

Long-term dissemination of an OXA-40 carbapenemase-producing *Acinetobacter baumannii* clone in the Iberian Peninsula

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Objective: The main objectives of this study were to assess the clonal relatedness of *Acinetobacter baumannii* carbapenem-resistant isolates recovered from the Iberian Peninsula and to investigate the production of carbapenemases.

Methods: One hundred and sixty-two imipenem-resistant *A. baumannii* isolates were collected from 1998 to 2003 in three Portuguese university hospitals. An imipenem-resistant isolate (988FFP strain) recovered in 1995 from a smaller hospital unit, was also included, as well as an OXA-40-producing *A. baumannii* Spanish strain (SM28). Susceptibility tests were carried out by disc diffusion and Etest methods. DNA fingerprints were obtained by PFGE of *Apal*-digested chromosomal DNA. Carbapenemase activity was determined by a bioassay and spectrophotometry. The detection of the *bla*_{OXA-40} gene was conducted through PCR analysis, cloning and nucleotide sequencing.

Results: All the isolates presented a similar multi-resistance pattern, including imipenem (MIC >32 mg/L). The Iberian isolates showed an identical PFGE pattern with minor band variations, including isolate 988FFP collected in 1995. PCR results revealed a *bla*_{OXA}-type gene in 65 isolates and nucleotide sequence analysis revealed the presence of the *bla*_{OXA-40} gene in seven representative Portuguese isolates from the various geographically dispersed hospitals.

Conclusions: Our results indicate that a multi-resistant epidemic clone of *A. baumannii*, carrying *bla*_{OXA-40}, is disseminated in the Iberian Peninsula, persisting in Portugal since 1995.

Keywords: *Acinetobacter* spp., epidemic clones, oxacillinases, β -lactamases

Introduction

Oxacillinases (Ambler class D) with carbapenemase activity, namely OXA-23, OXA-24, OXA-25, OXA-26, OXA-27 and OXA-40, have been found mainly in *Acinetobacter baumannii* isolates from the UK, Spain, Belgium and Singapore.^{1–4} *A. baumannii* is recognized as an important cause of nosocomial infections mainly observed in intensive care units. Numerous outbreaks of *Acinetobacter* spp. infections have been reported, often associated with the spread of multi-resistant strains.^{5,6}

Some outbreaks due to imipenem-resistant *A. baumannii* isolates occurred between 1998 and 2003 in various Portuguese hospitals located in distinct cities. These isolates were resistant

to most of the alternative antimicrobials. Clonal outbreaks of infection caused by carbapenem-resistant strains of *A. baumannii* have also been reported in Spain.^{4–6} The purposes of this study were to assess the clonal relatedness of *A. baumannii* imipenem-resistant isolates collected from Iberian Peninsula hospitals and to investigate the production of carbapenemases.

Materials and methods

Bacterial isolates

One hundred and sixty-two multi-resistant *A. baumannii* isolates were collected between 1998 and 2003, from different patients

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Dissemination of an *Acinetobacter baumannii* clone

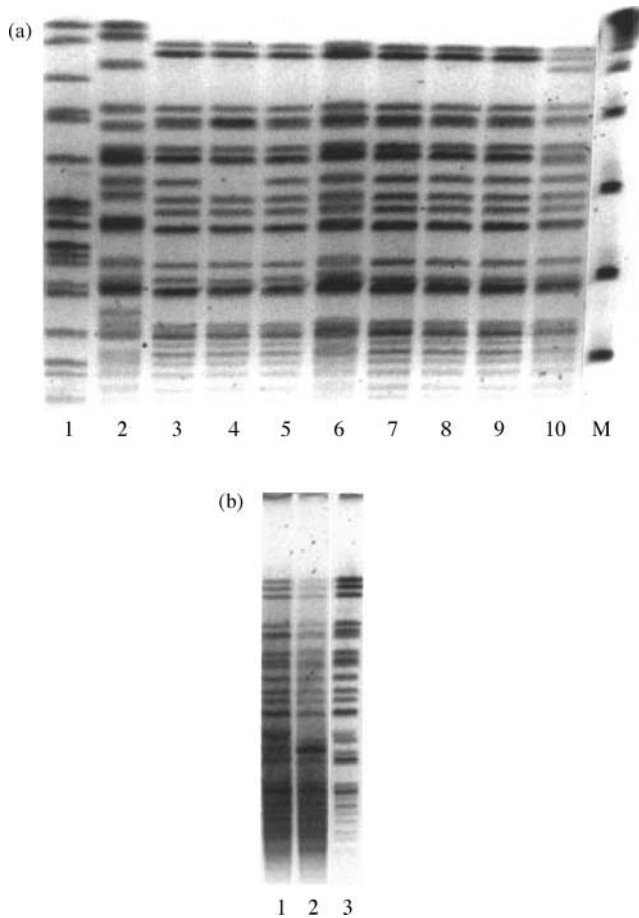


Figure 1. PFGE of genomic DNA from representative *A. baumannii* isolates, recovered from different Portuguese university hospitals, after digestion with *ApaI* (inverse image). (a) and (b) are from different gels. (a) Lane 1, ATCC 19606 strain; lane 2, imipenem-susceptible isolate; lanes 3–5 and 7–9, isolates from HUC (Coimbra), including 141FFc and 150FFC (lanes 3 and 5, respectively); lane 6, isolate from HSA (Porto); lane 10, isolate from HSM (Lisboa); lane M, lambda DNA concatemers. (b) lanes 1 and 2, isolates from HSM; lane 3, isolate SM28.

Discussion

A. baumannii is an emergent nosocomial pathogen, in part due to the capability of acquiring resistance to multiple antimicrobial agents. Clonal outbreaks due to imipenem-resistant strains of *A. baumannii* have been reported in particular hospitals.^{3,5,6}

Since 1998, a high imipenem resistance incidence has been observed among nosocomial *A. baumannii* isolates in Portugal. Therefore, it was our purpose to ascertain if the resistance was due to the dissemination of an imipenem resistance determinant and/or to the spread of one *A. baumannii* clone.

Microbiological and spectrophotometric assays showed that imipenem was hydrolysed by enzymic extracts. An alteration of carbapenemase activity was not observed in the presence of zinc and EDTA, suggesting the production of a non-metallo- β -lactamase. OXA Class D carbapenemases have been identified mainly in *A. baumannii* strains collected in Europe.^{1–4} OXA-24, OXA-25 and OXA-40 β -lactamases have been found in isolates from Spain associated with outbreaks. H er tier *et al.*¹⁰ have recently characterized biochemically and genetically the

OXA-40 carbapenemase in a single *A. baumannii* isolate, recovered from a Portuguese patient who was transferred directly to France. This study shows that *A. baumannii* isolates collected from the three main Portuguese university hospitals between 1998 and 2003 and the 988FFP strain, isolated in 1995, carried the *bla*_{OXA-40} gene, just as the Spanish isolate SM28.⁴ Among the isolates tested, three isolates did not significantly hydrolyse imipenem, although they carried the *bla*_{OXA-40} gene, as shown by PCR, and in 141FFC strain, confirmed by cloning and sequencing results. The data indicate that detection of *bla*_{OXA-40} by PCR does not always correlate with carbapenemase production and may over-report the frequency of OXA carbapenemase producers. Other resistance mechanisms, like reduced permeability of the outer membrane and alteration in PBPs, may also contribute to carbapenem resistance.⁵

The clonal relatedness of *A. baumannii* Portuguese isolates and SM28 strain was evaluated by antibiotyping and PFGE. All the isolates presented an identical antibiotic resistance profile, only variable for aminoglycosides, and a very similar *ApaI* pattern (Figure 1), differing by no more than three fragments. Such a correspondence of phenotypic and genotypic characteristics can be explained by a common clonal origin. They are likely to represent genotypic variants of the same clone, which, over time, suffered minor rearrangements. Curiously, the Spanish strain SM28 exhibited an identical DNA profile to that of the Portuguese isolates. This observation suggests that the OXA-40-producing strain might be dispersed in hospitals throughout the Iberian Peninsula. Moreover, the results reveal that the spread of this clone dates to at least 1995.

Geographical spread, at a national or international scale, may be an important feature in the epidemiology of *A. baumannii*. Increased resistance among *A. baumannii* isolates has been generally observed in the last decades. However, because epidemiological relatedness of strains was not assessed in most of these studies, susceptibility data may have been influenced by the unrecognized inclusion of epidemic strains that are often multi-drug resistant. Our results indicate that the observed carbapenem resistance among Portuguese *A. baumannii* isolates is due to the emergence of a resistant strain under antimicrobial selective pressure and the spread of an OXA-40-producing epidemic clone. This observation emphasizes the importance of having effective infection control measures in our hospitals, such as early detection of colonized patients, isolation procedures and a judicious use of antibiotics.

In summary, the present work reports for the first time the dissemination among Portuguese hospitals of an *A. baumannii* strain carrying *bla*_{OXA-40}, a clone endemic for several years in this country, and genetically related to the SM28 strain from Spain.

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