Occurrence of Carbapenem-Resistant Acinetobacter baumannii Clones at Multiple Hospitals in London and Southeast England

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Received 3 April 2006/Returned for modification 29 June 2006/Accepted 29 July 2006

Acinetobacters are important nosocomial opportunists, particularly in intensive care and other specialist units. Common infections include ventilator-associated pneumonias and bacteremias; less frequent sites include burn wounds and the urinary tract (3). Most infections are caused by Acinetobacter baumannii, a species resilient to drying and commonly multiresistant to antibiotics.

A. baumannii strains notoriously cause hospital outbreaks, and a few lineages achieve “epidemic” status, reaching multiple hospitals or countries. By convention, these are termed “clones” rather than “strains” when their relatedness is inferred on the basis of DNA profiles, without proven chains of site-to-site transmission. Examples include (i) the European clones I, II, and III, which are widespread in continental Europe (8, 25), (ii) a clone with the VEB-1 cephalosporinase that spread in northeast France and Belgium in late 2003 to 2004 (21), (iii) a clone with OXA-40 (OXA-24) carbapenemase which is prevalent at numerous hospitals in Spain and Portugal (7), and (iv) the southeast (SE) clone prevalent in southern England since 2000 (24). Disturbingly, several successful clones are now also carbapenem resistant and, as noted by the Infectious Disease Society of America, Acinetobacter is “a prime example of the mismatch between unmet medical need and the current antimicrobial research and development pipeline” (22).

Among consecutive A. baumannii isolates collected at 54 United Kingdom hospitals in 2000 more than 85% were resistant to cephalosporins, 43% were resistant to gentamicin, and 46% were resistant to quinolones, leaving the carbapenems as the only standard antibiotics active against more than 90% of isolates in vitro (11). However, the SE clone, which began to become prevalent shortly after the study period for this survey is “a prime example of the mismatch between unmet medical need and the current antimicrobial research and development pipeline” (22).

From late 2003 to the end of 2005, the Health Protection Agency’s national reference laboratories received approximately 1,600 referrals of Acinetobacter spp., including 419 and 58 examples, respectively, of two carbapenem-resistant Acinetobacter baumannii lineages, designated OXA-23 clones 1 and 2. Representatives of these clones were obtained from 40 and 8 hospitals, respectively, in London or elsewhere in Southeast England. Both clones had blaOXA-23-like genes, as well as the intrinsic (but downregulated) blaOXA-51-like carbapenemase genes typical of A. baumannii. Both were highly multiresistant: only colistin and tigecycline remained active versus OXA-23 clone 1 isolates; OXA-23 clone 2 isolates were also susceptible to amikacin and minocycline. These lineages increase the burden created by the southeast (SE) clone, a previously reported A. baumannii lineage with variable carbapenem resistance contingent on upregulation of the blaOXA-51-like gene. Known since 2000, the SE clone had been referred from over 40 hospitals by the end of 2005, with 627 representatives received by the reference laboratories. The OXA-23 clone 2 is now in decline, but OXA-23 clone 1 continues to be referred from new sites, as does the SE clone. Their spread is forcing the use of unorthodox therapies, principally colistin and tigecycline, although the optimal regimens remain uncertain.

<table>
<thead>
<tr>
<th>Yr and clone</th>
<th>No. of isolates</th>
<th>No. of patients</th>
<th>No. of Hospital Trusts</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>158</td>
<td>24</td>
</tr>
<tr>
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<td>12</td>
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<tr>
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<td>38</td>
<td>37</td>
<td>5</td>
</tr>
<tr>
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<td></td>
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<tr>
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<td>25</td>
</tr>
<tr>
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<td>20</td>
<td>6</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>52</td>
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</tr>
<tr>
<td>OXA-23 clone 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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* A Hospital Trust may include one or more hospital sites.

TABLE 1. Reference submissions of multiresistant A. baumannii isolates from 2003 to 2005
was completed, has variable carbapenem resistance contingent on activation, by IS\textit{Aba}1, of bl\textit{a}\textit{OXA-51} (23, 23), encoding a chromosomal carbapenemase that is intrinsic to all \textit{A. baumannii} isolates (12), but which is poorly expressed by most. Consistent carbapenem resistance arises in lineages with additional OXA or metallo (IMP and VIM)-carbapenemases (5). We report here the dissemination, at multiple hospitals in London and Southeast England, of two further carbapenem-resistant clones, each with OXA-23 carbapenemase.

FIG. 1. PFGE profiles of Apal-digested genomic DNA from bl\textit{a}\textit{OXA-23}-positive isolates and representative members of the SE clone. A bl\textit{a}\textit{OXA-23}-negative isolate (H26) of OXA-23 clone 1 is also included, as are some OXA-23 producers (labeled sporadic 1 to 3) with unique PFGE profiles. Isolates were from hospitals H1 to H26 (OXA-23 clone 1) and 1 to 7 (OXA-23 clone 2). Letters A to C represent isolates from different patients at the same hospital.
Isolate collection. Isolates were received by the Centre for Infections’ reference laboratories from hospital laboratories in the United Kingdom for outbreak investigation and for analysis of antibiotic resistance. The Centre for Infections has consistently sought, but cannot compel, submission of Acinetobacter spp. from suspected outbreaks and those with carbapenem resistance. Submissions are accompanied by a variable amount of clinical detail.

Isolate characterization. DNA fingerprinting was by pulsed-field gel electrophoresis (PFGE) of Apal-digested genomic DNA (24). Isolates were identified to the genospecies level by amplified rRNA gene restriction analysis (10, 26) and rRNA spacer fingerprinting (10) or (mostly) on the basis of having PFGE profiles corresponding to known A. baumannii clones. bla\textsubscript{OXA-23}-like genes were sought by PCR using primers and conditions described elsewhere (1, 4, 6) or in parallel with those for other OXA carbapenemases (bla\textsubscript{OXA-24}, bla\textsubscript{OXA-51}, and bla\textsubscript{OXA-58}) using a multiplex assay (27). Sequencing of bla\textsubscript{OXA-23}-like genes was performed on a CEQ 8000 (Beckman-Coulter, High Wycombe, United Kingdom) apparatus as described previously (7). Susceptibility testing was done on IsoSensitest agar (Oxoid, Basingstoke, United Kingdom) according to British Society for Antimicrobial Chemotherapy guidelines (2).

RESULTS

From January 2000 to December 2005, the Centre for Infection received approximately 3,000 isolates of Acinetobacter spp. for strain typing or analysis of resistance mechanisms, with 1,600 arriving between late 2003 and the end of 2005. Examples of the SE clone (24), with its variable carbapenem resistance contingent on the regulation of the bla\textsubscript{OXA-51}-like gene (23), were first received in April 2000. By the end of the study period, these totalled 627 isolates obtained from 531 patients at 42 hospitals (Table 1), mostly in the London and Southeast Government and Health Regions. One exceptional representative of the SE clone also carried a bla\textsubscript{OXA-23}-like gene.

In November 2002 and July 2003, respectively, we began to receive numerous examples of two further multiresistant A. baumannii clones, each from multiple hospitals (Table 1). These were distinct from each other and from the SE clone in PFGE profile (Fig. 1). Nearly all representatives were positive for the bla\textsubscript{OXA-23}-like gene, as well as the bla\textsubscript{OXA-51}-like gene, and the two clusters were designated OXA-23 clones 1 and 2. The bla\textsubscript{OXA-23}-like gene was sequenced from three representatives of each, from different sites, and was found to have the classical sequence, as in A. baumannii 6B92, collected in Scotland in 1985 (9).

By the end of 2005 we had received 419 and 58 examples of OXA-23 clones 1 and 2, respectively, with the former from 40 Hospital Trusts and the latter from 8. The maximum numbers of isolates from single trusts were 71 (from 63 patients) for clone 1 and 23 (from 20 patients) for clone 2. Five exceptional isolates, in addition to these totals, had PFGE profiles corresponding to OXA-23 clone 1 but lacked bla\textsubscript{OXA-23}. In all, and since 2000, at least 56 hospital trusts have been affected by one or more of the three clones discussed here.

Both OXA-23 clones were distinct in PFGE profile from the original OXA-23-producing isolate (6B92; labeled sporadic 1 on Fig. 2) and from an OXA-23-producing Brazilian strain (6), included as a control. Both were also distinct from 13 further Acinetobacter isolates that were PCR positive for the bla\textsubscript{OXA-23}-like gene and that were received from United Kingdom laboratories during the study period. Most of these were sporadic strains and did not cause outbreaks (e.g., sporadic 2 and 3 in Fig. 1); they split into nine distinct clones by PFGE.
that the meropenem; PIP, piperacillin; PTZ, piperacillin-tazobactam; SUL, sulbactam; MIN, minocycline; TIG, tigecycline; COL, colistin. The British Society for Antimicrobial
Acinetobacter
Acinetobacter
has no current breakpoint for minocycline or sulbactam versus
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that the carbapenem resistance. These conclusions are in keeping
between representatives from different centers.
dent selection from a widespread common ancestor (8), a hypoth-
mains possible that they have arisen at different sites by indepen-
dent selection. The international literature reports generally good outcomes with intravenous colistin (polymyxin E) against infections caused by multiresistant Acinetobacter spp., except in pneumonia, where a 75% failure rate was noted (16). The poor performance with pneumonia probably reflects poor penetration to the lung (16), and there is some evidence that this limitation can be overcome by increased dosage (19) or by the coadministration of nebulized drug (14, 20). However, neither of these approaches has been validated in formal trials, and higher intravenous doses may increase the risk of nephrotoxicity. It should also be stressed that pharmacodynamic analyses for polymyxins remain very limited and that much of the pharmacokinetic analysis is old and needs to be reevaluated by modern methodologies (17). In the case of tigecycline, we await analysis of the compassionate-use program, under whose ambit several patients infected with these clones were treated; anecdotally, we are aware both of successes with tigecycline in acinetobacter infections and of a few instances where resistance emerged during therapy but cannot, as yet, relate these to the clone type (18).

With this background of uncertainty it is unclear as yet whether tigecycline or colistin should be the preferred therapy for infections due to the present clones or other similarly resistant A. baumannii strains; nevertheless, it does seem ap-
tibility to sulbactam; otherwise, they were as multiresistant as blaoxa-23-positive representatives of the clone.

**DISCUSSION**

Acinetobacter spp. are notorious both for their ability to acquire antibiotic resistance and for the ability of some strains, mostly strains of A. baumannii, to cause nosocomial outbreaks (3). Nevertheless, prior to 2000, virtually all A. baumannii isolates in the United Kingdom were susceptible to carbapenems (11), and very few genotypes appeared to occur in multiple hospitals. These patterns changed with the multicentric isolation of the SE clone, with its variable resistance to imipenem and meropenem (24). The two OXA-23-producing clones described here represent a further ratcheting of the problem, and meropenem (24). The two OXA-23-producing clones described here represent a further ratcheting of the problem, being more consistently resistant to carbapenems.

The origins of these clones remains unclear, as do the reasons for their epidemic success. It seems likely, particularly in London, that their spread among centers has been via patient transfers, but there is no direct epidemiological proof of this view, and it remains possible that they have arisen at different sites by independent selection from a widespread common ancestor (8), a hypothesis that may explain the observed variation in PFGE profiles between representatives from different centers.

Extracted OXA-23 enzyme has very weak activity against carbapenems; nevertheless, MIC comparisons for members of the OXA-23 clone 1 with or without the blaoxa-23-like gene (Tables 2 and 3) suggest that the enzyme was responsible for the carbapenem resistance. These conclusions are in keeping with studies showing that carbapenem resistance cotransferred with blaoxa-23 in Acinetobacter (13), and with data showing that the blaoxa-23-like gene is not activated by ISAba1 in the OXA-23 clone 1, indicating that it is unlikely to be the real source of resistance (23, 23).

Although OXA-23 clone 2 now seems to be in decline (Table 1), the reference laboratories continue to receive referrals of OXA-23 clone 1, as well as of the SE clone. Both are causing therapeutic as well as infection control problems. Treatments being used include intravenous colistin, often with nebulized colistin added in pneumonia cases, or tigecycline. Local outcome analyses are in progress. The international literature reports generally good outcomes with intravenous colistin versus Acinetobacter spp., and has adopted the European Committee on Susceptibility Testing's (EUCAST; http://www.eucast.org) view that there is as yet “insufficient evidence” to set breakpoints for tigecycline versus Acinetobacter spp. NT, not tested.

### Table 2. Summary of MIC and resistance data for OXA-23 clone 1 and 2 isolates and comparator groups

<table>
<thead>
<tr>
<th>Specimen</th>
<th>AMK (8/16)</th>
<th>AMP (8/16)</th>
<th>CIP (0.5/1)</th>
<th>CTX (1/1)</th>
<th>CAZ (2/2)</th>
<th>GEN (2/4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-23 clone 1 (131–134)</td>
<td>53.6 (0.5–64)</td>
<td>$&gt;64$ ($&gt;64$)</td>
<td>$&gt;8$ ($&gt;8$)</td>
<td>108.2 (16–256)</td>
<td>69.6 (4–256)</td>
<td>28.4 (0.5–32)</td>
</tr>
<tr>
<td>OXA-23 clone 2 (19–22)</td>
<td>2.4 (1–32)</td>
<td>$&gt;64$ ($&gt;64$)</td>
<td>$&gt;8$ ($&gt;8$)</td>
<td>122 (64–256)</td>
<td>70 (32–256)</td>
<td>25.4 (4–32)</td>
</tr>
<tr>
<td>SE clone (53–54)</td>
<td>11.9 (4–64)</td>
<td>$&gt;64$ ($&gt;64$)</td>
<td>$&gt;8$ ($&gt;8$)</td>
<td>21.6 (0.25–64)</td>
<td>11.3 (0.25–64)</td>
<td>25.4 (0.25–64)</td>
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</tbody>
</table>

### Table 3. MICs for OXA-23 clone 1 isolates lacking blaoxa-23

<table>
<thead>
<tr>
<th>Specimen</th>
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<th>CEF</th>
<th>CTX</th>
<th>GEN</th>
<th>IPM</th>
<th>MEM</th>
<th>PIP</th>
<th>PTZ</th>
<th>SUL</th>
<th>MIN</th>
<th>TIG</th>
<th>COL</th>
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<tbody>
<tr>
<td>H121</td>
<td>1</td>
<td>$&gt;64$</td>
<td>$&gt;8$</td>
<td>128</td>
<td>32</td>
<td>0.25</td>
<td>1</td>
<td>0.5</td>
<td>$&gt;64$</td>
<td>NT</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>H472</td>
<td>$&gt;64$</td>
<td>$&gt;64$</td>
<td>$&gt;8$</td>
<td>$&gt;256$</td>
<td>128</td>
<td>$&gt;32$</td>
<td>1</td>
<td>0.5</td>
<td>$&gt;64$</td>
<td>NT</td>
<td>4</td>
<td>4</td>
<td>0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>H170</td>
<td>2</td>
<td>$&gt;64$</td>
<td>$&gt;8$</td>
<td>128</td>
<td>64</td>
<td>$&gt;32$</td>
<td>1</td>
<td>8</td>
<td>$&gt;64$</td>
<td>NT</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>H403</td>
<td>$&gt;64$</td>
<td>$&gt;64$</td>
<td>$&gt;8$</td>
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<td>$&gt;64$</td>
<td>$&gt;64$</td>
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<td>0.5</td>
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<td>4</td>
<td>2</td>
<td>0.5</td>
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</table>

*Abbreviations are as defined in Table 2, footnote a.

b Isolate included in Fig. 1.
propriate that microbiology laboratories serving affected hospitals should test both of these agents, as well as sulbactam, which retains better activity against other resistant lineages than those prevalent in London and southeast England (16). The Health Protection Agency knows of several hospitals that have brought problems with the OXA-23 clones under control by rigorous infection control measures involving the cohorting of infected patients and their care staff, along with major cleaning and, in some cases, bed-closure programs. In light of both the growth of the problem and the associated therapeutic difficulties and uncertainties, such approaches remain critical, and national infection control guidance for Acinetobacter infections have been published by the Health Protection Agency (http://www.hpa.org.uk/infections/topics_az/acinetobacter_b/guidance.htm).

ACKNOWLEDGMENTS

We are grateful to all Trust Laboratories that have sent us isolates and to AstraZeneca for sponsorship of Juliana Coelho’s Ph.D. student, under whose ambit much of this work was undertaken, and to Wyeth for provision of the tigecycline.

REFERENCES