

# First Report of an OXA-23 Carbapenemase-Producing *Acinetobacter baumannii* Clinical Isolate Related to Tn2006 in Spain

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**A carbapenem-resistant *Acinetobacter baumannii* clinical isolate belonging to European clone II and sequence type 2 was recovered from a patient in the Son Espases hospital in Mallorca, Spain. Genetic analysis showed the presence of the *bla*<sub>OXA-23</sub> gene in association with the widely disseminated transposon Tn2006. This is the first reported identification of *A. baumannii* carrying *bla*<sub>OXA-23</sub> in Spain.**

*Acinetobacter baumannii* is responsible for hospital outbreaks, and it is considered one of the most important nosocomial pathogens causing bacteremia, pneumonia, and other respiratory and urinary tract infections (1, 2). The abilities to acquire resistance mechanisms and to persist in the environment for long periods of time make this pathogen difficult to eradicate from the clinical setting (3, 4). The resistance of *A. baumannii* isolates to carbapenems is on the rise mainly in association with a variety of combined mechanisms, among which the production of acquired carbapenem-hydrolyzing OXA-type class D β-lactamases (CHDLs), OXA-23 in particular, has been identified worldwide (2).

Here we report the first case of OXA-23 in an *A. baumannii* clinical isolate in Spain and in association with the widely spread Tn2006 transposon.

OXA-23 was identified in a clinical isolate from a 59-year-old male patient admitted to the Department of Pneumology at the Son Espases hospital in Mallorca, Spain, in February 2010 because of increased dyspnea, coughing, chills, and purulent secretions during the previous 3 days. The patient was born in Portugal and had lived in Palma de Mallorca since 1998, although he had traveled to Portugal and France during the previous year. The patient had been hospitalized in Lisbon between 7 and 23 December 2009 and then again in another hospital between 26 December 2009 and 8 January 2010. He was a former smoker and had needed multiple hospital admissions because of exacerbations of chronic obstructive pulmonary disease. His regular medication included bronchodilators but no antibiotics. On the second day after admission, *A. baumannii* was isolated from his sputum and the patient was empirically treated with levofloxacin (500 to 750 mg/day) but switched to intravenous colistin (2 to 3 MU/8 h) after 6 days because of susceptibility test results. The patient improved and was discharged for specialized home care. After the initial isolation, periodic sputum cultures were taken for several months with no further isolation of the strain.

*A. baumannii* isolate AB 308 was initially identified by MicroScan and confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics GmbH, Leipzig, Germany). Antibiotic susceptibilities were determined by MicroScan (Siemens) for most antimicrobial agents, except colistin and tigecycline, which were determined by Etest (AB bioMérieux, Solna, Sweden). These results were interpreted according to CLSI guidelines (5). The AB 308 isolate was resistant

to amikacin (>256 μg/ml), ceftazidime (>128 μg/ml), tetracycline, ciprofloxacin, gentamicin, netilmicin (>64 μg/ml), piperacillin (>512 μg/ml), imipenem, and meropenem (>16 μg/ml). The MIC of both tigecycline and colistin was 0.5 μg/ml.

PCR detection of metallo-beta-lactamase and CHDL genes (6–8) was only positive for *bla*<sub>OXA-51</sub> and *bla*<sub>OXA-23</sub>. The insertion sequence IS*Aba1* was found upstream of *bla*<sub>OXA-23</sub> but not upstream of *bla*<sub>OXA-51</sub>.

The genetic location of *bla*<sub>OXA-23</sub> was determined by S1 nuclease pulsed-field gel electrophoresis (PFGE) and Southern blot analysis with a digoxigenin-labeled probe (Roche, Barcelona, Spain) matching the *bla*<sub>OXA-23</sub> gene. S1 PFGE revealed a band of ca. 100 kb with no signal hybridization with the probe. However, we detected a positive signal matching the bacterial chromosome indicating the chromosomal location of *bla*<sub>OXA-23</sub> (Fig. 1).

Characterization of the genetic structures surrounding the *bla*<sub>OXA-23</sub> gene was performed by inverse PCR (9) with primers OXA23invF (5'-GGAAAGACTGGTTGGGCAATGG-3') and OXA-23invR (5'-CACCTCAGGTGTGCTGGTTATTTC-3') and revealed genetic structures neighboring the *bla*<sub>OXA-23</sub> gene consistent with the arrangement of the previously described composite transposon Tn2006 (10, 11).

AB 308 was included within European clone II (EC-II) (12), and multilocus sequence typing performed according to the scheme devised by Nemec et al. (13) allowed the allocation of the AB 308 strain to sequence type 2 (ST2) (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html>).

In this report, we describe the first identification of an *A. baumannii* clinical isolate carrying *bla*<sub>OXA-23</sub> associated with Tn2006 in Spain. Until recently, *A. baumannii* isolates bearing CHDLs in Spain were related mainly to OXA-24 (14, 15). Interestingly, the emergence and spread of several outbreak or sporadic *A. baumannii* strains producing OXA-23 related to EC-II and ST2 have been reported around the world (4, 11, 16–18).

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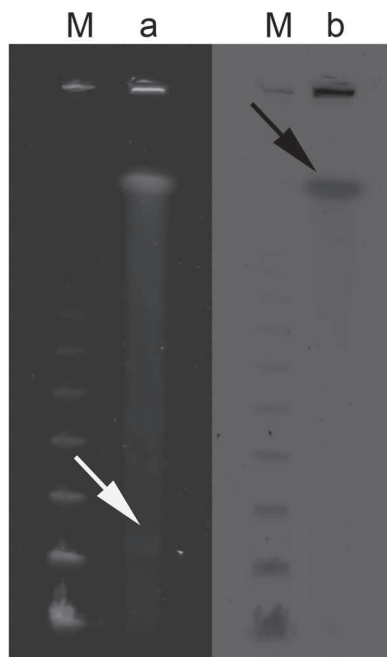
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**FIG 1** Southern blot assay. Lane a, PFGE analysis of the AB 308 strain by digestion with S1 nuclease. Lane b, hybridization with a *bla*<sub>OXA-23</sub> probe. The black arrow indicates the chromosomal location with positive hybridization with the *bla*<sub>OXA-23</sub> probe. The white arrow indicates a plasmid band with no hybridization with the probe. Lanes M, lambda ladder PFGE marker (New England BioLabs).

Moreover, in countries in the Mediterranean region, there seems to be a trend toward the replacement of OXA-58/OXA-24-producing *A. baumannii* by OXA-23 producers, also associated with *A. baumannii* strains belonging to EC-II and ST2 and carrying Tn2006. In central Italy, an interesting replacement of *bla*<sub>OXA-58</sub> with *bla*<sub>OXA-23</sub> belonging to EC-II has recently been established (18, 19) and a similar abrupt shift has also been reported in Greece and Algeria, where 72.4% and 92% of the carbapenem-resistant isolates tested harbored the *bla*<sub>OXA-23</sub> gene (20, 21), while the *bla*<sub>OXA-58</sub> gene was predominant in those countries half a decade ago. All of the *bla*<sub>OXA-23</sub> genes from Italy and Greece were located within Tn2006 transposons, either chromosomally or in plasmids.

In Portugal, carbapenem-resistant *A. baumannii* isolates have typically been associated with OXA-24 but a predominance of *bla*<sub>OXA-23</sub> (63%) has been detected since 2006. All of the isolates belonged to EC-II and ST2 and carried Tn2006 chromosomally (17). While the replacement of OXA-58 by OXA-23 might be explained by the selective advantage associated with the higher carbapenemase activity of OXA-23, the selective advantage of OXA-23 over OXA-24 producers is not clear but could be linked to additional features within the carrying clone (i.e., extensive multidrug resistance), which might explain why OXA-23 in Italy and Greece is associated with epidemiologically diverse clones carrying either chromosomal or plasmidic *bla*<sub>OXA-23</sub>, while in Portugal, OXA-23 dissemination is caused by a particular clone (17).

The AB 308 strain described in this study, however, was not related to any outbreak in Spain and represented the only OXA-23-producing *A. baumannii* strain out of 456 *Acinetobacter* strains collected in a nationwide multicenter study in Spain performed

during 2010. Therefore, it is not likely that OXA-23 producers were disseminating throughout Spain in 2010 and AB 308 was probably acquired during a recent trip to either France or Portugal. Nevertheless, recent investigations have reported two OXA-23 outbreaks in Spain in 2012 (J. Vila and G. Bou, personal communication); therefore, in the years to come, we might expect an eruption of *A. baumannii* strains carrying OXA-23 in this country as well.

The genomic plasticity of the strains carrying *bla*<sub>OXA-23</sub> associated with the composite transposon Tn2006 allows mobilization or transposition events to occur more easily, since insertions can occur in different locations on the chromosome or even in plasmids, and the emerging spread of carbapenem-resistant *A. baumannii* strains associated with transposon structures and the worldwide dissemination of EC-II and ST2 represent a worrying threat, as well as an important challenge for surveillance and infection control measures.

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