

## Genetic background of carbapenem resistant *Acinetobacter baumannii* in a health care setting in Alexandria, Egypt

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**Abstract:** *Acinetobacter baumannii* (*A. baumannii*) is recognized as a major pathogen causing nosocomial infections, particularly in patients admitted to intensive care units (ICU), and many widespread strains are resistant to almost all antibiotics currently in use. Carbapenemases belonging to molecular class D (OXA enzymes) have emerged globally as the main mechanism responsible for this resistance. This work aimed at detecting the spread of OXA carbapenemases in *A. baumannii* in ICU patients and to identify the susceptibility patterns of strains to polymyxin E (colistin), polymyxin B, and tigecycline as a promising option for treatment. Twenty seven clinical isolates of *A. baumannii*, collected from Alexandria Main University Hospital, were included in this study. *A. baumannii* was isolated from different clinical samples from ICU patients. All 27 isolates were subjected to complete identification and antimicrobial susceptibility testing. The identified carbapenem resistant *A. baumannii* strains were tested for the presence of *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-58</sub> and *bla*<sub>OXA-51</sub> genes using multiplex PCR assay. Colistin and polymyxin B were found to be active upon the majority of identified resistant *A. baumannii* strains, where around 70% of the strains were sensitive to both of them, while tigecycline was found to be more effective as 92.5% of strains were sensitive to it. Resistance to carbapenems was mediated by both OXA-51 (100%) and OXA-23 (92.5%) for the tested *A. baumannii* strains.

**Keywords:** *Acinetobacter baumannii*, carbapenemases, polymyxins, colistin, tigecycline, OXA genes.

### INTRODUCTION

*Acinetobacter baumannii* (*A. baumannii*) is a Gram-negative organism that is increasingly recognized as a major pathogen causing nosocomial infections, including bacteremia and ventilator-associated pneumonia, particularly in patients admitted to intensive care units (ICU).<sup>(1)</sup> It is associated with multiple antibiotic resistance, and many widespread strains are resistant to almost all antibiotics currently in use, leaving few therapeutic options remaining.<sup>(2)</sup> *A. baumannii* persistence in hospital environments and propensity to cause outbreaks are contributed to its increasing

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resistance to antimicrobial drugs, including carbapenems, and resistance to desiccation and disinfectants.<sup>(3, 4)</sup> Several studies have shown the geographically widespread occurrence of multidrug-resistant *A. baumannii* strains, which suggested a clonal relatedness of these strains.<sup>(3)</sup>

Carbapenemases belonging to molecular class D have emerged globally as the main mechanism responsible for this resistance. The OXA carbapenemases of *Acinetobacter* spp. are divided into four phylogenetic subgroups: OXA-23-like; OXA-24-like; OXA-51-like; and OXA-58.<sup>(5)</sup> Recently it has been suggested that enzymes belonging to the OXA-51-like subgroup are intrinsic to *A. baumannii*, occurring in most or all strains, although they are very variably expressed. A significant contribution to resistance by OXA-51-like enzymes therefore requires the presence of an insertion element *ISAbal* upstream of the gene, able to act as a strong transcriptional promoter.<sup>(6)</sup>

The *bla*<sub>OXA-23</sub> gene has been increasingly reported worldwide. Clonal outbreaks of carbapenem-resistant and OXA-23–producing *A. baumannii* have been reported in many countries, such as Bulgaria, China, Brazil, Iraq, and Afghanistan.<sup>(7-10)</sup> Carbapenemases OXA-58 were first described in Europe, where they are widely disseminated.<sup>(11, 12)</sup> In South America, the presence of the gene was described in Argentina, commonly associated with resistance to carbapenems in *Acinetobacter* spp. outbreaks. The occurrence of this carbapenemase has also been observed in Asia, showing that it is widely distributed throughout the world.<sup>(3,13,14)</sup>

The emergence of multidrug resistant (MDR) Gram-negative bacteria has led to the re-administration of polymyxin B and polymyxin E (colistin) as "salvage" therapy in critically ill patients. Recent studies demonstrated acceptable effectiveness and considerably less toxicity than reported in older studies of polymyxins.<sup>(15)</sup> Colistin,

an old antibiotic also known as polymyxin E, has attracted more interest because of its significant activity against multi-resistant *Pseudomonas aeruginosa*, *A. baumannii* and *Klebsiella pneumoniae*, and the low resistance rates to it. Our knowledge of this drug is limited because its use was displaced by the potentially less toxic aminoglycosides in 1970s.

This work underlines the spread of OXA carbapenemases in *A. baumannii* in ICU patients admitted to *one of the main hospitals in Alexandria* and to identify the susceptibility patterns of strains to polymyxin E (colistin), polymyxin B, and tigecycline as a promising option for treatment.

## **MATERIAL AND METHODS**

### ***A. baumannii* isolates:**

From September 2011 to March 2012, 27 non repetitive clinical isolates of imipenem non-susceptible *A. baumannii*, based on the results of disk diffusion tests, were collected from patients (20 males and 7 females) admitted to the ICU of the

Alexandria Main University Hospital. Samples included endotracheal aspirates (n=15), sputum (n=3), pus (n=5), blood (n=2), -pleural effusion (n=1) and aspirates from hepatic catheter (n=1)). Samples were cultured on Blood and MacConkey's agar plates (Oxoid), and incubated at 37°C aerobically for 24 hours. The strains that were non-lactose fermenters on MacConkey's agar and identified as Gram negative bacilli (that appeared in a diploid form or in chains of variable length) by Gram stain and were oxidase negative were further identified by biochemical tests as shown in table 1.

### **Antimicrobial Susceptibility Testing**

Identified *A. baumannii* strains were screened for their antimicrobial susceptibility using single disc diffusion method described by Bauer *et al.*<sup>(17)</sup> The test was done on Mueller Hinton agar plates (Oxoid), using the selected antibiotic discs with various concentrations including amoxicillin/ clavulanic acid, piperacillin,

ampicillin/ sulbactam, meropenem, imipenem, ceftazidime, cefotaxime, ceftriaxone, aztreonam, gentamicin, tobramycin, amikacin, ciprofloxacin, levofloxacin, trimethoprim/ sulfmethoxazole. Inhibition zones were measured and susceptibility was interpreted as susceptible (S), intermediate (I) and resistant (R) according to standard tables published by Clinical Laboratory Standard Institute (CLSI). The inhibition zones for polymyxin E (colistin), polymyxin B zone and tigecycline were interpreted according to published recommendations. For polymyxin E (colistin) and polymyxin B, the zone diameter were (resistant  $\leq 12$  mm, intermediate = 13, susceptible  $\geq 14$  mm). Isolates were considered susceptible to tigecycline if they had an inhibition zone diameter of  $\geq 13$  mm.<sup>(18-21)</sup>

*A. baumannii* strains were considered to be carbapenem resistant when they were found to be resistant to all beta-lactams, including carbapenems (carbapenemase producing bacteria).

## **Detection of genes coding for carbapenemases production**

### **Multiplex PCR assay:**

All 27 identified resistant *A. baumannii* strains and carbapenem sensitive *A. baumannii* as a negative control isolated from the same ICU, were tested for the presence of *bla* OXA-23, *bla*OXA-24, *bla*OXA-58 and *bla*OXA-51 genes using multiplex PCR assay as follows:<sup>(22)</sup>

#### **1) DNA Extraction**

DNA was extracted from the 27 carbapenem resistant *A. baumannii* isolates by boiling five colonies of fresh organism culture in 20  $\mu$ l of sterile distilled water for 5 minutes, followed by centrifugation for 2 min at 8000 rpm, then supernatants were used for amplification.

#### **2) Amplification Reaction**

Multiplex PCR amplification for detection of OXA-23, OXA-24, OXA-58 and OXA-51 genes coding for class D carbapenemases were sought by PCR using Taq polymerase with specific primers

supplied by Eurofins (Table 2). A 50- $\mu$ l PCR reaction mixture containing 2  $\mu$ l extracted DNA, 250 pmole of each primer, 25  $\mu$ l of DreamTaq™ Green PCR Master mix (Fermentas). Amplification was performed in a Biocycler Tc-S Thermocycler (Boeco, Germany). The amplification profile started by 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 25 sec, annealing at 52°C for 40 sec, and extension at 72°C for 50 sec, followed by a final extension step of 6 min at 72°C. The amplification products were analyzed by agarose gel electrophoresis and ethidium bromide staining.

## RESULTS

In a 7 months period, a total of 27 *A.*

*baumannii* strains were isolated from different clinical samples collected from ICU patients admitted to Alexandria Main University Hospital. In this study, we examined *A. baumannii* resistant strains that were found to be resistant to all beta-lactams, including carbapenems (carbapenemase producing bacteria).

The isolates recovered were isolated from 20 (74%) male and 7 (26%) females respectively. They were distributed as: 3 (11.2%) from sputum samples, 5 (18.5%) from pus, 15 (55.5%) from endotracheal aspirates, 2 (7.4%) from blood and 1 (3.7%) from each of pleural effusion and hepatic catheter aspirate. (Table 3).

**Table 1: Biochemical reactions used for identification of *Acinetobacter* spp.**

Biochemical reaction	<i>Acinetobacter</i> spp.
Lactose fermentation	-ve
Gram stain	Gram -ve cocci in pairs
Oxidase test	-ve
Indole production	-ve
MR	-ve
VP	-ve
Citrate utilization	V
Motility	-ve
Urease production	V
TSI	No change H <sub>2</sub> S -ve, gas -ve

MR: Methyl red, VP: Voges proskauer, TSI: Triple sugar iron agar  
-ve: negative, V: variable

**Table 2: Oligonucleotides used as primers for testing *A. baumannii* strains by multiplex PCR assay.**

Primers	Sequence (5' to 3') <sup>(23)</sup>	Amplicon (nucleotide)
OXA-23	F: GAT CGG ATT GGA GAA CCAGA R: ATT TCT GAC CGC ATT TCC AT	501-bp amplicon
OXA-24	F: GGT TAG TTG GCC CCC TTA AA R: AGT TGA GCG AAA AGG GGATT	246-bp amplicon
OXA-58	F: AAG TAT TGG GGC TTG TGC TG R: CCCCTCTGCGCTCTACATAC	599-bp amplicon
OXA-51	F:TAA TGC TTT GATCGGCCTTG R:TGG ATT GCA CTT CAT CTT GG	353-bp amplicon

**Table 3: Distribution of the 27 *A. baumannii* strains isolated from different clinical samples, Alexandria.**

Type of Samples	<i>A. baumannii</i> strains	
	No.	%
Endotracheal aspirates	15	55.5
Pus (purulent discharge)	5	18.5
Sputum	3	11.2
Blood	2	7.4
Aspirate from hepatic catheter	1	3.7
Pleural effusion	1	3.7
Total	27	100

Regarding the resistance to antibiotics polymyxin B and tigecycline. Out of other than beta-lactams, *A. baumannii* 27 strains, 74.0% were sensitive to colistin, strains were resistant to all tested 70.3% to polymyxin B and 92.5% to antimicrobial agents except colistin, tigecycline. (Table 4)

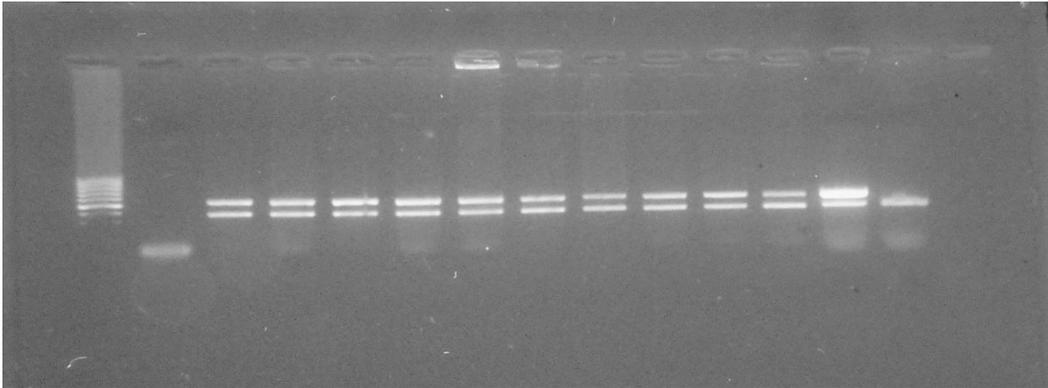
**Table 4: Susceptibility patterns of the 27 *A. baumannii* strains to colistin, polymyxin B and tigecycline.**

Susceptibility patterns Tested antimicrobials	Susceptible		Intermediate		Resistant		Total	
	No.	%	No.	%	No.	%	No.	%
Colistin	20	74.0	5	18.5	2	7.5	27	100
Polymyxin B	19	70.3	7	26	1	3.7	27	100
Tigecycline	25	92.5	0	0	2	7.5	27	100

All *A. baumannii* strains had the *bla*<sub>OXA-51</sub> gene and with the exception of 2 strains they all also harbored *bla*<sub>OXA-23</sub>. None of the tested strains was positive for *bla*<sub>OXA-58</sub> or *bla*<sub>OXA-24</sub> genes by PCR assay. (Table 5 Figure1)

**Table 5: Genotypic characteristics of carbapenem resistant *A. baumannii* isolated from ICU patients, Alexandria Main University Hospital.**

Primers	Positive strains	
	No.	%
OXA-23	25	92.5
OXA-24	0	0.0
OXA-58	0	0.0
OXA-51	27	100.0



**Figure 1: Multiplex PCR of *A. baumannii* strains with positive *bla*<sub>OXA-51</sub> and *bla*<sub>OXA-23</sub> genes at 353-bp and 501-bp.**

## DISCUSSION

The multidrug resistance is now a worldwide problem with the increasing antibiotic abuse that more and more selects for resistant strains. The prevalence of carbapenem resistance in *Acinetobacter* spp. has been on the increase during the past decade, and has emerged as a significant public health problem.<sup>(23, 24)</sup> *A. baumannii* has been a predominantly an opportunistic pathogen that is a main cause of nosocomial infections especially nosocomial and ventilator acquired pneumonia (VAP). Recently there is a rise in community acquired infections

caused by *A. baumannii*. The occurrence of *A. baumannii* among hospitalized patients depends on the hospital populations, types of performed interventions and procedures done.<sup>(25, 26)</sup>

In the present study, the most common site of *A. baumannii* isolation was endotracheal aspirates 15 (55.5%), followed by pus from wounds 5 (18.5%), and sputum 3 (11.2%). Similar findings were also reported by Nasr and Attalah,<sup>(27)</sup> who isolated *A. baumannii* from 3 ICU in Cairo, whereas 14 (70%) of isolates were from VAP, followed by

4 (20%) from postoperative wound infections. They also observed that all their isolates were MDR and were totally resistant to imipenem, ampicillin/sulbactam, ciprofloxacin, and cephalosporins, with a high resistance rate to amikacin and trimethoprim/sulfmethoxazole (90% each), and gentamicin (85%). These results suggests the role of *A. baumannii* as a major pathogen causing VAP that requires more attention in infection control programs. Another study in Alexandria, isolated *A. baumannii* which was found resistant to imipenem and 96% were MDR. The reason for this high level of resistance might be a nation wide problem due to the extensive use of carbapenems creating a selective antibiotic pressure and adding to the increasing trend of carbapenem resistant *A. baumannii*.<sup>(28)</sup> The increasing prevalence of resistant *A. baumannii* strains to all antibiotics, including carbapenems has led to the revival of interest in using polymyxins discovered more than 50 years ago, due to the limited choice of a suitable antibiotic drug of

choice.<sup>(29, 30)</sup>

Polymyxins in recent studies is shown promise in the treatment of infections caused by MDR Gram-negative bacteria, including *A. baumannii* infections. In the present study, the susceptibility pattern for colistin, polymyxin B and tigecycline were tested. It was found that 74% of isolated *A. baumannii* were sensitive to colistin and 70.3% were sensitive to polymyxin B (Table 4). Mohammed and Raafat<sup>(28)</sup> demonstrated higher sensitivity results where colistin and polymyxin retained their activity in 82.6 % and 91.3% of the tested isolates, respectively. High sensitivity to colistin were also reported, one study found that 98% of carbapenem-resistant *A. baumannii* were susceptible to colistin.<sup>(35)</sup> Noticeably, Frickmann et al<sup>(36)</sup> reported that colistin among 24 tested antibiotics was the only effective one against all MDR isolates.

Tigecycline is a relatively new antibiotic approved by the FDA in 2005. Tigecycline is a minocycline active that has potency

against both Gram-positive and Gram-negative bacteria including *Acinetobacter*.<sup>(25)</sup>

In the present study it was found to be more effective against *A. baumannii* than the polymyxins. Around 92.5% of strains were sensitive to tigecycline. While tigecycline gives good in vitro activity against *A. baumannii* isolates, the question remains of its effectiveness in vivo.<sup>(37)</sup> On the contrary to our results, a study conducted in Egypt found only 60% of *A. baumannii* strains to be sensitive to tigecycline.<sup>(38)</sup>

Based on sequence homology alone, class D carbapenemases can be divided into the following clusters: OXA-23 (includes OXA-27 and OXA-49); OXA-24 (includes OXA-25, OXA-26 and OXA-40), OXA-58 and an intrinsic chromosomal OXA-51.<sup>(6, 39)</sup> In the present study, all the carbapenem-resistant *A. baumannii* strains were tested for OXA-23, OXA-24, OXA-58 and OXA-51 genes using PCR assay. All strains (100%) were positive for *bla*<sub>OXA-51</sub> gene, and all except 2 were positive for *bla*<sub>OXA-23</sub> (Table 5). The

genes *bla*<sub>OXA-24</sub> and *bla*<sub>OXA-58</sub> were not detected in any of the isolates.

Nosocomial outbreaks or sporadic cases of carbapenemase producing *A. baumannii* producing OXA-23 enzymes have been reported worldwide.<sup>(40-42)</sup> Kusradze et al showed that all resistant *A. baumannii* isolates contained *bla*<sub>OXA-51</sub> in isolates collected from Iraq and Georgia. In accordance with our study, isolates from Iraq contained the *bla*<sub>OXA-23</sub> gene located on a plasmid, but the isolates from Georgia contained the *bla*<sub>OXA-24</sub> gene located on the chromosome and none of the isolates contained the *bla*<sub>OXA-58</sub>-encoding genes.<sup>(43)</sup>

In Greece, carbapenem resistance in *A. baumannii* was associated mainly with carriage of the *bla*<sub>OXA-23</sub> gene in 72.4% of the isolates, which represents a replacement of previously predominant OXA-58 positive *A. baumannii* strains.<sup>(44)</sup> The presence of resistant strains that carry only the *bla*<sub>OXA-51</sub> as the only resistance gene

can be explained by the presence of the insertion sequence ISAb<sub>a</sub>1 adjacent to *bla*<sub>OXA-51</sub> acting as its promoter that allows over production of carbapenemase enzyme which may lead to resistance.<sup>(45,46)</sup>

## CONCLUSIONS

- Tigecycline, colistin and polymyxin B were found to be active against carbapenem resistant *A. baumannii* isolates of this study. A further study of the intrinsic OXA-51 to look for the insertion element ISAb<sub>a</sub>1 upstream of the gene is recommended

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