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Prevalence of Carbapenem Resistant *Acinetobacter baumannii* (CRAB) in some Egyptian Hospitals: Evaluation of the Use of $bla_{\text{OXA-51-like}}$ Gene as Species Specific Marker for CRAB

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CARBAPENEM resistant *Acinetobacter baumannii* (CRAB) now leads the WHO's critical priority list of nosocomial pathogens worldwide. Carbapenem Hydrolysing Class D β-lactamases (CHDL) -"OXA-type β-lactamases"- is of great concern. In this study, bacterial isolates recovered from different clinical samples collected from three different hospitals in Cairo from October 2014 to May 2015. The isolates were identified using Vitek 2 and partial 16S rRNA gene sequencing. *Acinetobacter baumannii* isolates represented 16.1% of the total Gram negative isolates. Susptibility test revealed that 88.9% were CRAB, 65% of imipenem resistant isolates and 57.5% of meropenem resistant isolates had chromosomal mediated resistance. Forty CRAB isolates were identified and screened for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$ genes.

The most prevalent gene was $bla_{\text{OXA-51-like}}$ which was detected in 95% of the isolates followed by $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ in 90% of the isolates. However, $bla_{\text{OXA-58-like}}$ was not detected in any of the isolates. The absence of $bla_{\text{OXA-51-like}}$ in 5% of CRAB isolates proving the inconvenience of using $bla_{\text{OXA-51-like}}$ as a species specific region for identification of CRAB by PCR. In addition, all isolates harbored more than one OXA gene, 75% were $bla_{\text{OXA-23/OXA-24/OXA-51}}$ carriers while 25% harbored two OXA genes. This study revealed the emergence of $bla_{\text{OXA-24-like}}$ gene in CRAB for the first time in Egyptian hospitals.

Keywords: *A. baumannii*, Carbapenem resistance, Multiple bla_{OXA} genes, $bla_{\text{OXA-51-like}}$, Plasmid curing.

Introduction

Multidrug resistant nosocomial pathogens are a global threat to hospitalized patients constituting a strong challenge for treatment. Implementation of measures to achieve the containment of outbreaks of those pathogens has turned to be a global priority. WHO recommended that the surveillance should be directed to pathogens with high resistance index. *Acinetobacter baumannii* was listed as one of the top six priority pathogens (WHO, 2001).

Multidrug resistant *A. baumannii* has been increasingly reported worldwide. *A. baumannii* has been resistant to almost all antibiotics available. According to Magiorakos et al. (2012) multidrug resistant *A. baumannii* (MDR) are isolates resistant to at least one agent from three or more antimicrobial categories (aminoglycosides, carbapenems, fluroquinolones, cephalosporines, penicillins plus β lactamase inhibitors, polymyxins and tetracyclines). Extensively drug resistant (XDR) isolates are resistant to at least one agent in all of the above mentioned categories for MDR. Pan Drug Resistant *A. baumannii* (PDR) are XDR isolates resistant to all agents in all antibiotic categories (Magiorakos et al., 2012).

Carbapenems have been used in multi-drug resistant *A.baumannii* treatment (Li et al., 2015). Resistance against carbapenems is, in itself,

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considered sufficient to define the isolate of *A. baumannii*as highly resistant isolate (Poirel & Nordmann et al., 2006). Carbapenem Resistant *Acinetobacter baumannii* (CRAB) is now enlisted as number one in the critical priority of the "Global Priority Pathogen List" by WHO in February 2017 (WHO, 2007).

Resistance to carbapenems in *Acinetobacter* sp. is mainly mediated by serine oxacillinases (OXAs; Ambler class D), and metallo-β-lactamases (MBLs; Ambler class B). Carbapenem resistance in *A. baumannii* isolates is most frequently due to OXA production, whereas MBL production is more prevalent in non-*baumannii Acinetobacter* isolates (Queenan & Bush, 2007; Lee et al., 2009).

Over 220 OXA-type-β-lactamease has been identified to date. The main five phylogenetic OXA-type sub groups have been detected in CRAB including: $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$ and more recently $bla_{\text{OXA-143-like}}$ (Higgins et al., 2010). The largest sub group is $bla_{\text{OXA-51}}$, followed by $bla_{\text{OXA-23-like}}$. It has been reported that $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58}}$ are chromosomally or plasmid mediated while $bla_{\text{OXA-51}}$ is chromosomally encoded and intrinsic to *A.baumannii* (Evans & Amyes, 2014). Sequence-based typing of the full length of $bla_{\text{OXA-51-like}}$ have been used as a marker for *A.baumannii* in many reports (Rafei et al., 2015). However plasmid encoded OXA-51 have been reported in non-*A. baumannii* species (Lee et al., 2011) and in A. baumannii susptible to carbapenems (Karmostaji et al., 2013). Recently, Nigro & Hall (2018) reported that blaOXA-51-like on mobile IS element.

This study aimed to assess the prevalence of carbapenem resistant *A.baumannii* harbouring class D β-lactamases (OXA) genes in some hospitals in Egypt and evaluate the use of $bla_{\text{OXA-51-like}}$ gene as an intrinsic marker for *A.baumannii.*

Materials and Methods

Bacterial isolation and identification

A total of 279 non duplicate isolates of Gram negative bacilli were collected from health care units of three different hospitals in Cairo, International Medical Center (IMC), Kobry El-Kobba and Al-Ganzouri specialized hospital over 8-month period from October 2014 to May 2015. Isolates were recovered from various clinical specimens (sputum, blood, wound swab, urine, endotracheal tube (ETT), central venous line (CVL), broncoalveolar lavage (BAL), drain, pus, pleural fluid and cereprospinal fluid (CSF). The isolates were identified to the species level using Vitek 2 compact system (bioMérieux). *A. baumanii* isolates were identified by amplification of partial 16S rRNA gene using primer pairs PA, 5' -AGA GTT TGA TCC TGG CTC AG - 3' and 1492R, 5'-CGG TTA CCT TGT TAC GAC TT-3' (Edwards et al., 1989; Lane, 1991). PCR product of partial 16SrRNA gene (almost 1400bp in length) was purified and sequenced using primers PA, 1492 R and 907R, 5'-C CGT CAA TTC ATT TGA GTT T-3' (Lane et al., 1985). Multiple alignments were performed in BioEdit 7.0 and Clustal-W. Phylogenetic neighbor-joining tree was constructed using Phylip. 3.6. Boots trapping was performed by creating consensus tree out of 1000 trees using Seq boot. The 16S rRNA gene sequences of the two isolates A23 and A419 lacking $bla_{\text{OXA-51-like}}$ gene have been deposited to Gen Bank under accession numbers: MG983836 and MG983835.

Antibiotic susceptibility testing (AST)

Antibiotic susceptibility testing (AST) was performed by Vitek 2 compact System (bioMérieux). AST data results (MIC) are interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). Thirteen antimicrobial agents corresponding to nine different antimicrobial categories were used. The tested 13 antibiotics were: gentamicin (GN), piperacillin/ tazobactam (TZP), ampicillin/ sulbactam (SAM), imipenem (IPM), meropenem (MEM), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), moxifloxacin (MOX), tetracycline (TE), tigecycline (TGC), trimethoprim/ sulfamethoxazole (SXT) and Polymxin B (PB) was used as 300IU discs from Oxoid.

Multiple antibiotic resistance index (MAR) was determined using the formula MAR= *x/y,* where *x* was the number of antibiotics to which test isolate displayed resistance and *y* was the total number of antibiotics to which the test organism has been tested (Sandhu et al., 2016).

Determination of MIC for carbapenems (IPM and MEM)

Carbapenem resistant isolates were tested for

MIC by agar well diffusion method (CLSI, 2012). MICs of carbapenems IPM (Merck and Co., USA) and MEM (Astra Zeneca, UK) were determined. Breaking points were interpreted according to CLSI guidelines (CLSI, 2012a).

Plasmid curing

Plasmid curing was carried out using 0.10mg/ml of acridine orange (Yah et al., 2007; Akortha & Filgona, 2009). Antibiotic susceptibility testing and gel electrophoresis were carried out before and after curing to indicate the presence or absence of plasmid.

Antibiotic susceptibility test was carried out by Kirby-Bauer (KB) disk diffusion method according to CLSI (2012b) on Mueller Hinton agar (Oxoid, UK) for IPM and MEM (10µg discs, Oxoid, UK) before and after Plasmid curing to distinguish plasmids-mediated from chromosomal mediated resistance. Resistance patterns were interpreted according to CLSI guidelines (CLSI, 2013).

DNA extraction and amplification of $bla_{OXA\text{-}like}$ carbapenemases

Bacterial DNA was extracted using GeneJET Genomic DNA Purification Kit (Thermo Scientific, UK). Carbapenem resistant *A.baumannii* (CRAB) isolates were screened for four oxacillinases subgroups ($bla_{OXA\text{-}23\text{-}like,}$ $bla_{OXA\text{-}24\text{-}like,}$ $bla_{OXA\text{-}51\text{-}like\ and}$ $bla_{OXA\text{-}58\text{-}like}$) according to Woodford et al. (2006). The PCR mixture containing 3µl DNA template in a 25µl mixture containing 12.5µl Dream Taq green PCR master mix (Thermo Fisher Scientific), 2µl of 1µM oxacillinase primer (Biosearch technologies) and 5.5µl nuclease free water (Thermo Fisher Scientific). In each run, a positive control and a negative control containing nuclease free water was included. The PCR protocol was performed as initial denaturation at 94ºC for 5min, followed by 30 cycles of denaturation at 94ºC for 25sec, annealing as shown in Table 1 for 40sec, extension at 72ºC for 40sec and final extension step at 72ºC for 7min. PCR was conducted in 2725 Thermal Cycler (Applied biosystems). The PCR products were visualized in 1% agarose gel stained with ethidium bromide. Hyperladder 1Kb (Bioline, UK) was used DNA marker. All negative PCR samples were spiked for confirmation.

Results

A total of 279 Gram-negative bacterial pathogens were recovered from hospitalized patients with different nosocomial infections. Sputum specimens represented the majority of specimens 41.2 % (n= 115), followed by wound swabs 17.63% (n= 49), urine specimens 16.91% (n= 47), blood 14.03% (n= 39), endotracheal tube (ETT) 3.23% (n= 9), central venous line (CVL) 2.16% (n= 6), pus 1.80% (n= 5), drain 1.44% (n= 4), broncoalveolar lavage (BAL) 0.72% (n= 2), Pleural fluid 0.72% (n= 2) and cereprospinal fluid (CSF) 0.36% (n= 1).

A. baumannii infections represented 16.1% (n= 45/279) of the total Gram-negative infections, they predominated the respiratory tract infections with percentage 62.88% (n= 28/45), blood stream infections 17.8% (8/45) wound infection 15.6% (7/45) and 4.4% (2/45) from urinary tract infections.

TABLE 1. Primer sequences and the expected amplicon size according to Woodford et al. (2006).

Target gene	Amplicons (bp)	Annealing temp. (°C)	Primers	Sequences
OXA-23-like	501	53	Oxa-23-like-F	5'-GAT CGG ATT GGA GAA CCA GA-3'
			Oxa-23-like-R	5'-ATT TCT GAC CGC ATT TCC AT-3'
OXA-24-like	246	54	Oxa-24-like-F	5'-GGT TAG TTG GCC CCC TTA AA-3'
			Oxa-24-like-R	5'-AGT TGA GCG AAA AGG GGA TT-3'
OXA-51-like	353	53	Oxa-51-like-F	5'-TAA TGC TTT GAT CGG CCT TG-3'
			Oxa-51-like-R	5'-TGG ATT GCA CTT CAT CTT GG-3'
OXA-58-like	599	55	Oxa-58-like-F	5'-AAG TAT TGG GGC TTG TGC TG-3'
			Oxa-58-like-R	5'-CCC CTC TGC GCT CTA CAT AC-3'

Susptibility test revealed that 88.9% (n= 40) of *A.baumaannii* isolates were carbapenem resistant, all of them100% (n= 40) were multi-drug resistant (MDR) as they were resistant to more than three different antibiotic categories, the least MAR index value was 0.6. According to the multiple antibiotic resistance index 42.5% (n= 17) were XDRs as they were resistant to at least one agent of the nine antibiotic categories used, their MAR index values were between 0.77 and 0.92. Most of the isolates 52.5% (n= 21) were resistant to all the tested antimicrobial agents with MAR index value= 1 (Table 2).

TABLE 2. Genotypic characteristics and MIC of the carbapenem resistant *Acinetobacter baumannii*.

Isolate Code	(MAR)	MIC (µg/ml)		CHDL β-lactamase genes				Origin of resistance
		IPM	MEM	OXA-23-like	OXA-24-like	OXA-51-like	OXA-58-like	
A01	0.77	128	>128	+	+	+	-	C & P
A02	0.77	32	32	+	+	+	-	C & P
A03	0.85	32	32	-	+	+	-	C & P
A04	1	32	16	+	+	+	-	C & P
A07	1	64	64	+	+	+	-	C
A08	1	32	32	+	-	+	-	C
A09	0.92	32	32	+	+	+	-	C
A10	1	16	32	+	+	+	-	C
A11	0.62	64	32	+	+	+	-	C
A12	0.92	64	32	+	+	+	-	P
A13	0.62	16	32	+	+	+	-	P
A14	1	16	32	+	+	+	-	C & P
A16	0.92	16	32	+	+	+	-	C
A17	1	16	32	-	+	+	-	C
A18	0.77	32	64	+	-	+	-	C
A19	0.85	32	32	+	+	+	-	C
A20	1	16	32	+	+	+	-	P
A21	1	16	16	+	+	+	-	P
A22	1	16	32	+	+	+	-	C
A23	1	16	16	+	+	-	-	C
A24	0.92	64	64	+	+	+	-	C
A25	0.92	> 128	128	+	+	+	-	C
A26	0.92	128	32	+	+	+	-	C
A27	0.92	128	128	+	+	+	-	P
A28	1	32	32	+	+	+	-	C
A30	0.92	16	16	+	+	+	-	C
A31	0.85	128	128	+	+	+	-	P
A32	1	>128	128	+	+	+	-	C
A33	1	64	>128	+	+	+	-	C
A34	1	64	64	+	+	+	-	P
A35	1	64	128	+	+	+	-	C
A36	0.92	16	128	+	+	+	-	C&P
A37	1	16	16	+	+	+	-	C
A38	1	64	64	+	+	+	-	C
A39	1	128	128	+	+	+	-	C&P
A40	1	64	64	+	+	+	-	C
A41	1	32	32	+	+	-	-	C
A43	1	16	32	+	-	+	-	C
A44	1	64	64	-	+	+	-	C
A45	0.92	128	>128	-	+	+	-	C

MAR: Multidrug resistance index, IPM: Imipenem; MEM: Meropenem; C: Chromosomally mediated resistance; P: Plasmid mediated resistance.

All the isolates were resistant to IPM and MEM. MIC determination by agar well diffusion method was carried out, results showed that the breaking points for both IPM and MEM ranged between 16µg/ml and >128µg/ml (Table 2).

Plasmid curing followed by antibiotic susceptibility test revealed that 65% (n= 26) of IPM resistant isolates and 57.5% (n= 23) MEM resistant isolates exhibited no change in inhibition zone around IPM and MEM discs, indicating that these isolates have chromosomal mediated resistance.

Plasmid mediated resistance was detected in 35% (n= 14) of IPM resistant isolates and in 37.5% (n= 12) of MEM resistant isolates. Isolates A01, A03, A36, A39 were chromosomally mediated resistance to IPM and plasmid mediated resistance to MEM representing 10% (n= 4) of CARB isolates. Isolates A02, A04, A14 showed plasmid mediated resistance for IPM and chromosomal mediated resistance for MEM representing 7.5% (n= 3) CRAB isolates. The total number of only chromosomally mediated CRAB was 65% (n= 26), plasmid mediated CRAB were 17.5% (n= 7) and those chromosomally and plasmid CRAB were 17.5% (n= 7) (Table 2).

PCR of bla_{OXA} genes was carried out, single band of the right size was performed as shown in Fig. 1. The results showed that $bla_{OXA\text{-}51\text{-}like}$ gene was detected in 95 % (n= 38) of the isolates, $bla_{OXA\text{-}23\text{-}Like}$ and $bla_{OXA\text{-}24\text{-}like}$ genes in 90% (n= 36) of isolates while none of the isolates carried $bla_{OXA\text{-}58\text{-}like}$ gene.

The co-existence of OXA gene subgroups was detected in all of the isolates. Coexistence of 3 bla_{OXA} genes was detected in 75% (n=30) of CRAB isolates ($bla_{OXA\text{-}23\text{-}like}$/ $bla_{OXA\text{-}24\text{-}like}$/ $bla_{OXA\text{-}51\text{-}like}$), 10% (n=4) carry $bla_{OXA\text{-}23\text{-}like}$/ $bla_{OXA\text{-}51\text{-}like}$, 10% (n=4) carry $bla_{OXA\text{-}24\text{-}like}$/ $bla_{OXA\text{-}51\text{-}like}$), While 5% (n=2) carry $bla_{OXA\text{-}23\text{-}like}$/ $bla_{OXA\text{-}24\text{-}like}$. Chromosomally mediated resistance in CRAB at which bla_{OXA} genes ($bla_{OXA\text{-}23\text{-}like}$/ $bla_{OXA\text{-}24\text{-}like}$/ $bla_{OXA\text{-}51\text{-}like}$) were detected was found in 45% (n= 18), while 17.5% (n= 7) showed plasmid mediated resistance (Table 2).

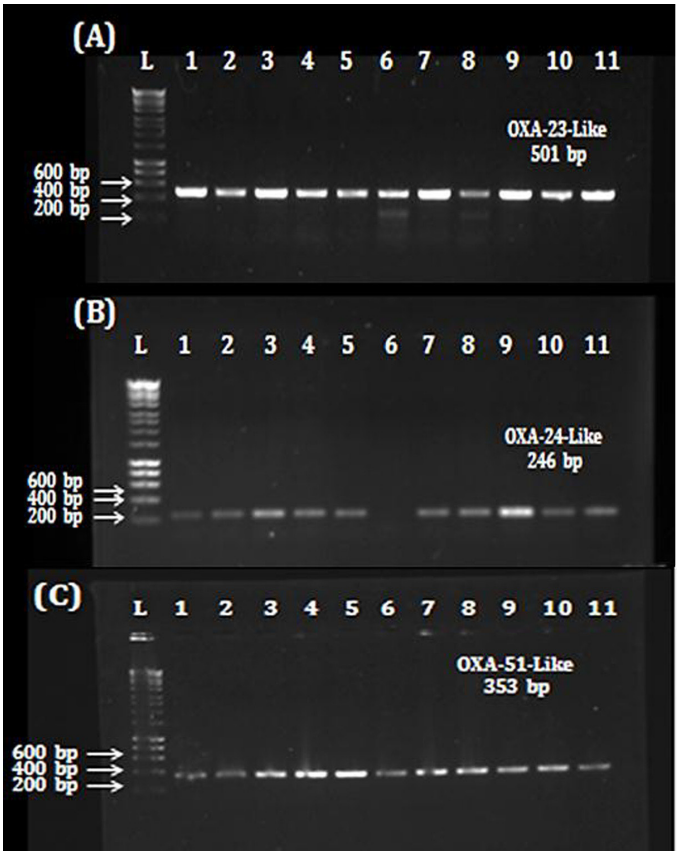

Fig. 1. PCR amplification of Oxacillinase genes; (A) Amplicons of $bla_{OXA\text{-}23\text{-}like}$ gene (501bp), (B) $bla_{OXA\text{-}24\text{-}like}$ gene (246bp), (C) $bla_{OXA\text{-}51\text{-}like}$ gene [Lanes 1 to 10 represent the PCR products for 10 carbapenem resistant *Acinetobacter baumannii* isolates, lane 11 positive control, "L" is 1kp" DNA ladder].

Discussion

In the current study, MDR *A. baumannii* isolates represented 88.8% of the total *A. baumannii* isolates, all were carbapenem resistant. Previous reports in Egypt demonstrated high resistance levels. Al-Hassan et al. (2013) demonstrated that carbapenem resistance in *A. baumannii* represented 73% of the total *A. baumannii* population in Egyptian hospitals. In 2016, a study conducted by Abdl-Hamid et al. (2016) revealed that 95.1% of *A. baumannii* isolates were MDR at which meropenem resistance was 93.9% and imipenem resistance was 87.8%. The previous data indicate the increase in carbapenem resistance by time among *A.baumannii* isolates (WHO, 2001).

In the present study, $bla_{\text{OXA-51-like}}$ gene was the most prevalent gene carried by 95% of isolates, while $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ were detected in 90% of the isolates. None of the isolates were positive for $bla_{\text{OXA-58-like}}$. Previous studies on the prevalence of CRAB in EGYPT conducted by Abdl-Hamid et al. (2016) demonstrated that $bla_{\text{OXA-51-like}}$ was detected in 93.9% of the isolates, $bla_{\text{OXA-23-like}}$ in 63.4% isolates, while $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were not detected in any isolate. Al-Agamy et al. (2014) reported that $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$were recorded in only 7.5 and 5% of the CRAB isolates, respectively. Recently Abdulzahra et al. (2018) and Ramadan et al. (2018) demonstrated that non of the studied CRAB isolates harbored $bla_{\text{OXA-24-like}}$ genes. Therefore, the emergence of $bla_{\text{OXA-24-like}}$ in 90% of carbapenem resistant *A. baumannii* isolates in this study is the first to be reported in Egypt.

By comparing different studies conducted in the Middle East by Bakour et al. (2014), Vali et al. (2015), Zowawi et al. (2015) and Rolain et al. (2016) in Yemen, Kuwait, Saudi Arabia, United Arab Emirates, Oman, Bahrain, Qatar, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ were the most prevalent except in Bahrain; $bla_{\text{OXA-24-like}}$ gene was the most prevalent, while $bla_{\text{OXA-58-like}}$ has not been detected to date. In Tahran, Karmostaji et al. (2013), Zanganeh & Eftkhar (2015), Azizi & Shkibaie (2016) reported the prevalence of $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$, while $bla_{\text{OXA-58-like}}$ was not detected.

Studies conducted in Asia, (Hong Kong, India, South Korea, Malaysia, Philippines, Singapore, Taiwan and Thailand and China) showed that $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ were the most prevalent except in Taiwan, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were the most frequent (Kim et al., 2013; Chang et al., 2015). While in China neither $bla_{\text{OXA-24-like}}$ nor $bla_{\text{OXA-58-like}}$ was detected (Chang et al., 2015). Similarly, in Pakistan $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ were the most prevalent while $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were not found (Hasan et al., 2013).

In South Africa, France and Turkey $bla_{\text{OXA-51-like,}}$ $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ were prevalent while $bla_{\text{OXA-58}}$ was reported occasionally (Bellomo et al., 2013; Jeannot et al., 2014; Ahmed et al., 2016).

The largest OXA beta-lactamase subgroup identified to date is $bla_{\text{OXA-51-like}}$ genes; it is considered intrinsic to *A. baumannii* and naturally found on the chromosome (Evans & Amyes, 2014). Although $bla_{\text{OXA-51-like}}$ is considered a species specific DNA region for carbapenem resistant *A. baumannii* and reported positive in all *A.baumannii* isolates in numerous studies such as Bellomo et al. (2013), Evans & Amyes (2014), Pournaras et al. (2014), El-Shazly et al. (2015), Rafei et al. (2015), Zowawi et al. (2015) and Dias et al. (2017), it was not detected in 5% of CRAB in this study. Isolates A23 and A41 were identified by Vitek 2 as *A. baumanii* and for more confirmation it was identified by partial 16S rRNA gene sequencing; their nucleotide sequence verified the 100% similarity to *A. baumannii*. Phylogenetic tree was constructed and the isolates A23 (MG983836) and A41(MG983835) lacking the $bla_{\text{OXA-51-like}}$ gene were grouped with *A. baumanii* strains (Fig. 2). These two isolates were confirmed phenotypically and genotypically as CRAB, however, they lack $bla_{\text{OXA-51-like}}$ gene. This finding refutes the idea of using it as a species specific marker for CRAB.

Similarly, a study in Iran (2015) showed that $bla_{\text{OXA-51-like}}$ gene was not reported in 41.37% of carbapenem resistant *A.baumannii* isolates (Azizi et al., 2016). In a study in Japan, $bla_{\text{OXA-51-like}}$ was not detected in 5.45% of carbapenem *A. baumannii* isolates (Endo et al., 2012). On the other hand, $bla_{\text{OXA-51-like}}$ gene was reported as a plasmid born gene in non-*A. baumannii* species in Taiwan (Lee et al., 2011).

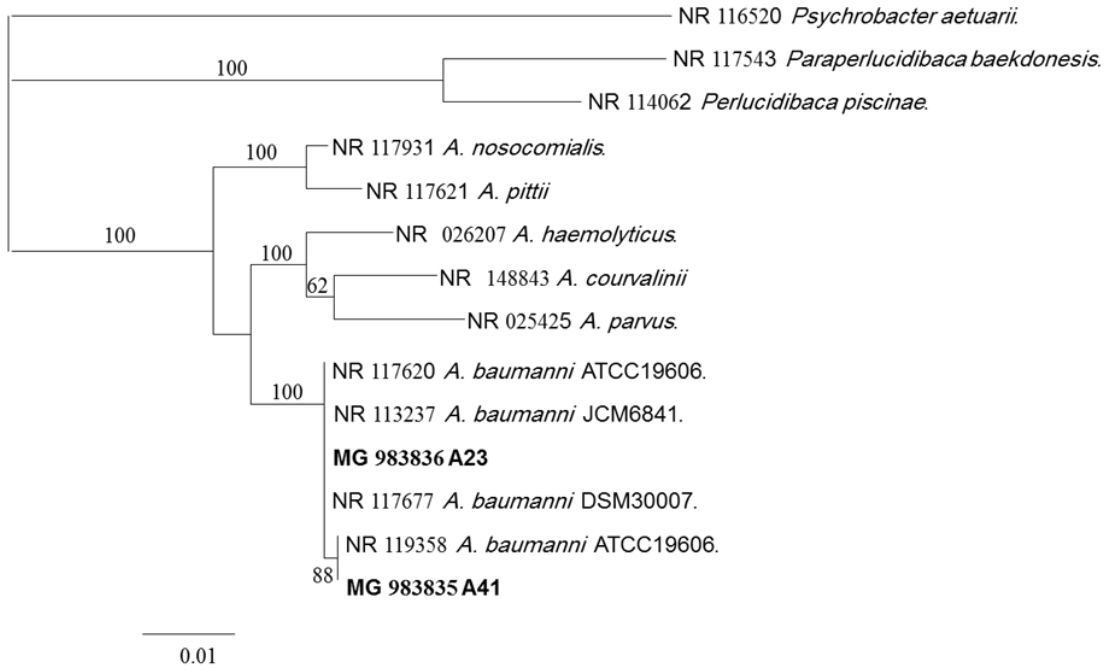

Fig. 2. Neighbour joining phylogenetic tree of 16Sr RNA gene sequence of isolates A23 and A41 [The numbers on the nodes are bootstrap values recovered from 1000 trees, the bar indicates 10% nucleotide substitution].

Moreover, the presence of $bla_{\text{OXA-51-like}}$ gene does not indicate resistance to carbapenems, since the expression and regulation of $bla_{\text{OXA-51-like}}$ gene is dependent on the presence of insertional sequence ISAba1, "mobile promoter". In contrast, the presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were consistent with resistance (Nigro & Hall, 2018). It was reported that $bla_{\text{OXA-51-like}}$ or *oxa*Ab has been shown to hydrolyse both meropenem and imipenem, but its affinity for these drugs is quite low (Corvec et al., 2007). In the current study $bla_{\text{OXA-51-like}}$ negative CRAB isolates possessed chromosomally mediated resistance and were $bla_{\text{OXA-23-like}}$/ $bla_{\text{OXA-24-like}}$ carriers. Another study reported $bla_{\text{OXA-51-like}}$ in 4 carbapenem susceptible *A. baumannii* isolates; 3 of these isolates were susceptible to IPM and MEM, implying the independence of $bla_{\text{OXA-51-like}}$ on carbapenem resistance (Karmostaji et al., 2013). All these reasons affect the credibility of $bla_{\text{OXA-51-like}}$ gene as a species specific identification tool for CRAB. From all the previous, it is recommended to identify the carbapenem resistant *Acinetobacter baumanii* phenotypically and genotypically by determination of MIC for IPM and MEM in addition to genotypic screening for all bla_{OXA} genes ($bla_{\text{oxa23, 24,51,85}}$) to confirm the identification of CRAB not depending on the presence of $bla_{\text{OXA-51}}$ only.

The co-existence of multiple OXA genes has been reported in many studies; in this study, 25% of the isolates carried two OXA genes in different combinations. The co-existence of $bla_{\text{OXA-51}}$/ $bla_{\text{OXA-23}}$ is widely spread worldwide, It represented 10% of CRAB in the study. The $bla_{\text{OXA-51}}$/ $bla_{\text{OXA-23}}$ carriers have been reported in an outbreak in Egypt (Fouad et al., 2013). It was also reported in Tahran, South Africa, Yemen, Switzerland, Brazil and Los angeles (Feizabadi et al., 2008; Bellomo et al., 2013; Bakour et al., 2014; Cherkaoui et al., 2015; Cortivo et al., 2015; El-Shazly et al., 2015).

Acinetobacter baumannii with $bla_{\text{OXA-51}}$/ $bla_{\text{OXA-24}}$ represented 10% of CRAB in this study and has been reported in Tahran, South Africa, and Switzerland (Feizabadi et al., 2008; Bellomo et al., 2013; Cherkaoui et al., 2015), while $bla_{\text{OXA-23}}$/ $bla_{\text{OXA-24}}$ gene carriers were detected in 5% of isolates and has been reported previously in Asia (Chang et al., 2015).

In the current study $bla_{\text{OXA-23-like}}$/ $bla_{\text{OXA-24-like}}$/ $bla_{\text{OXA-51-like}}$ gene carriers were the most prevalent, representing 75% (n= 30) of isolates. $bla_{\text{OXA-23-like}}$/ $bla_{\text{OXA-24-like}}$/ $bla_{\text{OXA-51-like}}$ gene carriers were reported in other studies in Taiwan and Lithuania (Kim et al., 2013; Povilonis et al., 2013).

Conclusion

The results demonstrate the emergence of CRAB harboring $bla_{\text{OXA-24-like}}$ for the first time in Egyptian hospitals, in addition to the existence of multiple OXA genes $bla_{\text{OXA-23-like}}$/ $bla_{\text{OXA-24-like}}$/ $bla_{\text{OXA-51-like}}$ carriers. Moreover, the study demonstrates the inconvenience of using $bla_{\text{OXA-51-like}}$ gene as a species specific region for CRAB identification.

However, further phenotypic and genotypic studies on bla_{OXA} genes from hospitals across Egypt and the neighboring countries are required to track carbapenem resistant *A.baumannii* and develop adequate infection control practices that enable the implementation of containment measures during infection outbreaks.

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مدى انتشار *Acinetobacter baumannii* المقاومة للكاربابينيم (CRAB) فى بعض المستشفيات المصرية: تقييم استخدام جين $bla_{\text{OXA-51-like}}$ كعلامة خاصة بأنواعها

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تتصدر *Acinetobacter baumannii* المقاومة للكاربابينيم (CRAB) الآن قائمة الأولويات الحرجة لمنظمة الصحة العالمية من مسببات عدوى المستشفيات في جميع أنحاء العالم. يعتبرمحللات الكربابنيم من نوع "OXA-β-lactamases - "Class D l-lactamases (CHDL - مصدر قلق كبير.

في هذه الدراسة، تم جمع العزلات البكتيرية من عينات سريرية مختلفة من ثلاثة مستشفيات مختلفة في القاهرة من الفترة من أكتوبر 2014 إلى مايو 2015. تم تعريف العزلات باستخدام Vitek 2 وتفاعل البلمرة المتسلسل لجين 16S rRNA الجزئي. تمثل عزلات *Acinetobacter baumannii* 16% من مجموع العزلات السالبة الجرام. وكشف اختبار الحساسية للمضادات الحيوية أن 88.9% كانت CRAB. و 65% من العزلات مقاومة للإيميبينيم و 57.5% من العزلات مقاومة للميروبينيم لديها مقاومة بوساطة الكروموسومات. تم التعرف على أربعين عزلة CRAB وتم فحص إحتوائها على جينات مثل $bla_{\text{OXA-23}}$، و $bla_{\text{OXA-24-like}}$، و $bla_{\text{OXA-51}}$، $bla_{\text{OXA-58-like}}$

كان الجين الأكثر شيوعًا هو $bla_{\text{OXA-51}}$، والذي تم اكتشافه في 95% من العزلات يليه $bla_{\text{OXA-23-like}}$ و $bla_{\text{OXA-24-like}}$ في 90% من العزلات. ولكن لم يتم اكتشاف $bla_{\text{OXA-58 like}}$ في أي من العزلات.

لم يتم العثور علي $bla_{\text{OXA-51}}$ في 5% من عزلات ال CRAB مما يثبت عدم ملائمة استخدام هذا الجين $bla_{\text{OXA-51}}$ كعلامة محددة للأنواع ال CRAB بواسطة ال PCR.

بالإضافة إلى ذلك، وجد أن جميع العزلات تحتوي على أكثر من جين OXA ، وكانت 75% منها تحوى $bla_{\text{OXA-23/OXA-24/OXA-51}}$ بينما تحتوي 25% على جينين من نوع OXA. كشفت هذه الدراسة عن ظهور جين $bla_{\text{OXA-24-like}}$ في ال CRAB لأول مرة في المستشفيات المصرية.