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Early detection of metallo-β-lactamase NDM-1- and OXA-23 carbapenemase-producing *Acinetobacter baumannii* in Libyan hospitals

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ABSTRACT

Acinetobacter baumannii is an opportunistic pathogen causing various nosocomial infections. The aim of this study was to characterise the molecular support of carbapenem-resistant *A. baumannii* clinical isolates recovered from two Libyan hospitals. Bacterial isolates were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Antibiotic susceptibility testing was performed using disk diffusion and Etest methods, and carbapenem resistance determinants were studied by PCR amplification and sequencing. Multilocus sequence typing (MLST) was performed for typing of the isolates. All 36 imipenem-resistant isolates tested were identified as *A. baumannii*. The bla_{OXA-23} gene was detected in 29 strains (80.6%). The metallo-β-lactamase bla_{NDM-1} gene was detected in eight isolates (22.2%), showing dissemination of multidrug-resistant (MDR) *A. baumannii* in Tripoli Medical Center and Burn and Plastic Surgery Hospital in Libya, including one isolate that co-expressed the bla_{OXA-23} gene. MLST revealed several sequence types (STs). Imipenem-resistant *A. baumannii* ST2 was the predominant clone (16/36; 44.4%). This study shows that NDM-1 and OXA-23 contribute to antibiotic resistance in Libyan hospitals and represents the first 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.

1. Introduction

Acinetobacter baumannii is an opportunistic pathogen that is being increasingly reported as the cause of nosocomial infections [1–4]. It is associated with a wide range of clinical complications, such as pneumonia, septicaemia, urinary tract infection, wound infection and meningitis, particularly in immunocompromised patients [5]. Carbapenems have been the mainstay of treatment for *Acinetobacter* infections for the past decade, but the emergence of carbapenem

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resistance is increasingly being documented worldwide in *A. baumannii* isolates leading to limited therapeutic options [1]. Several mechanisms are responsible for resistance of *A. baumannii* to carbapenems, including reduced outer membrane permeability, penicillin-binding protein alterations and, mostly, the production of carbapenemases [6]. Three types of enzymes capable of hydrolysing carbapenems have been reported in *A. baumannii*, belonging to class A (bla_{GES-14} and bla_{KPC}), class B (bla_{IMP}, bla_{VIM}, bla_{SIM-1} and bla_{NDM}) and class D ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$, $bla_{OXA-104}$, $bla_{OXA-143}$, $bla_{OXA-164}$ and $bla_{OXA-182}$) [6]. Outbreaks of carbapenem-resistant *A. baumannii* strains have been documented in diverse geographical areas including Europe, South America and Asia [6–11], but little information is available from North Africa [12–14]. 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 in a previous study in 2015 [15]. Unlike oxacillinase genes, the New Delhi metallo-β-lactamase 1 (NDM-1), one of the most recently discovered metallo-β-lactamases (MBLs) among various Gram-negative species including *A. baumannii*, has never been reported in Libya.

The aim of this study was to investigate the prevalence and to identify the molecular mechanism of carbapenem resistance in clinical imipenem-resistant *A. baumannii* strains collected in two Libyan hospitals located in the capital, Tripoli. Here we present the first report documenting the detection of multidrug-resistant (MDR) *A. baumannii* producing NDM-1 MBL and OXA-23 carbapenemase in autochthonous Libyan strains.

2. Materials and methods

2.1. Bacterial isolates

A total of 36 non-duplicate imipenem-resistant *A. baumannii* isolated in two Libyan hospitals [24 from Tripoli Medical Center (TMC) and 12 from the Burn and Plastic Surgery Hospital (BPSH) in Tripoli] isolated between January 2015 and May 2015 were identified using a BD Phoenix™ System (BD Diagnostics, Franklin Lakes, NJ) and a matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) method (Microflex; Bruker Daltonics, Bremen, Germany) and were confirmed using PCR amplification and sequencing of the intrinsic $bla_{OXA-51-like}$ gene.

2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility was determined on Mueller–Hinton agar (Oxoid Ltd., Basingstoke, UK) using the standard disk diffusion test as described by the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (http://www.sfm-microbiologie.org/). Twenty antibiotics were tested, including ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefotaxime, cefepime, aztreonam, sulbactam, amikacin, tobramycin, gentamicin, ciprofloxacin, rifampicin, trimethoprim/sulfamethoxazole, ertapenem, meropenem, imipenem, minocycline, tigecycline and colistin (Bio-Rad, Marnes-la-Coquette, France). The minimum inhibitory concentration (MIC) of imipenem was determined using the Etest method (AB BIODISK, Askim, Sweden). The results of antibiotic sensitivity testing were interpreted according to the CA-SFM breakpoints.

2.3. Carbapenemase assays

Imipenem-resistant isolates were screened for carbapenemase production using the modified Hodge test, the modified Carba NP test and the ethylene diamine tetra-acetic acid (EDTA) test as described previously [16–18].

2.4. Detection of antibiotic resistance genes

Real-time PCR and conventional PCR were performed to screen for the presence of bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-24}, bla_{NDM-1}, bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{GIM} genes.

2.5. DNA sequencing

Positive PCR products were sequenced using BigDye® Terminator chemistry on an ABI 3730 Automated Sequencer (Applied Biosystems, Foster City, CA). The sequences obtained were analysed using BlastN and BlastP against the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) and using ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) (http://en.mediterranee-infection.com/article.php?laref=283&titre=arg-annot-).

2.6. Molecular epidemiology

Sequence types (STs) and the clonal relatedness of the isolates were determined by multilocus sequence typing (MLST) using the internal fragments of seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB*) according to the schemes available at Institut Pasteur's MLST website (http://www.pasteur.fr/mlst).

3. Results

All 36 isolates (24 from TMC and 12 from the BPSH) were identified as *A. baumannii* both by Phoenix and MALDI-TOF/MS (score values >2.3 for all strains). Among these strains, 8 (22.2%) were isolated from the burn intensive care unit (BICU), followed by 6 (16.7%) from the medical intensive care unit (MICU). The majority of *A. baumannii* isolates were recovered from wounds (15/36; 41.6%). The results of antibiotic susceptibility testing demonstrated high-level resistance to all antibiotics tested (>72%) except minocycline, tigecycline and colistin (Table 1). All isolates showed high MICs for imipenem (>32 μg/mL) and were positive in the modified Hodge test, suggesting carbapenemase production. Moreover, β-lactamase activity was inhibited by EDTA in seven A. *baumannii* (three strains from TMC and four strains from BPSH), indicating the probable production of class B MBL.

Table 1
Antimicrobial resistance of carbapenem-resistant *Acinetobacter baumannii* isolated from two hospitals in Libya.

Antimicrobial agent	% resistant		
	TMC ($n = 24$)	BPSH ($n = 12$)	Total ($n = 36$)
Ticarcillin	100	100	100
Ticarcillin/clavulanic acid	100	100	100
Piperacillin/tazobactam	100	100	100
Ceftazidime	100	100	100
Cefotaxime	83	66	77
Cefepime	100	100	100
Aztreonam	79.1	58.3	72.2
Sulbactam	95.8	100	97.2
Amikacin	87.5	83.3	86.1
Rifampicin	58.5	91.6	77.7
Trimethoprim/sulfamethoxazole	83.3	91.6	86.1
Tobramycin	100	100	100
Gentamicin	100	100	100
Ciprofloxacin	100	100	100
Ertapenem	100	100	100
Meropenem	100	100	100
Imipenem	100	100	100
Minocycline	0	0	0
Tigecycline	0	0	0
Colistin	0	0	0

TMC, Tripoli Medical Center (Tripoli); BPSH, Burn and Plastic Surgery Hospital (Tripoli).

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