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Three distinct clones of carbapenem-resistant *Acinetobacter baumannii* with high diversity of carbapenemases isolated from patients in two hospitals in Kuwait

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Summary
Objectives: This study was undertaken to investigate the clonal relatedness of multidrug-resistant (MDR) *Acinetobacter baumannii* isolates collected from patients in two teaching hospitals in Kuwait.
Materials and methods: Clinically significant consecutive isolates of *A. baumannii* obtained from patients in the Mubarak (36) and Adan (58) hospitals over a period of 6 months were studied. These isolates were identified using molecular methods, and their antimicrobial susceptibility was determined by the Etest method. The mechanism of resistance to carbapenem was investigated by PCR, and pulsed-field gel electrophoresis (PFGE) was used to determine the clonal relatedness of MDR isolates.
Results: Of the 94 isolates investigated, 80 (85.1%) were multidrug resistant (MDR). The *A. baumannii* PFGE clone A and subclone A1 were the most prevalent in patients infected with MDR isolates. Fifty-five (94.8%) and 15 (41.7%) of the MDR isolates from the Adan and Mubarak hospitals, respectively, belonged to PFGE clone A; isolates in this group showed higher resistance rates to antibiotics than isolates form other groups. Of the 94 isolates, 40 (42.6%) were resistant to either imipenem or meropenem or to both (CRAB). Most CRAB isolates (29/40 or 72.5%) carried *bla* genes, which code for MBL (VIM-2 and IMP-1) enzymes. Two isolates harbored bla_{OXA-23}.
Conclusion: Three distinct clones of CRAB were isolated, providing evidence of a high diversity of carbapenemases among our geographically related isolates.
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Introduction

Interest in *Acinetobacter baumannii* has grown rapidly over the last two decades, primarily as a result of the emergence and outbreak of multidrug-resistant (MDR) strains [1]. *A. baumannii* represents an important cause of nosocomial infections including septicemia, ventilator-associated pneumonia and urinary tract infections [2]. The bacterium is usually intrinsically resistant to multiple antimicrobial agents, and resistance to β-lactam is most commonly associated with the production of high levels of naturally produced cephalosporinases [3]. However, acquired metallo-β-lactamase (MBL) enzymes, which confer resistance to the carbapenems, have been reported [4]. Recent reports have shown that MBLs, such as PER-1, VIM-2 and IMP-1, are prevalent in Turkey and Korea and are a growing source of resistance in these countries [5,6]. The dissemination of the OXA-23 and OXA-58 carbapenemases among *A. baumannii* isolates has also been reported in hospitals in Argentina [7,8]. In addition, hospitals in the UK have experienced outbreaks with three widespread clones: the ''South East'' clone, OXA-23 clone 1 and OXA-23 clone 2; the latter two are consistently resistant to imipenem and meropenem [9,10].

Recently, infections due to MDR *A. baumannii* strains have become a serious problem in some hospitals in Kuwait, particularly the Mubarak Al-Kabeer and Al-Adan hospitals, where these isolates are the second most frequently isolated pathogens, particularly in the ICUs [11]. The Mubarak Al-Kabeer hospital is the primary teaching hospital and serves as both a referral and secondary medical center, whereas Al-Adan hospital, also a teaching hospital, is a secondary medical center, located 16 km away, in the southern part of the country. This study was designed to investigate the clonal relatedness of the carbapenem-resistant *A. baumannii* strains isolated from infected patients in these hospitals.

Materials and methods

Bacterial isolates

All consecutive clinically significant *A. baumannii* isolates collected from patients with proven infections requiring antimicrobial therapy were collected from the Mubarak Al-Kabeer hospital (MKH, Kuwait) and the Al-Adan hospital (ADH, Kuwait) between May and October 2007. Only one isolate per patient was tested, and all duplicate strains were excluded. Primary identification was performed using the API 20 NE system (BioMerieux, l'Étoile, France) and confirmed by PCR using the following primers: 5′-CTAATAATTGATCTACTCAAG-3′ ($bla_{OXA\text{-}69A}{}^{f}$) and 5′-CCAGTGGATGGATGGATAGATTATC-3′ ($bla_{OXA\text{-}69}{}^{r}$) for the $bla_{OXA\text{-}69}$ gene, a naturally occurring oxacillinase in *A. baumannii*. Isolates were serially labeled with abbreviations reflecting the hospital at which they were collected (1—58 ADH and 59—94 MKH).

Susceptibility test

Susceptibility testing of the isolates was carried out by determining the minimum inhibitory concentrations (MICs) of 11 antibiotics commonly used in our hospitals to treat gram-negative infections using the Etest (AB Biodisk, Solana, Sweden). Briefly, a bacterial suspension with a turbidity of 1.0 McFarland was prepared by inoculating a single colony of an overnight (16—18 h) pure culture of the bacterium into sterile PBS. Using a sterile disposable cotton swab soaked in the inoculum, a 150 mm Mueller-Hinton agar (Becton Dickinson, Sparks, MD, USA) plate was inoculated in three directions and spread to completely cover the entire surface. Six Etest strips of the antibiotics were placed on each plate. The inoculated plates were then incubated at 37 °C for 24 h. An inoculated plate without antibiotics was also included in each run to check for the purity of the suspension. The MIC values were estimated based on the concentration of the antibiotics at which the elliptical zone of inhibition intersected the Etest strip. The results were interpreted according to criteria suggested by the CLSI [12]. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were included in each run as controls.

Detection of metallo-β-lactamase (MBL) and oxacillinase (OXA)

Strains showing non-susceptibility to the carbapenems were screened for production of MBLs using the disk approximation test as described by Lee et al. [13] and the MBL Etest (AB Biodisk, Sweden). Detection of the $bla_{IMP\text{-}1}$, $bla_{VIM\text{-}1}$, $bla_{VIM\text{-}2}$, $bla_{SPM\text{-}1}$, $bla_{OXA\text{-}23}$, $bla_{OXA\text{-}24}$ and $bla_{OXA\text{-}58}$ genes in carbapenem-resistant isolates was performed under standard PCR conditions using previously reported primers [14—16].

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