Short Communication

Role of Integrons in Antimicrobial Susceptibility Patterns of *Acinetobacter baumannii*

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**SUMMARY:** The relationship between the presence and types of integrons and the antimicrobial susceptibility patterns of *Acinetobacter baumannii* was investigated. A total of 134 non-duplicated *A. baumannii* isolates, 54.5% (*n* = 73) of which were subsequently found to carry class 1 integrons, were collected from a regional hospital in Taiwan between March and September 2007. Only two types of gene cassette array, *aacA4-catB8-aadA1* and *aacCI-orfP-orfP-orfQ-aadA1*, were identified. Susceptibility data showed that those strains carrying integrons were significantly more resistant to all antibiotics tested except ampicillin/sublactam and imipenem. An epidemiological study revealed that the same integron could be found in different unrelated strains. These findings suggest that the presence of integrons in *A. baumannii* is responsible for both the horizontal transfer of antibiotic-resistance genes related to aminoglycosides and chloramphenicol and also represents a marker of multidrug resistance and epidemic potential.

*Acinetobacter baumannii* has emerged recently as a major cause of health care-associated infections due to the extent of its antimicrobial resistance and its propensity to cause large nosocomial outbreaks (1). The multidrug resistance of *A. baumannii* to many commonly used antibiotics, such as aminoglycosides, fluoroquinolones, cephalosporins, and carbapenems, has been increasingly reported worldwide (2). The horizontal transfer of antibiotic-resistance genes within a shared gene pool may be one of the most important means by which the spread of antimicrobial resistance in a hospital environment is enhanced (3).

Integrons are genetic assembly platforms/DNA elements that acquire open reading frames embedded in exogenous gene cassettes, which are converted into functional genes upon correct expression (4). The dissemination and characterization of integrons in *A. baumannii* has been described previously (5,6). Most studies reported that integrons are associated with the multidrug resistance of *A. baumannii* (6,7), although one report from northern Spain indicated that no clear antibiogram difference could be correlated with the presence or absence of integrons (8). We therefore decided to undertake this study to investigate the relationship between the presence and types of integrons and the antimicrobial susceptibility patterns of *A. baumannii*. 

*Acinetobacter* spp. were collected consecutively from a regional teaching hospital (699 beds) in northern Taiwan between March and September 2007. Only the first isolate from each patient was included. Organisms were classified as belonging to the genus *Acinetobacter* using a Vitek system (Biomerieux Vitek, Hazelwood, Mo., USA). Identification of these isolates as *A. baumannii* was performed by one-tube multiplex PCR, based on the method of Chen et al. (9). Species other than *A. baumannii* were identified by 16S-23S rRNA gene interngenic spacer (ITS) sequence analysis, as described previously (10). The susceptibility to antimicrobial agents was determined by the disk diffusion method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (11). The agents tested included ampicillin/sublactam, piperacillin, piperacillin/tazobactam, cefepime, gentamicin, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, and imipenem. DNA fingerprinting was performed by repetitive-sequence-based (REP)-PCR, as described previously (12), and cluster analysis for REP-PCR DNA fingerprints was performed by construction of a dendrogram.

Class 1 and 2 integrase (*intI1* and *intI2*) genes and integron gene cassettes were detected by PCR, as described previously (13). One *A. baumannii* isolate with integron gene cassette amplicons was sequenced and then compared with those registered in the National Center for Biotechnology Information (NCBI) database. The gene cassettes in the other *A. baumannii* isolates were typed by nested-PCR. Internal primers specific for the gene cassette arrays were used for PCR am-
plification, with 10-fold dilutions of integron gene cassette amplicons serving as DNA templates. The expected sizes of the amplified DNA fragments confirmed the identity of the gene cassette arrays. Amplification fragments that were not of the expected sizes were subjected to further sequencing.

The susceptibility difference between integron-positive and integron-negative A. baumannii strains was analyzed using the chi-square or Fisher’s exact test, as appropriate. The differences between the two groups of isolates were considered significant at $P < 0.05$. Data entry and analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 15.0 (SPSS Inc., Chicago, Ill., USA).

A total of 149 non-duplicated Acinetobacter spp. isolates were collected and assigned to five genomic species groups based on their 16S-23S rRNA gene ITS regions. Of these, 134 were identified as A. baumannii according to the presence of an internal 208-bp fragment from the ITS region. The species groups of the remaining 15 isolates, which included Acinetobacter genomic species 13TU ($n = 11$), Acinetobacter calcoaceticus ($n = 3$), and Acinetobacter baylyi ($n = 1$), were identified on the basis of the highest similarity scores (88–99%) obtained by ITS sequencing.

Of the 134 A. baumannii isolates, intI1 genes were found in 73 (54.5%). None of the isolates possessed intI2 genes. Table 1 shows that the integron-positive strains were significantly more resistant to all of the tested antibiotics, except ampicillin/sulbactam and imipenem, than the integron-negative strains. The resistance ratios of all the tested antimicrobials, except ampicillin/sulbactam and imipenem, were over 50%. Over 70% of the isolates were resistant to piperacillin, piperacillin/tazobactam, cefepime, ciprofloxacin, and trimethoprim/sulfamethoxazole. Susceptibility to the three most effective antimicrobials, including ampicillin/sulbactam, amikacin, and imipenem, was used to classify the isolates into eight subgroups on the basis of their antibiograms (Table 2). Table 2 shows that the rates of integron carriage were substantially higher in the isolates resistant to amikacin. Furthermore, the integron carriage rates were much higher in two subgroups (1 and 3) of the isolates resistant to both amikacin and imipenem, although no correlation was identified with isolates resistant to ampicillin/sulbactam and imipenem.

Integron cassette genes were found in all intI1-containing isolates. Two dominant gene cassette fragments were found: 91.8% (67/73) of the integron-containing isolates possessed one gene fragment (2.3 kb) and 8.2% (6/73) contained two gene fragments (2.3 kb and 3.0 kb). The smaller gene fragment (2.3 kb) was identified as aacA4-catB8-aadA1 (Genbank accession no. AY557339) and the larger one (3.0 kb) as aacC1-orfP-orfQ-aadA1 (Genbank accession no. AY577724). Two sets of primers specific for these gene cassette arrays, aacA4-catB8-aadA1 (aacA4: 5'-TTGCAATGCTGAATGGAGAG-3' and aadA1: 5'-GTTCAGGAACCGGATCAAAG-3' and orfP: 5'-TGCGGTTATCGGGTTC-3') were designed to confirm their presence in the remaining 72 A. baumannii isolates by nested-PCR. Detection of an approximately 1.6-kb gene fragment confirmed the identity of the gene cassette array aacA4-catB8-aadA1, with a further two gene fragments (1.1 kb and 600 bp) confirming the presence of the gene cassette aacC1-orfP-orfQ-aadA1. Neither the metallo-β-lactamase nor the carbapenem-hydrolyzing class D β-lactamase gene-carrying cassette was found.

The dendrogram for the REP-PCR fingerprints of the 73 integron-containing A. baumannii strains showed eight genotypes (at an 80% similarity cut-off value), with 76.7% (56/73) clustering into genotype I (Table 3). The aacA4-catB8-aadA1 gene cassette array was spread

### Table 1. Susceptibility testing results of 134 integron-positive and -negative Acinetobacter baumannii isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Integron-positive ($n = 73$)</th>
<th>Integron-negative ($n = 61$)</th>
<th>Total (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin/sulbactam</td>
<td>28 (38.4)</td>
<td>19 (31.1)</td>
<td>47 (35.1)</td>
<td>0.384</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>71 (97.3)</td>
<td>38 (62.3)</td>
<td>109 (81.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>63 (86.3)</td>
<td>38 (62.3)</td>
<td>101 (75.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cefepime</td>
<td>65 (89.0)</td>
<td>36 (59.0)</td>
<td>101 (75.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>68 (93.2)</td>
<td>9 (14.8)</td>
<td>77 (57.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amikacin</td>
<td>68 (93.2)</td>
<td>8 (13.1)</td>
<td>76 (56.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>71 (97.3)</td>
<td>39 (63.9)</td>
<td>110 (82.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>71 (97.3)</td>
<td>41 (67.2)</td>
<td>112 (83.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Imipenem</td>
<td>30 (41.2)</td>
<td>19 (31.1)</td>
<td>49 (36.6)</td>
<td>0.234</td>
</tr>
</tbody>
</table>

1: Resistance included resistant (R) and intermediate (I) by CLSI.
2: A $P$ value of $<0.05$ is considered a significant difference in resistance between integron-positive and -negative isolates.

### Table 2. Association of integrons in different subgroups of Acinetobacter baumannii based on the antibiotic

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>IPM</th>
<th>SAM</th>
<th>AN</th>
<th>intI1</th>
<th>intI2</th>
<th>Total</th>
<th>Integron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>1</td>
<td>1</td>
<td>19</td>
<td>94.7</td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>2</td>
<td>11</td>
<td>13</td>
<td>81.8</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>1</td>
<td>11</td>
<td>12</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>31</td>
<td>5</td>
<td>36</td>
<td>86.1</td>
</tr>
<tr>
<td>6</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>12.5</td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>3</td>
<td>33</td>
<td>36</td>
<td>9.1</td>
</tr>
</tbody>
</table>

IPM, imipenem; SAM, ampicillin/sulbactam; AN, amikacin; intI1, class 1 integrase gene; R, resistant; S, susceptible.
throughout all the eight genotypically unrelated A. baumannii strains, and the aacC1-orfP-orfP-orfQ-aadA1 gene cassette array was detected in six strains (genotype 2); 93.2% (68/73) of the integron-containing isolates were simultaneously resistant to gentamicin and amikacin.

One study conducted in Korea (14) showed that A. baumannii was the most prevalent (61%) among Acinetobacter spp., followed by Acinetobacter genomic species 13TU (26%). Both these Acinetobacter spp. possessed distinct phenotypic and genotypic traits in terms of antimicrobial susceptibility, therefore herein we investigated the role of integrons in antimicrobial susceptibility only in A. baumannii. In comparison with the results of antimicrobial susceptibility testing among A. baumannii strains performed by Ko et al. (15), our data revealed astonishingly high resistance ratios of A. baumannii to antimicrobials in Taiwan, especially to imipenem and ciprofloxacin.

Our results also revealed that integrons are largely responsible for the carriage of cassette genes conferring aminoglycoside resistance and represent a marker of multidrug resistance. One previous study suggested that integron-associated multidrug resistance may either be directly involved in the carriage of specific resistance genes or indirectly involved by virtue of linkage to other resistance determinants (16). In our study, the higher resistance among the integron-positive A. baumannii isolates to antibiotics other than aminoglycosides did not appear to rely greatly upon the presence of integrons, thus suggesting that other resistance mechanisms are likely to be involved.

Resistance to carbapenem by carbapenem-hydrolyzing oxacillinase (17), metallo-β-lactamases (18), and extended-spectrum β-lactamase (19) has been reported to be conferred to A. baumannii strains via integrons. The whole genome pyrosequencing of an epidemic, multidrug-resistant A. baumannii strain belonging to the European clone II group showed the presence of an island containing seven resistance genes, including a class 1 integron carrying the aacA4 and blaOXA-20 β-lactamase genes (20). This correlation between the presence of integrons and certain resistance genes might explain the multidrug resistance of intI1-positive A. baumannii strains described in some previous studies. However, no resistance genes, such as blaoXA or blaIMP, were found in the integron gene cassettes of A. baumannii isolates in our study.

Only two cassette arrays were found in A. baumannii in this study. As shown in Table 3, there was no significant difference in terms of antimicrobial susceptibility patterns between these two groups of isolates with different gene cassette arrays (P = 0.357). The cassette array aacA4-catB8-aadA1, which had been previously documented in A. baumannii worldwide (5), was the most prevalent (100%) in this study, although another cassette array, aacCl-orfP-orfP-orfQ-aadA1, which was also found in this study, is widely distributed in isolates of European clones I and II from many countries (21). This cassette array has not, however, been reported in Taiwan. Although the number and types of integrons in A. baumannii were different among the previous two studies (6, 22) and ours, all three studies identified the cassette array aacA4-catB8-aadA1 in A. baumannii, thereby suggesting that aacA4-catB8-aadA1 is a prevalent integron gene cassette array in A. baumannii in Taiwan.

Horizontal gene transfer was clearly evident in this study for aacA4-catB8-aadA1, which was disseminated among clonally unrelated A. baumannii strains. Integron typing might therefore be helpful to prevent the further spread of strains with epidemic potential, as proposed in a previous study (23).

In conclusion, irrespective of whether or not resistance genes were contained within an integron, we have demonstrated a strong association between integron carriage and a reduced susceptibility to many classes of antibiotics. The results of this study also indicate that the presence of integrons in A. baumannii is responsible for both horizontal transfer of antibiotic-resistance genes related to aminoglycosides and chloramphenicol and also represents a marker of multidrug resistance and epidemic potential.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gene cassette array</th>
<th>No. of strains</th>
<th>Exhibiting resistance to</th>
<th>Gentamicin</th>
<th>Amikacin</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>aacA4-catB8-aadA1</td>
<td>56</td>
<td>R</td>
<td>R</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>aacCl-orfP-orfP-orfQ-aadA1</td>
<td>1</td>
<td>S</td>
<td>S</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>aacA4-catB8-aadA1</td>
<td>6</td>
<td>R</td>
<td>R</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>aacA4-catB8-aadA1</td>
<td>3</td>
<td>S</td>
<td>S</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>aacA4-catB8-aadA1</td>
<td>5</td>
<td>R</td>
<td>R</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>aacA4-catB8-aadA1</td>
<td>2</td>
<td>R</td>
<td>R</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>aacA4-catB8-aadA1</td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>aacA4-catB8-aadA1</td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

R, resistant; S, susceptible.

Table 3. Summary for REP-PCR typing, integron organization and antimicrobial susceptibility in integron-containing Acinetobacter baumannii isolates
Acknowledgments This study was supported by a grant from the North Region Alliance Department of Health Hospital in Taiwan. We thank Professor Tsung-Chain Chang at National Cheng Kung University, Taiwan, for providing reference Acinetobacter strains.

REFERENCES