Type 1 Integrons in Epidemiologically Unrelated Acinetobacter baumannii Isolates Collected at Spanish Hospitals

Acinetobacter baumannii is an opportunistic nosocomial pathogen, which is an important cause of pneumonia and bacteremia in patients in intensive care units (1). Increased resistance to all commercial antimicrobial agents, including colistin, in clinical isolates of A. baumannii has been reported (7, 12). An important factor for the development of multiresistance is the acquisition of genetic elements, such as integrons (6). Different reports have been published, identifying integrons as responsible for the presence and acquisition of antibiotic resistance in members of the genus Acinetobacter (2, 3, 5, 8, 9, 10, 13).

The aim of this study was to investigate the role of type 1 integrons in mediating antibiotic resistance in Spanish clinical isolates of A. baumannii. Moreover, the epidemiological relationship between Spanish isolates containing type 1 integrons and seven isolates from Italian hospitals containing the same integrons was determined.

For this purpose, 69 epidemiologically unrelated A. baumannii isolates were collected during November 2000 from 28 Spanish hospitals. All isolates were identified by amplified ribosomal DNA restriction analysis (11), and their epidemiological relationship was determined by pulsed-field gel electrophoresis (PFGE), following the method of Gautom (4).

PCR amplification of type 1 integrons was done using the set of primers described by Ploy et al. (8) following conditions and procedures that will be published elsewhere (9). DNA sequencing of the inserted gene cassettes was performed with the dRhodamine terminator cycle sequencing kit and was analyzed in an automatic DNA sequencer (ABI Prism 377; Perkin-Elmer, Emeryville, Calif.).

Of a total of 69 A. baumannii isolates, 19 (27.53%) possessed type 1 integrons. Fifteen of these 19 (79.94%) isolates showed the presence of a 700-bp band containing a single aadB allele (Table 1). One of the 19 isolates (5.26%) yielded an amplification product of approximately 2,400 bp (Table 1) with three gene cassettes, an aacC1 allele, an open reading frame (ORF) coding for a yet undetermined product named OrfO, and the blaOXA-20 gene (5, 8). Two of the 19 isolates (10.52%) gave an amplification product of approximately 800 bp (Table 1). Direct sequencing of this amplicon revealed the presence of a single gene cassette that contained an aacA4 gene, which was identical to that found in the integron mentioned above. Of the two isolates containing this integron, one was resistant to both tobramycin and amikacin, while the other isolate was resistant to tobramycin but was susceptible to amikacin. These results agreed with those found by Ploy et al. (8) who found two isolates with the same integron but susceptible to amikacin. Only one isolate (5.26%) showed an amplicon of approximately 2,800 bp containing four gene cassettes (Table 1), an aacC1 determinant, followed by two ORFs that code for unknown products and that are carried on two cassettes (5), and an aadA1 gene. Of course, this type of integron carrying four gene cassettes has been described only once and is found in Italian isolates (5).

The integrons of 800, 2,400, and 2,800 bp, were found in Italian A. baumannii isolates. In order to elucidate whether Italian isolates with the same type of integrons (5) possessed the same clonal origin as the Spanish clinical isolates of A. baumannii, a PFGE was performed. The results showed that all the isolates were not epidemiologically related.

In conclusion, our results reflect the potential risk of antimicrobial resistance dissemination, both within and between unrelated species. Moreover, we demonstrate that nonrelated isolates from different geographic areas are able to acquire common integrons, leading to the question of whether A. baumannii has a clear affinity for a specific type of integron.

A.R. has a fellowship from the Ministerio de Educación y Ciencia of Spain. This work has been supported in part by a research grant from Merck Sharp & Dohme in Madrid, Spain. We thank Lucilla Dolzani (Dipartimento di Scienze Biomediche, Sezione di Microbiologia, Università di Trieste, Trieste, Italy) for providing us with the Italian clinical isolates of A. baumannii. We also thank the Red Española de Investigación en Patología Infecciosa (Ministerio de Sanidad de Spain) for some financial support.

The members of the Spanish Group of Nosocomial Infections (GEIH) of the Spanish Society of Infectious Diseases and Clinical Microbiology are as follows: Javier Ariza, M. Angeles Domínguez, Miquel Pujol, and Fe Tubau (Hospital Universitario de Bellvitge, Barcelona, Spain); Juan Pablo Horcajada, Anna Ribera, and Jordi Vila (Hospital Clinic, Barcelona, Spain); Jordi Cuquet, Carmina Martí, and Dolores Navarro (Hospital General de Granollers, Barcelona, Spain); Francisco Alvarez Lema and Margarita Salvador (Hospital del Mar, Barcelona, Spain); Fernando Chaves and Antonio Sánchez Porto (Hospital de la Línea de la Concepción, Cadiz, Spain); Fernando Rodríguez López and Elisa Vidal (Hospital Universitario Reina Sofia, Córdoba, Spain); Alejandro Beceiro and German Bou (Hospital Juan Canalejo, A Coruña, Spain); Manuel de la Rosa (Hospital Virgen de las Nieves, Granada, Spain); Fernando Chaves and Manuel Lisazoain (Hospital Doce de Octubre, Madrid, Spain); Paloma García Hierro and Josefa Gómez Castillo (Hospital de Getafe, Madrid, Spain); Belén Perea (Complejo Hospitalario de Ourense, Ourense, Spain); Gloria Esteban and Begoña Fernández (Hospital Santa María de Nai, Orense, Spain); Nuria Borrell and Antoni Ramírez (Hospital Son Dureta, Palma de

TABLE 1. Integron gene composition related to the phenotype of resistance found in A. baumannii clinical isolates

<table>
<thead>
<tr>
<th>No. of isolates (n = 19)</th>
<th>Amplicon size (bp)</th>
<th>Resistance gene(s)</th>
<th>Resistance phenotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>700</td>
<td>aadB</td>
<td>GEN, TOB</td>
</tr>
<tr>
<td>1</td>
<td>2,400</td>
<td>aac4A, ORF O, blaOXA-20</td>
<td>AMK, TOB, β-Lactams</td>
</tr>
<tr>
<td>2</td>
<td>800</td>
<td>aac4A</td>
<td>TOB</td>
</tr>
<tr>
<td>1</td>
<td>2,800</td>
<td>aacC1, ORF X, ORF X', aadA1a</td>
<td>GEN</td>
</tr>
</tbody>
</table>

* Abbreviations: GEN, gentamicin; TOB, tobramycin; AMK, amikacin.

b Gene cassettes found in Italian isolates.
REFERENCES


