Acinetobacter baumannii International Clones and Acinetobacter nosocomialis Isolates

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The Acinetobacter baumannii clonal complex 113/79 (CC113/79) and class 2 integrons predominate in Latin America; a relationship between these characteristics was explored. The presence of integrons was determined in successive hospital Acinetobacter isolates (163 A. baumannii isolates and 72 Acinetobacter nosocomialis isolates). Most isolates had integrons, but class 1 and 2 integrons were present significantly more often in CC109/1 and CC113/79, respectively. The high prevalence of CC113/79 in Latin America may account for the predominance of class 2 integrons.

The dissemination of multidrug-resistant (MDR) Acinetobacter baumannii international clones (IC) and Acinetobacter nosocomialis has challenged health care (1, 2). The MDR phenotype in Acinetobacter has been related to class 1 and 2 integrons (3, 4, 5); class 2 integrons predominate in A. baumannii from Latin America (5, 6). This finding is possibly related to local clonal groups (6), but a hypothesis has not yet been addressed. The class 2 integron structure seems less diverse than that of class 1 (7) and is usually embedded within transposon Tn7 (5, 8).

Four A. baumannii and one A. nosocomialis IC characterized by multilocus sequence typing (MLST) have been described and are spread in Rio de Janeiro, Brazil (9, 10). The purpose of the present study was to explore the association of class 2 integrons and gene cassettes with these clonal lineages. Between 2007 and 2008, 163 A. baumannii and 72 A. nosocomialis hospital isolates were investigated (10); 153 (94%) of the A. baumannii and 21 (29%) of the A. nosocomialis isolates were MDR. Among the A. nosocomialis isolates, antimicrobial resistance was highest for trimethoprim-sulfamethoxazole (66%), cefepime (23%), and ciprofloxacin (23%) (10).

The presence of class 1 and 2 integrons was screened in all isolates by multiplex PCR with intI1 and intI2 gene-specific primers (11). The intI amplicons of two A. baumannii isolates were sequenced as controls. The variable region for intI2 gene-positive isolates was characterized by amplification with the primers Hep74 and Hep51 (12) and by sequence analyses with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Isolates with no amplification of the variable region were further studied by PCR mapping with a Hep74 primer combined with the dfra1, sat2, aadA1, and aadB reverse primers, as described previously (13). PCR was also performed for Tn7 transposition genes (tnsA, tnsB, tnsC, tnsD, and tnsE), where the 3’ conserved sequence region is located (7). One positive amplicon of each tns gene was sequenced as a control.

All isolates had been typed by randomly amplified polymorphic DNA (RAPD) PCR (10), and a few were typed by MLST (using University of Oxford [UO] and Institut Pasteur [IP] schemes) (9, 10). In the present study, MLST was performed for all A. baumannii isolates. The proportions were compared by the Fisher exact test or the chi-square test (see http://www.openepi.com); a P value of <0.05 was considered significant.

Integrase-encoding genes were found in 158 (67%) of the 235 isolates, with a slight predominance of intI1 in A. baumannii and intI2 in A. nosocomialis (Fig. 1). All A. baumannii isolates were clonal complex 113/79 (CC113/79) (according to UO/IP schemes), CC109/1, CC103/15, or CC110/25. A few A. baumannii isolates (5%) harbored both of the integrases, belonging to CC113/79 (n = 4) and CC110/25 (n = 1). The intI1 gene was predominant in CC109/1 (n = 30, 71%), and the intI2 gene was predominant in CC113/79 (n = 41, 41.7%) (P < 0.001 for CC109/1 versus CC113/79) (Fig. 2A). Altogether, CC109/1 accounted for 46% of class 1 integron-bearing isolates, and CC113/79 accounted for 89% of class 2 integron-bearing isolates. Among the 25 patients with intI2-positive CC113/79 isolates and the 11 patients with intI1-positive CC109/1 isolates, 7 (28%) and 6 (55%), respectively, were moved to the intensive care unit (ICU).

In A. nosocomialis, integrase-encoding genes were found in 53 (73%) isolates and intI2 was found in 39 (54%) isolates (Fig. 1). In RAPD-type A (CC260/71), intI1 and intI2 genes were each present in ≥50% of the isolates (Fig. 2B).

Five and three different cassette arrays were identified in class 2 integrons among A. baumannii and A. nosocomialis isolates, respectively (Table 1). Class 2 integrons In2-0, In2-1, In2-2, In2-4, and In2-6 were not particularly associated with any A. baumannii
or A. nosocomialis clonal type. However, In2-4 was found in 20 (50%) of 41 CC113/79 isolates.

The dfrA1, sat2, and aadA1 genes were identified in 61%, 76%, and 73%, respectively, of A. baumannii isolates carrying the class 2 integron and in 33%, 8%, and 8%, respectively, of A. nosocomialis isolates carrying the class 2 integron. At least one Tn7 transposition gene was present in 88% of A. baumannii and 18% of A. nosocomialis isolates (Table 1).

Among the 153 MDR A. baumannii isolates, 42 (28%) carried cassettes within the class 2 integron; all were resistant to trimethoprim-sulfamethoxazole, and 83% were resistant to aminoglycosides, indicating that resistance was related to the dfrA1 and aadA1 genes. MDR A. nosocomialis isolates were less frequent, but trimethoprim-sulfamethoxazole resistance was strongly associated with class 2 integrons. Nevertheless, five A. nosocomialis isolates with class 2 arrays (In2-1) were susceptible to all the agents tested (data not shown).

Here, we describe class 1 and 2 integrons that were strongly associated with CC109/1 (IC-I) and CC113/79 A. baumannii isolates, respectively. One concern with this study is that isolates were epidemiologically related, and some were from cross-infections. Nevertheless, the cohort study design provided accurate prevalence data; the cases moved to the ICU represented unrelated sources of these clones. This result may explain the high prevalence of intI2 in Latin America (5), where CC113/79 predominates (14). Indeed, in Latin America, the low prevalence of IC-I (<20%) (14, 15) can explain the low frequency of the class 1 integron among local A. baumannii isolates. In Europe, IC-I and class 1 integrons have been frequently found (3, 16).

Both integrons were found simultaneously in a few A. baumannii isolates of CC113/79 and CC110/25 in the present study. This finding has been described by others but only in isolates of undefined MLST clones (7, 17, 18). By in silico analysis of 11 A. baumannii genomes, including three IC-I and six IC-II, only class 1 integrons were found (19).

In other non-baumannii species of Acinetobacter, there have been few reports of integrons (20, 21). In the present study, a similar distribution of class 1 and 2 integrons was observed in A. nosocomialis, including in CC260/71 isolates. A class 1 integron containing a metallo-β-lactamase cassette was described in this clone from Japan and Korea (20, 21); however, we did not find such genes in A. nosocomialis (10).

In the present study, the In2-4 array was found in 46% of CC113/79 A. baumannii isolates. In Argentina, this array was also frequently observed (5), and this finding is in line with the report of the CC113/79 spread in this country (14). Similarly, the description of dfrA1, sat2, and aadA1 cassettes in Argentina, Chile, and Uruguay may also be explained by the CC113/79 spread in Latin America (5, 7). A single Brazilian study of MDR A. baumannii found class 2 integrons in 23% of isolates (22), which also carried the main cassette array (dfrA1-sat2-aadA1) found in our study.

The present study contributes to the understanding of the distribution of class 1 and 2 integrons in A. baumannii and A. nosocomialis isolates and of how the distribution is affected by the global dispersion of international clones in different regions of the world.

FIG 1 Distribution of intI genes among A. baumannii and A. nosocomialis isolates.

FIG 2 Frequency of intI genes in 163 Acinetobacter baumannii isolates according to MLST clonal complexes (CC) (A) and 72 Acinetobacter nosocomialis isolates according to RAPD types (B). The white and black columns are intI1- and intI2-positive isolates, respectively. The asterisk in panel A represents a P value of <0.001 (compared to A. baumannii isolates included in all other clonal types), and that in panel B represents a P value of 0.02 (for intI1 in A. nosocomialis type C compared to type A).
TABLE 1  A. baumannii clonal complexes and A. nosocomialis RAPD genotypes, class 2 integrons, and the variable regions

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<th>Species (no. of isolates)</th>
<th>Genotype (no. of isolates)</th>
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<th>Transposition gene(s) (no. of isolates)</th>
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Integrons in \textit{A. baumannii} and \textit{A. nosocomialis}