The resistance profile of Acinetobacter baumannii strains isolated from the Aberdeen Royal Infirmary

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Streptococcus sp. isolates identified and their Tn916-like elements. Organisms were identified to species level according to the nearest match in GenBank and were then sorted into strains according to sequence differences in the sodA gene.

<table>
<thead>
<tr>
<th>Species ID</th>
<th>No. of isolates</th>
<th>Elements present (no. of isolates)</th>
<th>Excision (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. anginosus</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. australis I</td>
<td>1</td>
<td>Tn6002</td>
<td>n</td>
</tr>
<tr>
<td>S. australis II</td>
<td>1</td>
<td>Tn6087</td>
<td>n</td>
</tr>
<tr>
<td>S. gordoni</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. infantis I</td>
<td>3</td>
<td>Tn6002 (1), Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. infantis II</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. infantis III</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. mitis</td>
<td>1</td>
<td>Tn6002</td>
<td>y</td>
</tr>
<tr>
<td>S. oralis I</td>
<td>1</td>
<td>Tn6002</td>
<td>y</td>
</tr>
<tr>
<td>S. oralis II</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. parasanguinis I</td>
<td>2</td>
<td>Tn6002</td>
<td>y</td>
</tr>
<tr>
<td>S. parasanguinis II</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius I</td>
<td>1</td>
<td>Tn3872 and Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius II</td>
<td>6</td>
<td>Tn6002</td>
<td>y (2)/(n (4)</td>
</tr>
<tr>
<td>S. salivarius III</td>
<td>12</td>
<td>Tn3872 (1), Tn916</td>
<td>y (Tn3872 ×6, Tn916)/(n (Tn3872 ×5)</td>
</tr>
<tr>
<td>S. salivarius IV</td>
<td>3</td>
<td>Tn3872</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius V</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius VI</td>
<td>1</td>
<td>Tn3872</td>
<td>n</td>
</tr>
<tr>
<td>S. salivarius VII</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius VIII</td>
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<td>Tn916</td>
<td>y</td>
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<tr>
<td>S. salivarius IX</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. salivarius X</td>
<td>1</td>
<td>Tn916</td>
<td>y</td>
</tr>
<tr>
<td>S. sanguinis I</td>
<td>2</td>
<td>Tn3872 (1), Tn6002</td>
<td>y (Tn3872)/n (Tn6002)</td>
</tr>
<tr>
<td>S. sanguinis II</td>
<td>2</td>
<td>Tn3872</td>
<td>y</td>
</tr>
<tr>
<td>S. sanguinis III</td>
<td>1</td>
<td>Tn6002</td>
<td>y</td>
</tr>
</tbody>
</table>

* y, yes; n, no.
* Southern blot analysis of isolate S. salivarius I was found to contain three copies of intTn, suggesting that an integrase gene may be present but not associated with a Tn916-like element.

the composition of the indigenous microbiota at various body sites.

Competing interests: None declared.

Ethical approval: UCL Ethics Committee (project no. 1364/001).

References


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22 November 2011

The resistance profile of Acinetobacter baumannii strains isolated from the Aberdeen Royal Infirmary

Sir,

The increase in carbapenem resistance in Acinetobacter baumannii is largely attributable to the Ambler class D β-lactamases, in particular enzymes related to OXA-23 and OXA-58. The purpose of this study was to analyse the resistance in A. baumannii strains isolated from Aberdeen Royal Infirmary (Aberdeen, UK) from 2006 to 2010.

Nine non-repetitive A. baumannii strains were chosen for this study. The strains were identified by polymerase chain reaction (PCR) of the blaOXA-51-like gene and by sequencing of the rpoB gene. Minimum inhibitory concentrations (MICs) were determined according to the guidelines of the British Society for Antimicrobial Chemotherapy (BSAC). The blaOXA-23-like, blaOXA-40-like, blaOXA-51-like, blaOXA-58-like, and blaOXA-143-like gene families were screened by multiplex PCR [1]. ISAAC1 and OXA-23R primers were used for the detection of ISAba1 upstream of the blaOXA-23-like gene [1,2]. Amino-glycoside resistance genes were identified by multiplex PCR [3]. Primers for amplification of the blaADC gene have been described previously [2], and primers FU (5′-CGG CCG TGA ATT CTT AAG TG-3′) and RU (5′-AGC CAT ACC TGG CCC ATC AT-3′) were used to amplify the integenic region upstream of the blaADC gene. PCR was performed for amplification of the quinolone resistance-determining regions (QRDRs) of the gyrA and parC genes [4], and gene fragments were sequenced for determining specific amino acid changes. PCR for detection of the class 1 integrase gene was performed as described previously [4].

A macrorestriction assay followed by pulsed-field gel electrophoresis (PFGE) was performed on all A. baumannii strains. Cluster analysis was performed by the unweighted pair-group method with mathematical averaging (UPGMA), and DNA relatedness was calculated using the band-based Dice coefficient with a tolerance setting of 1.5% band tolerance and 1.5% optimisation setting for the whole profile. Gel analysis was performed using BioNumerics v2.5 software (Applied Maths, Sint-Martens-Latem, Belgium). A value of ≥80% was chosen as the threshold for the establishment of clonal relatedness of the isolates.

SI nuclease (Promega, Southampton, UK) digestion using 10 U of enzyme with incubation at 37 °C for 45 min was performed for the PFGE plugs according to the manufacturer’s instructions. Plasmid curing was performed using acriflavine and with an elevated temperature of incubation. The strains were serially subcultured for 14 days at 47 °C.

Two novel variants of the blaOXA-51-like gene were found (Table 1): strain 14 had serine-14 (TCT) of the blaOXA-180 gene replaced by phenylalanine (TTT) and is now designated blaOXA-216; and strain 6 had threonine-255 (ACA) of the blaOXA-78 gene substituted by isoleucine (ATA) and is now designated blaOXA-217. Isolates 10 and 10n had variants of blaOXA-65 gene with synonymous mutations.
Strains 3 and 12 possessed the aac(3’)-Ia gene conferring gentamicin resistance and had high ceftazidime MICs owing to the presence of ISAba1 upstream of the blaADC gene. All of the remaining strains (except 10) had the blaADC gene without ISAba1 upstream and thus were susceptible to ceftazidime (Table 1). Strain 10 completely lacked the blaADC gene. All of the isolates were susceptible to colistin.

Strains 3 and 12 had amino acid changes at position 83 of GyrA (serine83 → leucine) and position 80 of ParC (serine80 → leucine) conferring ciprofloxacin resistance. Both strains possessed integrase genes and sequencing confirmed the presence of putative glucose dehydrogenase precursor that could be responsible for catabolism of glucose by oxidation.

The PFGE profiles of the strains showed that most were not clonally related as they had <80% similarity (data not shown). PCR for insertions causing disruption of carO (29 kDa outer membrane protein) was not detected for any of the strains. PFGE analysis revealed that strains 3 and 12 had 83% similarity; strain 3, isolated in the year 2006, was negative for the ISAba1–blaOXA-23 gene, whereas strain 12, isolated in the year 2008, was positive and was resistant to imipenem and meropenem (Table 1). Strains 3 and 12 had an identical blaOXA-51-like allele 1 corresponding to sequence group 1. In addition, strain 12 had the blaOXA-23 clone 1 allele, which belongs to European clone II. This indicates the A. baumannii is actively acquiring resistance genes, probably through plasmid transfer, although S1 nuclease digestion and plasmid extraction procedures did not detect any plasmids, and elimination studies with acriflavin did not remove the resistance determinants. This suggests that if blaOXA-23 was plasmid borne, it is now integrated in the host chromosome of strain 12 endowing it with a stable mechanism of carbapenem resistance.

These results show that the clinical situation in the hospital in Aberdeen is in a state of flux. New variant strains are emerging and, most importantly, a carbapenem-sensitive strain has become resistant through acquisition of the blaOXA-23 gene with an ISAba1 element upstream that carries a promoter allowing expression of the β-lactamase. The blaOXA-23 gene was first found in Scotland more than 20 years ago and it has remained the sole mechanism of carbapenem resistance until this point [5].

**Nucleotide accession numbers**

The blaOXA-216 and blaOXA-217 genes have been deposited in GenBank under the accession nos. FR865168 and JN603240, respectively.

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**Competing interests**: None declared.

**Ethical approval**: Not required.

**References**


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21 October 2011