ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2010, p. 578–579 Vol. 54, No. 1
0066-4804/10/$12.00 doi:10.1128/AAC.00861-09

Letters to the Editor

First Isolation of the bla_{OXA-23} Carbapenemase Gene from an Environmental *Acinetobacter baumannii* Isolate▽

Acinetobacter baumannii is frequently associated with nosocomial infections, and its increasing resistance to carbapenems may significantly reduce the choice of effective antibiotics (2). Since the first description of a carbapenem-hydrolyzing class D β-lactamase (CHDL), ARI-1 (renamed OXA-23), from a clinical isolate of *A. baumannii* in Scotland in 1995 (11), the corresponding bla_{OXA-23} gene has been detected in many *A. baumannii* clinical isolates worldwide (Brazil, Spain, Belgium, Singapore, Portugal, and France) and once in *Proteus mirabilis* in France (1). The bla_{OXA-23} gene can be plasmid or chromosome borne (12). Three main groups of oxacillinases (OXA-23, -40, and -58) possessing weak carbapenemase activity have been characterized for *A. baumannii* (6). All CHDL-positive isolates were found in hospital settings. In this report, we describe the first *A. baumannii* strain isolated in the environment and producing such acquired β-lactamase.

A. baumannii B9 was recovered from water of the Seine river in downtown Paris as a result of screening for multidrug-resistant isolates. Samples were collected and processed on the day of their collection. The sampling procedure consisted of filtrating 100 ml water through nitrocellulose membranes (0.45 μm; Millipore), resuspending the filters in 1 ml of sterile water, and plating 100-μl aliquots on imipenem (2 μg/ml)-containing MacConkey agar plates. Isolate B9 was identified by the API 32GN system (bioMérieux, Marcy l'Etoile, France) and by sequencing of the 16S rRNA genes. MICs were determined by Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar plates at 37°C. Isolate B9 was resistant to all β-lactams including carbapenems (Table 1). It was also resistant to quinolones and fluoroquinolones, chloramphenicol, tetracycline, and tigecycline and susceptible to aminoglycosides (tobramycin, amikacin, netilmicin, kanamycin, and gentamicin). PCR experiments, performed as previously described for screening of the presence of the bla_{OXA-23}, bla_{OXA-40}, and bla_{OXA-58} genes (6), followed by sequencing, showed that this isolate possessed the bla_{OXA-23} gene.

Repeated attempts to transfer the OXA-23 determinant from *A. baumannii* B9 to azide-resistant *Escherichia coli* J53 by mating-out assay (9) and to *A. baumannii* CIP7010 by electroporation (6) failed. In addition, plasmid extraction from *A. baumannii* B9 performed by the Kieser technique (7) did not yield any plasmid. These results suggested the chromosomal location of the bla_{OXA-23} gene, which was confirmed by a total DNA digest with the I-CeuI enzyme, its separation by pulsed-field gel electrophoresis, and hybridization with the bla_{OXA-23} and rRNA probes, as described previously (1). PCR amplification with primers for IS*Aba1* alone and in combination with the bla_{OXA-23} gene revealed a structure in which a single copy of this insertion sequence is located upstream of the bla_{OXA-23} gene but is absent on its downstream extremity as opposed to what has been observed for the composite transposon Tn*2006* (4).

This is the first isolation of an environmental *A. baumannii* strain producing an acquired CHDL. The progenitor of the bla_{OXA-23} gene had been identified as being *Acinetobacter radioresistens*, in which the bla_{OXA-23} gene is not expressed or is poorly expressed (10). That species is rarely involved in human infections but is known to be present in the environment. Therefore, it is hypothesized that genetic exchanges between the two *Acinetobacter* species may lead to acquisition and expression of the bla_{OXA-23} gene in *A. baumannii*. This might occur in aquatic environments, where *A. baumannii* and *A. radioresistens* could be in close contact. The *A. baumannii* B9 isolate was not recovered in the immediate vicinity of a hospital wastewater discharge site. Pulsed-field gel electrophoresis (PFGE) analysis of ApaI-restricted DNA from *A. baumannii* B9 and from clinical *A. baumannii* isolates from different geographical origins showed that *A. baumannii* B9 was clonally related to a human *A. baumannii* isolate previously identified in New Caledonia in June 2004 (8). Interestingly, *Acinetobacter* spp. have been previously used as an indicator of antimicrobial resistance in aquatic environments (13). This may indicate the presence of some selective pressure in the Seine river, although environmental *Acinetobacter* sp. strains are usually of the wild-type phenotype of resistance.

Our study suggests that the ongoing spread of CHDL-producing *A. baumannii* is currently occurring simultaneously in different environments and seems not to be restricted to the hospital setting. Although it does not provide evidence for the direct transfer of resistance elements from the soil resistome to pathogenic bacteria, this finding emphasizes the importance of a survey of environmental strains that may act as a source and/or reservoir of resistance genes with clinical relevance, as exemplified by D'Costa et al. (5). Additionally, *Shewanella*

TABLE 1. MICs of antibiotics for *A. baumannii* B9

Antibiotic	MIC (μg/ml) for *A. baumannii* B9
Amoxicillin	>256
Amoxicillin + CLA[a]	>256
Ticarcillin	>256
Ticarcillin + CLA	>256
Piperacillin	>256
Cephalothin	>256
Cefuroxime	>256
Ceftazidime	>256
Cefotaxime	>256
Cefepime	>256
Cefpirome	>256
Moxalactam	>256
Aztreonam	32
Imipenem	48
Ertapenem	>32
Meropenem	24
Nalidixic acid	>256
Pefloxacin	>256
Ofloxacin	>32
Ciprofloxacin	>32
Amikacin	8
Netilmicin	8
Gentamicin	8

[a] CLA, clavulanic acid.

algae, which is a Gram-negative waterborne bacterial species, has been shown to be the source of plasmid-mediated quinolone resistance *qnrA*-like genes in the *Enterobacteriaceae* (3).

This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA 3539), Université Paris XI, by grants from the European Community (DRESP2, LSHM-CT-2005-018705, and TROCAR, HEALTH-F3-2008-223031), and by the INSERM.

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Delphine Girlich
Laurent Poirel
Patrice Nordmann*
Service de Bactériologie-Virologie
INSERM U914 Emerging Resistance to Antibiotics
Assistance Publique/Hôpitaux de Paris
Faculté de Médecine et Université Paris-Sud
Hôpital de Bicêtre
94275 Le Kremlin-Bicêtre, France

*Phone: 33-1-45-21-36-32
Fax: 33-33-1-45-21-63-40
E-mail: nordmann.patrice@bct.aphp.fr

▽ Published ahead of print on 2 November 2009.