ORIGINAL ARTICLE

Emergence of multidrug-resistant *Acinetobacter baumannii* producing OXA-23 Carbapenemase in Qatar

J.-M. Rolain[1], L. Loucif[1,3], M. Al-Maslamani[2], E. Elmagboul[2], N. Al-Ansari[2], S. Taj-Aldeen[2], A. Shaukat[2], H. Ahmedullah[2] and M. Hamed[1,2]

1) *Unité de Recherche sur les Maladies infectieuses et Tropicales Emergentes (URMITE), UM63, CNRS7278, IRD 198, INSERM 1905, IHU Méditerranée Infection, Faculté de Médecine et de Pharmacie, Université de la méditerranée, Marseille, France,* 2) *Hamad Medical Corporation, Department of Microbiology and Infectious Diseases, Doha, Qatar and* 3) *Laboratoire de biotechnologie des molecules bioactives et de la physiopathologie cellulaire, Université El Hadj Lakhdar, Batna, Algeria*

Abstract

The objective of our study was to describe the molecular support of carbapenem resistance from randomly selected clinical isolates of multidrug-resistant (MDR) *Acinetobacter baumannii* as a pilot study from the Hamad Medical Corporation (HMC), Qatar. Results of our report will be used to study carbapenemases using molecular techniques in all isolated MDR *A. baumannii*. Forty-eight MDR *A. baumannii* were randomly selected from isolates preserved at HMC. Identification of all isolates was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Antibiotic resistance was tested phenotypically by Phoenix and confirmed by Etest. The molecular support of carbapenemases (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{NDM}) was investigated by real-time PCR. The epidemiologic relatedness of the isolates was verified by phylogenetic analysis based on partial sequences of *CsuE* and bla_{OXA-51} genes. All 48 isolates were identified as *A. baumannii* and were confirmed to be resistant to most antibiotics, especially meropenem, imipenems, ciprofloxacin, levofloxacin, amikacin, gentamicin and most of the β-lactams; they were sensitive to colistin. All the isolates were positive for bla_{OXA-23} and negative for the other tested carbapenemase genes. Clonality analysis demonstrated that different lineages were actually circulating in Qatar; and we suggest that an outbreak occurred in the medical intensive care unit of HMC between 2011 and 2012. Here we report the emergence of MDR *A. baumannii* producing the carbapenemase OXA-23 in Qatar.

Keywords: *Acinetobacter baumannii*, carbapenemase bla_{OXA-23}, clonality analysis, multidrug-resistant bacteria, Qatar
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Corresponding author: M. Hamed, Al Wakra Hospital, Hamad Medical Corporation (HMC), DLMP, Microbiology, Al Wakra, Qatar
E-mail: mhamed@hamad.qa

Introduction

Acinetobacter species are strictly aerobic nonfermenting Gram-negative coccobacilli if in the inactive phase or bacilli if in the rapid-growth phase. The *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex comprises *A. baumannii, A. pittii, A. nosocomialis, A. lwoffii* and the environmental species *A. calcoaceticus*. Common commercial tests cannot differentiate among these species, as they share the same phenotypic tests. For daily microbiology work, speciation adds clinical value, as *A. baumannii* is the only species in this genus that is clinically important [1,2].

For epidemiologic studies, it is important to know the identity of the main strains or clones of the same species causing infections, especially in outbreak investigations, by determining virulence or resistance genes using genotypic studies [3,4]. Different genotypic tests have been used to study the relatedness of the *Acinetobacter* isolates to learn the sources of outbreaks and epidemics and the modes of intrahospital or regional transmission. These tests include PCR-based methods, mainly pulsed-field gel electrophoresis (PFGE), amplified

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fragment-length polymorphism, PFGE followed by further subtyping using variable number of tandem repeat loci and finally multilocus sequence typing (clonality analysis), which is considered a powerful and discriminatory tool [1,5,6].

Mechanisms of carbapenem resistance in *A. baumannii* are mainly due to the production of carbapenemases, especially OXA-type carbapenem-hydrolyzing (class D) β-lactamases, which are either chromosomally located, like bla_{OXA-51}, which become expressed only when the insertion sequence ISAba1 element is inserted upstream of the gene, or acquired, mostly bla_{OXA-23}-like, bla_{OXA-24}-like and bla_{OXA-58}-like subfamilies, and metallo-β-lactamases (class B; bla_{IMP}, bla_{VIM}, bla_{NDM}) [7]. Rapid diagnosis of resistance helps clinicians adequately manage infection, contain the spread of infection and decrease the toxic adverse effects of antimicrobial drugs due to multiple trials of empirical treatment.

Qatar's population structure is complex and unique: approximately 93% of the population comprises expatriates coming from different countries, mainly other Arab nations and the Indian continent (http://www.gsdp.gov.qa/portal/page/portal/ppc/PPC_home/ppc_news/ppc_files_upload/populations_status_2012_en.pdf). The expatriate workforce is kinetic; many people travel frequently—more than once a year—to their home countries. This continuous travelling facilitates the transfer of many resistant bacteria. If one of these multidrug-resistant (MDR) organisms such as *A. baumannii* gained access to the hospital, it is not easy to eliminate it, as the hospital environment favours its growth and transmission either by colonizing or infecting healthcare workers or living within biofilms formed by the bacteria, thus protecting it from the effects of common disinfectants.

A recent study in Qatar described the mechanisms of carbapenem resistance in eight *A. baumannii* isolates [8]. Ours is the second study to describe the genetic causes of carbapenem resistance among 48 MDR *A. baumannii* isolates. The objective of our study was to determine carbapenem resistance genes in a randomly selected number of MDR *A. baumannii* from isolates stored at Hamad Medical Corporation (HMC), Qatar, and to learn whether they were genetically related. The outcome of our study will help inform a project studying the magnitude of MDR *A. baumannii* in the last few years, the common resistance genes and the dominant clones circulating in Qatar. HMC (comprising tertiary, general and continuing care hospitals) is the principal public healthcare provider for the state of Qatar.

Materials and Methods

Bacterial strains

Forty-eight MDR *A. baumannii* samples of the period 2011–2012 were randomly chosen. Isolates were taken from stored MDR isolates in the cryobank of HMC's microbiology department, which is Qatar's premier not-for-profit healthcare provider. Located in the state of Qatar, HMC manages eight hospitals (approximately 2600-bed capacity). All isolates were sent to Marseille in chocolate agar slants at room temperature.

Identification and antibacterial susceptibility testing

Forty-eight MDR *A. baumannii* were identified, and antibiotic susceptibility testing was performed by the broth microdilution method (BD Phoenix; Becton Dickinson, Franklin Lakes, NJ, USA) and confirmed for identification at URMITE (Unité des Maladies Infectieuses et Tropicales Emergentes), Marseille, France, by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) using a MS LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [9], with flex control software (Bruker) and MALDI Biotyper version 3.0. A score of $\geq$1.9 was considered positive for identification at the species level, as previously reported [9]. Confirmation of resistance by Etest (bioMérieux, Marcy l'Etoile, France) was performed. The susceptibility breakpoints used were those recommended in 2013 by the Clinical and Laboratory Standards Institute (M100-S23). Susceptibility testing by Etest was additionally done in Marseille to the following antibiotics: colistin, imipenem and sulbactam (AB Biodisk, Solna, Sweden).

The 48 isolates were collected mainly from respiratory specimens (n = 10); 11 of the patients were from the intensive care unit (ICU), two from the coronary care unit, 12 from the medical intensive care unit (MICU), one from the paediatric intensive care unit, five from the surgical intensive care unit and eight from the trauma intensive care unit (TICU) (Table 1). Twenty-seven patients had comorbidities, and 15 patients died, ten of whom were ICU patients.

TABLE 1. Demographic data of 48 patients

Characteristic	*n*
Patient age	
Adult	48
Child	1
Nationality	
Qatari	14
Indian	6
Other	29
Death	15
Length of stay	
>1 year	6
2–6 months	19
Treatment received	
Colistin	33
Tigecycline	5
Comorbidities	27
Diabetes mellitus	21
Polytrauma	6
Coinfection with *Pseudomonas aeruginosa*	17