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ORIGINAL ARTICLE

Characterization of Carbapenem-resistant *Acinetobacter baumannii* Isolated from Intensive Care Unit, Egypt

[1]Eman A. El-Masry*, [2]Hesham A. El- Masry
[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt
[2] Intensive care unit , Giza Chest Hospital , Egypt

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Corresponding Author:
Eman El- Masry
Department of Medical Microbiology and Immunology
College of Medicine
Menoufia University, Egypt
Tel: 01003591928
emanshma@yahoo.com

ABSTRACT

Background*: Carbapenem resistant Acinetobacter baumannii has emerged worldwide in the hospitals especially in Intensive care units (ICU).* ***Objectives:*** *to detect carbapenems resistance and characterization of some carbapenem resistant genes among acinetobacter baumannii isolated from patients in ICU unit in Giza Chest Hospital, Egypt.* ***Methodology:*** *This study was designed to detect the prevalence of carbapenem resistance among A. baumannii species isolated from patients having nosocomial infections in Egypt. Antimicrobial susceptibility pattern was done. Identification and susceptibility testing was confirmed by VITEK® 2 Compact system. Isolates were further tested by the modified Hodge test (MHT) to detect carbapenem resistance. PCR was used to detect some carbapenem resistant genes.* ***Results:*** *A. baumanniii represent 22(16.1%) of total isolates causing nosocomial infection in ICU. A. baumanniii were resistant to sulfamethoxazole-trimethoprim, amoxicillin/ clavulanicacid, ciprofloxacin, piperacillin/tazobactamandceftazidime 100%, 90.9%, 90.9, 90.9%, 81.8% and 81.8% respectively. Imipenem resistance was 14/22(63.6%). Colistin showed the highest activity against A. baumannii isolates; the resistance rate was 4.5%. Total number of carbapenem-resistant gram-negative isolates was (38.9%). Regarding A. baumanniii 14 out of 22(63.6%) were carbapenem resistant as detected by the antibiotic susceptibility test. According to this antibiogram result, 14 A. baumanniii isolates screened for carbapenemase production by MHT, carbapenemase activity was detected among 10(71.4%) of carbapenem-resistant A. baumanniii isolates. Molecular detection of carbapenem resistant genes showed that Bla_{OXA-23} was the common detected gene 6/14 (42.8%). Bla_{OXA-58} was detected among 1/14(7.1%) of isolates.* ***Conclusion:*** *A. baumannii with multidrug resistance become a problem especially in immunocompromised patient. Resistance among A. baumannii caused by several mechanisms. OXA-23-like carbapenemase-producing strains have been among the most detected patterns.*

INTRODUCTION

Nosocomial infections are common problems among different countries. One of the opportunistic nosocomial infections are caused by *Acinetobacter baumannii* which has emerged worldwide especially in Intensive Care Units (ICU)[1,2]. ICU patients usually are compromised by multiple factors like mechanical ventilation, invasive procedures, and frequent use of urinary catheters. These infections are usually life threatening among these patients [3].

In recent decades, Multidrug- resistant (MDR) *Acinetobacter baumannii* has emerges as a major cause of nosocomial infections and outbreaks including pneumonia, bacteremia, urinary tract infection and wound infection[4,5,6,7]. Infection caused by these serious multidrug resistant *A. baumannii* are treated by carbapenems which has been widely used as the first line of treatment but unfortunately carbapenems resistance also has been worldwide increased[8,9,10].

Consequence of this emerging resistance to carbapenems , new treatment options even with old antibiotics like colistin drug and recent combination like with tigecycline are used [9].

Carbapenem resistance and resistance of *A. baumannii* species occur due to several mechanisms including beta-lactamase production, loss of outer membrane porins, penicillin binding protein alteration, over expression of efflux pumps, and carbapenem-hydrolyzing oxacillinases [8]. Enzymatic degradation by beta lactams is the most prevalent mechanism of carbapenem resistance among *A. baumannii*. The Ambler class A, B, C, and D β -lactamases confer various resistance phenotypes, such as extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases[9,10]. (MBLs), carbapenem-hydrolyzing class D β-lactamases (CHDLs), and Acinetobacter-derived

cephalosporinases (ADCs)[11,12]. Regarding acquired resistance to carbapenem is mediated mostly by the CHDLs (OXA-23, OXA-24/40, OXA-58, and OXA-143) and less frequently by MBLs (IMP, VIM, SPM, GIM, and NDM), which are responsible for high levels of carbapenem resistance. Recently, the Ambler class A carbapenemase GES has been described in *A. baumannii*, which is responsible for a low level of carbapenem resistance[13,14]. Although metallo-beta-lactamase (MBL) has been carbapenem-hydrolyzing enzymes, OXA-23-like carbapenemase-producing strains have been among the most detected patterns [15].

The last option for treatment of carbapenems-resistant *A. baumannii* is colistin–tigecyclin but later on resistance to these antimicrobilas has also been reported and as a result, *A.baumannii* with résistance to carbapenems, colistin and tigecyclins makes treatment of these isolates difficult.[16,17].

The infection caused by these carbapenems-resistant bacteria have been associated with higher mortality rates. Because of that early detection of these organisms and strict application of infection control measures is important in reducing the transmission in hospitals[18]. The aim of this study is to detect carbapenems resistance and characterization of some carbapenems resistant genes among *acinetobacter baumannii* isolated from patients in ICUs in Giza Chest Hospital, Egypt.

METHODOLOGY

This study was conducted during the period from January 2016 to January 2018 Permission and signature from all patients included in the study and ethical approval from the Local Ethical Committee of Giza chest Hospital were obtained for the use of specimens. It included 132 patients admitted to ICU, Giza Chest Hospital with various nosocomial infections.

One hundred thirty-two gram-negative bacterial strains were isolated from different specimens including blood, urine, sputum, pus aspirates and endotracheal secretions and identified using a combination of conventional techniques[19]. A. *baumannii* isolates identification was confirmed by the automated VITEK 2 system (bioMérieux) according to the manufacture instructions.

Antibiotic susceptibility testing

Antibiotic susceptibility profile of isolates was determined by using a modified Kirby Bauer disk diffusion method on Muller Hinton agar Oxoid Ltd., Basingstoke, UK) according to the CLSI guidelines[20]. Antibiotics (Oxoid Ltd.) used are amoxicillin/clavulanic acid(30µg); piperacillin/tazobactam (110 µg); cefixime (5µg); ceftazidime (30µg); cefotaxime (30µg); (imipenem (10µg); amikacin (30µg);ciprofloxacin (5µg); sulfamethoxazole-trimethoprim (25µg); colistin (10µg) and tigecycline (15µg).

A. baumannii sensitivity was confirmed by the VITEK 2 system following the manufacture instructions as follow:

All isolates were cultured on blood agar then a liquid suspension was done for them. The suspension of isolates was loaded on the VITEK 2 system, left overnight. The VITEK 2 system was used for identification and for antibiltic sensitivity testing as followed by the manufacture instructions ((bioMérieux Inc., Durham, NC 27712, France). The VITEK machine deals automatically with the cards started from filling, sealing, transferring the card to the linked incubator at 35 °C, and then decoding the output report according to algorithmic system. The results were compared with the ID-GN databank.

A. baumannii strains were considered to be carbapenem resistant when they were resistant to all beta-lactam including carbapenems [21,22]. *A. baumannii* strains which have less sensitivity to Imipenem on modified Kirby Baure disk diffusion method were suspected as carbapenem resistant and were further tested by the modified Hodge test.

Modified Hodge Test (MHT)

This was done according to CLSI [23] guidelines for detection of carbapenem resistance among Enterobacteriaceae as follow:

We Prepared 0.5 McFarland of the negative control (*E. coli* ATCC 25922). Then made 1:10 dilution, this was swabbed onto Muller Hinton agar then the test organism was streaked from the edge of meropenem(mrp) disk (10 µg) as a straight line to the edge of the plate. Isolates were considered positive as carbapenems producer if there was indentation in the growth towards the imipenem disk on either side of the test organism *Klebsiella pneumoniae* BAA 1705 used as a positive control [24].

Molecular study

Detection of some genes coding for carbapenems producing *A .baumannii* isolates by polymerase chain reaction (PCR).

DNA extraction

A single colony of the isolate was inoculated into 2 mL of Mueller-Hinton broth and inoculated for 18 h at 37 °C. Cells from broth medium were harvested by centrifuging for 10 min in a microcentrifuge at 14,000 rpm. Cells were resuspended in Tris-EDTA (TE) buffer (1 mMTris, pH 7.5, and 0.5 mM EDTA, pH 8.0) and harvested by centrifuging for 10 min in a microcentrifuge. The bacterial pellet was then resuspended in TE buffer and boiled for 10 min, centrifuged at 10,000 rpm for 10 min. The DNA in the supernatant part was frozen at –80 °C until use. Primers used are shown in table (1).

Amplification reactions were performed in a final volume of 50 µL, containing 1X reaction buffer; 2.5

mM $MgCl_2$; 0.2 mM each of dATP, dCTP, dGTP, and dTTP; 0.5 U of Taq DNA polymerase (Thermo Scientific, Lithuania); 30 pM each of primers [25].

A multiplex PCR using Taq PCR Master Mix (Qiagen, Germany) was used for the identification of carbapenem resistance. Primer sets are described in [Table 1] were used [26-28]. The cycling conditions used included; one cycle denaturation at 94 °C for 5 min, 94 °C for 25 s for 30 cycles, annealing at 52 °C for 40 s and extension at 72 °C for 50 s, and a final extension at 72 °C for 6 min using Mycycler ™ Thermal cycler (BioRad, USA). A ladder of 15.0-1000.0 bp was used to estimate allele sizes after the PCR products were separated in 0.8% agarose gel, staining with ethidium bromide and visualization under UV light [25].

RESULTS

Total number of isolates from patients admitted to Intensive Care Unit was 136. *A. baumannii*i represent 22(16.1%) of total isolates causing nosocomial infection in ICU.

Total number of carbapenem-resistant gram-negative isolates was 53(38.9%). Regarding *A. baumannii*i 14 out of 22(63.6%) were carbapenem resistant as detected by the antibiotic susceptibility test.

Antibiotic resistant pattern of 22 A. baumanniii. Isolates showed that they were resistant to sulfamethoxazole-trimethoprim,amoxicillin/ clavulanicacid,ciprofloxacin,piperacillin/tazobactamand ceftazidime100%,90.9%,90.9%,90.9%,81.8% and 81.8% respectively .Imipenem resistance was 14/22(63.6%). Colistin showed the highest activity against *A. baumannii* isolates; the resistance rate was 4.5% (1/22).

According to the antibiogram result, the 14 carbapenem resistant *A. baumannii*i isolates screened for carbapenemase production by MHT, carbapenemase activity was detected among 10(71.4%) of carbapenem-resistant *A. baumannii*i isolates.

Molecular detection of carbapenem resistant genes among carbapenem resistant *A. baumannii*i showed that Bla_{OXA-23} was the common detected gene 6/14 (42.8%). Bla_{OXA-58} detected among 1/14(7.1%) of isolates while Bla_{NDM} was not detected among carbapenem resistant *A. baumannii*i

Table 1: PCR primer sequences used for carbapenem genes

	Target Primer	Sequence (5'-3')	Product size
***bla*$_{OXA-23}$**	OXA-23-F	5`-ATGGAAGGGCGAGAAAAGGT-3	361 bp
	OXA-23 R	` 5`-ATCCATTGCCCAACCAGTCT-3`	
***bla*$_{OXA-58}$**	OXA58A	(5′-CGA TCA GAA TGT TCA AGC GC-3′)	743- bp
	OXA-58B	5′-ACG ATT CTC CCC TCT GCG C-3′)	
***bla*$_{NDM}$**	NDM Fm	(5′GGT TTG GCG ATC TGG TTT TC3′)	1058-bp
	NDM Rm	(5′-CGC AAT GGC TCA TCA CGA TC-3′)	

Table 2. Number and percentage of carbapenem-resistant gram-negative isolates

Isolated Gram-negative bacilli			Number (%)of carbapenem-resistant isolates	
	No	(%)	No	(%)
Klebsiellapneumoniae	30	(22.05)	12	(22.6)
Acinetobacterbaumannii	22	(16.1)	14	(26.4)
Escherichia coli	24	(17.6)	9	(16.9)
Pseudomonas aeruginosa	34	(25)	10	(18.8)
Proteus spp	10	(7.3)	3	(5.6)
Other gram –negative	16	(11.7)	5	(9.4)
Total	136		53	(38.9)

Table 3: Antimicrobial susceptibility profile of 22 *Acinetobacter baumannii* isolates

Antibiotic	Resistance pattern, *no* (%)		
	R	I	S
amoxicillin/clavulanic acid	20(90.9)	2(9.1)	0(0)
piperacillin/tazobactam	18(81.8)	2(9.1)	2(9.1)
Cefixime	22(100)	0(0)	0(0)
ceftazidime	18(81.8)	0(0)	4(18.2)
cefotaxime	20(90.9)	0(0)	2(9.1)
imipenem	14(63.6)	0(0)	8(36.3)
amikacin	10(45.4)	5(22.7)	7(31.8)
ciprofloxacin	20(90.9)	0(0)	2(9.1)
sulfamethoxazole-trimethoprim	22(100)	0(0)	0(0)
colistin	1(4.5)	2(9.1)	19(86.4)
tigecyclin	3(13.6)	0(0)	19(86.4)

R, resistant; I, intermediate; S, susceptible.

Table 4: Carbapemem production by the modified Hodge test among 14 carbapenem- resistant Acinetobacter baumannii

Antimicrobial susceptibility		***Modified Hodge test(MHT)***	
No	***(%)***	***No***	***(%)***
14 /22	**(63.6)**	**10/14**	**(71.4)**

Table 5: Distribution of 53 carbapenem-resistant gram-negative isolates according to site of infection

	Blood No. (%)	**Urine No. (%)**	**Respiratory samples No. (%)**	**Wound swabs No. (%)**	**Total No. (%)**
Klebsiella pneumoniae	0(0%)	3 (25%)	6(50%)	3(25%)	12 (22.6)
Acinetobacter baumannii	3(21.4%)	4(28.5%)	6(42.8%)	1(7.1%)	14 (26.4)
Escherichia coli	1 (11.1%)	5(55.5%)	2(22.2%)	1(11.1%)	9 (16.9)
Pseudomonas aeruginosa	1(10%)	4 (40%)	3 (30%)	2(20%)	10(18.8)
Proteus spp	0(0%)	1(33.3%)	2(66.6%)	0(0%)	3(5.6)
Other gram –negative	2(33.3)	1(16.6)	2(33.3)	0(0)	5 (9.4)
Total	7(13.2)	18(33.9)	21(39.6)	7(13.2)	53(38.9)

Table 6: Distribution of carbapenem resistance genes among carbapenem-resistant Acinetobacter baumannii isolates (no=14).

	***Bla*$_{OXA-23}$ No. (%)**	***Bla*$_{OXA-58}$ No. (%)**	***Bla*$_{NDM}$ No. (%)**
A. baumannii	6 (42.8%)	1(7.1%)	0(0%)

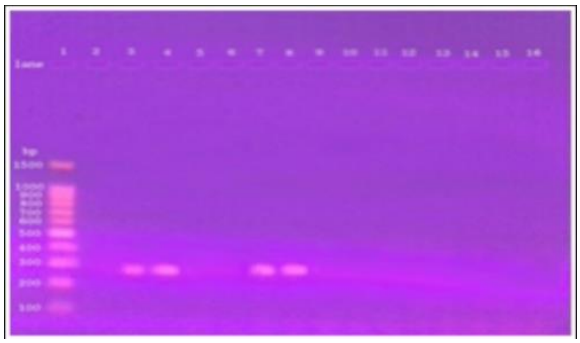

Fig. 1: Agarose gel electrophoresis of PCR products of ***Bla*$_{OXA-23}$** gene (361bp) in Acinetobacter baumannii isolates. Lanes 3,4,7,8 were gene positive isolates. Lane 2,14 are negative control

DISCUSSION

*A. baumannii*i is a common organism isolated from hospitals especially from ICUs as patients in the ICU usually have serious co-morbid conditions and under invasive procedures, such as mechanical ventilation, surgery, and frequent use of urinary catheters, or vascular catheters[29]. *Carbapenem resistant A. baumannii*i is commonly isolated from respiratory tract specimens. Similar reports detected it in respiratory tract specimens [30] and endotracheal secretions [31].

Antimicrobial resistance of *A. baumannii*i to antibiotics has become a problem worldwide. This resistance causes difficulty in treating infections caused by such organisms. In this study, *A. baumannii*i were resistant to sulfamethoxazole-trimethoprim, amoxicillin/ clavulanic acid, ciprofloxacin, piperacillin/ tazobactam and ceftazidime 100%, 90.9%, 90.9%, 90.9%, 81.8% and 81.8% respectively. It was highly sensitive to colistin 4.5% resistance rate and tigecycline, with a resistance rate 13.6%. Amikacin was effective in treating such organism, resistance rate was (45.4%).

These results were nearly consistant with other previous studies. In Al-Agamy et al study,100% of the isolates were resistant to amoxicillin–clavulanate, aztreonam, cefepime, cefotaxime, and ceftazidime [32].

The drug of choice for treatment of nosocomial infections caused by *A. baumannii* is carbapenems, the broadest spectrum β-lactams, which is considered as the last treatment choice for treatment of such serious infections caused by *A. baumannii* as they are not affected by most β-lactamases, however carbapenem-resistant strains have been reported recently. In the present study, 63.6% of isolates were imipenem resistant indicating carbapapenems production and this was confirmed by a modified Hodge test. Higher rates of resistance to carbapenems was observed in previous studies in Egypt ranging from 75% to 100% for imipenem[33-36]. In Al-Agamy et al for example the resistance rate to imipenem was high (70%) among *A. baumannii* isolates[32]. The resistance rate of *A. baumannii* to imipenem was 65% in Saudi Arabia [37] and 47.9% in Algeria [38].

This resistance to imipenem reflects that this problem might be due to extensive misuse of carbapenems. In this study, carbapenemase activity was detected in 45.4% of the carbapenem resistant isolates using MDH. Fouad et al.[36]. Also revealed 82% of carbapenem resistant isolates of *A.baumnannii* showed positive MHT. These finding support that carbapenemase production strongly contributed to carbapenem resistance and the negative MHT carbapenem-resistant *A.baumnannii* indicated that carbapemen resistance can be caused by other mechanisms [28].

The most prevalent carbapenem resistant mechanism in *A. baumannii* is degradation by carbapenem hydrolyzing B- lactamases and the most widespread carbapenems are CHDLs then MBL and class A carbapenems. The CHDLs are divided into subgroups the intrinsic $bla_{\text{OXA-51}}$-like and the acquired carbapenemase genes $bla_{\text{OXA-23}}$-, $bla_{\text{OXA-24/40}}$-, and $bla_{\text{OXA-58}}$-like [11].

In our study the most prevalent gene in *A. baumannii* was ***bla*** $_{\textbf{\textit{OXA-23}}}$, with 42.8% prevalence rate. Numerous studies also reported that **bla** $_{\textbf{OXA-23}}$ is the most frequent type of carbapenems among *A. baumannii*[39]. An Egyptian study reported the prevalence of OXA-23 as 55.8% [35] .This resistance mechanism due to class D OXA-type enzymes make the choice very limited and based mainly on polymyxin combination with other antibiotics.

The bla $_{\text{OXA-58}}$ in our study.is detected in only 7.1% isolate. Abdel Hamid et al [40]. In an Egyptian study also reported that blaOXA58 genes were not detected in any isolate

Resistance due to MBL is characterized by rapid dissemination because it is plasmid mediated. Because of its ability to spread, it has a serious concern [32]

CONCLUSION

A. baumannii. With multidrug resistance, become a problem especially in immunocompromized patient. Infection caused by Carbapenem resistant- *A. baumannii*.is a major challenge because the treatment options in such organism is limited. Resistance among *A. baumannii*. Caused by several mechanisms. OXA-23-like carbapenemase-producing strains have been among the most detected patterns. Such spread of this strain has serious consequences and so strict infection control measures should be applied.

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