Detection in Italy of two clinical *Enterococcus faecium* isolates carrying both the oxazolidinone and phenicol resistance gene optrA and a silent multiresistance gene cfr

Andrea Brenciani¹, Gianluca Morroni¹, Chiara Vincenzi¹,², Esther Manso², Marina Mingoia³, Eleonora Giovanetti³ and Pietro E. Varaldo¹*

¹Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy; ²Clinical Microbiology Laboratory, Torrette Regional Hospital, Ancona, Italy; ³Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

*Corresponding author. Tel: +39-071-2206294; Fax: +39-071-2206293; E-mail: pe.varaldo@univpm.it

Sir,

In a century in which the issue of emerging antibiotic resistance is being dominated by severe concerns chiefly regarding Gram-negative organisms, the multiresistance gene cfr is probably the greatest emerging problem in Gram-positive pathogens, particularly staphylococci and enterococci. The concern over this problem is motivated not only by the fact that the resistance involves linezolid—widely used in serious infections caused by MDR Gram-positive organisms, often as a last-resort drug—but also, and critically, by the fact that the frequent location of cfr on conjugative plasmids makes the resistance transferable. Now, the report in China of a second plasmid-borne transferable cfr, conferring oxazolidinone resistance, on conjugative plasmids makes the resistance transferable. The antibiotic MICs and other features for the two isolates are displayed in Table 1. Both isolates had a relatively low linezolid MIC, 4 mg/L, a value that is regarded as ‘susceptible’ according to EUCAST® and ‘intermediate’ according to CLSI. Both had a tedizolid MIC of 2 mg/L; breakpoints for resistance have recently been established by EUCAST for staphylococci and β-haemolytic streptococci (>0.5 mg/L) and for viridans group streptococci (>0.25 mg/L). The two isolates were also examined for mutations in 23S ribosomal RNA (not detected) and for the penicillin exporter genes fexA and fexB (not detected).

The two isolates exhibited closely related Smal-PFGE profiles; one (E35048) was investigated for molecular traits. Sequencing demonstrated that optrA and cfr displayed high-level DNA identities (98% and 99%, respectively) to the respective reference sequences (accession numbers KP399637 and AJ579366). Three amino acid changes were detected in the protein sequence of cfr and 21 (4 of which were already reported in Chinese isolates) in the protein sequence of optrA compared with the respective reference sequences. The results of long PCR assays seeking a possible linkage between optrA and cfr were negative. The genetic contexts of both genes proved capable of undergoing excision in circular form, and were completely sequenced. The sequence of the minicircle containing optrA (3350 bp), deposited under accession no. KT892063, included a transposase gene downstream of optrA. This transposase gene exhibited 70% DNA identity and 65% amino acid identity to a chromosomal transposase from *Clostridium sticklandii* (accession no. FP568809). The minicircle (3405 bp) containing the cfr gene and one intact IS, ISEfA5, was almost identical to a cfr genetic context described in *Staphylococcus lentus* (accession no. KFO49005).

Considering the low MICs of linezolid, florfenicol and chloramphenicol (Table 1), in spite of the presegence of two resistance genes acting by different mechanisms (cfr perturbing the ribosome function and optrA providing for active efflux), RT–PCR experiments were performed to check the actual transcription of the two genes (Figure S1). We found that optrA was transcribed, whereas cfr was not. Although the exact mechanism of non-transcription is still being investigated, preliminary data indicate a 52 bp deletion in the regulatory region upstream of cfr. Interestingly, a cfr gene failing to mediate resistance to oxazolidinones and phenicols has been described in a porcine *E. faecalis* isolate in China.

Our collection of Enterococcus blood isolates is still in progress, and the overall results of the survey will be assessed and
described later. The present, partial data are reported in advance to alert the scientific community to at least three important new facts: (i) that the *optrA* gene just described in enterococci in China is also found in enterococci isolated in Italy; (ii) that its genetic context may be unstable and yield a minicircle, a cause for concern in view of possible resistance spread; and (iii) that the *optrA* gene can be found in enterococcal strains that also carry *cfr*.

Although in this first report *cfr* was silent, meaning that only *optrA* accounted for the oxazolidinone MICs, we cannot exclude the possibility that strains carrying both functional genes are circulating.

### Table 1. Demographic data, antibiotic MICs and susceptibility profiles for the two *E. faecium* clinical isolates under study

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Isolation data</th>
<th>MIC (mg/L)</th>
<th>Genetic resistance markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em> E20818</td>
<td>April 2015, blood, CVC oncology</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>AMP</strong></td>
<td><strong>SAM</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤2</td>
<td>≤2</td>
</tr>
<tr>
<td><em>E. faecium</em> E35048</td>
<td>June 2015, blood, ICU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥32</td>
<td>≥32</td>
</tr>
</tbody>
</table>

AMP, ampicillin; SAM, ampicillin/sulbactam; CXM, cefuroxime; IPM, imipenem; GEN (HLAR), gentamicin (high-level aminoglycoside resistance); STR (HLAR), streptomycin (high-level aminoglycoside resistance); ERY, erythromycin; CLI, clindamycin; Q/D, quinupristin/dalfopristin; LZD, linezolid; TGD, tedizolid; TEC, teicoplanin; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole; CVC, central venous catheter.

*Gentamicin MIC: ≤128 mg/L denotes no high-level resistance.*

*Streptomycin MIC: >512 mg/L denotes high-level resistance.*

### References


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### Transparency declarations

None to declare.