Dissemination of two international linezolid-resistant Staphylococcus epidermidis clones in Greek hospitals


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Sir, The growing number of infections caused by multidrug-resistant Staphylococcus epidermidis has necessitated the use of new antimicrobials, such as linezolid, and enhanced the emergence of linezolid-resistant S. epidermidis strains. To date, mutations of region V of 23S rRNA (G2447T, T2504A, C2534T, G2576T, G2630T and G2631T) have been associated with the expression of linezolid resistance among clinical staphylococcal isolates.1–3 Additionally, the presence of the cfr gene, as well as changes in riboprotein L4, have been detected in linezolid-resistant S. epidermidis.4–6

From October 2008 to October 2009, 26 S. epidermidis isolates exhibiting resistance to linezolid were isolated from blood and central venous catheter cultures from 26 individual inpatients hospitalized in the intensive care units (ICUs) of four tertiary care Greek hospitals [Sismanoglion General Hospital of Athens (hospital A), University Hospital of Patras (hospital B), University Hospital of Larissa (hospital C) and University Hospital AHEPA (hospital D)]. Demographic data (age, gender and location) and clinical information (reason for admission and antibiotic therapy) for each patient were collected. The identification of the isolates to species level was primarily performed using the Vitek II Advanced Expert System (bioMérieux, Marcy l’Étoile, France), followed by a molecular method based on the tuf gene.1 Susceptibility testing was performed using the Vitek II Advanced Expert System. MICs of oxacillin, vancomycin, teicoplanin, linezolid, dalfo- pristin and tigecycline were determined using the CLSI microdilution method and Etest (Biodisk, Solna, Sweden).5

The isolates were primarily tested for the presence of any mutations of region V of 23S rRNA by PCR using the following pair of primers: 5′-gcggtccgcccttaaag-3′ (corresponding to bases 2278–2296 of the S. epidermidis 23S rRNA gene, GenBank accession no. CR000029) and 5′-ccgcctctccgacta-3′ (corresponding to bases 2679–2696 of the S. epidermidis 23S rRNA gene, GenBank accession no. CR000029). The PCR products were purified and sequenced, while the sequence data were analysed using Chromas (www.techneiyium.com.au/chromas.html). The determination of the number of mutated copies of each isolate was assessed as described previously.1 The presence of the cfr gene was tested by PCR.4 The possible presence of mutations in ribosomal protein L4 was investigated by PCR followed by sequence analysis as described previously.3 The clonal relatedness of isolates was investigated by multilocus sequence typing (MLST) as previously described (http://sepidermidis.mlst.net).5

All isolates were identified as S. epidermidis by conventional and molecular methods and exhibited a multiresistant phenotype, including resistance to gentamicin, tobramycin, erythromycin, clindamycin, oxacillin, cephalosporins, penicillin, ciprofloxacin, linezolid and fusidic acid, while remaining susceptible to quinupristin/dalfopristin, tetracycline, rifampicin, vancomycin, teicoplanin, daptomycin and tigecycline. The mechanism of linezolid resistance was associated with the presence of various mutations in region V of 23S rRNA; the isolates did not carry the cfr gene or mutations in the L4 riboprotein. The sequencing analysis of region V of 23S rRNA revealed that three isolates carried only a C2534T mutation, 11 carried both C2534T and G2576T mutations and 12 isolates carried both C2534T and T2504A mutations. The positions of mutations and the number of mutated alleles are described in Table 1. The strains belonged to two clones, ST2 and ST22 (where ST stands for sequence type). In Greece, 67% of clinically significant S. epidermidis strains belong to these clones (E. Petinaki and I. Spiliopoulou, unpublished data).

Table 1 clearly demonstrates that the MICs of linezolid, ranging from 8 to 1024 mg/L, were correlated not only with the number of mutated alleles, but also with the position of the mutation. Strains with C2534T had significantly lower MICs than the rest; interestingly, higher MICs were detected among strains carrying the T2504A mutation.

All patients were male, with a mean age of 56.9 years (range 21–76 years). The median length of ICU stay prior to S. epidermidis
Table 1. MICs of linezolid in correlation with molecular characteristics of linezolid-resistant S. epidermidis

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No. of isolates</th>
<th>MIC of linezolid (mg/L)</th>
<th>Mutation and location of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>8–10</td>
<td>rrlD, rrlF</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>16–24</td>
<td>rrlD, rrlF, rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>1024</td>
<td>rrlD, rrlF</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>1024</td>
<td>rrlD, rrlF, rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>16–24</td>
<td>rrlD, rrlF, rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>8</td>
<td>rrlD, rrlF, rrlA, rrlD, rrlE, rrlF</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>64</td>
<td>rrlD, rrlF, rrlA, rrlD, rrlE, rrlF</td>
</tr>
</tbody>
</table>

aA, Sismanoglion General Hospital of Athens; B, University Hospital of Patras; C, University Hospital of Larissa; D, University Hospital AHEPA.

isolation was 39.6 days (range 8–150 days). Reasons for admission were polytraumatism, brain trauma, respiratory failure, acute renal failure, peritonitis, necrotic pancreatitis, spontaneous brain haemorrhage and subarachnoid haemorrhage. Twelve of 26 strains were from patients with <2–12 weeks of exposure to linezolid.

This study describes the molecular epidemiology and the mechanism of linezolid resistance in S. epidermidis strains from Greece. Two international clones (ST2 and ST22) were identified that carried known mutations of region V of 23S rRNA. Mutations G2576T and C2534T have been previously reported in strains arising from different institutions; mutation T2504A was detected for the first time in Greece1 and has also been recognized in the USA.3 The finding that strains from the USA with the T2504A mutation belong to different STs from ours indicates a continuous potential for the emergence of linezolid-resistant staphylococci.

Dissemination of two common linezolid-resistant S. epidermidis clones among hospitals located in different areas of Greece (northern, central and south-western) is most likely due to linezolid exposure and patient-to-patient spread. This is more obvious in the ICUs of two university hospitals (hospitals B and D), where linezolid-resistant S. epidermidis strains were recovered within a 2–3 month period. Clones such as ST2 and ST22 that predominate in the hospital environment become linezolid resistant under selective pressure. After linezolid treatment, such resistant staphylococci colonize the skin, becoming the dominant cutaneous flora causing infections in critically ill patients. In order to preserve the usefulness of linezolid as a therapeutic agent, judicious use of antibiotics and application of strict infection control measures are essential.

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Transparency declarations

None to declare.

References


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Isolation of multidrug-resistant Klebsiella oxytoca carrying bla\textsuperscript{IMP-8}, associated with OXY hyperproduction, in the intensive care unit of a community hospital in Spain

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