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Regional Occurrence of Plasmid-Mediated Carbapenem-Hydrolyzing Oxacillinase OXA-58 in *Acinetobacter* spp. in Europe

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The spread of the plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 was detected in *Acinetobacter* sp. clinical isolates from southern Europe, the Balkans, and central Turkey. It may contribute significantly to the emergence of carbapenem resistance in *Acinetobacter* spp., at least in this part of the world.

Reports of carbapenem resistance in *Acinetobacter baumannii* have accumulated worldwide (11, 14, 21). Most of these studies showed that β-lactamase-mediated resistance is the most common mechanism for carbapenem resistance in that species. Four groups of carbapenem-hydrolyzing oxacillinases (Ambler class D β-lactamases) in *A. baumannii* have been described (1, 2, 6–8, 15). A first group consists of OXA-23, OXA-27, and OXA-49 (GenBank accession number AY288523) which have 99% amino acid identity and also share 60% identity with a second group of oxacillinases (OXA-24, OXA-25, OXA-26, and OXA-40), with the latter group of enzymes differing by a few amino acid substitutions. β-Lactamase OXA-51, which shares less than 63% amino acid identity with the two latter groups, has been recently identified in *A. baumannii* isolates from Argentina and would define a third group of those oxacillinases (3). In addition, we have recently characterized OXA-58 from France which belongs to a novel fourth group of those oxacillinases (7, 15). The $bla_{\text{OXA-58}}$ gene was found to be plasmid located, and the activity of OXA-58 was inhibited by NaCl, as opposed to the other oxacillinases possessing some carbapenemase activity (15).

The present study was designed to analyze the geographical spread of this novel carbapenemase among carbapenem-resistant *Acinetobacter* sp. isolates in continental Europe. Forty-eight nonreplicate isolates of *Acinetobacter* spp. were included in this retrospective study selected on the criterion of nonsusceptibility to carbapenems (MIC $\geq$ 8 μg/ml). They had been collected from February 1997 to March 2004 from patients hospitalized in intensive care units, medicine, and surgery wards and corresponded to clinical isolates from urine, blood, skin ulcer swabs, and bronchoalveolar specimens (colonizing isolates excluded). The isolates were from 12 cities located in six European countries (France, Greece, Italy, Romania, Spain, and Turkey) (Table 1). Strains from Romania were collected from the same pediatric unit from October to December 2003. Species identification was confirmed by the biochemical API32GN test (Biomérieux, Marcy-l'Etoile, France) and sequencing of 16S rRNA genes (16); all but one of the isolates were *A. baumannii* isolates, and a single *Acinetobacter junii* isolate was identified. Antibiotic-containing disks were used for the detection of imipenem susceptibility, along with Mueller-Hinton agar plates and a disk diffusion assay (www.sfm.fr) (Sanofi-Diagnostics-Pasteur, Marnes-La-Coquette, France). MICs were determined by an agar dilution technique, and results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) (13). Five isolates were of intermediate susceptibility to imipenem, and the others were resistant (Table 2). Most of the isolates were multidrug resistant, including being resistant to aztreonam, ceftazidime, amikacin, and ciprofloxacin.

Carbapenem-hydrolyzing β-lactamase was screened by measuring specific hydrolytic activity against imipenem of culture extracts, as previously described (8). Forty-three out of the 48 isolates hydrolyzed imipenem significantly (Table 1). Oxacillinase and metallo-β-lactamase genes were identified by using a standard PCR technique with primers specific for $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, and $bla_{\text{OXA-58}}$ genes (primers OXA-58A [5′-CGA TCAGAATGTTCAAGCGC-3′] and OXA-58B [5′-ACGATT CTCCCCTCTGCGC-3′]) and for the metallo-β-lactamase bla_{VIM} and bla_{IMP} genes (7, 8, 14). PCRs were positive for the $bla_{\text{OXA-58}}$ gene for 22 out of 42 carbapenem-hydrolyzing *A. baumannii* isolates and also for the single *A. junii* isolate (Table 1). PCR amplicons were sequenced with an Applied Biosystems sequencer (ABI 3100) and an identical $bla_{\text{OXA-58}}$ gene was identified in all cases. The carbapenem-hydrolyzing oxacillinase gene $bla_{\text{OXA-23}}$ was also identified in two *A. baumannii* isolates and the *A. junii* isolate collected from Romania (Table 1). In addition, $bla_{\text{OXA-40}}$ was found in several isolates recovered from Barcelona and Madrid (Table 1), strengthening the notion of endemicity of oxacillinase OXA-40 in Spain (5, 12).

Genotyping of the 23 OXA-58-positive strains was carried out by pulsed-field gel electrophoresis (PFGE), as described previously (20). After digestion by ApaI, restriction fragments were separated in a CHEF-DRII apparatus (16). Isolates were considered related if their PFGE patterns differed by six frag-

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TABLE 1. Distribution of imipenem nonsusceptible isolates of *Acinetobacter* spp. and pulsotypes of OXA-58-positive isolates

Country	City	No. of tested isolates	Species (no. of isolates)	No. of isolates					Pulsotype(s)
				Imipenem hydrolysis[a]	bla_{OXA-23}	bla_{OXA-40}	bla_{OXA-58}	bla_{VIM} or bla_{IMP}	
France	Toulouse	2	*A. baumannii* (2)	2	0	0	2	0	E
	Lille	1	*A. baumannii* (1)	1	0	0	1	0	I
	Mâcon	1	*A. baumannii* (1)	1	0	0	1	0	J
	K.-Bicêtre	1	*A. baumannii* (1)	1	0	0	1	0	K
	Suresnes	1	*A. baumannii* (1)	1	0	0	1	0	K
Spain	Seville	7	*A. baumannii* (7)	4	0	0	4	0	E, F, G
	Barcelona	12	*A. baumannii* (12)	12	0	8	0	0	—[b]
	Madrid	1	*A. baumannii* (1)	1	0	1	0	0	—
Italy	Rome	3	*A. baumannii* (3)	2	0	0	0	1	—
Greece	Athens	1	*A. baumannii* (1)	1	0	0	0	0	—
Turkey	Ankara	2	*A. baumannii* (2)	2	0	0	2	0	H
Romania	Iasi	15	*A. baumannii* (14)	14	2	1	10	0	B, C, D
			A. junii (1)	1	1	0	1	0	A
Total		47	*A. baumannii* (46)	42	2	10	22	1	—
			A. junii (1)	1	1	0	1	0	—

[a] Imipenem hydrolysis is expressed in units. One unit is defined by the enzymatic activity that hydrolyzes one μmol of imipenem per min per mg of protein.
[b] —, not applicable.

ments or fewer (20). A total of 11 PFGE profiles were identified; the *A. junii* isolate corresponded to pulsotype A, whereas *A. baumannii* isolates corresponded to pulsotypes B to K (Fig. 1; Table 1). Eight out of 10 *A. baumannii* isolates from Romania corresponded to a single clone (pulsotype C), whereas two others were of different genotypes (B and D). Pulsotype E included four isolates collected in Seville (1997) and Toulouse (2003). Pulsotypes F and G were both represented by single isolates collected in Seville in 1997. Pulsotype H consisted of two isolates collected in Ankara in 1998. Pulsotypes I and J were represented by single isolates collected in France (2003). Pulsotype K included two isolates collected in France in Suresnes and K.-Bicêtre (suburbs of Paris) in 2004.

To determine whether the bla_{OXA-58} gene was plasmid borne, plasmid DNA of a representative of each pulsotype was extracted by using the Kieser technique (9). Southern transfer was performed on a nylon membrane (Hybond N^+; Amersham Pharmacia Biotech, Orsay, France), as previously described (17). The membrane was successively UV cross-linked (Stratalinker; Stratagene, Amsterdam, The Netherlands) and hybridized (enhanced chemiluminescence nonradioactive labeling and detection kit; Amersham Pharmacia Biotech, Orsay, France) with a PCR-generated probe for bla_{OXA-58}. A plasmid location for the bla_{OXA-58} gene was confirmed in 10 out of the 13 *A. baumannii* isolates (*A. baumannii* isolates of pulsotypes C to F and H2 to K) and for the *A. junii* isolate (Fig. 2). However, after repeated attempts, conjugation experiments performed as previously described (7) failed to demonstrate the transferability of such plasmids. Only the use of electrotransformation gave *A. baumannii* transformants (15). Despite the use of the I-CeuI digestion technique that helps to determine plasmid or

TABLE 2. In vitro susceptibilities of carbapenem nonsusceptible clinical isolates of *Acinetobacter* spp.

Antibiotic	MIC range (μg/ml)	No. of isolates (%)[a]		
		S	I	R
Piperacillin-tazobactam	64–>128	0	5 (10)	43 (90)
Ceftazidime	2–>128	1 (2)	12 (25)	35 (73)
Cefepime	8–>128	0	4 (8)	44 (92)
Aztreonam	64–>128	0	0	48 (100)
Imipenem	8–>64	0	5 (10)	43 (90)
Meropenem	8–>64	0	17 (35)	31 (65)
Amikacin	2–>128	11 (23)	0	37 (77)
Ciprofloxacin	1–>128	1 (2)	0	47 (98)

[a] S, susceptible; I, intermediate; R, resistant.

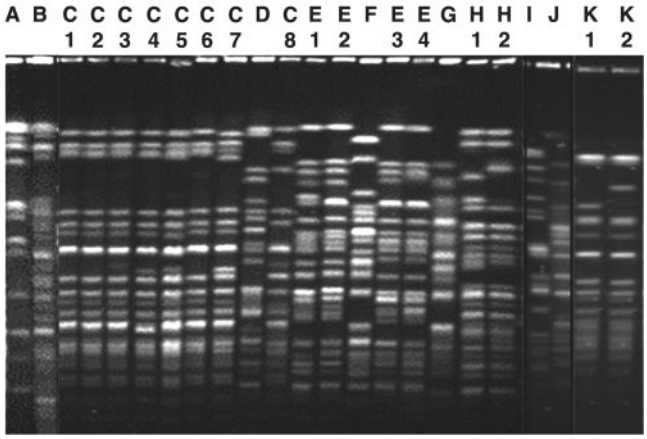

FIG. 1. PFGE patterns of OXA-58-producing *Acinetobacter* sp. isolates. Lane A, pulsotype of the unrelated *A. junii* isolate; lanes B to K, 10 pulsotypes obtained by pulsed-field gel electrophoresis after digestion with ApaI. Lanes C_1 to C_8, E_1 to E_4, H_1 and H_2, and K_1 and K_2 correspond to subtypes of pulsotypes C, E, H, and K, respectively.

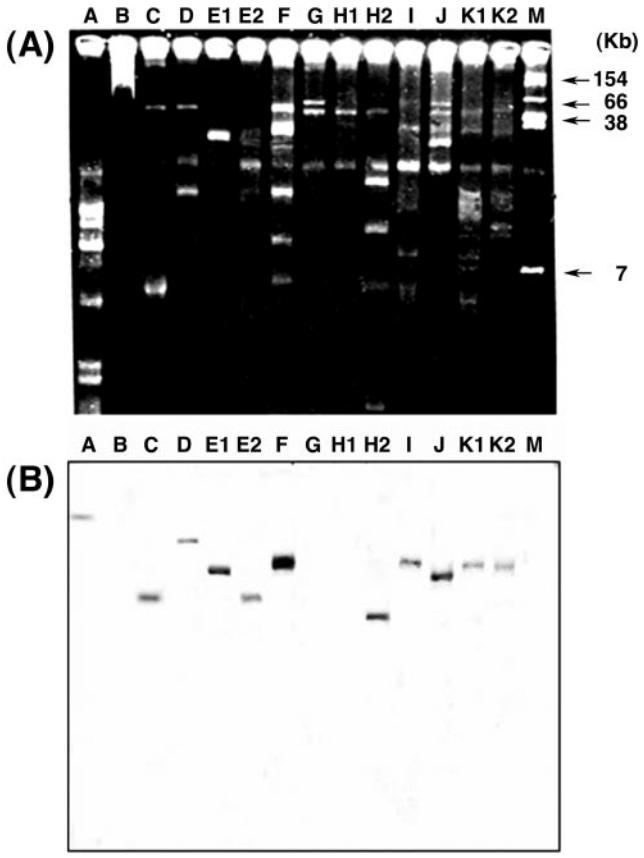

FIG. 2. Plasmid analysis of OXA-58-positive *Acinetobacter* sp. isolates. (A) Lanes A to K, 14 isolates of pulsotypes A to K (and subtypes E_1, E_3, H_1, and H_2) are shown. Results were identical for strains C1 to C8 and are shown here as lane C. Lane M, plasmids of reference size (12). (B) Hybridization with a probe specific for the bla_{OXA-58} gene.

chromosome location (16), the genetic location of bla_{OXA-58} remained uncertain for the last three isolates (data not shown). Sizes of OXA-58-positive plasmids ranged from 10 to 150 kb. A plasmid location of the bla_{OXA-23} gene was also confirmed in the *A. junii* isolate. The bla_{OXA-23} and bla_{OXA-58} probes gave the same hybridization profile for the *A. junii* isolate, with a single 150-kb signal, suggesting that the same 150-kb plasmid harbored both bla_{OXA-58} and bla_{OXA-23} genes. Conjugations using this *A. junii* isolate as the donor and *A. baumannii* CIP7010^T (Institut Pasteur, Paris, France) as the recipient strain failed.

We showed that OXA-58 was widespread among carbapenem-nonsusceptible *Acinetobacter* sp. isolates from southern Europe, central Turkey, and the Balkans. It is the most frequently distributed oxacillinase with carbapenemase activity in those isolates. Interestingly, several isolates had imipenem-hydrolyzing activity without detection of genes coding for known carbapenemases, suggesting additional undiscovered enzymes.

Whereas most of the other carbapenem-hydrolyzing oxacillinase genes are chromosomally located (14), the bla_{OXA-58} gene was located on nonconjugative plasmids, which, in most of the cases, differed in size. It is tempting to speculate that the plasmid location of bla_{OXA-58} has contributed to high levels of carbapenem resistance in *A. baumannii* at least in that part of the world (although isolates from Romania are overrepresented in our study). Indeed, we have shown recently that OXA-58 activity contributes significantly to carbapenem resistance in *A. baumannii*, especially when additional efflux mechanisms are associated (C. Héritier, L. Poirel, and P. Nordmann, unpublished data). Other factors, such as porin deficiency and overexpression of the chromosome-encoded cephalosporinase of *A. baumannii* with weak carbapenemase activity may contribute to carbapenem resistance also.

Whereas metallocarbapenemases in *A. baumannii* are reported mostly from Asia and South America and rarely from southern Europe (10, 18, 19), the carbapenem-hydrolyzing oxacillinases seem to have a wider distribution (1–7, 14). Further work should analyze the spread of those carbapenem-hydrolyzing oxacillinases, including OXA-58, in carbapenem-resistant *A. baumannii* isolates from the United States, a country where carbapenem-hydrolyzing oxacillinases have not yet been detected.

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