Aminoglycoside-Streptothricin Resistance Gene Cluster

**aadE–sat4–aphA-3 Disseminated among Multiresistant Isolates of Enterococcus faecium**

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Seventy-two *Enterococcus faecium* isolates of different origins highly resistant to nourseothricin and streptomycin were studied. Sequencing of a genomic fragment from two isolates identified a gene cluster, **aadE–sat4–aphA-3**, which has been isolated recently in staphylococci and *Campylobacter coli*. Patterns of digested PCR products of **aadE–sat4–aphA-3** were identical for all isolates.

Resistance to streptothricin antibiotics has been reported from gram-negative bacteria following use of nourseothricin as an antimicrobial feed additive on industrial animal farms in the former East Germany (12, 16). Streptothricins are antibiotics consisting of a streptolidine ring, a gulosamine, and a polylyside side chain (6). Resistance is due to N acetylation of lysine (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gram-negative bacteria via different streptothricin acetyltransferases, Sat1 to Sat4 (5, 8–10). In (11) and is mediated in gramm...
streptothricin resistant. E. faecium displays a higher level of intrinsic resistance to nourseothricin than S. aureus and gram-negative bacteria such as E. coli and Campylobacter coli (ca. 2 mg/liter).

Among the above-mentioned 95 E. faecium isolates, 35 were nourseothricin resistant and high-level streptomycin resistant (MIC of streptomycin of 2048 mg/liter). We also included 37 nonrelated, isolates resistant to high levels of streptomycin (and vancomycin) in our study which originated from our strain culture collection of human E. faecium isolates (Table 1). These isolates were also known to be nourseothricin resistant. Distribution of MICs of nourseothricin for all 72 nourseothricin-resistant E. faecium isolates were as follows: 1 isolate with a MIC of 128 mg/liter (PCR positive for sat4), 11 isolates with a MIC of 1,024 mg/liter, 30 isolates with a MIC of 2,048 mg/liter, and 30 isolates with a MIC of 2,048 mg/liter. Except for five isolates with a streptomycin MIC of 2,048 mg/liter, all the others exhibited a MIC of >2,048 mg/liter. All isolates were unrelated based on different antibiotic resistance phenotypes and macrorestriction patterns resolved by pulsed-field gel electrophoresis (14; unpublished data).

Cloned genomic DNA fragments of E. faecium UW1965 and UW786, which encoded kanamycin resistance in E. coli transformants, revealed identical nucleotide sequences (GenBank accession number AF330699). Three open reading frames were identified which showed almost complete identity with the resistance genes aadE, sat4, and aphA-3 (Fig. 2). All putative proteins from sat4 of C. coli BE/G4 (5), S. aureus BM3505 (3), and the two enterococcal isolates described here consist of 180 amino acids. Due to a point mutation at nucleotide 949 (A→G) in the two sequences described here, as well as at the corresponding position in the staphylococcal sat4 gene on plasmid pP1718 in S. aureus BM3505, the amino acid Glu (GAG) at position eight in the putative C. coli protein is changed to Gly (GGG). This indicates a close relationship between the sat4 alleles in this Staphylococcus and in the two investigated enterococcal isolates. The complete nucleotide sequence of the aadE–sat4–aphA-3 cluster of UW786 and UW1965 showed 100% identity with a cluster which has been isolated recently from different canine S. intermedius isolates (1). In contrast to this, a number of corresponding clusters in other staphylococci possessed a truncated and nonfunctional sat4 allele (3, 4). In most staphylococci, the gene cluster of aadE–sat4–aphA-3 is integrated into a transposon structure, Tn5405, flanked by two

![FIG. 1. Distribution of MICs of nourseothricin among 95 E. faecium isolates.](http://aac.asm.org/)

**TABLE 1. Seventy-two nourseothricin-resistant E. faecium isolates investigated in this study**

<table>
<thead>
<tr>
<th>Isolate type</th>
<th>No. of isolates</th>
<th>Origin</th>
<th>Genotype (PCR)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal and sewage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Poultry manure</td>
<td></td>
<td>vat(E), sat4, aadE-aphA, erm(B), cat</td>
</tr>
<tr>
<td>5</td>
<td>Pig manure</td>
<td></td>
<td>vat(E), sat4, aadE-aphA, erm(B)</td>
</tr>
<tr>
<td>2</td>
<td>Poultry meat</td>
<td></td>
<td>vat(E), sat4, aadE-aphA, erm(B)</td>
</tr>
<tr>
<td>4</td>
<td>Sewage</td>
<td></td>
<td>vat(E), sat4, aadE-aphA, erm(B)</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Stool samples from outpatients</td>
<td></td>
<td>vat(E) [8]/vat(D) [1], sat4, aadE-aphA, erm(B)</td>
</tr>
<tr>
<td>7</td>
<td>Hospitalized patientsb</td>
<td></td>
<td>vat(E) [3]/vat(D) [1], sat4, aadE-aphA, erm(B)</td>
</tr>
<tr>
<td>37</td>
<td>Hospitalized patientsc</td>
<td></td>
<td>vanA, sat4, aadE-aphA, erm(B)</td>
</tr>
</tbody>
</table>

* Gene names indicate a positive PCR product according to a resistance phenotype: aadE codes for streptomycin resistance, aphA codes for kanamycin resistance, cut codes for chloramphenicol resistance, erm(B) codes for macrolide-lincosamide-streptogramin B resistance, sat4 codes for streptothricin (nourseothricin) resistance, vanA codes for vancomycin resistance, and vat(D) and vat(E) codes for streptogramin A resistance. The number of positive isolates is given in brackets; if no number is indicated, all isolates were positive. Primers are given in the text or were according to reference 16.

* From stool samples and colonizations.

* 14 from stool samples, 23 from infections.

* Four isolates with an unknown genotype for streptogramin A resistance.
copies of the IS element IS182. Whether the aadE-sat4-aphA-3 cluster in the described enterococcal isolates is also integrated into a Tn5405-like element has not yet been investigated and is the subject of ongoing studies.

Arrangement of resistance genes in the other 70 E. faecium isolates was investigated by PCR for aadE-sat4-aphA-3 followed by a restriction digestion with endonuclease DdeI. All the isolates investigated showed an identical pattern, suggesting a highly conserved gene cluster among nonrelated E. faecium of different origins (not shown).

Four of five nourseothricin-resistant E. faecium transferred their nourseothricin resistance determinant into a recipient isolate, 64/3 (mating frequencies from $6.36 \times 10^{-8}$ to $1.44 \times 10^{-3}$ per recipient). One transconjugant per mating experiment was characterized in more detail. All transconjugants were resistant to fusidic acid (MIC $\geq$ 16 mg/liter) and rifampin (MIC $\geq$ 4 mg/liter) and to quinupristin-dalfopristin, erythromycin, clindamycin, oxytetracycline (all MICs $\geq$ 8 mg/liter), as well as to high levels of nourseothricin and streptomycin (MIC $\geq$ 2,048 mg/liter). A PCR for the sat4 gene and the aad-sat4-aphA-3 cluster using genomic DNA from the transconjugants gave positive results for both fragments (not shown). The transconjugants exhibited SmaI macrorestriction patterns that were different from those of the donors and related to the pattern of recipient 64/3 (not shown).

It remains unclear why enterococci still harbor resistance determinants against antibiotics that were not or only rarely used for treating enterococcal infections. Streptothricins have never been used for veterinary or human therapy in Middle European countries. A selective pressure resulting from use of aminoglycosides is more reasonable. However, the standard aminoglycoside treatment for enterococcal infections in humans in Europe involves gentamicin, for which the genes aadE and aphA-3 do not confer resistance. It is still not known if the described gene cluster is integrated into a larger composite element, where other as-yet-unknown determinants may promote dissemination among enterococci. This hypothesis is being investigated.

**Nucleotide sequence accession number.** The identical genomic fragments of *E. faecium* UW786 and UW1965 have been assigned GenBank accession no. AF330699.

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**REFERENCES**


