

Globally Expanding Carbapenemase Finally Appears in Spain: Nosocomial Outbreak of *Acinetobacter baumannii* Producing Plasmid-Encoded OXA-23 in Barcelona, Spain

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Resistance of *Acinetobacter baumannii* clinical isolates to carbapenems is on the rise worldwide mainly in association with the production of OXA-23. Until recently, however, OXA-23 was absent in Spain. In this work, we report the molecular characterization of a hospital outbreak of OXA-23-producing *A. baumannii* in Barcelona caused by a multidrug-resistant (MDR) clone belonging to international clone IC-II/sequence type ST85 between October 2010 and May 2011. *bla*_{OXA-23} was carried in a plasmid of 90 kb and located within the composite transposon Tn2006.

Acinetobacter baumannii is considered an important opportunistic pathogen causing pneumonia, bacteremia, and other respiratory and urinary tract infections (1, 2), and it is also responsible for nosocomial outbreaks worldwide, especially in intensive care units (ICUs) (3). Eradication of nosocomial infections caused by *A. baumannii* is particularly troublesome due to the ability of this bacterium to rapidly acquire antimicrobial resistance (4) and persist in the environment for long periods of time (5). Increased resistance to carbapenems in *A. baumannii* has raised special concerns during the last decade, especially since it is associated mostly with the production of acquired carbapenemases belonging to either class B metallo- β -lactamases or carbapenem-hydrolyzing OXA-type class D β -lactamases (2).

Outbreak multidrug-resistant (MDR) isolates causing hospital infections across Europe usually belong to one of the three main international clones or lineages (IC-I, IC-II, and IC-III) (6), with recent studies reporting the spread of genetically related epidemic clones of *A. baumannii* producing OXA-23 and belonging to IC-II within the Mediterranean region (7–11). Until very recently, however, OXA-23-producing *A. baumannii* was absent in Spain (12). In this work, we report the molecular characterization of an OXA-23-producing *A. baumannii* strain causing a hospital outbreak in Barcelona between October 2010 and May 2011.

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In October 2010, the first carbapenem-resistant *A. baumannii* isolate associated with this outbreak was identified by Vitek (bioMérieux, Marcy l'Etoile, France) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics GmbH, Leipzig, Germany) (13) from a bronchial aspirate from a 36-year-old male patient admitted to the ICU of the Hospital del Mar, Barcelona, Spain, because of cardiac arrest due to cocaine overdose. The patient had a record of multiple psychiatric stays during the previous 2 years and was already colonized upon ICU admission. Until May 2011, subsequent screenings recovered carbapenem-resistant *A. baumannii* isolates from 17 different patients and 3 environmental dry surfaces within the ICU. In 10 of the 17 patients (59%), *A. baumannii* was

recovered from rectal samples and its presence was considered colonization, whereas in the remaining patients (41%) it was considered infection and was recovered from different sites/sources (bronchial aspirates, blood culture, urine, or samples from skin and soft tissue infections). The implementation of infection control measures, such as reinforcement of hand hygiene, environmental and equipment cleaning, and cohort isolation of patients, led to outbreak containment, and no additional isolates were recovered after May 2011.

Antimicrobial susceptibility testing performed by Vitek (bioMérieux, Marcy l'Etoile, France) and Etest (AB bioMérieux, Solna, Sweden) interpreted according to CLSI guidelines (14) showed all isolates to be resistant to ceftazidime, cefotaxime, (MIC > 256 μ g/ml), cefepime, imipenem, meropenem, ciprofloxacin (MIC > 32 μ g/ml), and amikacin (MIC > 64 μ g/ml), intermediate to tetracycline (MIC, 8 μ g/ml), and susceptible to tobramycin (MIC, 1 μ g/ml), gentamicin (MIC, 0.38 μ g/ml), and colistin (MIC, 0.125 μ g/ml). MIC values of kanamycin, aztreonam and chloramphenicol were >256 μ g/ml, and those of tigecycline were 1 to 2 μ g/ml.

The analysis of the isolates by pulsed-field gel electrophoresis (PFGE) profiles of ApaI-digested genomic DNA (15) revealed that all isolates were clonally related (Fig. 1). Multilocus sequence typing (MLST) following the Pasteur scheme (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html>) assigned all isolates to sequence type ST85, and multiplex PCR to identify clonal lineages was positive for group 1 (IC-II) (16). Additional typing following the 3ST scheme (http://www.hpa-bioinformatics.org.uk/AB/ab_type1.php) provided allele sequences matching

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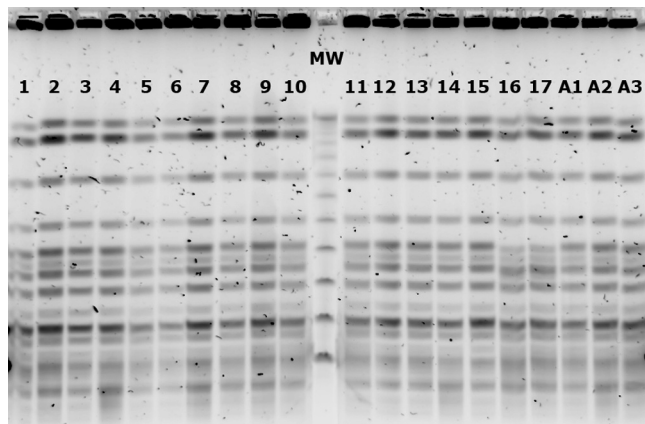


FIG 1 ApaI PFGE patterns of OXA-23-producing *A. baumannii* isolates. Lanes 1 to 17, isolates recovered from patients; lanes A1 to A3, environmental isolates; MW, lambda ladder PFGE molecular weight marker.

those of ST85 isolates from France (allele sequence numbers for *ompA*, *csuE*, and *bla*_{OXA-51} being 8, 11, and 10, respectively) (17).

PCR detection of metallo- and carbapenem-hydrolyzing OXA-type β -lactamase genes (18–20) was positive only for *bla*_{OXA-51} and *bla*_{OXA-23}. The insertion sequence *ISAbal* was found upstream of both *bla*_{OXA-23} and the chromosomal *bla*_{ADC} gene but not upstream of *bla*_{OXA-51}. DNA sequencing confirmed that the *bla*_{OXA-23} sequences displayed 100% identity to that of the original *bla*_{OXA-23} (GenBank accession number AJ132105.1). S1 nuclease digestion followed by pulsed-field gel electrophoresis and Southern hybridization with a digoxigenin (DIG)-labeled probe against the *bla*_{OXA-23} gene demonstrated the genetic location of *bla*_{OXA-23} within a plasmid of circa 90 kb. Plasmid replicon typing performed according to the scheme devised by Bertini et al. (21) was positive for the *repAci6* gene, in agreement with previous studies showing a positive correlation between the presence of this replicon group (GR6) and *bla*_{OXA-23} carriage (22). Further characterization of the genetic environment of *bla*_{OXA-23} by inverse PCR (12) revealed its inclusion within a canonical Tn2006 composite transposon (23).

PCR amplification and sequencing of the quinolone resistance-determining region (QRDR) also showed missense mutations in both the *gyrA* (Ser83→Leu) and *parC* (Ser80→Leu) genes, concomitant with high-level resistance to ciprofloxacin, as previously described (24). PCR detection of aminoglycoside-modifying genes and 16S rRNA methylases was positive only for *aphA6*, which is commonly associated with high-level resistance to amikacin (25).

Plasmid transfer to a rifampin-resistant *A. baumannii* ATCC 17978 strain was successfully achieved by both transformation (26) and conjugation (27) upon selection on LB agar plates containing 8 μ g/ml imipenem and 75 μ g/ml rifampin. Selected colonies were positive for both *bla*_{OXA-23} and *aphA6*, showing coexistence of the two genes in the same plasmid, as well as for the *repAci6* gene. Acquisition of the plasmid containing the *bla*_{OXA-23} and *aphA6* genes turned the ATCC 17978 strain resistant to carbapenems (imipenem and meropenem MICs increased from 0.38 and 0.5 μ g/ml, respectively, to >32 μ g/ml), kanamycin (MIC increased from 6 μ g/ml to >256 μ g/ml), and amikacin (MIC increased from 3 μ g/ml to 48 μ g/ml).

Although outbreak MDR *A. baumannii* isolates are usually included within one of the three major international clones (IC-I, IC-II, and IC-III), published data seem to indicate that isolates belonging to IC-II possess a considerable ability for epidemic clonal spread (28). During the last decade, genetically related OXA-23-producing *A. baumannii* isolates belonging to IC-II have also been shown to disseminate within the Mediterranean region and other European countries, with a high percentage of isolates also being included within the widespread clonal complex (CC) CC92/CC2 (7, 9–11, 29–32). It has been suggested that such isolates are progressively replacing previously predominant clones carrying alternative OXA-type genes (7, 9, 10, 29). Until recently, however, carbapenem-resistant *A. baumannii* in Spain was mostly attributed to OXA-40 and, to a lesser extent, OXA-58 (33), whereas the presence of OXA-23-producing *A. baumannii* was limited to a single IC-II/ST2 isolate described in Mallorca and most likely imported from Portugal (12).

In the present study, we have described an outbreak caused by a single *A. baumannii* MDR clone producing OXA-23 and belonging to IC-II that rapidly disseminated among inpatients and environmental samples within one hospital in Barcelona. MLST typing assigned this clone to ST85, which is unrelated to CC92/CC2 but included in a different CC together with ST6 (17) and, therefore, cannot be associated with the OXA-23-producing ST2 epidemic clones emerging in Portugal, Italy, or Greece. ST85 has previously been linked to NDM-1 (34) and OXA-23 in France (35), the latter enzyme being responsible for a small outbreak in Marseille, but it has also been reported in carbapenem-susceptible isolates in Greece (17).

Of note, the composite transposon Tn2006, characteristically harboring the *bla*_{OXA-23} gene in *A. baumannii* isolates belonging to CC92/CC2, has consistently been found in the chromosome, where it might be contained within *AbaR4*-like resistance islands (8, 10, 12, 23, 31), although IC-II isolates bearing both a chromosomal copy and a plasmid-encoded copy and IC-I isolates containing only a plasmid-encoded OXA-23 have also been described (10, 23). The presence of a plasmid-carried Tn2006 in the present study reflects the potential dissemination of *bla*_{OXA-23} into epidemic clones other than those within CC92/CC2.

These findings reveal that the emergence of *bla*_{OXA-23}-positive isolates in the Mediterranean region could be attributed to the horizontal mobilization of Tn2006 among several genetically related outbreak MDR clones belonging to IC-II with an increased capability to disseminate and persist within the hospital setting. The identification of such outbreak lineages in Spain might represent the final expansion of OXA-23 throughout the Mediterranean region and, perhaps, the end of OXA-40 hegemony within the Iberian Peninsula as well.

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