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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 (78.94%) 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 *aacA4* allele, an open reading frame (ORF) coding for a yet undetermined product named OrfO, and the $bla_{\text{OXA-20}}$ 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 *aadA1a* gene. To our knowledge, this type of integron carrying four gene cassettes has been described only once and is found in Italian isolates (5).

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

No. of isolates (n = 19)	Amplicon size (bp)	Resistance gene(s)	Resistance phenotype[a]
15	700	*aadB*	GEN, TOB
1	2,400[b]	*aacA4*, ORF O, $bla_{\text{OXA-20}}$	AMK, TOB, β-Lactams
2	800[b]	*aacA4*	TOB
1	2,800[b]	*aacC1*, ORF X, ORF X′, *aadA1a*	GEN

[a] Abbreviations: GEN, gentamicin; TOB, tobramycin; AMK, amikacin.
[b] Gene cassettes found in Italian isolates.

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.

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REFERENCES

1. **Bergogne-Berezin, E., and K. J. Towner.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148–165.
2. **Chu, Y.-W., M. Afzal-shah, E. T. S. Houang, M.-F. I. Palepou, D. J. Lyon, N. Woodford, and D. Livermore.** 2001. IMP-4, a novel metallo-β-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob. Agents Chemother. **45:**710–714.
3. **Gallego, L., and K. J. Towner.** 2001. Carriage of class 1 integrons and antibiotic resistance in clinical isolates of *Acinetobacter baumannii* from northern Spain. J. Med. Microbiol. **50:**71–77.
4. **Gautom, R. K.** 1997. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Microbiol. **35:**2977–2980.
5. **Gombac, F., M. L. Riccio, G. M. Rossolini, C. Lagatolla, E. Tonin, C. Monti-Bragadin, A. Lavenia, and L. Dolzani.** 2002. Molecular characterization of integrons in epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* from Italian hospitals reveals a limited diversity of gene cassette arrays. Antimicrob. Agents Chemother. **46:**3665–3668.
6. **Hall, R. M., and C. M. Collis.** 1998. Antibiotic resistance in gram-negative bacteria: the role of gene cassettes and integrons. Drug Resist. Updates **1:**109–119.
7. **Levin, A. S., A. A. Barone, J. Penco, M. V. Santos, I. S. Marinho, E. A. Arruda, E. I. Manrique, and S. F. Costa.** 1999. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Clin. Infect. Dis. **28:**1008–1011.
8. **Ploy, M. C., F. Denis, P. Courvalin, and T. Lambert.** 2000. Molecular characterization of integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron. Antimicrob. Agents Chemother. **44:**2684–2688.
9. **Ruiz, J., M. M. Navia, C. Casals, J. M. Sierra, M. T. Jiménez de Anta, and J. Vila.** 2003. Integron-mediated antibiotic multiresistance in *Acinetobacter baumannii* clinical isolates from Spain. Clin. Microbiol. Infect. **9:**907–911.
10. **Segal, H., and B. G. Elisha.** 1997. Identification and characterization of an *aadB* gene cassette at a secondary site in a plasmid from *Acinetobacter*. FEMS Microbiol. Lett. **153:**321–326.
11. **Vaneechoutte, M., L. Dijkshoorn, I. Tjernberg, A. Elaichouni, P. de Vos, G. Claeys, and G. Verschraegen.** 1995. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. **33:**11–15.
12. **Vila, J., M. A. Marcos, F. Marco, S. Abdalah, Y. Vergara, R. Reig, R. Gómez-Lus, and M. T. Jiménez de Anta.** 1993. In vitro antimicrobial production of β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase and susceptibility of clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **37:**138–141.
13. **Yum, J. H., K. Yi, H. Lee, D. Yong, K. Lee, J. M. Kim, G. M. Rossolini, and Y. Chong.** 2002. Molecular characterization of metallo-β-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new integrons carrying the bla_{VIM-2} gene cassettes. J. Antimicrob. Chemother. **49:**837–840.

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