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Genomic mapping of ST85 bla_{NDM-1} and bla_{OXA-94} producing *Acinetobacter baumannii* isolates from Syrian Civil War Victims

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ABSTRACT

Objectives: The rapid emergence of carbapenem-resistant *Acinetobacter baumannii* is a global health concern. A comparative genomic analysis was performed on two ST85 *A. baumannii* strains harboring bla_{NDM-1} and bla_{OXA-94} collected in Lebanon from Syrian Civil War victims.
Methods: Genome sequencing data of ACMH-6200 and ACMH-6201 were used for *in silico* extraction of multilocus sequence types (MLST), resistance genes, and virulence factors. Plasmids were genetically mapped *in silico* and using PCR-based replicon typing (PBRT). The genetic environment of bla_{NDM-1} and bla_{OXA-94} was determined, and whole-genome single nucleotide polymorphism (wgSNP) analysis in comparison with 41 publicly available *A. baumannii* genomes was performed.
Results: Tn*125* carrying bla_{NDM-1} was truncated by the insertion of IS*Aba14* downstream of *dct*, generating ΔTn*125*. bla_{OXA-94} was upstream of IS*Aba13* and IS*Aba17*. Resistance to ceftazidime could be attributed to AmpC cephalosporinase encoded by bla_{ADC-25}, and to bla_{NDM-1} on plasmids. GyrA (S83L) and ParC (S80L) substitutions conferred resistance to fluoroquinolones. wgSNP analysis separated the isolates based on their sequence types.
Conclusions: The role of refugees in the transmission of antimicrobial resistance in developing countries is understudied. As such, this study sheds light on the correlation between population mobility and the importation of drug-resistant pathogens. It also highlights the manifold mechanisms underlying antibiotic resistance in *A. baumannii*.

Introduction

Multidrug-resistant (MDR) *Acinetobacter baumannii* is a major cause of hospital-acquired infections, with the pan drug-resistant strains making *A. baumannii* infections virtually untreatable (Durante-Mangoni et al., 2014). *Acinetobacter spp* are strictly aerobic, non-motile bacilli normally found in water and soil. They are isolated as commensal organisms from skin, throat, and different discharges of healthy individuals (Peleg et al., 2008). Pathogen infection is particularly notorious in burns, open wounds, and trauma patients and in patients requiring mechanical ventilation (Davis et al., 2005). *A. baumannii* is responsible for up to 10% of hospital-acquired infections, increasing mortality rates by up to 70% (Peleg et al., 2008). Infections caused by *A. baumannii* have been reported in military personnel suffering from war-related trauma in Iraq and Afghanistan (Scott et al., 2007).

A. baumannii can be divided into eight worldwide clonal lineages (Zarrilli et al., 2013). These include three groups involved in epidemics worldwide referred to as European clones I–III (Zarrilli et al., 2013). The worldwide clonal lineages correspond to three multilocus sequence type (MLST) clonal complexes (CCs), each consisting of a central genotype and a few single locus variants (Diancourt et al., 2010). Other major CCs have been identified recently, including the 'pan-American clone' (Zarrilli et al., 2013).

The amplitude of mobile genetic elements (MGEs), such as plasmids, insertion sequences (ISs), transposons, and pathogenicity islands, contributes to the rapid development of multi-drug resistance in *A. baumannii* (Peleg et al., 2008). Plasmids in *A. baumannii* represent powerful routes for the transfer and

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evolution of antimicrobial resistance (Towner et al., 2010). These are very diverse and have recently been described as 'enigmatic' (Lean and Yeo, 2017). Thus, an in-depth molecular characterization of the genetic environment surrounding resistance genes on plasmids is important to understand the evolution of carbapenem-resistant *A. baumannii* (CRAB).

Carbapenem resistance in *A. baumannii* is attributed to the production of oxacillinases (OXAs) and less frequently to metallo-β-lactamases (Poirel and Nordmann, 2006). The first is linked to four main OXA groups: the chromosomally encoded OXA-51-like group and three acquired groups, OXA-23-like, OXA-40-like, and OXA-58-like (Poirel and Nordmann, 2006; Zarrilli et al., 2013). OXA-143 and OXA-235 have also been identified in *A. baumannii* (Poirel and Nordmann, 2006; Higgins et al., 2013). The latter is attributed to New Delhi metallo-β-lactamase 1 (NDM-1), encoded by $bla_{\text{NDM-1}}$ (Yong et al., 2009).

$bla_{\text{OXA-94}}$ has previously been identified in *A. baumannii* 85 (Pournaras et al., 2014). In Lebanon, $bla_{\text{OXA-94}}$ associated with ST85 has been detected in *A. baumannii* from Syrian patients (Rafei et al., 2014) and in non-human sources such as chicken (Rafei et al., 2015). ST6, a single locus variant of ST85 belonging to CC85, has also been identified among Lebanese patients (Rafei et al., 2014). Moreover, other studies have also reported the NDM-1-producing ST85 *A. baumannii* clone being recovered from Syrian refugees in Turkey (Heydari et al., 2015) and in France from patients hospitalized in Algeria, Tunisia, and Egypt (Bonnin et al., 2013; Decousser et al., 2013). Accordingly, the spread of NDM-1-producing ST85 *A. baumannii* poses a major health concern, especially in light of its spread through population migration (Soto, 2009).

Previously, Rafei et al. (2014) analyzed the pulsed-field gel electrophoresis (PFGE) patterns, sequence types (STs), and resistance profiles of four $bla_{\text{NDM-1}}$-producing *A. baumannii* isolated in Lebanon from Syrian patients wounded during the Syrian Civil War and revealed the presence of a unique $bla_{\text{OXA-94}}$ variant (Rafei et al., 2014). A corresponding genome announcement of two of these isolates was also published (Tokajian et al., 2016). The present study was conducted to complement what has been done previously, by revealing the repertoire of plasmids involved in the transmission of $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-94}}$, in order to better understand the genetic environment of carbapenem resistance in ACMH-6200 and ACMH-6201, in an attempt to limit spread and transmission.

Materials and methods

This study was approved by the Ethics Committee of Azm Center under authorization number 07/2012.

The two *A. baumannii* isolates studied were obtained from civilians wounded in 2012 during the Syrian Civil War while being treated in the government hospital of Kobbe, Tripoli, in northern Lebanon.

Antimicrobial testing

Antimicrobial susceptibility testing by disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing recommendations (http://www.eucast.org), was performed for imipenem, meropenem, doripenem, ceftazidime, ciprofloxacin, gentamicin, amikacin, colistin, tigecycline, rifampicin, and doxycycline, as described previously by Rafei et al. (2014).

bla_{NDM-1} *and* bla_{OXA} *PCR*

Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen), according to the manufacturer's instructions. $bla_{\text{NDM-1}}$ PCR was performed on the extracted plasmid DNA using NDM-F (5′-GGTTTGGCGATCTGGTTTTC-3′) and NDM-R (5′-CGGAATGGCTCATCACGATC-3′) primers under conditions described previously by Nordmann et al. (2011). bla_{OXA} PCR was performed as described previously (Tokajian et al., 2018).

Plasmid typing

PCR-based replicon typing (PBRT) of resistance plasmids in the isolates was performed using 19 primer pairs in six multiplex reactions based on the *A. baumannii* (AB-PBRT) scheme, according to Bertini et al. (2010).

Conjugation experiments

Conjugation was performed as described previously (Karthikeyan et al., 2010). Putative transconjugants were grown on 2 mg/l meropenem-containing Mueller–Hinton medium.

DNA isolation and genome sequencing

Genomic DNA (gDNA) was used as input for library preparation using the Illumina Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). The kit was used to simultaneously fragment and tag the library, as per the manufacturer's instructions. The library was normalized by bead-based affinity and then sequenced using the MiSeq v3 600-cycle Kit (Illumina) to perform 300-bp paired-end sequencing on the MiSeq instrument (Illumina), according to the manufacturer's instructions.

Genome assembly

Genome assembly was performed de novo using an A5-MiSeq assembly pipeline that automates data cleaning, trimming, error correction, contig assembly, scaffolding, and quality control with default parameters (Tritt et al., 2012).

Genome analysis

The assembly was uploaded and annotated using the RAST server (http://rast.nmpdr.org) (Larsen et al., 2012). The numbers of tRNAs and rRNAs were identified using ARAGORN v1.2.36 (Laslett and Canback, 2004) and the RNAmmer Prediction Server 1.2 (Lagesen et al., 2007), respectively. Resfinder was used for resistance gene identification with a threshold of 98.00% identity (Zankari et al., 2012). Amino acid substitutions conferring resistance to fluoroquinolones in the quinolone resistance-determining region (QRDR) were determined by comparing the sequences of GyrA and ParC with reference GenBank protein sequences of GyrA (GenBank accession number **CAA57655**) and ParC (GenBank accession number **CAA65085**), respectively, produced by quinolone-susceptible isolates as controls (Vila et al., 1997). Protein sequence alignment was performed using the EMBOSS Needle tool available on the EMBL-EBI website (http://www.ebi.ac.uk/Tools/). MLSTs were determined using the MLST 1.8 server (Larsen et al., 2012) available on the Center for Genomic Epidemiology website (http://www.genomicepidemiology.org) for two different MLST schemes. eBurst was used to determine the CCs (Spratt et al., 2004). PHAST was used to determine putative phage sequences (Zhou et al., 2011). IslandViewer 3 was used to identify putative genomic islands (Dhillon et al., 2015). ISs were identified and annotated using IS-finder (Siguier et al., 2006). The virulence factors database (VFDB) was used to detect the presence of virulence factors in the isolates by performing a BLAST search