

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

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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, imipenem, 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.

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Keywords: *Acinetobacter baumannii*, carbapenemase *bla*_{OXA-23}, clonality analysis, multidrug-resistant bacteria, Qatar

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Introduction

Acinetobacter species are strictly aerobic nonfermenting Gram-negative coccobacilli in the inactive phase or bacilli 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

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 ISAbal 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 health-care 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 ≥ 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 subactam (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 <i>Pseudomonas aeruginosa</i>	17

Molecular detection of carbapenemase-hydrolyzing oxacillinases

DNA was extracted using the EZ1 advanced XL extractor with DNA bacteria card and the EZ1 DNA Tissue Kit (Qiagen, Courtaboeuf, France) according to the manufacturer’s instructions. All 48 *A. baumannii* isolates were screened for the presence of resistance genes *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-58} and *bla*_{NDM} by real-time PCR (C1000 Thernal Cycler; Bio-Rad, Hercules, CA, USA). Amplification was performed using the following thermocycler conditions: initial denaturation step at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds and extension at 60°C for 1 minute. Negative and positive controls were included in each assay.

Clonality analysis

The 48 isolates were subjected to molecular typing by clonality analysis. Genotyping was performed using the genes *bla*_{OXA51-like} and *CsuE*, as previously described [10]. PCR amplification, sequencing and correction of gene sequences were performed as previously described [10]. For each of the two genes, the DNA sequences obtained from our isolates were aligned with those of the reference strains by Clustal W software. The phylogenetic relationship of our strains together with the

reference strains was then determined by MEGA 5 software with the Kimura two-parameter model and bootstrap analyses based on 1000 replications [11].

Results

All isolates were confirmed as *A. baumannii* by MALDI-TOF Microflex (Bruker).

All isolates were resistant to imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, sulfamethoxazole/trimethoprim, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime and ceftazidime; 39 isolates were resistant to sulbactam.

The colistin resistance rate was 0 (0/48), so treatment of these patients with colistin would be suitable unless otherwise contraindicated. All 48 isolates were positive for *bla*_{OXA-23}; none was positive for *bla*_{OXA-24}, *bla*_{OXA-58} or *bla*_{NDM}.

Clonality analysis typing revealed the existence of two predominant genotypes (Fig. 1). The first genotype was the most frequent (20 isolates), six of which were isolated from the MICU in 2011. The second most frequent genotype was isolated from ten isolates (after excluding one wrongly included

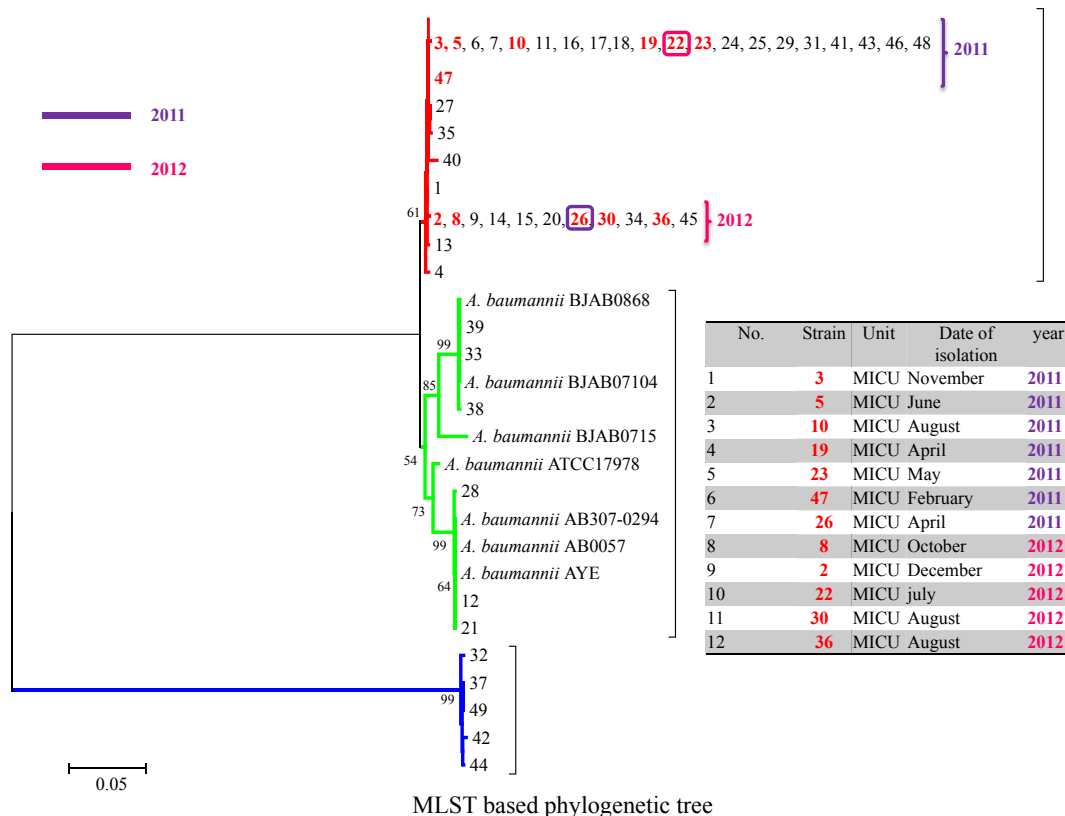


FIG. 1. Phylogenetic tree of concatenated partial sequences of *CsuE* and *bla*_{OXA-51} genes.

sample); five were from the MICU in 2012. This finding suggests the existence of an outbreak in the MICU, but because the isolates were chosen randomly, confirmation of an outbreak cannot be done except after a clonality study of all isolated *A. baumannii* during the 2 years to determine the isolation of certain genotypes from the MICU.

Discussion

MDR *A. baumannii* has emerged globally in both community and hospital settings, causing many outbreaks, especially in ICUs [12]. In our study, 28 of 48 patients were from ICUs. Resistance to carbapenems among these isolates left no options for treatment except colistin, which has many adverse effects, including nephrotoxicity and neurotoxicity which affect mainly debilitated persons [13,14]. In HMC, approximately 60% of *A. baumannii* isolated from clinically significant samples are MDR; in our study, we found 48 MDR *A. baumannii* clinical isolates which were randomly selected during the period 2011–2012 from isolates stored in the cryobank of HMC as a pilot study. In addition to describing the molecular characterization of carbapenems resistance in *A. baumannii*, we also studied the genetic relatedness of these isolates. Results of our report will be used to study all MDR *A. baumannii* isolated in the previous years to describe the magnitude of resistance in this organism and to learn which common genotypes are circulating in Qatar.

All 48 isolates were positive for *bla*_{OXA-23}. We detected no New Delhi metallo- β -lactamase (NDM) in the studied isolates even though Qatar's population structure is composed of different nationalities, including many expatriates coming from the Indian subcontinent. Studying all *A. baumannii* stored in the previous years is expected to reveal the presence of NDM. Our results are comparable with a recent study of *A. baumannii* which included seven hospitals in the Gulf Cooperation Countries, including eight isolates from Qatar, all of which were positive for *bla*_{OXA-23}, as well as many other countries in the region, including all isolates from Kuwait, Oman and United Arab Emirates and 90% of isolates from Riyadh, Saudi Arabia; 38% from Bahrain were positive for *bla*_{OXA-23} [8].

NDM was detected in many countries in the Middle East in *Enterobacteriaceae* and *A. baumannii*, including the United Arab Emirates, Oman, Saudi Arabia and Kuwait [15–18].

By comparing different studies which were conducted in the Middle East (Kuwait, Saudi Arabia, Egypt, Algeria, Yemen), no specific carbapenemase was found to be confined to the region. In Egypt, three acquired class D carbapenemases (*bla*_{OXA-23} in 72%, *bla*_{OXA-40} in 4%, *bla*_{OXA-58} in 20%) were identified among studied carbapenem-resistant *A. baumannii*

strains in two Egyptian centres [19]. In Saudi Arabia, *bla*_{OXA-23}, *bla*_{OXA-24} and *bla*_{OXA-58} were detected in 72.5, 45 and 37.5% respectively in isolated *A. baumannii* strains [20]. *bla*_{OXA-23} was detected in Riyadh and the Eastern Province, with 53 and 79.5% respectively among nonsusceptible *A. baumannii* (A. Ribeiro, paper presented at 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 2012) [21].

In the United Arab Emirates, one study found *bla*_{OXA-23}-like and *bla*_{OXA-64}-like genes in all three isolates under study [22]. In Abu Dhabi, it was found that the *bla*_{OXA-23}-like gene was detected in 73.6% of all strains included in the study [23], while the main oxacillinases in Bahrain were *bla*_{OXA-40}-like, which is a subgroup of the *bla*_{OXA-72}-like gene (5/8). Found less frequently were the *bla*_{OXA-58}-like (1/8) and *bla*_{OXA-23}-like (2/8) genes [24]. A Yemeni study identified three MDR *A. baumannii*; the *bla*_{OXA-51}-like gene and the acquired carbapenemase *bla*_{OXA-23}-like gene were detected in all three strains [25].

Clonality analysis typing revealed high diversity within our isolates, with predominance of two genotypes. The high diversity is explained by the diversity of the population of Qatar. Clonal dissemination was observed in the MICU during 2011–2012; nonclonal spread occurred in whole hospital wards and outpatient clinics.

In 1998, a Qatari study in an ICU in HMC revealed that all *A. baumannii* were carbapenem susceptible, while another study, performed between 2007 and 2008 on blood culture isolates, found carbapenem resistance in more than one third of the isolated *A. baumannii* (41.5%). In 2002, an outbreak in a TICU in HMC occurred by carbapenem-resistant *A. baumannii* which involved 20 patients admitted to the TICU during a 6-month period. A typing study was not performed at that time, but by comparing antibiograms of patient isolates to those isolated from the environment, it was found that all isolates shared the same antibiogram [26–28]. Given the occurrences of MDR *A. baumannii* in HMC, especially in the MICU, it is clear that stringent infection control measures need to be implemented. Further, studying the occurrence of all *A. baumannii* in previous years will provide insight into the presence of this organism's endemicity or epidemicity; there is also a need to screen these wards to compare the environmental clones to those isolated from patients.

In conclusion, studying molecular resistance genes and molecular the clonal relatedness provides insight into the transmission of MDR *A. baumannii* within HMC. This study has revealed the need to study all *A. baumannii* isolated in previous years to confirm the occurrence of the MICU outbreak of MDR *A. baumannii* during the 2011–2012 period and to confirm the presence or absence of other resistance genes, such as NDM or other metallo- β -lactamase resistance genes.

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Conflict of Interest

None declared.

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