Increasing imipenem resistance and dissemination of the ISAb1-associated \textit{bla}_{OXA-23} gene among \textit{Acinetobacter baumannii} isolates in an intensive care unit

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The antibiotic susceptibility of \textit{Acinetobacter calcoaceticus}–\textit{Acinetobacter baumannii} complex strains recovered from the intensive care unit (ICU) of West China Hospital, Sichuan, PR China, from 2006 to 2009 was investigated. The identification of \textit{A. baumannii} and analysis of carbapenemase-encoding genes and their relationship with ISAb1 were performed by PCR. Furthermore, a DiversiLab repetitive extragenic palindromic sequence-based PCR (rep-PCR) microbial typing system and a multilocus sequence typing (MLST) scheme were applied to assess the genetic relationship of the isolates. The results showed that the antibiotic susceptibility of the \textit{A. calcoaceticus}–\textit{A. baumannii} complex isolates changed and imipenem resistance increased rapidly between 2006 and 2009. The \textit{bla}_{OXA-51-like} and ISAb1-associated \textit{bla}_{OXA-23} genes were prevalent in the imipenem-resistant \textit{A. baumannii} isolates. However, the \textit{bla}_{OXA-58-like} gene was found in only one isolate and no metallo-\(\beta\)-lactamase genes were detected. The representative multidrug-resistant \textit{A. baumannii} isolates were identified as one cluster by rep-PCR fingerprinting and belonged to the clonal complex 92 (CC92) according to MLST. These findings indicate a situation of increasing resistance and wide distribution of class D \(\beta\)-lactamase genes, especially the acquired ISAb1-associated \textit{bla}_{OXA-23} gene, in \textit{A. baumannii} isolates in the ICU of West China Hospital, probably caused by expansion of the CC92 clone.

INTRODUCTION

\textit{Acinetobacter baumannii} is increasingly involved in nosocomial infections. Moreover, the wide dissemination of multidrug-resistant (MDR) \textit{A. baumannii} strains is an increasing threat in hospitals (Dijkshoorn et al., 2007; Livermore, 2003), especially for patients admitted to the intensive care unit (ICU) (Dijkshoorn et al., 2007; Hanberger et al., 1999; Husni et al., 1999; Peleg et al., 2008; Perez et al., 2007).

Carbapenems are a common choice for treating nosocomial infections caused by MDR \textit{A. baumannii} strains (Dijkshoorn et al., 2007). However, carbapenem resistance has been increasing in the past decade, and this is due mainly to the production of carbapenemases, including class D \(\beta\)-lactamasers (OXA-type carbapenemases) and class B metallo-\(\beta\)-lactamasers (Poirel & Nordmann, 2006; Poirel et al., 2007). Among the genes encoding OXA-type carbapenemases, three subgroups of acquired \textit{bla}_{OXA-23-like} \textit{bla}_{OXA-24-like} and \textit{bla}_{OXA-58-like} are scattered among \textit{Acinetobacter} species, whilst a fourth subgroup, \textit{bla}_{OXA-51-like} appears to be intrinsic to \textit{A. baumannii} (Turton et al., 2006a, b). Moreover, ISAb1, the most common insertion sequence (IS) in \textit{A. baumannii}, may enhance the expression of OXA-type carbapenemase genes and mobilize these genes among the strains (Mugnier et al., 2009; Turton et al., 2006b).

In West China Hospital, Sichuan, the largest regional hospital in the west of China with 4300 beds, members of the \textit{Acinetobacter calcoaceticus}–\textit{Acinetobacter baumannii} complex have been among the most common bacterial isolates identified in the ICU in recent years, causing approximately 12–15% of bacterial bloodstream infections in patients admitted to the ICU. An outbreak of carbapenem-resistant \textit{A. baumannii} in the medical ICU ward during December 2005 to January 2006 was reported previously (Zong et al., 2008). However, the overall situation regarding antibiotic susceptibility and the molecular epidemiology of \textit{A. baumannii} isolates in ICUs (including several wards such as medical, surgical,
neurological and transplant ICUs) in recent years has not been reported previously to our knowledge. Therefore, the current study focused on the susceptibility changes and presence of genes linked to imipenem resistance in A. baumannii isolated from the ICU. Furthermore, a new molecular typing method, the DiversiLab repetitive extragenic palindromic sequence-based PCR (rep-PCR) microbrial genotyping system, was conducted to assess the genetic relationship of resistant isolates. Multilocus sequence typing (MLST) was also performed to determine the clonal type of the isolates.

METHODS

Bacterial isolates and susceptibility testing. The bacterial isolates were recovered from clinical specimens according to standard methods. A. calcoaceticus–A. baumannii complex was identified using an automated Vitek 2 system (bioMérieux).

MICs for the isolates were determined according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2006). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as reference strains for susceptibility testing. MDR A. baumannii was defined as an isolate that was resistant to three or more classes of antimicrobial agents (Falagas & Karageorgopoulos, 2008).

Identification of A. baumannii. A. baumannii was identified from the A. calcoaceticus–A. baumannii complex using a one-tube multiplex PCR method (Chen et al., 2007). The template for PCR was prepared by boiling and centrifugation of pure bacterial suspensions.

PCR analysis of carbapenemase genes and IS elements. The genes encoding OXA carbapenemases and metallo-β-lactamases were screened by PCR (Ellington et al., 2007; Woodford et al., 2006). ISAba1 was detected using primers ISAba1F and ISAba1R (Segal et al., 2005). Whether ISAba1 preceded the OXA carbapenemase genes was determined using PCR mapping experiments by combinations of the ISAba1F and reverse primers designed for the blaOXA-23-like, blaOXA-51-like and blaOXA-58-like genes (Turton et al., 2006b). The entire blaOXA-23-like coding regions were amplified and sequenced (Corvec et al., 2007).

Rep-PCR fingerprinting using the DiversiLab system. The DNA of each isolate was extracted using two 1 µl loops of plated culture and an UltraClean Microbial DNA Isolation kit (MO BIO Laboratories) according to the manufacturer’s instructions. The rep-PCR system was prepared using a DiversiLab Acinetobacter kit (Bacterial Barcodes) and the amplification reaction was performed using a DNA Engine Peltier Thermal Cycler Engine (Bio-Rad Laboratories) according to the manufacturer’s instructions. The products of amplification were loaded in a microfluidics DNA LabChip and run using a 2100 Bioanalyzer (Agilent Technologies) according to the manufacturer’s instructions. When the run was completed, the results were sent via an individual secured website (https://huaxi.diversilab.com/). Data were analysed with DiversiLab software version 3.4, which uses the Pearson correlation coefficient to determine distance matrices and the unweighted pair group method with arithmetic averages to create dendrograms. Reports were automatically generated and included the dendrogram, electropherogram, virtual gel images, scatter plots and selectable demographic fields to aid in data interpretation (Fontana et al., 2008; Healy et al., 2005).

Determination of clonal type by MLST. Clonal types of the isolates were determined by MLST as described previously (Bartual et al., 2005). Briefly, the internal fragments of seven housekeeping alleles of A. baumannii (gyrB, gpi, rpoD, recA, cpn60, gpi and rpoD) were amplified and sequenced. The sequence of each allele was compared with existing sequences in the PubMLST database (http://pubmlst.org/abaumannii/). Sequence types (STs) of the isolates were designated according to the allelic profiles. With the eBURST version 3 algorithm (http://eburst.mlst.net/), the genetic relationship of STs was assessed using the most stringent definition of the groups as sharing alleles at six of seven loci, and clonal complexes (CCs) were assigned (Feil et al., 2004).

RESULTS AND DISCUSSION

Bacterial isolates and antibiotic susceptibility

A. calcoaceticus–A. baumannii complex was the most frequently isolated bacterial species, accounting for 22,

Fig. 1. Changes in antibiotic susceptibility of Acinetobacter calcoaceticus–Acinetobacter baumannii complex isolates in the ICU of West China Hospital from 2006 to 2009. IPM, Imipenem; PIP, piperacillin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; CIP, ciprofloxacin; GEN, gentamicin; TMP-SMZ, trimethoprim/sulfamethoxazole; TOB, tobramycin.
27, 31 and 34% of the overall bacterial strains recovered from clinical specimens in the ICU of West China Hospital in 2006, 2007, 2008 and 2009, respectively. These results showed a different situation from that in multiple Canadian and European ICUs where Staphylococcus aureus was the most prevalent isolate (Lepape & Monnet, 2009; Zhanel et al., 2008).

As shown in Fig. 1, antibiotic susceptibility to the common antibiotics among A. calcoaceticus–A. baumannii complex isolates changed from 2006 to 2009. The rates of resistance increased dramatically in 2009. In particular, imipenem resistance increased rapidly from 58.5% in 2006 to 88.4% in 2009. According to the resistance profiles, 87.3, 86.6, 90.3 and 95.4% of the isolates were MDR A. baumannii in 2006, 2007, 2008 and 2009, respectively. There were a very small number of cases where the level of resistance of isolates from the same patient increased over time, or where MDR isolates emerged during treatment. However, usually the first isolate from each patient was a MDR strain.

**Identification of A. baumannii**

A. baumannii, Acinetobacter genomic species 13TU and Acinetobacter genomic species 3 are the three clinically relevant species of Acinetobacter, but they cannot be differentiated by automated identification systems. Therefore, we identified A. baumannii by multiplex PCR and found that it accounted for 92.5% (98/106) of imipenem-resistant A. calcoaceticus–A. baumannii complex isolates. All of the imipenem-resistant A. baumannii isolates (100.0%) were MDR.

**Carbapenemase genes and IS elements in A. baumannii isolates**

The blaOXA-23 gene, increasingly reported worldwide (Kohlenberg et al., 2009; Mugnier et al., 2010; Stoeva et al., 2008; Wang et al., 2007; Zhou et al., 2007), was the most prevalent (97/98, 99.0%) acquired carbapenemase gene among 98 imipenem-resistant A. baumannii isolates, which is different from the result of a recent study showing that the blaOXA-58-like gene was epidemic in several Italian ICUs (Donnarumma et al., 2010). In addition, the presence of the ISAba1 element and its association with blaOXA-23 was identified among all the blaOXA-23-positive isolates. This result agreed with the findings of a molecular epidemiological investigation on imipenem-resistant Acinetobacter species collected from 1999 to 2005 at 11 teaching hospitals (not including West China Hospital) in China (Wang et al., 2007).

It is noteworthy that only one (1/98, 1.0%) A. baumannii isolate was positive for a blaOXA-58-like gene. To our knowledge, this is the first time that this gene has been identified in western China. The spread of this gene among isolates deserves further attention.

All 98 A. baumannii isolates carried the blaOXA-51-like gene. However, the PCR results showed that there was no linkage between ISAba1 and the blaOXA-51-like gene in these strains, perhaps because they had acquired ISAba1-associated blaOXA-23, so there was no need to activate the expression of the chromosomal blaOXA-51-like gene by insertion of the IS element. However, a recent report described that most A. baumannii isolates from a Taiwan hospital contained an ISAba1-activated blaOXA-51-like gene (Lin et al., 2010). These contrasting results indicate that there are different mechanisms for carbapenem resistance among A. baumannii isolates recovered from different regions of China (south-west area and south-east coastal area, respectively).

Additionally, blaOXA-24-like and class B metallo-β-lactamase genes were not found. Therefore, our data showed that the acquisition of ISAba1-associated blaOXA-23 was the main mechanism for imipenem resistance among the A. baumannii isolates in the ICU of our hospital.

**Genetic relationship of MDR A. baumannii isolates**

Rep-PCR fingerprinting of 30 representative MDR A. baumannii isolates was carried out using the DiversiLab system. All of these isolates carried the blaOXA-51-like and ISAba1-associated blaOXA-23 genes. Using a similarity threshold of 95% to define a cluster, all were found to belong to one cluster, as shown in Fig. 2.

According to MLST analysis, ST92 (1-3-3-2-2-7-3), ST137 (1-3-3-2-2-12-3), ST138 (1-3-3-2-2-50-3) and the novel gyrB allele (gyrB1) and ST profile (STn-1: 1-B1-3-2-2-16-3) were found in these 30 representative isolates. Compared with the gyrB12 allele, there was only one mutation (G→A) at nt 380 of the novel gyrB1 allele. Using the eBURST version 3 algorithm, all these STs were assigned to CC92 (originally CC22), which has been found in multiple cities in China (Fu et al., 2010). This result indicated the further dissemination of CC92 into the west of China.

It appeared that the ISAba1-associated blaOXA-23-producing A. baumannii CC92 had disseminated in the ICU of our hospital, as expected given the close relationship of the resistance mechanisms found in the isolates. Measures are needed to prevent and control colonization, transmission and infection among patients with such a predominant clone. The surveillance and early recognition of resistant clones and genes might influence the choice of antibiotic treatment and infection control. The DiversiLab rep-PCR system offers a rapid, reliable and highly discriminatory means of identifying and comparing A. baumannii strains for this purpose (Fontana et al., 2008; Grisold et al., 2010).

In conclusion, our findings illustrate further the challenge of increasing antibiotic resistance in A. baumannii isolates in China. According to the molecular epidemiological data in this study, we suggest that the emergence of ISAba1-
associated class D carbapenemase-encoding genes and dissemination of resistant CC92 have played important roles in bringing about the current situation of a high prevalence of imipenem-resistant *A. baumannii* in the ICU. Enhanced isolation procedures, strict policies on antibiotic treatment and laboratory-based surveillance systems are necessary to prevent the dissemination of such resistant strains.

**ACKNOWLEDGEMENTS**

We thank Huili Chen and other colleagues for recovering and identifying *A. baumannii*. We appreciate the suggestions given by M. D. Zhiyong Zong at the Department of Infectious Diseases, West China Hospital.

**REFERENCES**


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**Fig. 2.** Rep-PCR-based similarity analysis and gel images for 30 multidrug-resistant *Acinetobacter baumannii* isolates from the ICU of West China Hospital. According to a similarity level of >95%, all these isolates belonged to one cluster.


