PHENOTYPIC AND GENOTYPIC INVESTIGATION OF OXA23 AND OXA51 CARBAPENEMASES PRODUCING ACINETOBACTER BAUMANNII IN TRIPOLI HOSPITALS

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ABSTRACT

Acinetobacter baumannii is an opportunistic pathogen causing various nosocomial infections. The aim of this study was to characterize the molecular support of carbapenem-resistant *A. baumannii* clinical isolates recovered from four hospitals in Tripoli, Libya. Bacterial isolates were identified and antibiotic susceptibility testing was performed using automated system. Carbapenem resistance determinants were studied phenotypically using two different techniques: E-test; chromogenic culture media. Polymerase chain reaction (PCR) amplification was used to determine the presence of *bla* $_{OXA23}$ and *bla*$_{OXA51}$ genes among isolates. A total of 119 isolates were characterized, overall the resistance prevalence was extremely high for aminoglycosides (79-96.6%), fluoroquinolones (94-96%), cephalosporins (96.6-100%) and carbapenemes (93.2-100%), all isolates were susceptible to colistin. In addition, 97.5% of isolates were identified as multidrug resistance (MDR). Varying degree of phenotypic detection of carbapenemes was determined; highest levels of carbapenemes were detected using chromogenic media (76.5%) compared with E-test (45.4 %). The carbapenem resistance-encoding genes detected were *bla*$_{OXA23}$ (84%) and *bla*$_{OXA51}$ (73.1%); the highest occurrence of *bla*$_{OXA23}$ was demonstrated in Tripoli's Central Hospital (5/5; 100%) then in Tripoli Medical Center (44/51; 86.27%). The co-occurrence of these genes was demonstrated in (75/119; 63%) showing dissemination of carbapenemes resistance MDR *A. baumannii* in hospitals. This study shows that the high prevalence of OXA-23 contribute to antibiotic resistance in Libyan hospitals and represents the high incidence of the association of these two carbapenemases in an autochthonous MDR *A. baumannii* isolated from patients in Libya, indicating that there is a longstanding infection control problem in these hospitals.

KEY WORDS: *bla*$_{OXA23}$, *bla*$_{OXA51}$, *A. baumannii*, Tripoli, Libya.

INTRODUCTION

A. baumannii is an opportunistic pathogen mainly involved in healthcare-associated infections, with increased mortality and morbidity[(1)]. It is associated with a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, particularly in immunocompromised patients[(2)]. The serious concern associated with this bacterium is the increasing prevalence of multidrug resistant isolates, especially carbapenem resistant ones. Outbreaks of carbapenem resistant *A. baumannii* strains have been documented in diverse geographical areas including Europe, South America and Asia[(3-5)], but little information is available from North Africa[(6,7)]. In Libya, dissemination of carbapenemases, such as the *bla*OXA-23-like and *bla*OXA-24-like genes, among *A. baumannii* isolates has been reported previously[(8)].

Carbapenem resistance in *Acinetobacter* species is most commonly caused by the production of OXA-type carbapenemases[(9)]. The OXA-type carbapenemases comprise four broad groups: *bla*OXA-23-like, *bla*OXA-40-like, *bla*OXA-58-like and an intrinsic *bla*OXA-51-like[(10,11)]. In Libya, limited numbers of epidemiological studies concerning *A. baumannii* have been reported[(7,8)]. Such information is important in guiding clinicians to select the best alternative drug(s) to treat serious infections associated with carbapenem resistant *A. baumannii*. The aim of this study was to characterize *A. baumannii* molecular epidemiology in Tripoli-Libya.

MATERIALS AND METHODS

Identification and antibiotic susceptibility testing of isolates

During 2013-2014, Specimens were collected from different anatomical sites including (urine, stool, sputum, cerebro spinal fluid, blood), swabs (wound exudates, ear, throat, rectal, axilla, nasal), endotracheal tube tip, central line tube, urine catheter, execration in naso gastric tube. All specimens were taken as part of the clinical workup was included in this laboratory-based surveillance study. Demographic data, age, gender of the patients, in/out patients, department, and type of specimens were recorded from four major teaching hospitals: Tripoli Medical Centre (TMC); Tripoli Pediatric Hospital (TPH); Burn and Plastic Surgery Hospital (BPSH) and Tripoli Central Hospital (TCH). All isolates were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by the BD Phoenix Automated Microbiology System (USA) according to the manufacturer's instructions. *A. baumannii* isolates that showed resistance to at least three classes of antibiotics such as fluoroquinolones, aminoglycosides, and cephalosporins were defined as multidrug resistant (MDR) in accordance to the definitions provided by Magiorakos and colleagues[(12)].

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Phenotypic detection of carbapenem-hydrolysing oxacillinases

Carbapenem resistance determinants were studied phenotypically using two different techniques: chromogenic culture media, this screening medium (Chromatic CRE) used for detection carbapenem-resistant *Enterobacteriaceae* and non-fermentative Gram negative bacilli (Liofilchem, Italy) and E-test (Liofilchem, Italy) according to manufacturer's instructions and as previously described[13].

Pseudomonas aeruginosa ATCC 27853 and *Escherichia coli* ATCC 25922 were used as controls for susceptibility testing. In this investigation, specimens were collected under approved ethical standards and the study was reviewed and approved by the Faculty of Pharmacy, University of Tripoli and hospitals participating in this study.

Molecular detection of *bla*$_{OXA23}$ and *bla*$_{OXA51}$ genes

Polymerase chain reaction (PCR) amplification was used to determine the presence of carbapenem-hydrolysing oxacillinases *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ genes among isolates. The primers used for PCR amplification of the carbapenemase genes are listed in (table 1).

(Table 1) Primers used in the amplification of selected carbapenemase genes

Name	Nucleotide sequence (5′ → 3′)	Product size (bp)	Location
OXA-23-like	F-GATGTGTCATAGTATTCGTCGT R- TCACAACAACTAAAAGCAC-TGT	1064	*bla*OXA-23
OXA-51-like	F- TAATGCTTTGATCGGCCTTG R- TGGATTGCACTTCATCTTGG	353	*bla*OXA-51

All isolates were screened for the presence of genes encoding *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ by PCR using previously reported primers[14]. The plasmids were isolated using the QIAGEN Plasmid Mini Kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions. The reaction mixture contained a total of 25 µl: 5 µl of 5X Red Load Taq Mix composed of Taq Polymerase, 0.05 u/µl dNTPs (200 µM) (dATP, dCTP, dGTP, dTTP) reaction buffer with KCl and MgCl2 (1.5 mM) red dye, gel loading buffer, stabilizers (Metabion, Martinsried- Germany); 0.5 µl of 10pmol/µl of each primer, 2-50ng of the extracted plasmid DNA. The thermal profile included one cycle of initial denaturation at 95ºC for 2 min followed by 35 cycles at 95ºC for 30 sec, annealing at 52ºC for 30 sec, and extensions at 72ºC for 45 sec. The PCR reaction was carried out with TC-412 thermocycler (Techne, Duxford, Cambridge, U.K.). Five µl of the PCR amplification products were electrophoresed in agarose (2% m/v) containing 0.5 ug/mL ethidium bromide.

The amplified PCR products were visualized under UV light and electronically documented with a gel documentation system (MultiDoc-It Digital Imaging System UVP, Cambridge, UK). A 100bp DNA ladder (Metabion, Martinsried- Germany) was used as a molecular size marker.

RESULTS

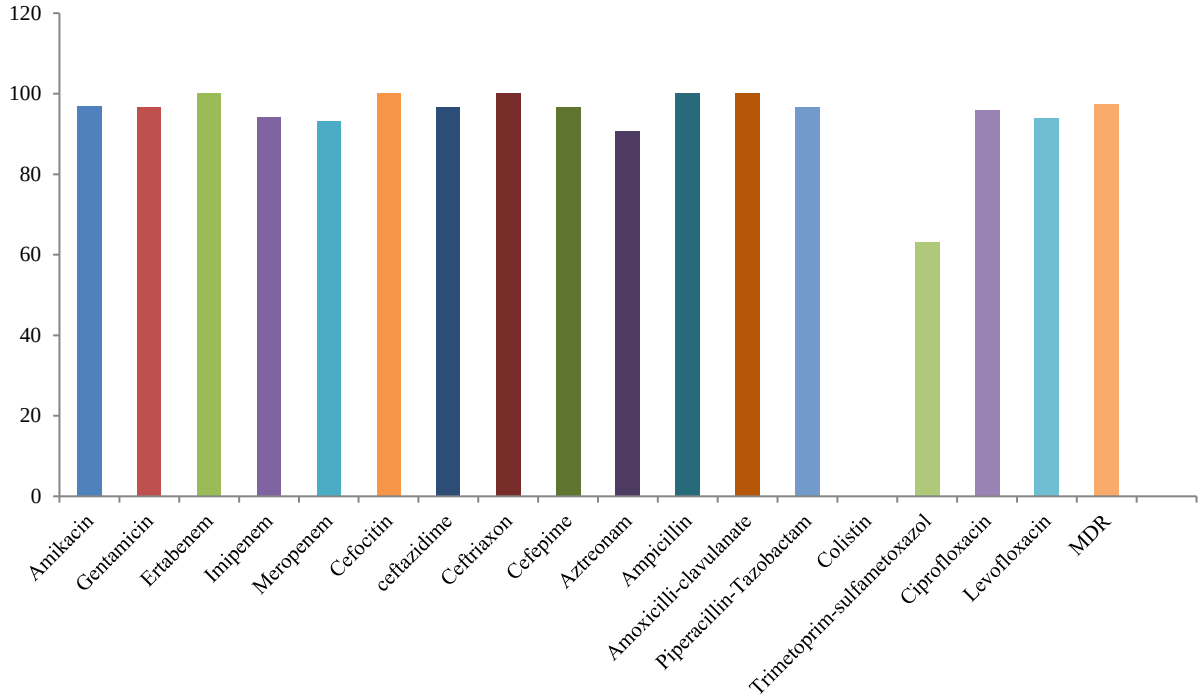
A total of 119 isolates *A. baumannii* isolates were characterized, the majority was isolated from patients at BPSH (57; 48%) and TMC (51; 42.8%) compared with other hospitals; TPH (6; 5%) and TCH (5; 4.2%). A 98.2% of strains were isolated from in-patients specimens primarily from burn wounds (61.5%) and less frequently from tips (ETT and central line etc.) (16.5%); blood (8.2%) and urine (5.5%). Most (85.3%) were obtained from patients hospitalized in ICUs (burn, neonatal, surgical etc.) and the remaining from patients housed in other hospital sectors.

Overall the resistance prevalence was extremely high for aminoglycosides (79-96.6%), fluoroquinolones (94-96%), cephalosporins (96.6-100%) and carbapenemes (93.2-100%), all isolates tested were susceptible to colistin . Over ninety percent of isolates showed resistance to imipenem and meropenem and exhibited minimum inhibitory concentration (MIC) >8µg/ml. In addition, 97.5% of isolates were identified as MDR (figure 1). Varying degree of phenotypic detection of carbapenemes was determined; highest levels of carbapenemes were detected using chromogenic media (76.5%) compared with E-test (45.4%) (Table 2).

(Table 2) Phenotypic and genotypic detection of carbapenem resistant *A. baumannii*

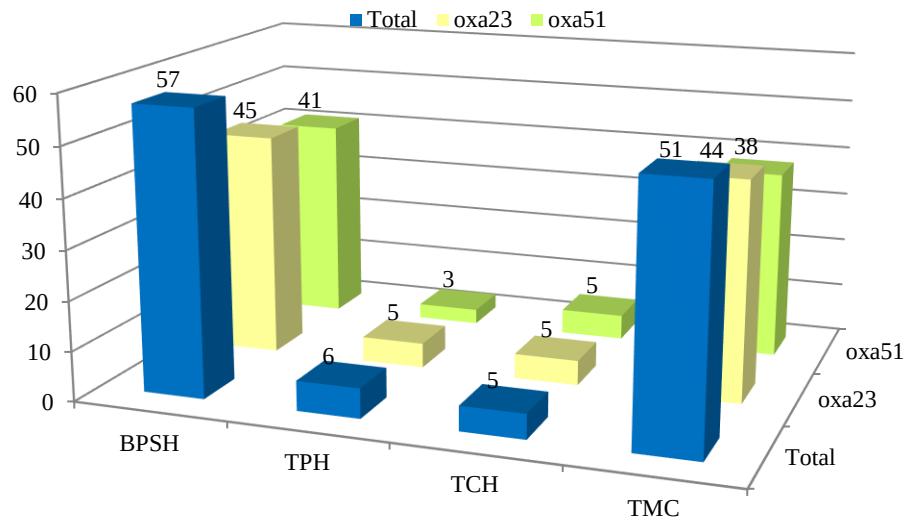
Isolate	phenotype		Genotype	
A. Baumannii	E test MBL No (%)	Chromogen media No (%)	OXA-23 No (%)	OXA-51 No (%)
	54 (45.4)	91 (75.5)	100 (84)	87 (73.1)

High level of carbapeneme resistance-encoding genes were detected *bla*$_{OXA23}$ (84%) and *bla*$_{OXA51}$ (73.1%). *A. baumannii* harboring carbapeneme resistance-encoding genes were mainly detected in ICUs (93/119; 78.1%); the highest was demonstrated in TCH (5/5; 100%) and BPSH (42/57; 73.7%) (Figure 2). The co-occurrence of *bla*$_{OXA23}$ and *bla*$_{OXA51}$ were demonstrated in (75/119; 63%) showing dissemination of carbapenemes resistance MDR *A. baumannii* in hospitals.



(Figure 1) Antibiotic resistance of *A. baumanni* isolated from different clinical specimens

Antibiotic	***A.Baumanniin* = 119 (%)**
Amikacin	94 (79)
Gentamicin	115 (96.6)
Ertapenem	119 (100)
Imipenem	112 (94.1)
Meropenem	111 (93.2)
Cefoxitin	119 (100)
Ceftazidime	115 (96.6)
Ceftriaxone	119 (100)
Cefepime	115 (96.6)
Ciprofloxacin	114 (96)
Levofloxacin	112 (94)
Amoxicillin-clavulanate	119 (100)
Piperacillin-Tazobactam	106 (89.1)
Colistin	0 (0)
Trimetoprim-sulfametoxazol	73 (61.3)
Aztreonam	119 (100)
Ampicillin	119 (100)
MDR	116 (97.5)
ESBL	115 (96.6)



(Figure 2) *A. baumannii* harbouring carbapenem resistance-encoding genes in four Tripoli hospitals.

DISCUSSION

The widespread of carbapenem-resistant *A. baumannii* constitutes a global public health threat. Molecular characterization of mechanisms and epidemiology of MDR is a remarkable step to monitor its spreading and develop therapeutic strategies. Over ninety percent of *A. baumannii* strains collected from four hospitals in Tripoli were resistant to imipenem and meropenem, higher than that previously reported in Libya[15]. Carbapenem resistance has rapidly increased worldwide and prevalence of imipenem-resistant strains has reached 100% in some countries[16].

All our imipenem-resistant strains were MDR, as commonly reported worldwide[17,18], this is may be inherent to the accumulation of mutations selected by various antibiotics before introduction of carbapenems, and to the multiple mechanisms of carbapenem resistance in *A. baumannii* conferring simultaneous resistance to antibiotics of other classes[19]. Overall, MDR *Acinetobacter* strains remain susceptible to colistin. Besides imipenem, it also should be noted the very high prevalence (100%) of aztreonam resistance in our strains. This antibiotic, which normally is not or weakly hydrolyzed by OXA-carbapenemases types, constitutes a therapeutic solution in combination with a large broad spectrum serine beta-lactamases inhibitor[20]. In agreement with our study, the prevalence of trimethoprim/sulfamethoxazole resistance (61.3%) in *A. baumannii* is high in many geographic regions[21]. Trimethoprim resistance in *Acinetobacter* can be related to housekeeping *dfr* genes and to efflux systems[22,23].

Carbapenem resistance was investigated by phenotypic and genotypic tests of all strains. Although few *A. baumannii* strains were found to be positive by phenotypic tests used in the study, while carbapenem resistance genes were not detected, this might be adequately explained by the fact only two genes associated with carbapenems were investigated. Different studies have reported positive results by MBL phenotypic tests, but MBL resistance genes that could not be identified in *Acinetobacter* strains. Suggesting that carbapenem resistance genes, which are common in the region, should be investigated to evaluate the phenotypic test results correctly[24].

Carbapenem resistance in *A. baumannii* is most often associated with class D β-lactamases (OXA-23-like, OXA-40-like and OXA-58-like). OXA-23-like is the most prevalent of carbapenamases with a global distribution and was described as cause of nosocomial outbreaks[25-27]. We found that OXA-23-type was the major (84%) carbapenemase mechanism responsible for the resistance phenotype. This finding is similar to data previously reported from the Gulf region[28,29] and our region (Egypt, Algeria and Tunis)[30-33]. Class D carbapenemases blaOXA-23 was identified in 72%, 72.5%, 67.02% and 90% of studied carbapenem-resistant *A. baumannii* strains in two Egyptian centres[34], Saudi Arabia[35], Algeria[19] and Lebanon[13], respectively. Hammoudi and co-workers suggested that the predominance and dissemination of OXA-23 in Lebanon is consistent with the worldwide epidemiology of OXA-23 and with reports from neighboring countries[13]. It is frequently detected in isolates from Asia and Europe, and in most cases, it is found concomitantly with the *bla*OXA-51-like gene[36,37]. In this study, the co-occurrence of bla_{OXA23} and bla_{OXA51} gene was detected in 63%; 75/119 of isolates. Through molecular methods this study has indicated that 73.1% of the *A. baumannii* isolates contained the OXA-51 gene. Although it is clear that blaOXA-51-like genes are present in at least the vast majority of isolates of *A. baumannii*, there has been some debate as to whether they are present in all isolates of this species[38].

CONCLUSION

The high prevalence of OXA-23 and OXA-51 among *A. baumannii* contributes to antibiotic resistance in Libyan hospitals with a great potential for spread in ICUs, warrants the attention of a nationwide surveillance programme to contain the spread, and represents the high incidence of the association of these two carbapenemases in an autochthonous MDR *A. baumannii* indicating that there is a longstanding infection control problem in these hospitals and emergence of MDR GNB harboring genes coding for carbapenemases will undoubtedly limit the use of carbapenems in treating serious infectious in the country and also in the nearby countries.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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