ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2008, p. 2955–2958 Vol. 52, No. 8
0066-4804/08/$08.00+0 doi:10.1128/AAC.00072-08

Characterization of the Carbapenem-Hydrolyzing Oxacillinase Oxa-58 in an *Acinetobacter* Genospecies 3 Clinical Isolate▽

Sara Marti,[1] Javier Sánchez-Céspedes,[1] M. Dolores Blasco,[2] Marc Ruiz,[1] Paula Espinal,[1] Verónica Alba,[1] Felipe Fernández-Cuenca,[3] Alvaro Pascual,[3] and Jordi Vila[1]*

Servei de Microbiologia, Centre de Diagnóstic Biomèdic, Hospital Clínic, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain[1]; Departament de Microbiologia i Ecologia, Universitat de Valencia, Valencia, Spain[2]; and Departamento de Microbiología, Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain[3]

Received 17 January 2008/Returned for modification 20 March 2008/Accepted 16 May 2008

Based on imipenem resistance in an *Acinetobacter* genospecies 3 clinical isolate, we were able to identify, for the first time in this genomic species, a plasmid-encoded bla_{OXA-58} gene that was 100% homologous to the same gene in *Acinetobacter baumannii*.

Since 1986 members of the genus *Acinetobacter* are determined by DNA-DNA hybridization. Genospecies 1 (*Acinetobacter calcoaceticus*), 2 (*A. baumannii*), 3, and 13TU are genetically closely related and are commonly known as the *A. calcoaceticus-A. baumannii* complex. With the exception of genospecies 1, the other members of this complex have been involved in nosocomial infections and have the ability to spread in hospitals (3, 9, 19, 23, 25, 26). Treatment of these nosocomial infections is becoming a problem because increasing resistance to antibiotics, especially in the case of *A. baumannii*. In the last decade, carbapenem resistance in *Acinetobacter* spp. has been reported worldwide (3, 16, 23), mostly associated with the synthesis of carbapenem-hydrolyzing β-lactamases, reduced outer membrane permeability and, occasionally, modification of penicillin-binding proteins (7, 16, 22, 27). The most prevalent carbapenemases in *Acinetobacter* spp. are the carbapenem-hydrolyzing class D β-lactamases, which are divided into four phylogenetic subgroups: OXA-23, OXA-24, and OXA-58 with all their variants and the OXA-51 family, which is intrinsic to *A. baumannii* (16, 27).

OXA-58 confers reduced susceptibility to carbapenems, but it produces high-level resistance to carbapenems when additional efflux mechanisms are expressed (12, 15). It was first identified in France in 2003 and, at present, is found worldwide in *A. baumannii* isolates (13, 16, 20, 21), as well as in *A. junii* isolates from Romania and Australia (12, 14).

The clinical isolates Ac057 (*Acinetobacter* sp. strain G3) and Ac058 (*A. baumannii*) were obtained from the same hospital in November 2000 and were identified by amplified ribosomal DNA restriction analysis (ARDRA) (6, 24). The epidemiological difference was corroborated by pulsed-field gel electrophoresis (PFGE) with ApaI (Promega, Madrid, Spain) under conditions described elsewhere (11).

Antimicrobial susceptibility analysis was performed by Etest according to the manufacturer's instructions (AB Biodisk, Sölna, Sweden) and determined that both strains had an imipenem MIC of >32 μg/ml (Table 1). The breakpoints for imipenem were those proposed by the Clinical and Laboratory Standards Institute (5).

PCR analysis with specific primers for all class D β-lactamases (Table 2) determined the presence of the bla_{OXA-58} gene in both strains; *A. baumannii* strain Ac058 was also positive for the bla_{OXA-51} gene. Additional primers were designed at the beginning and end of the bla_{OXA-58} gene (Table 2) to amplify the whole fragment. This gene presented 100% homology with the bla_{OXA-58} gene from *A. baumannii* listed in GenBank.

Plasmid DNA identification was attempted by using genomic mapping with I-CeuI (10) and by digestion with the S1 nuclease (1). I-CeuI cuts a 26-bp site in the *rrl* gene (23S rRNA), shearing the bacterial genome into an analyzable number of fragments (10). The S1 nuclease transforms supercoiled plasmids into linear molecules (1). Digested genomic DNA and plasmids were sepa-

TABLE 1. MICs for the clinical isolates used in this study

Strain	MIC (μg/ml)[a]																
	AMP	PIP	CEF	FOX	CAZ	FEP	SAM	IMP	MEM	CIP	GEN	TOB	AMK	DOX	AZM	RIF	PMB
Ac057	256	512	256	256	8	8	8	>32	8	32	<1	0.25	0.5	<0.5	4	2	2
Ac058	256	512	256	256	256	64	64	>32	8	64	16	64	256	16	128	2	1

Abbreviations: AMP, ampicillin; PIP, piperacillin; CEF, cephalothin; FOX, cefoxitin; CAZ, ceftazidime; FEP, cefepime; SAM, ampicillin-sulbactam; IMP, imipenem; MEM, Meropenem; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; DOX, doxycycline; AZM, azithromycin; RIF, rifampin; PMB, polymyxin B.

* Corresponding author. Mailing address: Servei de Microbiologia, Centre de Diagnóstic Biomèdic, Hospital Clínic, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain. Phone: 34 93 227 55 22. Fax: 34 93 227 93 72. E-mail: jvila@ub.edu.

▽ Published ahead of print on 27 May 2008.

TABLE 2. Oligonucleotide sequences used in this study

Nucleotide	Sequence (5′–3′)	Size (bp)	Use
OXA51-1	AACAAGCGCTATTTTTATTTCAG	641	Detection bla_{OXA-51} variants
OXA51-2	CCCATCCCCAACCACTTTT		
OXA23-1	GATGTGTCATAGTATTCGTCGT	641	Detection bla_{OXA-23} variants
OXA23-2	TCACAACAACTAAAAGCACTGT		
OXA24-1	ATGAAAAAATTTATACTTCCTATATTCAGC	825	Detection bla_{OXA-24} variants
OXA24-2	TTAAATGATTCCAAGATTTTCTAGC		
OXA58-1	AGTATTGGGGCTTGTGCT	453	Detection bla_{OXA-58} variants
OXA58-2	AACTTCCGTGCCTATTTG		Southern blot probe[a]
OXA58-1TOT	ATGAAATTATTAAAAATATTG	843	Total gene amplification
OXA58-2TOT	TTATAAATAATGAAAAACACC		Total gene amplification
OXA58-inv1	GAGCGCAGAGGGGAGAATCGTC		Genetic surrounding
OXA58-inv2	CTCAGCACAAGCCCCAATACT		Genetic surrounding
OXA58-inv3	AAGCCATGCAAGCATCTACA		Genetic surrounding
OXA58-inv4	CATCTCTTTCACTTGTTGCTGAA		Genetic surrounding

[a] Primers OXA58-1 and OXA58-2 were used for detecting the bla_{OXA-58} gene and also to generate the probe for Southern blot analysis.

rated by PFGE (Fig. 1). Probes were marked with the PCR DIG probe synthesis kit (Roche, Barcelona, Spain), and detection was performed with anti-digoxigenin antibody conjugated to alkaline phosphatase and the color substrates NBT/BCIP (Roche) according to the manufacturer's instructions. In Fig. 1a, the most intense bands would represent fragments of genomic DNA, and the faded bands represent plasmid DNA. Hybridization with probes for the bla_{OXA-58} gene (Fig. 1c) and the 23S rRNA gene (Fig. 1b) suggest that in both isolates the bla_{OXA-58} gene is present in a plasmid. With the S1 nuclease (Fig. 2a), the highest band would be the genomic DNA and the remaining bands would be linear plasmids. Hybridization with the probe for the OXA-58 gene (Fig. 2c) gives the same pattern as obtained with I-CeuI. The hybridization signal with the probe for the 23S rRNA gene was only observed in the undigested genomic DNA (Fig. 2b). Although conjugation experiments did not show any plasmid transfer between strains, Southern blot analysis suggests that the bla_{OXA-58} gene could be present in a plasmid in both strains, and the plasmid from *A. baumannii* is possibly different from the plasmid in the *Acinetobacter* genospecies 3 isolate.

In order to determine the genetic structure surrounding of the bla_{OXA-58} gene, DNA from both isolates was digested with MspI "C*CGG" (Promega). The fragments obtained were autoligated overnight at 16°C using a T4 DNA ligase (Promega).

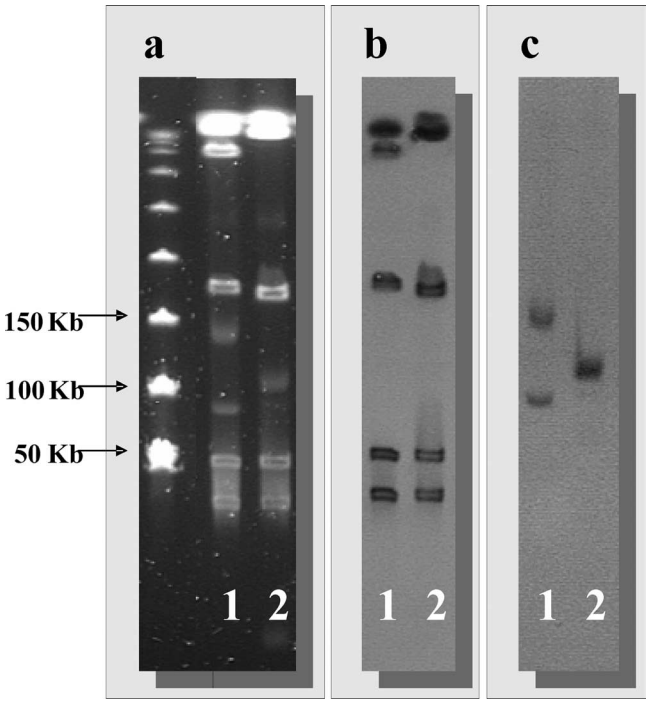

FIG. 1. Plasmid identification by genomic mapping with I-CeuI. (a) PFGE gel. (b) Hybridization with probe for the 23S rRNA gene. (c) Hybridization with probe for the OXA-58. Lane 1, *A. baumannii* strain Ac058; lane 2, *Acinetobacter* genospecies 3 strain Ac057.

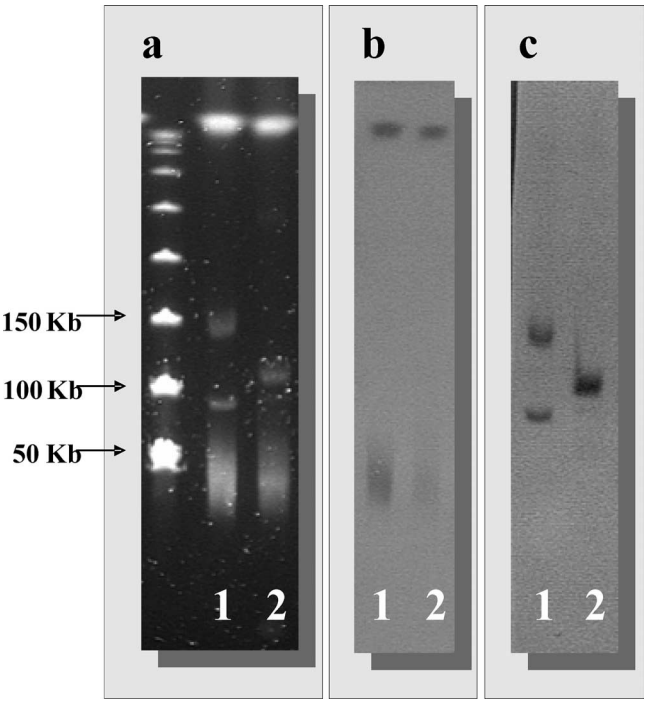

FIG. 2. Plasmid identification by digestion with S1 nuclease. (a) PFGE gel (b). Hybridization with probe for the 23S rRNA gene. (c) Hybridization with probe for the OXA-58. Lane 1, *A. baumannii* strain Ac058; lane 2. *Acinetobacter* genospecies 3 strain Ac057.

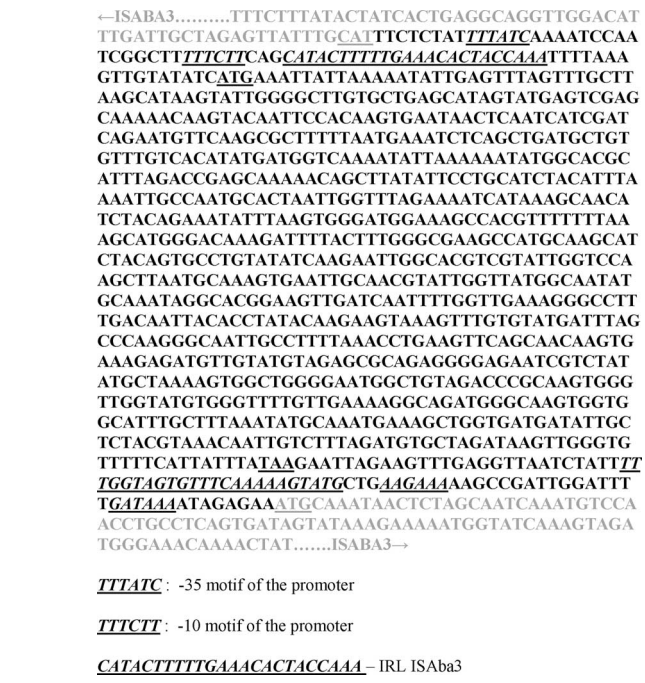

FIG. 3. Structure of the genetic surrounding in *Acinetobacter* genospecies 3 strain Ac057, which is structurally identical to the one described by Poirel et al. (17).

The fragment of DNA containing the bla_{OXA-58} gene was used as a template for a PCR with inverse primers designed from the bla_{OXA-58} gene sequence (Table 2). All PCR fragments were sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom) and analyzed in an automatic DNA sequencer (3100 Genetic Analyzer; Applied Biosystems).

Analysis of the genetic surrounding confirms that both plasmids are different (Fig. 3). In Ac057, the bla_{OXA-58} gene is surrounded by two copies of the Insertion Sequence ISAba3; the copy downstream has the same direction as the bla_{OXA-58} gene, and the upstream copy has the opposite direction (Fig. 3). This structure has already been described in *A. baumannii* by Poirel et al. (17, 18).

The presence of the OXA-58 alone does not account for the level of resistance to imipenem of these isolates (MIC of >32 μg/ml). Further work is needed to determine whether additional efflux pumps or porin modifications are involved.

A. baumannii is certainly the most frequently isolated species in hospitals and also the microorganism of greatest clinical interest in this genus. However, *Acinetobacter* genospecies 3 and 13 are also nosocomial pathogens, and they should be considered in hospital settings. Previous studies in *Acinetobacter* genospecies 3 have revealed the presence of AmpC (2), IMP-4 (4), and bla_{VIM-2} (28). In addition to these previously described enzymes, we report here, for the first time, the presence of the bla_{OXA-58} in this microorganism. The main reason for the lack of interest on non-baumannii *Acinetobacter* isolates is probably their susceptibility to antimicrobial agents (9). However, as suggested by Horrevorts et al. (8), the clinical significance of genospecies 3 can be underestimated because the resistant strains can be erroneously classified as *A. baumannii*.

Nucleotide sequence accession number. The GenBank accession number for the bla_{OXA-58} in *Acinetobacter* genospecies 3 is EU642594.

This study has been supported by grant SGR050444 from the Departament d'Universitats, Recerca I Societat de la Informació de la Generalitat de Catalunya, Spain, and by the Spanish Ministry of Health (FIS 04/0068 to J.V.). This study was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008), as well.

REFERENCES

1. **Barton, B. M., G. P. Harding, and A. J. Zuccarelli.** 1995. A general method for detecting and sizing large plasmids. Anal. Biochem. **226:**235–240.
2. **Beceiro, A., L. Dominguez, A. Ribera, J. Vila, F. Molina, R. Villanueva, J. M. Eiros, and G. Bou.** 2004. Molecular characterization of the gene encoding a new AmpC β-lactamase in a clinical strain of *Acinetobacter* genomic species 3. Antimicrob. Agents Chemother. **48:**1374–1378.
3. **Bergogne-Berezin, E., and K. J. Towner.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148–165.
4. **Chu, Y. W., M. Afzal-Shah, E. T. S. Houang, M. F. I. Palepou, D. J. Lyon, N. Woodford, and D. M. 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.
5. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing: 17th informational supplement M100–S17. CLSI, Villanova, PA.
6. **Dijkshoorn, L., A. Nemec, and M. Vaneechoutte.** 2007. Identification of *Acinetobacter* genomic species by means of amplified rDNA restriction analysis (ARDRA). http://users.ugent.be/~mvaneech/ARDRA/Acinetobacter.html.
7. **Fernandez-Cuenca, F., L. Martinez-Martinez, M. C. Conejo, J. A. Ayala, E. J. Perea, and A. Pascual.** 2003. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of *Acinetobacter baumannii*. J. Antimicrob. Chemother. **51:**565–574.
8. **Horrevorts, A., K. Bergman, L. Kollee, I. Breuker, I. Tjernberg, and L. Dijkshoorn.** 1995. Clinical and epidemiological investigations of *Acinetobacter* genomospecies 3 in a neonatal intensive care unit. J. Clin. Microbiol. **33:**1567–1572.
9. **Lim, Y. M., K. S. Shin, and J. Kim.** 2007. Distinct antimicrobial resistance patterns and antimicrobial resistance-harboring genes according to genomic species of *Acinetobacter* isolates. J. Clin. Microbiol. **45:**902–905.
10. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-Ceu I, an intron-encoded endonuclease specific for genes for rRNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
11. **Marcos, M. A., M. T. Jimenez de Anta, and J. Vila.** 1995. Correlation of six methods for typing nosocomial isolates of *Acinetobacter baumannii*. J. Med. Microbiol. **42:**328–335.
12. **Marque, S., L. Poirel, C. Heritier, S. Brisse, M. D. Blasco, R. Filip, G. Coman, T. Naas, and P. Nordmann.** 2005. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. J. Clin. Microbiol. **43:**4885–4888.
13. **Peleg, A. Y., J. M. Bell, A. Hofmeyr, and P. Wiese.** 2006. Inter-country transfer of gram-negative organisms carrying the VIM-4 and OXA-58 carbapenem-hydrolyzing enzymes. J. Antimicrob. Chemother. **57:**794–795.
14. **Peleg, A. Y., C. Franklin, L. J. Walters, J. M. Bell, and D. W. Spelman.** 2006. OXA-58 and IMP-4 carbapenem-hydrolyzing beta-lactamases in an *Acinetobacter junii* blood culture isolate from Australia. Antimicrob. Agents Chemother. **50:**399–400.
15. **Poirel, L., E. Lebessi, C. Heritier, A. Patsoura, M. Foustoukou, and P. Nordmann.** 2006. Nosocomial spread of OXA-58-positive carbapenem-resistant *Acinetobacter baumannii* isolates in a paediatric hospital in Greece. Clin. Microbiol. Infect. **12:**1138–1141.
16. **Poirel, L., and P. Nordmann.** 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. **12:**826–836.
17. **Poirel, L., and P. Nordmann.** 2006. Genetic structures at the origen of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene bla_{OXA-58} in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **50:**1442–1448.
18. **Poirel, L., S. Marque, C. Heritier, C. Segonds, G. Chabanon, and P. Nordmann.** 2005. OXA-58, a novel class D β-lactamase involved in resistance to

carbapenems in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**202–208.
19. **Ribera, A., F. Fernandez-Cuenca, A. Beceiro, G. Bou, L. Martinez-Martinez, A. Pascual, J. M. Cisneros, J. Rodriguez-Bano, J. Pachon, and J. Vila.** 2004. Antimicrobial susceptibility and mechanisms of resistance to quinolones and beta-lactams in *Acinetobacter* genospecies 3. Antimicrob. Agents Chemother. **48:**1430–1432.
20. **Ruiz, M., S. Marti, R. Fernandez-Cuenca, A. Pascual, and J. Vila.** 2007. High prevalence of carbapenem-hydrolyzing oxacillinases in epidemiologically related and unrelated *Acinetobacter baumannii* clinical isolates in Spain. Clin. Microbiol. Infect. **13:**1192–1198.
21. **Salazar, D., V. B. Nievesm, M. Ruiz, J. Ruiz, J. Vila, A. Maria, and V. Elsa.** 2007. Molecular epidemiology and characterization of resistance mechanisms to various antimicrobial agents in *Acinetobacter baumannii* isolated in Merida, Venezuela. Med. Sci. Monit. **13:**BR89–BR94.
22. **Vahaboglu, H., F. Budak, M. Kasap, G. Gacar, S. Torol, A. Karadenizli, F. Kolayli, and C. Eroglu.** 2006. High prevalence of OXA-51-type class D beta-lactamases among ceftazidime-resistant clinical isolates of *Acinetobacter* spp.: co-existence with OXA-58 in multiple centres. J. Antimicrob. Chemother. **58:**537–542.
23. **Van, L. M., and H. Goossens.** 2004. Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin. Microbiol. Infect. **10:**684–704.
24. **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.
25. **Vila, J., S. Marti, and J. Sanchez-Cespedes.** 2007. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J. Antimicrob. Chemother. **59:**1210–1215.
26. **Weaver, R. E., and L. A. Actis.** 1994. Identification of *Acinetobacter* species. J. Clin. Microbiol. **32:**1833.
27. **Woodford, N., M. J. Ellington, J. M. Coelho, J. F. Turton, M. E. Ward, S. Brown, S. G. Amyes, and D. M. Livermore.** 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents. **27:**351–353.
28. **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.