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ORIGINAL ARTICLE

Emergence of carbapenem-resistant *Acinetobacter baumannii* ST787 in clinical isolates from blood in a tertiary teaching hospital in Northern Taiwan

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Abstract *Background/Purpose:* The purpose of this study is to investigate the predominant clones of carbapenem-resistant *Acinetobacter baumannii* (CRAB) in our hospital in Taiwan by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) technique.
Methods: We collected 108 non-duplicate *A. baumannii* clinical blood isolates from January 2012 to December 2013 in MacKay Memorial Hospital. PFGE and MLST were used for typing the *A. baumannii* isolates and for investigation of the predominant clones. Bacteria isolates were screened by polymerase chain reaction for the presence of the carbapenemase-encoding genes.
Results: All 108 isolates were classified as 33 pulsotypes by PFGE. The predominant clones were pulsotype 10 (12.04%) in 2012 and pulsotype 8 (16.67%) in 2013, respectively. The 31 predominant pulsotype isolates were typed by MLST, and ST787 (54.84%) and ST455 (45.16%) were identified. All isolates carried $bla_{\text{OXA-51-like}}$ genes, and $bla_{\text{OXA-23-like}}$ genes was founded in 101 isolates (93.52%). Other identified resistance genes included $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-IMP}}$.

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Conclusion: To the best of our knowledge, this study is the first to describe the microbiological characteristics of CRAB ST787, which carried high genetic resistance to carbapenem, but remained susceptible to colistin. CRAB ST787 was the predominant clone in our hospital in the study period.

Introduction

Acinetobacter baumannii has emerged as an important nosocomial pathogen across the globe.[1,2] Carbapenem resistance of *A. baumannii* are increasingly reported worldwide.[3] Resistance rates of *A. baumannii* to meropenem and imipenem were 57.4% and 47.9% in Europe.[4,5] In New York, carbapenem-resistant *A. baumannii* (CRAB) comprised 53% of all clinical isolates.[6] There has been a rapid increase in *A. baumannii* complex-related infections in Taiwan since 1990, and they have become a major cause of hospital-acquired bacteremia.[7] The carbapenem-resistance rate among AB complex in Taiwan increased from 3.4% in 2002 to 58.7% in 2010.[8] Taiwan Nosocomial Infections Surveillance (TNIS) data shows that the percentage of carbapenem-resistant AB complex infections in intensive care unit stays increased from <20% in 2003 to 70% in 2011. CRAB is difficult to eradicate and treat, and its presence can lead to hospital-wide outbreak, leading to increased morbidity, mortality, and hospital costs.[9,10] Abbo et al. determined that CRAB bacteremia is associated with a mortality rate of more than 50%.[11]

CRAB was discovered in England in 1985[1] from a group of *A. baumannii* 6B92 mutants.[12] The novel beta-lactamase (ARI 1) was subsequently named as OXA-23. Most CRAB isolates are able to produce OXA-type (oxacillinase) carbapenemase.[1,13] The most frequently CRAB OXA-type groups are OXA-24, OXA-58, and especially OXA-23.[14,15] Beyond the OXA-type enzymes, metallo-beta-lactamases, including VIM, IMP, and SIM are also able to hydrolyze broad spectrum carbapenems, including imipenem or meropenem.[16]

We found that the carbapenem-resistance rates of AB complex isolates from different clinical samples increased from 59.98% (781/1302) in 2005 to 90.01% (2099/2332) in 2011 in our hospital in despite of enhanced infection control, such as strict hand hygiene, contact precaution, proper environment cleaning and appropriate antimicrobial strategy. Due to the increasing significance of CRAB, we designed this study for investigation of the epidemiology of resistance for better control. We performed PFGE to determine the relationship of CRAB clinical isolates from blood in a tertiary teaching hospital in Northern Taiwan in 2012 and 2013, and then performed MLST for the predominant clones for long-term surveillance and comparison with international data. Then, we screened the carbapenemase-encoding genes.

Methods

Collection of bacterial isolates

From January 2012 to December 2013, we collected carbapenem-resistant *A. baumannii* complex blood isolates in MacKay Memorial Hospital (MMH). MMH is a 2200-bed medical center in Northern Taiwan. The study protocol (16MMHIS070) was reviewed and approved by the Institutional Review Boards of MMH, Taipei, Taiwan. The carbapenem resistance is defined as MIC for imipenem ≥8 mg/L.[17] All of the isolates were non-duplicate from blood cultures of different patients. We identified these isolates using the Vitek 2 system, and further species identification was confirmed by polymerase chain reaction (PCR)-base technique.[18]

Species identification

PCR amplification and sequencing for species identification were performed by analysis of the RNA polymerase β subunit (*rpoB*) gene and flanking spaces according to the protocol by La Scola et al.[18]

Gene detection: polymerase chain reaction (PCR)

The *A. baumannii* blood isolates were screened using PCR and sequencing for the carbapenemase-producing genes bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{GES}, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, and $bla_{OXA\text{-}58\text{-}like}$.[19–22] The bacteria were boiled in sterile water for 10 min, and the supernatant was collected for use in PCR as DNA sources. The 25-μl reaction mixture consisted of 1X S-T Gold buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 20 pmol of each primer, and 0.4 units of Super-Therm polymerase. Products were visualized on an agarose gel. Sequence analysis of the resulting amplicons was performed with an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequence similarity searches were performed with the basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Pulsed-field gel electrophoresis

The *A. baumannii* blood isolates with resistance to imipenem (MIC ≥ 8 mg/L) were typed by PFGE following digestion of intact genomic DNA with *Apa*I (Biolabs, Beverly, MA, USA). The DNA fragments were separated on 1% (w/v) SeaKem GTG agarose gels in 0.5% Tris—borate—EDTA buffer in a CHEF Mapper apparatus (Bio-Rad, Hercules, CA, USA) with a potential of 6 V/cm pulsed from 5 s to 20 s for 23 h at 14 °C. The completed gels were stained with ethidium bromide and photographed with UV light.

The *Apa*I restriction profiles were initially compared with each other by visual inspection, and isolates were considered to be closely related if they showed differences

of less than three bands.[23] Computer-assisted analysis was also performed using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was performed by the unweighted pair group method with mathematical averaging, and DNA relatedness was calculated using the band-based Dice coefficient with a tolerance setting of 1.5% band tolerance and 1.5% optimization setting for the whole profile. Isolates were considered to belong to the same PFGE type when the similarity coefficient reached 87%[16]

MLST

The predominant pulsotypes by PFGE were analyzed by MLST for international database and literature comparison. MLST was performed using Bartual's scheme.[24] Seven housekeeping genes included *gltA* (coding for citrate synthase), *gyrB* (coding for DNA gyrase subunit B), *gdhB* (coding for glucose dehydrogenase B), *recA* (coding for homologous recombination factor), *cpn60* (coding for 60-kDa chaperonin), *gpi* (coding for glucose-6-phosphate isomerase), and *rpoD* (coding for RNA polymerase 70 factor) were checked. Sequences were compared with the *A. baumannii* database at the MLST database (http://pubmlst.org/abaumannii/).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the Vitek 2 system. The sensitivities of tested antibiotics and minimum inhibitory concentrations (MICs) were interpreted according to Clinical and Laboratory Standards Institute guidelines.[17] The used MIC breakpoints for tigecycline are $\leqq$2 mg/L for susceptible and $\geqq$8 mg/L for resistant, respectively.[25] The significance of differences in proportions of resistance isolates was calculated by Chi square test. $P \leq 0.05$ was considered significant.

Results

Collection of bacterial isolates and species identification

There were 136 non-duplicate clinical isolates of blood identified as carbapenem-resistant *A. baumannii* complex by Vitek 2 system in MMH from January 2012 to December 2013. Seventy-nine isolates (58.08%) were collected in 2012, while 57 isolates (41.91%) were found in 2013. Among the 136 clinical blood isolates of CRAB, 108 isolates was confirmed as *A. baumannii* by PCR and sequencing in a research laboratory. Sixty isolates (55.56%) were collected in 2012, and 40 isolates (44.44%) were found in 2013.

PFGE

The 108 clinical isolates were classified into 33 PFGE pulsotypes (Fig. 1). The most common was pulsotype 8 (16.67%, 18/108), followed by pulsotype 10 (12.04%, 13/108). Pulsotype 10 was predominant in 2012, and pulsotype 8 was predominant in 2013. The other 77 isolates were defined as non-predominant pulsotypes.

MLST

MLST was performed using Bartual's scheme for predominant pulsotypes for the total number of 31 isolates. 17 isolates (54.84%) were identified as ST787, and 14 isolates (45.16%) were typed as ST455. All of the pulsotype 10 belonged to ST787, and the pulsotype 8 corresponded to both ST454 (77.78%, 14/18) and ST787 (22.22%, 4/18) (Fig. 1). ST787 was predominant in 2012, and ST455 was predominant in 2013.

The allelic profile of ST787 was 1-87-3-2-2-157-3, and the ST455 was 1-87-3-2-2-83-3. These two sequence types shared six alleles out of seven loci, except for *gpi*.

Carbapenemase genes

There were 108 isolates in total. All of them carried $bla_{\text{OXA-51-like}}$, 101 (93.5%) carried $bla_{\text{OXA-23-like}}$, five (4.6%) carried $bla_{\text{OXA-24-like}}$, and one (0.9%) carried $bla_{\text{OXA-IMP}}$ gene. All 108 isolates were negative for genes bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{GES}, $bla_{\text{OXA-48-like}}$, and $bla_{\text{OXA-58-like}}$.

Among the 31 predominant pulsotypes isolates, all carried both $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ genes, and one isolate (3.2%) also harbored $bla_{\text{OXA-IMP}}$ (Fig. 1). All ST787 isolates carried $bla_{\text{OXA-23-like}}$. All ST455 isolates carried $bla_{\text{OXA-23-like}}$, and one isolate also harbored $bla_{\text{OXA-IMP}}$ (7.14%).

Among 77 non-predominant pulsotypes isolates, all carried $bla_{\text{OXA-51-like}}$ isolates, 70 isolates carried $bla_{\text{OXA-23-like}}$ (90.9%), while five had $bla_{\text{OXA-24-like}}$ (6.49%). No $bla_{\text{OXA-IMP}}$ was found.

Antibiotics susceptibility

All isolates were susceptible to colistin. The predominant clones had significantly higher tigecycline resistance than non-predominant clones (90.3%, 28/31 vs 32.5%, 25/77, $p < 0.0001$). The resistance rate of predominant clones was 96.77% to gentamicin and ampicillin-sulbactam, and 100% to other antibiotics. The resistance rate of non-predominant clones was 94.81 to gentamicin, 98.70% to ceftazidime and ciprofloxacin, and 100% to other antibiotics.

Discussion

In this study, 33 PFGE pulsotypes were classified from 108 non-duplicate *A. baumannii* clinical blood isolates. The most common were pulsotype 8 (16.67%) and pulsotype 10 (12.04%). We denoted pulsotype 8 and 10 as predominant pulsotypes. No outbreak was observed by the active surveillance of infection control team during the study period. In order to compare the predominant clones in our study to the reports from other hospital in Taiwan and worldwide, MLST was performed for the predominant pulsotypes. Some recent studies have found higher discriminatory power of the Bartual's scheme compared to the Pasteur.[26–28] However, there are limited reports for MLST analysis using Bartual's scheme in Taiwan.[27] Therefore, we used the Bartual's PubMLST scheme to classify the sequence types.

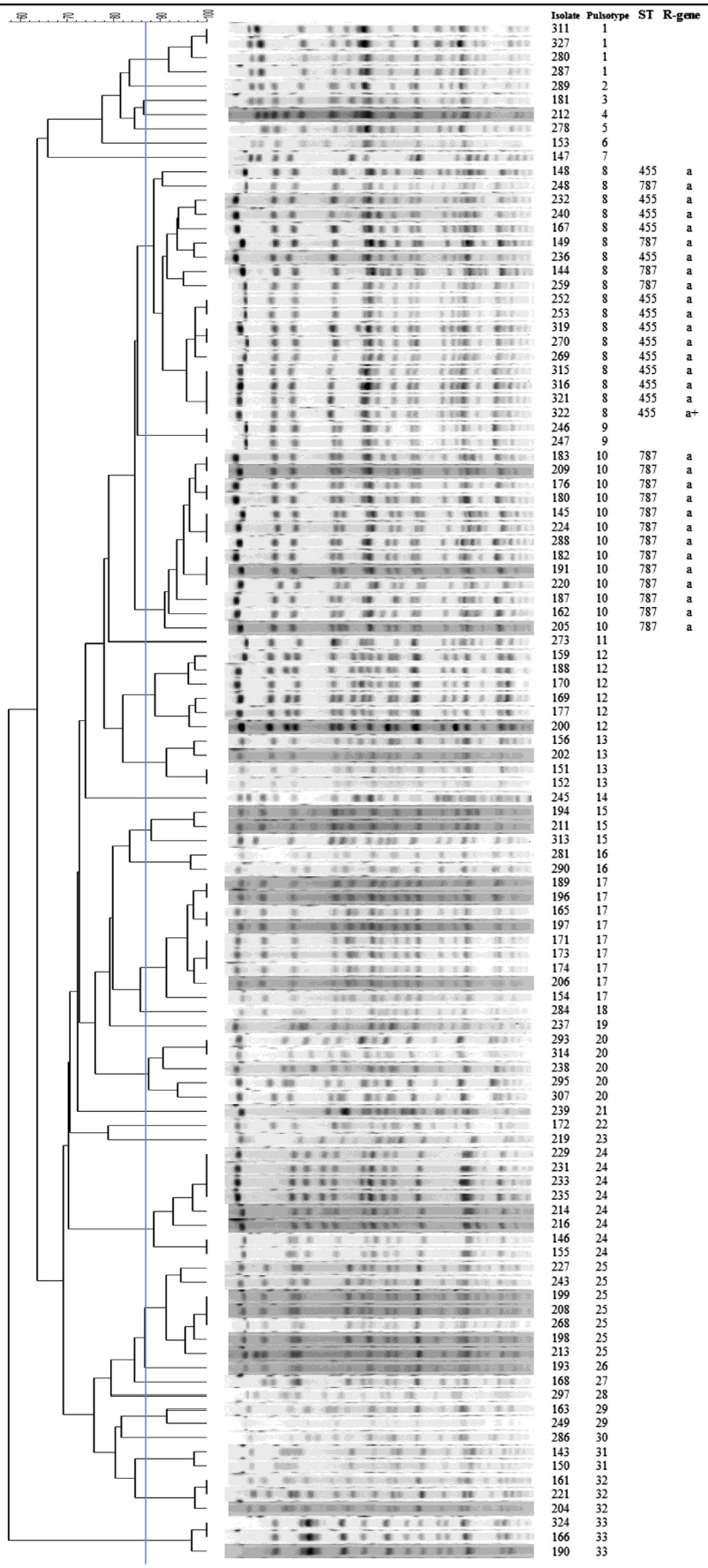

Figure 1. Pulsed-field gel electrophoresis, multilocus sequence typing (MLST), and resistance genes of carbapenem-resistant *A. baumannii* blood isolates. R-gene: Resistant gene; a: harboring $bla_{\text{OXA-23-like}}$, and $bla_{\text{OXA-51-like}}$; a+: harboring bla_{IMP}, $bla_{\text{OXA-23-like}}$, and $bla_{\text{OXA-51-like}}$.

The results showed that while similar, the products of PFGE and MLST analyses were not directly correlated with each other, as isolates of the same PFGE pulsotype could have different MLST classification. The discordance between MLST and PFGE typing has been described in a previous study.[26] Therefore, MLST should be regarded as a complementary typing method to PFGE, rather to substitute PFGE.[26,29]

In order to decrease the emergence of antibiotics resistance and the spread of CRAB, infection control strategies and antimicrobial stewardship programs are important.[30] Rational use of antimicrobials with microbiology laboratory support and dedicated use of carbapenem are essential to control the increase of CRAB.[31] Strategies for control of the spread of CRAB include strict adherence to hand hygiene, use of contact precautions, proper environmental cleaning and sterilization of reusable medical equipment.[30]

We found that all of the predominant pulsotype isolates harbored both $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes. Howard et al. showed that *A. baumannii* has intrinsic class D oxacillinase belonging to the OXA-51-like group, which is an important marker in the identification of genomic species. OXA-51-like enzymes are able to hydrolyze carbapenems, but very weakly.[32] During 1999 to 2009, the predominant oxacillinase-encoding gene was $bla_{\text{OXA-58}}$ in several Mediterranean countries including Italy, Greece, Lebanon and Turkey.[33] Since 2009, $bla_{\text{OXA-23}}$-positive *A. baumannii* isolates has been increasing in many European countries, and has rapidly "replaced" the $bla_{\text{OXA-58}}$-positive isolates in Greece.[33] Liakpoulos et al. showed that carbapenem resistance was associated with the $bla_{\text{OXA-23}}$ gene in 72.4% of isolates.[33] Similarly, the $bla_{\text{OXA-23-like}}$ was the major resistance gene in this study.

Colistin was the only antibiotic to which all *A. baumannii* were susceptible. Our findings resembled those reported by Villalon et al.[29] The resistance for tigecycline was significantly higher in predominant clones (90.3%) than non-predominant clones (32.5%) in this study ($p < 0.0001$). The development of tigecycline resistance after treatment for *A. baumannii* infection has been observed. Ni et al. reviewed that the four single-arm studies (362 patients) investigated the susceptibility changes to tigecycline after treatment for *A. baumannii* infection. The pooled rate of resistance emergence of four studies was 12.47% ($p = 0.006$).[34] According to the antimicrobial susceptibility surveillance in our hospital, the overall tigecycline resistance for AB complex is 45% in 2012 and 18% in 2013, respectively. The tigecycline resistance for CRAB complex is 60% in 2012 and 59% in 2013, respectively. There was no increase in tigecycline resistance for AB complex in our hospital during the study period. Because of the low serum concentration of tigecycline after intravenous administration, tigecycline use alone for *A. baumannii* bloodstream infection is not recommended.[35] However, since there was limited choice of antibiotics for CRAB, the tigecycline remain an important antibiotic that plays a role in combination therapy and in patients which with intolerance to colistin. Further larger studies focus on the tigecycline resistance of CRAB are warranted.

Therefore, the high tigecycline resistance in predominant clones of *A. baumannii* isolates from blood might not be a significant clinical dilemma for the treatment for *A. baumannii* bacteremia.

A. baumannii ST455 has been reported in Japan and Taiwan,[27,36] and has been described as the predominant clone in a tertiary hospital in northern Taiwan.[27] Lee et al. stated that the most of ST455 isolates carried bla_{OXA-23}, had high antibiotics resistance, and led to high mortality.[27] In our study, all of ST455 carried $bla_{\text{OXA-23-like}}$. The predominance of this resistance gene was consistent with the previous study.[27]

To the best of our knowledge, the *A. baumannii* ST787 was first registered to MLST database (http://pubmlst.org/abaumannii/) in 2014 by Yang; however, our study is the first to describe the microbiological characteristics of ST787. The high rate of carriage of $bla_{\text{OXA-23-like}}$ and the relatively high prevalence in CRAB bacteremia make it important and warranted further studies in the future.

There were some limitations in this study. First, this study is a single-center study for a period of 2 years. Second, the sample size is relatively small. Third, there were not all isolates typed by MLST.

In conclusion, infection caused by carbapenem-resistant *Acinetobacter baumannii* has become a major issue in nosocomial infection, with difficulty in treatment and infection control. Carbapenem-resistant *A. baumannii* ST787 was identified as the predominant clone in our hospital in the study period, and had high genetic resistance to carbapenem, but remained susceptible to colistin. To the best of our knowledge, our study is the first to describe the microbiological characters of carbapenem-resistant *A. baumannii* ST787. Further studies with larger number of isolates and longer duration are warranted.

Conflicts of interest statement and funding/support statement

The authors declare that they have no conflicting interests. This study was supported by grants MMH-103-61 and MMH-105-16 from Mackay Memorial Hospital, Taiwan.

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