Molecular Epidemiology of Imipenem-Resistant Acinetobacter haemolyticus and Acinetobacter baumannii Isolates Carrying Plasmid-Mediated OXA-40 from a Portuguese Hospital

Major outbreaks of multidrug-resistant Acinetobacter baumannii associated with nosocomial infections have been increasingly reported worldwide (1, 10, 12). The endemicity of an OXA-24/40-producing A. baumannii clone associated with mortality events in Portugal has been observed at numerous hospitals within the Iberian Peninsula (5, 6, 10). Inversely, Acinetobacter haemolyticus, isolated only occasionally from clinical samples (9), usually presents susceptibility to different antibiotics, including β-lactams (13). The isolation of two carbapenem-resistant A. haemolyticus strains prompted us to assess the relative contribution of clonal spread to the observed high rate of carbapenem-resistant Acinetobacter spp. in a general hospital in Porto, Portugal.

Between January 2001 and October 2004, 224 imipenem-resistant Acinetobacter spp. were collected from several specimen sources and different hospital wards, where A. baumannii was associated with nosocomial infections and colonizations for several months (Table 1). Imipenem resistance significantly increased from 2001 to 2002 and from 2002 to 2003. Macrorestriction analysis of genomic DNA by pulsed-field gel electrophoresis (5) and 16S rRNA gene sequencing, performed for each clone and species representative, showed that, with the exception of two clonally related A. haemolyticus isolates, the remainder were A. baumannii isolates, distributed among three different pulsotypes. Clonal dissemination of two major pulsotypes (A and B), widespread throughout the hospital, contributed to the observed A. baumannii imipenem resistance, which has persisted since at least 2001 despite several elimination attempts, including the use of polymyxin. Pulsotype B was predominant from 2001 to 2002, after which clone A emerged as the dominant type (Table 1). This clone was found to be identical to the previously described Iberian OXA-24/40-producing clone (5). Pulsotype C, with only two isolates, seemed to represent a sporadic event within the observed prevalence of clones A and B. Antimicrobial susceptibilities varied among isolates according to clones (Table 2). A. haemolyticus isolates presented resistance to all β-lactams, with the exception of cepemine, ceftazidime, and aztreonam. All Acinetobacter sp. isolates were resistant to ciprofloxacin, whereas susceptibility to aminoglycosides was variable. Only 11 isolates (including the two A. haemolyticus isolates) showed a colistin MIC of ≥4 μg/ml (2). However, when the recently updated CLSI susceptible interpretative criterion of ≤2 μg/ml (3, 8) was applied, the susceptibility rate dropped from 96.1% to 92.1%. Detection of carbapenemase production, ulteriorly identified as an OXA-40 enzyme, was performed as previously described (5) and was positive only for clone A. A. baumannii isolates and, for the first time, A. haemolyticus isolates. Hybridization assays after...

### TABLE 1. Clinical data for imipenem-resistant Acinetobacter spp.

<table>
<thead>
<tr>
<th>Yr</th>
<th>% Imipenem resistance (no. of isolates)</th>
<th>Clone (no. of isolates)</th>
<th>Ward(s) (no. of isolates)</th>
<th>Main specimen source(s) (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>32 (47)</td>
<td>A (14)</td>
<td>ICU (8), ICU-P (2), ICU-S (1), NC (2), NK (1)</td>
<td>Respiratory tract (12), urine (1), NK (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (33)</td>
<td>ICU (6), ICU-P (14), CET (3), surgery 12B (3), Med A, B, and D (3), OBS (2), neurology (1), orthopedics (1)</td>
<td>Respiratory tract (13), urine (9), pus (4), catheter (3), blood (3), CSF (1)</td>
</tr>
<tr>
<td>2002</td>
<td>53 (31)</td>
<td>A (6)</td>
<td>ICU (4), Med B and C (2), Med B and D (7), ICU (5), ICU-P (6), neurology (2), orthopedics (1), urology (1)</td>
<td>Respiratory tract (5), urine (5), Respiratory tract (8), urine (10), pus (1), blood (2), catheter (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (22)</td>
<td>Endocrinology (1), Med B (1)</td>
<td>Pus (1), urine (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (9)</td>
<td>Surgery 2 (2), PED (1), Med (5), ICU (1)</td>
<td>Respiratory tract (3), urine (5), blood (1), Catheter (1), urine (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C (2)</td>
<td>Med D (1), ICU-P (1)</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>98 (42)</td>
<td>A (9)</td>
<td>ICU (4), surgery (1), NC (1), Med (1), OBS (1), oncology (1)</td>
<td>Respiratory tract (6), blood (1), NK (2), Urine (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (1)</td>
<td>GIN (1)</td>
<td></td>
</tr>
</tbody>
</table>

* ICU, intensive care unit; Med, medical unit(s); ICU-P, polyvalent ICU; ICU-S, post-surgical ICU; OBS, observation; NC, neurosurgery; PED, pediatrics; GIN, gynecology; CET, cranium-encephalic traumatism; CSF, cerebrospinal fluid; NK, origin not known.

* Number of pulotyped isolates (in 2003 and 2004, only representative isolates from the different hospital units were included). Clones are designated by capital letters and refer to A. baumannii isolates.

* The respiratory tract includes sputum, bronchial secretions, and tracheal aspirate.

* P was <0.01 for the difference between the two values (after Bonferroni’s adjustment) for 2001 and 2002, and P was <0.001 for the difference between the two values for 2002 and 2003. No significant differences were observed between 2003 and 2004 (P = 0.73)
both S1 nuclease digestion and I-CeuI digestion, performed as previously described (7), revealed that although some clone A
Acinetobacter baumannii isolates showed a chromosome-positive signal
(ca. 150 kb) for the blaOXA-40 probe, most also presented a positive hybridization in plasmidic bands of ca. 180 kb and ca.
30 kb. Similar hybridization signals were observed in the A.
haemolyticus isolates. Further studies on plasmid characterization,
assessing the homology among different plasmids, are ongoing.

We describe, for the first time, the presence of an OXA
24/40 enzyme in an A. haemolyticus clinical isolate. Although the spread of OXA-24/40, both in the Iberian Peninsula and in France, has been correlated with the progressive dissemination
of a single A. baumannii clone, the observation of this enzyme in a
different, previously unreported, genomic species, A. haemolyticus,
poses new questions on OXA-24/40 dissemination. It now seems reasonable to suspect a horizontal dissemination
of the blaOXA-40 gene between different species, an ability
supported by the observation of this enzyme, previously de-
scribed as chromosomally encoded (7), in a plasmid. Notwith-
standing, the dissemination of “successful” clones may possibly contribute to the high rates and persistence of imipenem-res-
istant A. baumannii isolates (4).

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REFERENCES

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