<sup>13</sup> in Turkey reported that longer duration of hospital ICU stay was a significant risk factor for *Acinetobacter* infections ( $p \leq 0.05$ ).

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.<sup>10</sup> 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.<sup>14</sup>, Hernández et al.<sup>15</sup> and Ellis et al.<sup>16</sup> who reported that use of previous antibiotic treatment is one of the significant risk factors in *Acinetobacter* infection. Arafa et al.<sup>10</sup> 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.<sup>7</sup> 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.<sup>17</sup> reported that out of 305 *Acinetobacter spp.* isolates 44.6% and 18% were recovered from sputum and urine samples, respectively.

Zowawi et al.<sup>18</sup> 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, Tawfeeq et al.<sup>19</sup> 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.<sup>7</sup>.

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.<sup>20</sup> 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.<sup>7</sup> reported that 88.9% of *A. baumannii* isolates were carbapenem resistant, all of them 100% were MDR

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

Tawfik et al.<sup>21</sup> 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 Carbapeneme-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 *bla*OXA23 carbapenemase gene and one strain only (4%) had both *bla*OXA58 gene and *bla*OXA23 gene.

In agreement with the present study, Josheghani et al.<sup>20</sup> found that 90% of CRAB were positive for the *bla*OXA-23 gene, and None were positive for *bla*OXA-58.

Also, a study done in Egypt by Tolba et al.<sup>7</sup> revealed that *bla*OXA-23- Like gene was detected in 90% of isolates while none of the isolates carried *bla*OXA-58-like gene.

Tawfeeq et al.<sup>19</sup> found that *bla*OXA-23-like gene was existed in 90.74% of *A. baumannii* isolates and *bla*OXA58 gene was found in 2 (3.70%) of all *A. baumannii* isolates.

Upon the current results, the production of *bla*OXA-23-like gene is the dominant carbapenems resistance gene in *A. baumannii* isolates. This may be explained by the fact that *bla*OXA23 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.<sup>22</sup>

Much lower results for *bla*OXA-23 gene were detected by Kuo et al.<sup>23</sup> who found that (52.6%) only had *bla*OXA-23-like and (1.1%) only had *bla*OXA-58-like. and Simo Tchuinte et al.<sup>24</sup> who found that *bla*OXA-



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.<sup>25</sup>

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.<sup>26</sup> 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.<sup>27</sup>

## 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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