Dissemination of multidrug-resistant Acinetobacter baumannii carrying Bla<sub>OXA-23</sub> from hospitals in central Taiwan

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**KEYWORDS**
Acinetobacter baumannii; Bla<sub>OXA-23</sub>; Carbapenem resistance; Molecular epidemiology

**Background:** Imipenem-resistant Acinetobacter baumannii (IRAB) poses a great threat to healthcare systems. Production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) is the major mechanism for imipenem resistance. In this study, we found a high prevalence of IRAB carrying a gene encoding CHDL, Bla<sub>OXA-23</sub>, in central Taiwan and elucidated the molecular characteristics and possible mechanisms of the spread of these isolates.

**Methods:** During 2007, we collected 291 nonrepetitive A baumannii isolates from 10 teaching hospitals in Taiwan. The antimicrobial susceptibility of the isolates was determined by agar dilution or Etest. The genes encoding carbapenemase and related structure were detected by polymerase chain reaction mapping and sequencing, and the clonal relationship of the isolates was analyzed by pulsed-field gel electrophoresis. Plasmid localization of Bla<sub>OXA-23</sub> was determined by extraction of plasmid with commercial kit and Southern blot analysis.

**Results:** Among 142 IRAB isolates, 30 harbored the Bla<sub>OXA-23</sub>. The prevalence of IRAB with Bla<sub>OXA-23</sub> was highest in central Taiwan compared to other areas [24.8% (27/109) vs. 1.6% (3/182); p < 0.001]. These IRAB with Bla<sub>OXA-23</sub> were also resistant to other antimicrobial
agents, except colistin. The PCR methods showed the presence of blaOXA-51 in all isolates. We could exclude clonal spreading due to the diversity of the pulsotype. The blaOXA-23 gene was detected in the plasmids of 6 isolates. Tn2006 was present in 22 (73.3%) isolates, and Tn2008, in 6 other isolates (26.7%). Two strains had blaOXA-23-ΔATPase but lacked upstream ISAba1.

Conclusion: The high prevalence of blaOXA-23-harboring IRAB in central Taiwan might be attributed to the transposition event of Tn2006.

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Introduction

Acinetobacter baumannii has emerged as a major pathogen causing a multitude of infections, especially in critically ill, immunosuppressed patients and those treated with broad-spectrum antibiotics.¹,² Owing to the ability of bacteria to develop different mechanisms of resistance and the increasing number of vulnerable hosts, the prevalence of multidrug resistant A baumannii (MDRAB) rose in the past few decades. Unfortunately, the treatments of choice are limited.³ The resistance to carbapenem, which was one of the few therapeutic options available, is now a worldwide problem.⁴–⁶ In Taiwan, in 2000, the imipenem-resistant rate in the intensive care units of 5 major hospitals was 22%,³ whereas in 2010, it was 66.8%.⁷ Although resistance to carbapenem has been associated with the loss of outer-membrane porins or overexpression of efflux pumps, production of Ambler class B metallo-β-lactamasases and carbapenemase-hydrolyzing class D β-lactamasases (CHDLs) are the most common mechanisms.⁸ Three (blaIMP-like, blaVIM-like, and blaSIM-1) of the 6 known metallo-β-lactamasases have been identified in IRAB, but these are less prevalent than CHDLs, which include blaOXA-23-like, blaOXA-24-like, blaOXA-51-like, and blaOXA-58-like.⁹,¹⁰ blaOXA-51-like is intrinsic to A baumannii, while other CHDLs genes were acquired. The distribution of A baumannii carrying these different acquired CHDLs genes varies among different regions and even different hospitals.¹¹,¹² The most common acquired CHDL gene of imipenem-resistant A baumannii (IRAB) in many Asia-Pacific countries is blaOXA-23.¹³–¹⁶ and a high prevalence of bacterial strains carrying blaOXA-23 has been reported in hospitals in central Taiwan.¹⁷,¹⁸ The blaOXA-23 gene can be carried by transposons such as Tn2006, Tn2007, and Tn2008.¹⁹ In this study, we aimed to investigate the distribution and characteristics of A baumannii carrying blaOXA-23 in Taiwan. We also propose possible ways of spreading of blaOXA-23-carrying stains.

Materials and methods

Bacterial isolates and identification

We collected 367 nonrepetitive isolates of Acinetobacter species from 10 teaching hospitals located in different areas in Taiwan from June 2007 to September 2007 (Fig. 1). Four hospitals are located in northern (N) Taiwan, 3 in central (C) Taiwan, and 3 in southern (S) Taiwan. The isolates were initially stored at −70 °C in trypticase soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 15% glycerol. The A baumannii species was identified at the Taipei Veterans Hospital, using a multiplex polymerase chain reaction (PCR) method for detection of specific 16S–23S rRNA intergenic spacer present in A baumannii as described previously.²⁰,²¹ Those confirmed as A baumannii were selected for further studies.

Antimicrobial susceptibility testing

The minimal inhibitory concentration (MIC) of isolates was determined by the agar dilution method according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI)²² or Etest (bioMérieux, Marcy-L’Étoile, France). Susceptibility was interpreted based on CLSI breakpoints or manufacturer’s instructions. Antimicrobials including meropenem, imipenem, colistin, tigecycline, sulbactam, amikacin, ticarcillin, piperacillin, ceftazidime, cepafime, and ciprofloxacin, were tested.

Pulsed-field gel electrophoresis

The clonality of isolates carrying blaOXA-23 was determined with pulsed-field gel electrophoresis (PFGE) as described

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Figure 1. Prevalence of Acinetobacter baumannii carrying blaOXA-23 from 10 teaching hospitals in Taiwan. Percentage was presented as the number of A baumannii carrying blaOXA-23 divided by the number of all A baumannii isolates in different hospitals.
previously. Briefly, after digestion with Apal, the DNA fragments were subjected to PFGE in 1% SeaKem Gold agarose gels (Cambrex Bio Science, Rockland, ME, USA) in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA, pH 8.0). The stained gel was photographed and analyzed by BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) to generate a dendrogram of relatedness among these isolates. Isolates with >85% similarity were grouped as the same clone.

Identification of CHDLs

PCR with primers targeting blaOxa-23-like, blaOxa-24-like, blaOxa-58-like, and blaOxa-51-like were used to detect genes encoding common CHDLs. Primers for ISAb1F and OXA-likeR were used to detect the presence of ISAb1 upstream of different carbapenemases genes.

Determination of the plasmid localization of blaOxa-23

The plasmid was extracted with plasmid DNA Miniprep Kit (Biomax, Taipei, Taiwan) or a plasmid Maxi Kit (Qiagen, Valencia, CA, USA). The localization of blaOxa-23 was detected by Southern blot. After hybridization with a PCR-generated probe derived from primers targeting blaOxa-23 (5′-TTTACCTTGCTTGGTTG-3′ and 5′-CATTCTGACCGCATTTCT-3′), the band was visualized by digoxigenin (DIG) DNA labeling and detection kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s instructions.

Identification of Tn2006, Tn2007, and Tn2008

PCR mapping was used to detect Tn2006, Tn2007, and Tn2008. Primer locations are shown in Fig. 2. The common region of Tn2006 and Tn2008 (923 bp, from blaOxa-23 to ΔATPase) was amplified using primers P3 (Tn2006OXA23; 5′-GTCTATCACGAACTTGGCCG-3′) and P5 (Tn2008ATPase; 5′-GGCTTACATGCGTACTAAGT-3′). PCR for Tn2006 was performed with primers P3 and P4 (Tn2006ISAb1; 5′-GCAAGGCTTTAGTGTCGG-3′) to amplify the region (2237 bp) between blaOxa-23 and ISAb1 in Tn2006. PCR targeting ISAb4 (Tn2007) was performed as previously described. Amplified DNA products were resolved by electrophoresis in agarose gels (2% w/v), stained with ethidium bromide, purified according to the manufacturer’s instruction, (Geneaid Biotech Ltd, Taipei, Taiwan), and processed for DNA sequencing by a commercial company (Mission Biotech, Taipei, Taiwan).

Concentration of imipenem and meropenem per 100 person-days in different areas

The National Health Insurance program covers 99% of the population in Taiwan. Computerized claims data are collected and stored in the National Health Insurance Research Database by the National Health Research Institute. We retrospectively retrieved inpatients’ claim data from 2007. We calculated for each patient the dose of imipenem and meropenem and the total person-day to obtain the defined daily dose per 100 person-day in northern, central, and southern areas, which were defined according to the location of the hospitals. The northern area included Taipei and New Taipei City; the central area included Taichung City and its county and Changhua; and the southern area included Tainan City and its county and Kaohsiung City and its county.

Results

Of 291 isolates confirmed to be A baumannii, 142 (48.8%) were resistant to imipenem. The imipenem-resistance rate in northern, central, and southern Taiwan was 39.3% (42/107), 56.2% (62/110), and 50.7% (38/75), respectively. The defined daily doses of carbapenem per 100 person-days in northern, central, and southern areas were 1.43, 1.33, and 1.50, respectively. Among IRAB isolates, 30 (21.1%) harbored blaOxa-23. The prevalence of IRAB with blaOxa-23 was highest in central Taiwan (Fig. 1), accounting for 24.8% (27/109) of all isolates, compared to that in the northern (2.8%, 3/107) and southern areas (0%, 0/75; both p < 0.001).

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests (Supplementary Table 1) revealed that strains carrying blaOxa-23 were also resistant to many other commonly used antimicrobial agents. The resistance rates to ticarcillin, sulbactam, amikacin, ceftazidime, ceftazidime, cefepime, pipacillin, imipenem, meropenem, and ciprofloxacin were all higher than 90%. Colistin-resistant isolates were not found. However, the MICs of tigecycline among 9 isolates (30%) were more than 2 mg/L by using Etest.

Detection of other carbapenemase genes and surrounding genetic structure of blaOxa-23

Most of the isolates carrying blaOxa-23 (28, 93.3%) had ISAb1 upstream the blaOxa-23 gene. All isolates contained blaOxa-51 but only 3, which did not belong to the same clone but were present in the same hospital, had ISAb1 upstream of blaOxa-51 (Fig. 3). A strain, which did not have ISAb1 preceding the blaOxa-23 or blaOxa-51, expressed blaOxa-58, and the MICs of imipenem and meropenem were
both 32 mg/L. Using primers described in Materials and Methods, we found that the common region of Tn\textsubscript{2006} and Tn\textsubscript{2008} was detected in all strains, and the region specific for Tn\textsubscript{2006} was positive in 22 (73.3%) isolates (Fig. 3). Tn\textsubscript{2008} was present in 6 other isolates (26.7%), whereas \textit{bla}\textsubscript{OxA-23}--\textit{ATPase} but not upstream IS\textsubscript{Aba1} was present in 2 isolates. No IS\textsubscript{Aba4} was found, indicating there was no Tn\textsubscript{2007} in any of these strains.

**Clonal relationship and plasmid study of the isolates**

The PFGE analysis showed no major clustering among the isolates (Fig. 3). Five of the 6 isolates with Tn\textsubscript{2008} were collected from the same hospital, and 4 of them belonged to the same clone. Twenty-four isolates were positive for the plasmid, as determined using standard extraction methods. Electrophoresis showed 11 different plasmid patterns (Fig. 3). Isolates with the same plasmid pattern (e.g., Groups 1, 3, and 4) did not necessarily belong to the same pulsotype, but isolates with the same plasmid pattern were present in the same hospital. Using of the methods described above, the \textit{bla}\textsubscript{OxA-23} gene was detected in plasmids of Group 1, 11, and 6 (data not shown).

**Discussion**

Our study revealed that \textit{bla}\textsubscript{OxA-23} was mostly found in IRAB isolates collected in central Taiwan, whereas the prevalence of isolates with \textit{bla}\textsubscript{OxA-23} was low in other areas. The presence of Tn\textsubscript{2006} and Tn\textsubscript{2008} in these isolates together with the diversity of the pulsotypes indicated that the preferred mechanism of spread of \textit{bla}\textsubscript{OxA-23} was via transposons. Clonal spread played a minor role, especially in isolates harboring Tn\textsubscript{2008} in Hospital C3. The spread of \textit{bla}\textsubscript{OxA-23} via plasmid dissemination cannot be excluded with the use of current method.

As observed in other Asian countries,\textsuperscript{29} our study revealed that the prevalence of IRAB in Taiwan increased in 2007 compared to the rates reported in 2003\textsuperscript{30} and 2005.\textsuperscript{30} These strains were also resistant to other antimicrobial agents. This resistance resulted in an increased risk of administering inappropriate therapy and therefore was associated with a poor prognosis.\textsuperscript{31} Colistin and tigecycline were the main choices for the treatment of infection caused by these IRAB.\textsuperscript{9} Although colistin-resistant strains have been emerging in Korea,\textsuperscript{32} our survey revealed that colistin retained its activity against these IRAB.

\textit{A. baumannii} strains carrying \textit{bla}\textsubscript{OxA-23} have been discovered worldwide\textsuperscript{8} and are prevalent in Asian-Pacific regions.\textsuperscript{13--16} In Taiwan, the epidemiology of acquired CHDL genes differed among areas\textsuperscript{11,12} In line with previous studies performed in a single hospital of central Taiwan,\textsuperscript{17,18} \textit{bla}\textsubscript{OxA-23} was the most prevalent CHDL gene in all 3 teaching hospitals in this area and was rarely found in other areas. Although naturally occurring and chromosome-located \textit{bla}\textsubscript{OxA-51} was discovered in all isolates, IS\textsubscript{Aba1} carrying the promoter was essential for its contribution to carbapenem resistance.\textsuperscript{25} Only three isolates in our study had IS\textsubscript{Aba1}-\textit{bla}\textsubscript{OxA-51}. In contrast, most strains (93.3%) carrying \textit{bla}\textsubscript{OxA-23} had preceding IS\textsubscript{Aba1}, which has been shown to enhance the expression of \textit{bla}\textsubscript{OxA-23}.\textsuperscript{33}

PFGE studies revealed a minor role of clonal spreading in the dissemination of isolates with \textit{bla}\textsubscript{OxA-23} in \textit{A. baumannii} in Taiwan. In agreement with previous studies,\textsuperscript{19} our study
indicates that the dissemination of blaOXA-23 is attributed to transposons. Indeed blaOXA-23 can be mobilized by Tn2006, Tn2007 and Tn2008. Pauline et al. reported that Tn2006 was associated with 20 OXA-23—producing A. baumannii clinical isolates obtained from 15 countries, including Thailand and Vietnam. Our study also showed that the blaOXA-23 genes were embedded in Tn2006 in most of our isolates. Although Tn2008 has been identified only in one isolate from Libya, it has recently been recognized as the major vehicle carrying blaOXA-23 in China. However, in our study, isolates with this genetic structure only accounted for the minority. Two strains carrying blaOXA-23 had similar structure of Tn2008 but lacked upstream ISAb1. Further studies are warranted for delineating the sequence of the upstream region.

Four isolates with Tn2008 had identical pulsotype and were collected from the same hospital (hospital C3), indicating a clonal spreading. Interestingly, all the isolates bearing Tn2008 had blaOXA-23 detected in plasmids. However, the localization of blaOXA-23 in a large plasmid of other isolates cannot be detected using the currently method. Therefore, the spread of blaOXA-23 via plasmid dissemination cannot be totally excluded.

In conclusion, in this study, we observed a high prevalence of blaOXA-23 in central Taiwan compared with other areas. The blaOXA-23 is disseminated via complex routes. The majority of the strains might acquire blaOXA-23 through the transposition of Tn2006. Clonal spreading played a minor role in the spread of blaOXA-23. The role of plasmid dissemination needed to be validated.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jmii.2012.08.006.

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