Integrons and Gene Cassettes in the *Enterobacteriaceae*

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Integrons were detected in 59 of 120 (49%) urinary isolates of *Enterobacteriaceae* by PCR using degenerate primers targeted to conserved regions of class 1, 2, and 3 integrase genes. PCR sequencing analysis of the cassette arrays revealed a predominance of cassettes that confer resistance to the aminoglycosides and trimethoprim.

Dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates of bacteria (20). The spread of resistance genes is greatly enhanced when they form part of a mobile gene cassette, since this provides for horizontal transfer by several mechanisms. These mechanisms include (i) mobilization of individual cassettes by the integron-encoded integrase (9), (ii) movement when the integrin containing the cassette relocates—probably by targeted transposition (6, 10, 19), (iii) dissemination of larger transposons such as Tn21 carrying integrons (16), and (iv) movement of conjugal plasmids containing integrons among different bacterial species. It is therefore not surprising that many of the antibiotic resistance genes found in clinical isolates of gram-negative microorganisms are part of a gene cassette inserted into an integron (21). The four classes of integron so far identified (classes 1, 2, 3, and 4) are distinguished by their respective integrase (*int*) genes (1, 18, 21). Class 4 is a distinctive class of integrons located in the *Vibrio cholerae* genome and is not known to be associated with antibiotic resistance (18).

Gene cassettes consist of a gene flanked by a recombination site, known as a 59-base element, which is recognized by the integron-encoded site-specific recombinase (IntI). Gene cassettes can exist as free circular molecules (9) and are transcribed only when captured and inserted into an integron, usually at the *attI* recombination site 104 bp upstream of the *intI* gene (13). New cassettes are continually being discovered, and now over 60 cassettes that confer resistance to a range of antimicrobial agents have been identified (14, 21, 23).

Despite the detailed understanding of the molecular relationship between gene cassettes and integrons (13, 21), there is a paucity of information on how widespread these elements are in a hospital setting. Only a few studies have suggested that integrons are widespread in both animal and human clinical bacterial isolates (3, 15, 17, 22). In this study we have determined the incidence of integrons and their class and characterized the cassette arrays in a collection of random isolates collected from nine clinical settings in Sydney, Australia.

Clinical isolates. One hundred and twenty urinary isolates identified as belonging to the *Enterobacteriaceae* were studied. The isolates were randomly selected during a six-month period (August 1998 to January 1999) from patients in 46 wards of seven hospitals and two community clinics. These organisms were identified as *Escherichia coli* (n = 90), *Proteus* sp. (n = 13), *Klebsiella* sp. (n = 15), and *Enterobacter* sp. (n = 2) using standard biochemical criteria (2). The diversity of the 120 isolates was assessed by calculating Simpson's index, using identification of bacterial genus (n = 4) and antibiotic profiles (n = 52). The probability of selecting two strains at random from different genera with different antibiotic profiles was calculated to be 94.2%.

Antibiotic resistance profiles. Sensitivity profiles were established by agar diffusion using the calibrated dichotomous sensitivity test (4, 5). Isolates were tested for susceptibility to a panel of 18 antibiotics, representing 11 classes (Table 1). Integrons were strongly associated with multiple-antibiotic-resistant strains, with integron-positive strains demonstrating a greater predilection for antibiotic resistance than integron-negative strains (Table 1). Ninety-seven of 120 (81%) strains were resistant to one or more antibiotic. High levels of resistance were found to antibiotics which have been available therapeutically for a long time including ampicillin (64%), sulfafurazole (59%), streptomycin (48%), tetracycline (39%), and trimethoprim (36%) (Table 1).

Incidence of integrons in 120 isolates of *Enterobacteriaceae*. DNA was extracted from bacteria using standard techniques for the isolation of plasmid DNA, and integrons were detected by PCR with the degenerate primers hep35 (5' TGGCGGT YAARGATBTKGATT3') and hep36 (5' CARCACATGC GTRARAT 3'), which hybridize to conserved regions of integron-encoded integrase genes *intI1*, *intI2*, and *intI3* (23). Fifty-nine of the 120 isolates (49%) were integron positive. The class of the integron was determined by analyzing integrase PCR products by restriction fragment length polymorphism (RFLP) following digestion using either *RsaI* or *Hinfl* restriction enzyme (Table 2). Restriction analysis revealed that 36% contained a single class 1 integron, 3% contained two class 1 integrons (as determined by the presence of two class 1 cassette PCR products), 6% contained class 1 and 2 integrons, and 4% contained a class 2 integron (Table 3). In total, 58 class 1 integrons and 12 class 2 integrons were identified in 59 of the 120 isolates. No class 3 integrons were detected.

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Characterization of cassette arrays. Class 1 integron cassette regions were amplified using hep58 and hep59 as described previously (23). Class 2 integron cassette regions were amplified using hep74, which binds to attI2, and hep51, which binds to orfX, which is situated downstream of the cassette region within Tn7 (GenBank accession number AJ002782). Cassette PCR products were restricted with RsaI and Hinfl. Two representative products of each distinct RFLP were purified by polyethylene glycol precipitation (11) and sequenced. Analysis of 67 integron cassette regions identified a total of 104 cassettes (Table 3). The cassette regions of three class 1 integrons could not be amplified by PCR, possibly due to the lack of a 3′-conserved segment. Class 1 integrons harbored 11 different cassette arrays (Table 3). The most common types of cassette carried by class 1 integrons were those conferring resistance to streptomycin and spectinomycin. These cassettes represented 53% of all cassettes found and included aadA1 (39% of cassettes), aadA2 (9%), and aadA5 (5%).

A second aminoglycoside adenylyltransferase gene cassette (aadB), encoding resistance to gentamicin, kanamycin, and tobramycin, was detected exclusively in seven Klebsiella isolates. Interestingly, five of these seven isolates exhibited extended-spectrum β-lactamase activity, indicating that aadB was also associated with this resistance. dfr cassettes (dfrA1, dfrA2, dfrA12, dfrA17, and dfrB2) that confer resistance to trimethoprim represented 27% of cassettes detected (Table 3). It is likely that selection for cassettes carrying dfr genes has occurred in this population because trimethoprim is used to treat urinary tract infections, and this could therefore account for their high prevalence. Other individual cassettes identified in class 1 integrons were orfA and ereA2 (resistance to erythromycin).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Date of discovery</th>
<th>% Resistance of int-positive strains (total no. of isolates)</th>
<th>% Resistance of int-negative strains (total no. of isolates)</th>
<th>% Resistance of total (total no. of isolates)</th>
<th>Association with integron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1944</td>
<td>81.4 (48)</td>
<td>16.4 (10)</td>
<td>48.3 (58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>1957</td>
<td>22.0 (13)</td>
<td>1.6 (1)</td>
<td>11.7 (14)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1963</td>
<td>16.9 (10)</td>
<td>3.3 (2)</td>
<td>10.0 (12)</td>
<td>0.0151</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1968</td>
<td>16.3 (9)</td>
<td>3.3 (2)</td>
<td>9.2 (11)</td>
<td>0.0283</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1972</td>
<td>3.4 (2)</td>
<td>1.6 (1)</td>
<td>2.5 (3)</td>
<td>0.6155</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>1976</td>
<td>3.4 (2)</td>
<td>1.6 (1)</td>
<td>2.5 (3)</td>
<td>0.6155</td>
</tr>
<tr>
<td>Antifolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfafurazole</td>
<td>1932</td>
<td>91.5 (54)</td>
<td>27.9 (17)</td>
<td>59.2 (71)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1961</td>
<td>62.7 (37)</td>
<td>9.8 (6)</td>
<td>35.8 (43)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Carbapenem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>1983</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>1969</td>
<td>11.9 (7)</td>
<td>6.6 (4)</td>
<td>9.2 (11)</td>
<td>0.5257</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1976</td>
<td>11.9 (7)</td>
<td>6.6 (4)</td>
<td>9.2 (11)</td>
<td>0.5555</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>1981</td>
<td>1.7 (1)</td>
<td>1.6 (1)</td>
<td>1.7 (2)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1961</td>
<td>89.8 (53)</td>
<td>39.3 (24)</td>
<td>64.2 (77)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Augmentin</td>
<td>1977</td>
<td>8.5 (5)</td>
<td>8.2 (5)</td>
<td>8.3 (10)</td>
<td>0.3910</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1947</td>
<td>30.5 (18)</td>
<td>4.9 (3)</td>
<td>17.5 (21)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>52.5 (31)</td>
<td>26.2 (16)</td>
<td>39.2 (47)</td>
<td>0.0048</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>1953</td>
<td>23.7 (14)</td>
<td>19.7 (12)</td>
<td>21.7 (26)</td>
<td>0.6606</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>1980</td>
<td>10.2 (6)</td>
<td>1.6 (1)</td>
<td>5.8 (7)</td>
<td>0.1111</td>
</tr>
</tbody>
</table>

* Data obtained from reference 12.

* Total number of int-positive bacteria, 59.

* Total number of int-negative bacteria, 61.

* Significant values are in bold.

TABLE 2. RFLP classification of integrase PCR products

<table>
<thead>
<tr>
<th>PCR product</th>
<th>Enzyme</th>
<th>No. of fragments</th>
<th>Fragment size(s) (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>int1</td>
<td>RuI</td>
<td>1</td>
<td>491</td>
</tr>
<tr>
<td></td>
<td>Hinfl</td>
<td>1</td>
<td>491</td>
</tr>
<tr>
<td>int2</td>
<td>RuI</td>
<td>2</td>
<td>334, 157</td>
</tr>
<tr>
<td></td>
<td>Hinfl</td>
<td>2</td>
<td>300, 191</td>
</tr>
<tr>
<td>int3</td>
<td>RuI</td>
<td>3</td>
<td>97, 104, 290</td>
</tr>
<tr>
<td></td>
<td>Hinfl</td>
<td>2</td>
<td>119, 372</td>
</tr>
<tr>
<td>Integrons and cassette arrays</td>
<td>Site of collection</td>
<td>Incidence by organism</td>
<td>Total</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------</td>
<td>-----------------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>Klebsiella sp.</td>
</tr>
<tr>
<td>Class 1 integrons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>aadA1</strong></td>
<td>H1 (17), H2, H3, H4, H5 (3), C2</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td><strong>dfA17-aadA5</strong></td>
<td>H1 (2), H2, H4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>dfA12-aadA2</strong></td>
<td>H1, H2 (2)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>dfA5</strong></td>
<td>H1, H2, H3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>aadB</strong></td>
<td>H1, H2 (2)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>dfA1-aadA1</td>
<td>H1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aadA1-aadA5</td>
<td>H5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>dfB2-orfA</td>
<td>H1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>dfA12</td>
<td>H1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Class 1 (undetermined)</td>
<td>H1 (2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Two class 1 integrons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>aadB + aadA2</strong></td>
<td>H1 (2), H3 (2)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Class 1 and 2 integrons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aadA1 + dfA1-sat1-aadA1</td>
<td>H3, H4, H6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>dfA12-aadA2 + dfA1-sat1-aadA1</td>
<td>H1, C1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>dfA5-ereA2 + dfA1-sat1-aadA1</td>
<td>H1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Class 1 (undetermined) + dfA1-sat1-aadA1</td>
<td>H2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Class 2 integron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dfA1-sat1-aadA1</td>
<td>H1, H3, H4 (2), C1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total (% of total)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>44 (74.6)</td>
<td>9 (15.2)</td>
</tr>
</tbody>
</table>

- Class 2 cassettes are underlined.
- For identification of cassettes, accession numbers from references 21 and 24 were used.
- Numbers of organisms isolated are shown in parentheses if more than one. H, hospital; C, community clinic.
- Bold, antibiotic relevance to integron-associated resistance genes. AM, ampicillin; CM, chloramphenicol; GM, gentamicin; KM, kanamycin; SM, streptomycin; SU, sulfafurazole; TC, tetracycline; TP, trimethoprim.
All 12 class 2 integrons carried the same three cassettes as those found in Tn7, namely, dfrA1, sat1, and aadA1 (Table 3). This could be explained by the fact that the class 2 integrase gene (intI2) contains an early stop codon resulting in a truncated form of the enzyme. The resultant integrase is therefore unable to excise existing cassettes or insert new ones.

**Associations between integrons and antibiotic resistance.**

Integrons were significantly associated with resistance to certain antibiotics including gentamicin, kanamycin, streptomycin, tobramycin, sulfafurazole, trimethoprim, ampicillin, chloramphenicol, and tetracycline (Table 1). However, resistance to only streptomycin, sulfafurazole, and trimethoprim, and to some extent gentamicin, kanamycin, and tobramycin, could be directly related to the presence of resistance genes within the integron (Table 3). The association of the other older antibiotics ampicillin, chloramphenicol, and tetracycline with the presence of an integron is likely to be due to genetic linkage between integrons and conjugative plasmids and transposons.

**Conclusion.** Gene cassettes conferring resistance to nearly every major class of antibiotics have been identified, with the notable exception of the quinolones. Despite this, the current impact of integrons on resistance in Sydney, Australia, and indeed in other countries (17), appears to be focused towards the usual array of gene cassettes. Antimicrob. Agents Chemother. 39:155–162.

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**REFERENCES**


