Colonization of a Medical Center in Southern Taiwan by Epidemic Strains of Carbapenem- and Multidrug-Resistant Acinetobacter baumannii and the Genetic Organization of Their Integrons

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SUMMARY: A total of 46 carbapenem- and multidrug-resistant (CR- and MDR-) Acinetobacter baumannii bacteremic isolates from a Taiwanese medical center were investigated over the period 2000 to 2006 using randomly amplified polymorphic DNA (RAPD) profiling and by analysing the genetic organization of their integrons. The results of RAPD patterns revealed that before 2003 each CR- and MDR-A. baumannii bacteremic isolate was independent, but after 2003 the isolates appeared to belong in four epidemic strains and persisted in the hospital. All the CR- and MDR-A. baumannii strains harbored class I integron (intI1) genes. PCR amplification and nucleotide sequencing showed that the cassette genes of intI1 were found to form four different antibiotic-resistant gene alignments in those strains. The blaTEM-1 gene in the cassette genes of intI1 was identified in a clone, which raised great concern that clonal spread of this strain or of an integron-mediated horizontal gene may have occurred.

Carbapenem-resistant (CR-) or multidrug-resistant (MDR-) Acinetobacter baumannii has emerged as a major nosocomial pathogen worldwide. The prevalence of nosocomial infection in hospital respiratory care wards due to CR- or MDR-A. baumannii was 32.1 or 8.9%, respectively, of all Gram-negative bacterial infection in Taiwan (1). Such infections are beset by the problem that these bacteria offer very few therapeutic options and sometimes can develop into an epidemic strain, which produces repeated outbreaks over a long time period in a hospital (2). Developing MDR strains have been suggested to acquire their antibiotic-resistant genes via class I integrons (intI1) that carry within them a single or multiple gene cassette(s); most often among MDR-A. baumannii isolates, these are found in the internal variable regions between the integron’s two conserved regions (the 5’-CS and the 3’-CS) (3). Aminoglycoside resistance genes, such as aac (acetyltransferase), aph (phosphotransferase), and aad (adenyllytransferase), which contribute to the enzymatic inactivation of the aminoglycoside antibiotics, are the genes usually found among the cassette genes (4). In special instances, A. baumannii can give rise to the spread of β-lactam antibiotic drug resistance in hospital if the bacteria harbor a bla gene variant among the cassette genes (5,6). In this report, we identified colonization by epidemic strains of CR- and MDR-A. baumannii in a 1,400-bed teaching hospital, Kaoshiung Veterans General Hospital (KVGH), and investigated the prevalence of antibiotic-resistant genes within the IntI1 cassettes from those isolates.

A total of 46 CR-A. baumannii strains were isolated from individual bacteremic patients over the period 2000 to 2006. The medical records of 40 of these patients were available for review, and 87.5% (35/40) of cases were mechanical ventilation users. All the CR-A. baumannii bacteremic isolates were also MDR strains as defined. Those strains were confirmed by an automatic system (BD Phoenix 100 Automated Microbiology System; Becton, Dickinson and Company, Franklin Lakes, N.J., USA) as well as by the nucleotide sequences of their 16S-23S rRNA gene spacer region (7). An antimicrobial susceptibility test was performed against a range of commercially available antibiotics, and the identification of this resistance profile followed the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (8). MDR strains were defined as resistant to at least four of the five following groups: (i) gentamicin or amikacin; (ii) cephalosporin or ceftazidime; (iii) piperacillin, piperacillin–tazobactam or ticarcillin–clavulanic acid; (iv) ciprofloxacin or levofloxacin; (v) meropenem or imipenem.

Each strain was grown in 3 ml of Luria-Bertani (LB) broth medium at 37°C. After 16 h, the genomic DNA of the bacteria was isolated using a genomic DNA extraction kit (Promega, Madison, Wis., USA). The primer designs for randomly amplified polymorphic DNA (RAPD) (5’-CCTCATGACCC-3’) and differential integron (intI1f: 5’-CAGTTGGACATAACGCCTGTTC-3’ and intI1r: CCCGAGGCATAGACTGTA; intI1f: 5’-TTGGGAGTAGATCCCTGAGGTCG-3’ and intI1r: TTGCAGTATCCACATCTGGATTTAAGCCTG-3’) and PCR conditions were followed as standard protocols (9). The re-producible PCR-RAPD amplicon profiles were visualized by 1.5% agarose electrophoresis by UV illumination. The RAPD profiles were captured and analyzed using the Quantity One software, version 4.4.0 (BioRad, Hercules, Calif., USA). If the isolates had the same RAPD profiles, the second random primer (5’-TGCGCGCGGGG-3’) was used to confirm their identical genetic backgrounds.
The CR- and MDR- \textit{A. baumannii} bacteremic strains isolated at KVGH included as few as four isolates per year before 2003, but this dramatically increased to over 10 after 2003. A total of 46 individual isolates of CR- and MDR- \textit{A. baumannii} were obtained over the period from 2000 to 2006 and were analyzed for genetic differentiability (Fig. 1). The results indicated that the RAPD patterns of each CR- and MDR- \textit{A. baumannii} isolate before 2003 were independent, but after 2003, the isolates appeared to be grouped into four identical patterns (A-D). An epidemic strain (pattern A) initially appeared in 2003, reached a peak in 2004, but then decreased in 2005. The epidemic strain (pattern B) was found in 2004 and continuously appeared in the hospital. Two emerging epidemic strains (patterns C and D) only occurred in 2006 (Table 1). These results indicate that the origin of the CR- and MDR- \textit{A. baumannii} strains in KVGH changed over time.

Integron-specific PCR amplification revealed that all the CR- and MDR- \textit{A. baumannii} strains harbored class 1 integrons and that no class 2 integrons were present in the chromosomes of these strains. The cassette genes found within those strains were shown to form four types by PCR amplification and nucleotide sequencing (Table 1). All types harbored \textit{aadA1a}, and Types I and IV harbored \textit{aacA4}. This result was associated with the known resistance of these \textit{A. baumannii} isolates to aminoglycoside antibiotics. The open reading frames coding for as-yet-undetermined products were presented in all types. An open reading frame \textit{orfC} in Type III was identical to the sequence of an unknown functional protein which was found in environmental isolates of \textit{Acinetobacter} spp. (3). It is noted that one strain harbored an \textit{intI1} encoding the \textit{bla\textsubscript{IMP-1}} gene.

Our group and others (10) have reported that MDR strains harboring \textit{bla\textsubscript{IMP-1}} existed where this gene forms part of the \textit{intI1} cassette genes found in \textit{A. baumannii}. This finding raised great concern because integron-associated imipenem-resistant \textit{A. baumannii} has been repeatedly found in Taiwanese hospitals, and it suggested that antibiotic resistance might be disseminated throughout populations by the epidemic spread of this clone or through the exchange of genetic material. However, due to natural transformation, loss or altered outer-membrane

![Fig. 1. Dendrogram and RAPD patterns of \textit{A. baumannii} isolates. The PCR-RAPD amplicon profiles were amplified with random primer (5′-CCTCATGACC-3′). The RAPD profiles were captured and analyzed using the software of Quantity One, version 4.4.0 (BioRad). The typical RAPD patterns (A-D) of related strains and the profiles of independent strains (strain number) were presented in this figure.](image)

<table>
<thead>
<tr>
<th>Isolation Year</th>
<th>Isolation No.</th>
<th>Pattern A</th>
<th>Pattern B</th>
<th>Pattern C</th>
<th>Pattern D</th>
<th>Independent strain (Strain no. integron type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12\textsuperscript{a} 3\textsuperscript{iv}</td>
</tr>
<tr>
<td>2001</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4\textsuperscript{m} 3\textsuperscript{m}</td>
</tr>
<tr>
<td>2002</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>2</td>
<td>\textit{9}\textit{m*} 1\textit{m**}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>13</td>
<td>11\textsuperscript{m} 13\textsuperscript{m} 15\textsuperscript{m}</td>
<td>23\textsuperscript{ii}</td>
<td></td>
<td></td>
<td>12\textsuperscript{m} 18\textsuperscript{m} 21\textsuperscript{m}</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
<td>2\textsuperscript{m} 2\textsuperscript{m} 30\textsuperscript{m} 31\textsuperscript{m}</td>
<td>25\textsuperscript{m} 26\textsuperscript{m} 32\textsuperscript{m}</td>
<td></td>
<td></td>
<td>24\textsuperscript{m} 28\textsuperscript{m} 33\textsuperscript{m}</td>
</tr>
<tr>
<td>2006</td>
<td>15</td>
<td></td>
<td>34\textsuperscript{m} 35\textsuperscript{m} 36\textsuperscript{m} 39\textsuperscript{m}</td>
<td>46\textsuperscript{m} 47\textsuperscript{m} 49\textsuperscript{m}</td>
<td>40\textsuperscript{m} 42\textsuperscript{m}</td>
<td></td>
</tr>
</tbody>
</table>

*Roman letters indicated the genetic organization of integron type following as:

- Type I: \textit{IntI1} \textit{aroA3} \textit{accaA4} \textit{orfC} \textit{aadA1a} \textit{qacE\textsubscript{Al}}
- Type II: \textit{IntI1} \textit{bla\textsubscript{IMP-1}} \textit{orfC} \textit{aadA1a} \textit{qacE\textsubscript{Al}}
- Type III: \textit{IntI1} \textit{dsrA1} \textit{orfC} \textit{aadA1a} \textit{qacE\textsubscript{Al}}
- Type IV: \textit{IntI1} \textit{accA4} \textit{catB8} \textit{orfC} \textit{aadA1a} \textit{qacE\textsubscript{Al}}

**The underline indicated that the clinical isolates were obtained from mechanical ventilator users.
brane protein and endogenous metallo-β-lactamases variants, CR strains were probably generated (11-13). Cross-transmission of colonized MDR- \textit{A. baumannii} between patients who are hospitalized in intensive-care units has been well documented (14). In this study, we reported an epidemic strain that caused a hospital-wide outbreak over the period 2003 to 2005. The results of molecular typing revealed that the origin of epidemic strains after 2005 may have changed in this hospital. The restriction of antibiotics use, the intensification and modification of the cleaning procedures for contaminated equipment and the cohorting of patients infected or colonized with MDR- \textit{A. baumannii} need to be seriously considered as control measures to confine the spread of these bacteria.

**ACKNOWLEDGMENTS**

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**REFERENCES**