Co-existence of $bla_{\text{OXA-23}}$ and $bla_{\text{VIM}}$ in carbapenem-resistant Acinetobacter baumannii isolates belonging to global complex 2 in a Chinese teaching hospital

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Abstract

Background: Carbapenem-resistant Acinetobacter baumannii (CRAB) have been a challenging concern of health-care associated infections. The aim of the current study was to investigate the molecular epidemiology and clonal dissemination of CRAB isolates in a Chinese teaching hospital.

Methods: Non-duplicate clinical A. baumannii isolates were collected from inpatients, and we measured the minimal inhibitory concentrations to determine antimicrobial susceptibility. Polymerase chain reaction (PCR) and sequencing were performed to detect carbapenem-resistance genes and occurrence of transposons among CRAB isolates. Moreover, the genetic diversity among isolates and clonal dissemination were determined by repetitive element PCR-mediated DNA fingerprinting (rep-PCR) and multilocus sequence typing (MLST).

Results: A total of 67 CRAB isolates displayed resistance to most of the antibiotics tested in this study, except tigecycline. We detected $bla_{\text{OXA-23}}, bla_{\text{OXA-51}},$ and $bla_{\text{VIM}}$ genes in 94.0%, 100.0%, 1.5%, and 80.6% of the CRAB isolates, respectively. Nevertheless, 74.6% of the CRAB isolates co-harbored the $bla_{\text{OXA-23}}$ and $bla_{\text{VIM}}$. Only one type of transposons was detected: Tn2008 (79.1%, 53/67). Although 12 distinctive types (A-L) were determined (primarily A type) ST195 was the most prevalent sequence type (ST). ST168, ST210, ST90, ST829, and ST136 were also detected, and all belonged to clonal complex 208 (CC208) and global complex 2 (GC2).

Conclusion: The $bla_{\text{OXA-23}}$ and $bla_{\text{VIM}}$ genes contributed to the resistance among CRAB isolates collected in our study. Notably, most of the CRAB strains co-harbored $bla_{\text{OXA-23}}$ and $bla_{\text{VIM}}$ genes, as well as Tn2008, which could contribute to clonal dissemination. The prevalence of such organisms may underlie hospital acquired infections.

Keywords: Acinetobacter baumannii; $bla_{\text{OXA-23}}$ and $bla_{\text{VIM}}$; rep-PCR; Transposons

Introduction

Acinetobacter baumannii (A. baumannii) is prevalent in the hospital environment and is the source of contamination in various clinical settings. A. baumannii has emerged as one of the most significant opportunistic pathogens of healthcare-associated infections [HAI]. Other than bacteremia and secondary meningitis, A. baumannii is responsible for numerous infections of the urinary and respiratory tract, skin, soft tissues, solid organ transplant, and burn wounds. Rapid emergence of carbapenem-resistant A. baumannii (CRAB) has been reported as a global issue. A rapid increase of HAI caused by CRAB, from 4.0% in 2003 to 62.0% in 2008 ($P < 0.0001$), was reported in Taiwan, China. A US national surveillance study conducted in 2010 reported a prevalence of 44.7% and 49.0% among A. baumannii isolates resistant to imipenem and meropenem, respectively. A dramatic increase in the mortality rate, from 16.0% to 76.0%, has been reported in cases of infections caused by CRAB compared to an increase from 5.0% to 53.0% in cases of infections caused by carbapenem susceptible pathogens. Furthermore, high mortality rates recently reported in Taiwan, China (70%), and Korea (79.8%) have been attributed to CRAB infections in the blood, whereas lower mortality (24.5%) has been observed in similar infections where imipenem susceptible A. baumannii were involved.

As previously described, carbapenemases are responsible for the acquisition of carbapenem-resistance in A. baumannii. According to the Ambler classification,
Carbapenemases mainly belong to class A, B, and D β-lactamases, and class D β-lactamases are commonly disseminated in A. baumannii, which are comprised of OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like, and OXA-23, which is the primary cause of carbapenem resistance in A. baumannii.[14,15] Another important type of carbapenemases, plasmid-mediated metallo-β-lactamases (MBLs), which hydrolyze carbapenems and all β-lactams except monobactams, were also increasingly reported in Acinetobacter spp. Presently, few reports have been published on the prevalence of MBLs in A. baumannii worldwide.[16-18]

In the current study, we investigated if two genes, bla_{OXA-23} and bla_{VIM}, were co-harbored among CRAB isolates collected from patients admitted to different clinical departments at a Chinese teaching hospital. Furthermore, the molecular epidemiology and clonal dissemination of these isolates were determined.

**Methods**

**Ethics approval**

This study was approved by the Ethics Committee of Xiangya Hospital, Changsha, China. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

**Bacterial isolates**

Non-repetitive A. baumannii isolates were collected consecutively from inpatient units between January 1st and December 31st in 2016 at Xiangya Hospital Central South University, a teaching hospital with a 3500-bed capacity located in Changsha, Hunan Province, China. Isolates were collected from different types of specimens from one patient or from the same specimen, but the interval collection time was at least 1 week. All isolates were identified by MALDI-TOF-MS (Bruker Daltonics GmbH, Bremen, Germany) and confirmed by polymerase chain reaction (PCR) by detecting 16S rRNA.[19]

**Antimicrobial susceptibility testing**

Minimal inhibitory concentrations (MICs) were measured using 10 representative antimicrobial agents, including imipenem, amikacin, ceftriaxone, piperacillin/tazobactam, ceftazidine, gentamicin, cefepime, ciprofloxacin, tigecycline, and minocycline. The MICs were determined using VITEK 2 Compact (BioMérieux, Missouri, France), except for tigecycline, which was measured using E test (BioMérieux). Pseudomonas aeruginosa ATCC 27853 and Escherichia coli (E. coli) ATCC 25922 were used as the control organisms. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[20] For tigecycline, interpretive criteria was based on the United States Food and Drug Administration, where isolates having MIC ≥8, 4, and ≤2 μg/mL were considered resistant, intermediate, and sensitive, respectively.[21]

**Phenotypic tests for MBLs production**

MBLs production was performed using the double-disc synergy test (DDST) and the imipenem-EDTA combined disc test (CDT). In the DDST method, isolates (turbidity was adjusted to 0.5 McFarland) were cultured on Mueller-Hinton agar plates as recommended by the CLSI. After drying, two discs (one disc contained imipenem [10 μg], and the other contained only 10 μL of 0.5 mol/L EDTA [Sigma Chemicals, USA]) were placed on the culture plate at 20 mm from the center of the discs. After overnight incubation, the presence of even a small synergistic inhibition zone was interpreted as positive.[22] The CDT was carried out as follows: two discs (one disc contained imipenem [10 μg] and 5 μL of 0.5 mol/L EDTA [Sigma Chemicals], and the other disc contained only imipenem [10 μg]) were placed about 20 mm apart on a Mueller-Hinton agar plate inoculated with each test strain. The result was considered positive, meaning there was MBL hydrolysis activity, when the zone diameter around the imipenem-EDTA disc was >7.0 mm of the imipenem alone disc.[23]

**PCR and sequencing of carbapenemase genes**

PCR was conducted to detect the presence of carbapenemase genes in 67 CRAB isolates. bla_{OXA-23-like}, bla_{OXA-24-like}, bla_{OXA-51-like}, bla_{OXA-58-like}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA-23-like} were detected using previously described primers.[24] All PCR products were sequenced by Sangon Biotech (Sangon Biotech, Shanghai, China) and analyzed for similarity searches with the BLAST website (https://blast.ncbi.nlm.nih.gov/blast.cgi).

**Repetitive element PCR-mediated DNA fingerprinting (rep-PCR)**

Extraction of bacterial genomic DNA and rep-PCR was performed with the GeneAmp PCR System 2720 (Applied Biosystems ABI, USA) using previously described methods. The primers were (REP1: 5'-IIIIGGCCGGCGATCGGC-3', REP2: 5'-ACGTCTTATCAGGCCTAC-3', I represents hypoxanthine).[25,26] PCR-banding patterns were analyzed and interpreted using NTsys-2.10 software (Exeter Software, Stauket, NY, USA) for rep-PCR types.[26]

**Multilocus sequence typing (MLST)**

MLST was performed for molecular typing of 67 CRAB isolates by sequencing seven housekeeping genes of A. baumannii (cpn60, fusA, gltA, pyrG, recA, rplB, and rpoB) as previously described.[27] Primer sequences are available at http://pubmlst.org/abaumannii/. The sequencing results were compared to the sequence types (STs) available on online databases at http://pubmlst.org/databases/. For investigation of genetic relationships and clonal complexes (CCs), all sequence data of the isolates included in this study were analyzed with eBURST.
Genetic environment of the blaOXA-23 gene

Genetic characterization of blaOXA-23-carrying transposons, Tn2006, Tn2007, Tn2008, and Tn2009, was conducted using PCR and sequencing with previously described primers. The presence of insertion sequence ISAba1 upstream of transposons was also confirmed by PCR using previously described primers (F: 5’-CATTGGCATATAGGAGAAA-3’, R: 5’-TTGGAAATGGGGAAAAACGAA-3’).

Results

Samples and bacterial isolates

A total of 67 CRAB isolates were collected from 67 inpatients, including 47 male and 20 female patients. Approximately 70.1% (47/67) of the CRAB isolates were identified in patients from the intensive care unit (ICU), and the remaining isolates were acquired from patients in the emergency (9.0%), burn (6.0%), pancreatic surgery (3.0%), nose and skull base surgery (3.0%), infectious diseases (3.0%), rehabilitation (3.0%), brain trauma specialist (1.5%), and cerebrovascular surgery (1.5%) departments. The proportions of specimens included in this study were 35.8% each for blood and cerebrospinal fluid, followed by 11.9%, 7.5%, 7.5%, and 1.5% for pleural effusion, tissue block, ascitic fluid, and bile, respectively, as shown in Figure 1.

Antimicrobial susceptibility testing and detection of carbapenemase genes

The MICs of 10 antimicrobial agents were determined against all clinical isolates of A. baumannii. The resistant rates of imipenem, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, ceftazidime, gentamicin, amikacin, cefepime, ciprofloxacin, and minocycline were 100.0%, 53.7%, 98.5%, 95.5%, 86.6%, 77.6%, 98.5%, 83.6%, and 67.1%, respectively. Although only two isolates showed resistance to tigecycline, 40.3% of CRAB isolates exhibited an intermediate phenotype to tigecycline. Details of isolate susceptibility are shown in Table 1.

The DDST and imipenem-EDTA CDT were used to determine the prevalence of MBLs among CRAB; 77.6% of the isolates were positive for DDST, while 76.1% were positive for CDT as shown in Figure 1.

blaOXA-23, blaOXA-51, and blaVIM genes were detected in 94.0%, 100.0%, and 80.6% of the 67 CRAB isolates, respectively, whereas only one isolate carried the blaOXA-58 gene. None of these isolates harbored blaOXA-24, blaIMP, blaVIM, and blaNDM. Notably, 74.6% of the CRAB isolates carried both the blaOXA-23 and blaVIM genes [Figure 1]. Moreover, isolates harboring blaVIM genes were positive in the DDST and CDT analysis, except for two isolates (CS24 and CS53).

Homology analysis

Based on rep-PCR results, 12 (A–L) genetically different profiles were observed among 67 CRAB isolates. The 56.6% of the isolates were linked to the type A profile. However, type A and I were further divided into three subtypes. Similarly, type H, J, and K were distributed into two subtypes, whereas type B into G and L was not divided into subtypes. A large number of isolates from each profile was derived from the ICU samples [Figure 1].

Among the 67 CRAB isolates, six STs – ST195 (41.8%), ST368 (13.4%), ST210 (3.0%), ST90 (3.0%), ST829 (11.9%), and ST136 (3.0%) – were detected by MLST. The eBURST analysis indicated that all STs belonged to the CC208 clonal complex and originated from the CC92 clonal complex, corresponding to global clone 2 (GC2) [Figure 2].

Genetic environment of the blaOXA-23 gene

Among the four common transposons tested in this study, only Tn2008 was identified in 53 isolates by PCR analysis. Nevertheless, insertion sequence ISAba1 located upstream of the transposons was characterized among all the CRAB isolates.

Discussion

A. baumannii is a significant opportunistic pathogen associated with HAIs. A. baumannii has the ability to adapt to its environment and acquire drug resistance genes from its surroundings. As such, A. baumannii can lead to multiple drug-resistant (MDR) and even extensively drug-resistant (XDR) isolates in the hospital setting. Carbapenemase production is considered the primary underlying cause of the CRAB, oxacillinase (OXA), especially OXA-23. However, MBLs are another common carbapenemase in the Acinetobacter calcoaceticus-A. baumannii complex (ACB), but few reports of MBL prevalence in A. baumannii have been found. In New Delhi in 2009, Metallo-β-lactamases (NDM, common MBL) was first discovered in Klebsiella pneumoniae. Since then, it has disseminated to other species including A. baumannii, E. coli, and Enterobacter cloacae. However, another MBL gene, blaVIM, has rarely been identified in A. baumannii. In this study, we identified MDR A. baumannii isolated in patients from different clinical departments of a teaching hospital. Moreover, we investigated the molecular characteristics and clonal dissemination of these isolates.

In the current study, three types of OXA genes were detected, including OXA-23 (94.0%), OXA-51 (100.0%), and OXA-58 (1.5%), in the CRAB isolates. The occurrence of OXA-23 was considered the main reason of carbapenem resistance in A. baumannii CRAB isolates carrying-blaOXA-23 genes are disseminated worldwide due to the transposons. Five transposons have been identified, Tn2006, Tn2007, Tn2008, Tn2008B, and Tn2009, among bacterial isolates; however, Tn2006 and Tn2008 were globally disseminated, and Tn2006 was the most prevalent transposon in China. In contrast, we identified Tn2008, rather than Tn2006, in 79.1% of CRAB isolates, in accordance with Chen et al. indicating that Tn2008 could be involved in the transmission of the blaOXA-23 gene among CRAB isolates, and also closely
Figure 1: Dendrogram based on rep-PCR profiles with clinical features and molecular characterization of carbapenemases and MLST. +, positive; –, negative; ND, not determined; Sa, specimens; RDs, resistance determinants; bl, blood; bi, bile; bv, blood vessel; ti, tissue; sf, cerebrospinal fluid; ep, empyema pleural; fn, aspirate fine-needle; E, emergency department; P, pancreatic biliary surgery; Q, cerebrovascular surgery; R, nose and skull base surgery; T, the department of rehabilitation; U, burns surgery; V, brain trauma specialist; A-L, rep-PCR types; rep-PCR: Repetitive element PCR-mediated DNA fingerprinting; MLST: Multilocus sequence typing.
related to the molecular epidemiological characteristics of the strains in this area of China. Tn2008B is the most recently identified structure containing bla\textit{OXA-23}. To date, Tn2008B has not been reported in China; however, we did not detect Tn2008B in our study. In addition, we found that IS\textit{Aba1} was located upstream of the \textit{bla\textit{OXA-23}} gene, which could facilitate the mobilization of \textit{bla\textit{OXA-23}} and provide promoters for its expression.[37] The \textit{bla\textit{OXA-51}} gene is unique to \textit{A. baumannii} and may be used as a marker to identify this species.[38] Moreover, some previous studies reported that OXA-51 carbapenemases had minimal ability to hydrolyze carbapenems when IS\textit{Aba1} was upstream of the \textit{bla\textit{OXA-51}} gene.[39] In contrast, we showed in this study that 88.9% of the CRAB isolates carried IS\textit{Aba1} upstream of the \textit{bla\textit{OXA-51}} gene. Hence, there may be contribution of OXA-51 in CRAB. Isolates carrying \textit{bla\textit{OXA-24/58}}-like genes are typically resistant to carbapenems. Fortunately, they have not been commonly identified in China. In the current study, only one isolate carried OXA-58. Our results also demonstrated that \textit{bla\textit{OXA-24}}, \textit{bla\textit{NDM}}, \textit{bla\textit{SPM}}, and \textit{bla\textit{IMP}} were not expressed in our isolates. However, due to their plasmid location, the distribution of these genes in \textit{A. baumannii} should be monitored early.

Notably, MBLs, which are rarely reported in \textit{A. baumannii}[16,17] widely existed in the CRAB isolates included in this study, and 80.6% of these CRAB isolates harbored \textit{bla\textit{VIM}} genes and produced MBLs confirmed by DDST and CDT, which have been considered the standardized methods for detecting MBL production.[40] Notably, 74.6% of the CRAB isolates co-harbored \textit{bla\textit{OXA-23}} and \textit{bla\textit{VIM}}. However, the globally disseminated NDMs, including \textit{K. pneumoniae}, \textit{A. baumannii}, \textit{E. coli}, and \textit{E. cloacae},[31] were not detected in any of the isolates included in this study.

We analyzed 67 CRAB isolates using rep-PCR, which has been widely used in molecular typing of \textit{A. baumannii}[41]. Additionally, 12 distinctive types of profiles obtained by rep-PCR indicated that genetically diversified CRAB isolates are prevalent in our teaching hospital. Three European clones (EC I, II, and III) of \textit{A. baumannii} have been reported[42] EC I (GC1) and EC II (GC2) are globally propagated.[43] Of particular interest, six STs (ST195, ST368, ST210, ST90, ST829, and ST136) were found by MLST, all of which belong to CC208 and are generally linked to GC2. Although ST195 (41.8%) was the predominant ST in the current study, ST92 is among the most prevalent CRAB isolates in China.[44] We did not detect ST92 in the current study; we detected ST208 using the Institute Pasteur scheme (compared to ST92; Oxford scheme). Actually, ST92 may not exist.[45]

![Figure 2: Identification of clonal complex by eBURST analysis of CRAB isolates. ST195, ST368, ST210, ST90, ST829, and ST136, all of which were detected in this study, belong to the CC208 clonal complex. CRAB: Carbapenem-resistant Acinetobacter baumannii.](image_url)
There are also some limitations in this study. We did not analyze the plasmid where the drug resistance gene is located. In addition, we only studied the CRAB isolated from our hospital and did not include other hospitals in our region.

In summary, bla_OXA-23, bla_OXA-51, and bla_VIM may be responsible for CRAB. ST195 was the most prevalent clonal complex found in our teaching hospital and could disseminate in this area of China. Remarkably, the clonal dissemination of GC2 isolates co-harboring bla_OXA-23 and bla_VIM genes among A. baumannii strains may be responsible for the rapid acquisition of carbapenem resistance. Furthermore, the detection of Tn2008 suggests that dissemination of bla_OXA-23 might be facilitated by these transposons. Therefore, some infection control measures should be reinforced to reduce the further spread of A. baumannii, including rapid identification of bla_OXA-23, hand hygiene, and environmental disinfection.

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Conflicts of interest

None.

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