Case Report

Colonization of the tip of a thoracic catheter by Enterococcus faecalis resistant to vancomycin and linezolid

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We report the isolation of Enterococcus faecalis resistant to vancomycin and linezolid from the tip of a thoracic drainage catheter in an elderly patient. He was treated with vancomycin for a pleural empyema due to a meticillin-resistant Staphylococcus aureus but never received linezolid. A surveillance rectal swab yielded both linezolid-susceptible and -resistant strains, and the two isolates were not genotypically related. Careful monitoring for linezolid-resistance is critical to avoid potential therapy failure and transmission of resistant E. faecalis.

Introduction

Enterococcus faecalis has dramatically emerged in recent decades as one of the most common nosocomial pathogens. Intrinsic resistance to many β-lactams and low level aminoglycosides, and the facility to acquire resistances to other common drugs, are a huge issue in hospital settings, causing therapy failure, high mortality rates and high costs. Vancomycin has been the drug of choice for multidrug-resistant enterococci since the development of resistance following the use of glycopeptide. The increasing emergence of vancomycin-resistant enterococci has been recently overcome by the approval for clinical use (in 2001 in Italy) of linezolid, the first of a new class of antibiotics, the oxazolidinones.

In vivo, resistance to linezolid usually develops after a prolonged therapy, and only a few cases have been reported in the absence of treatment (Marra et al., 2006; Kainer et al., 2007; Bonora et al., 2006a; Rahim et al., 2003). Although E. faecalis is more likely than Enterococcus faecium to acquire resistance to linezolid in vitro (Prystowsky et al., 2001), E. faecalis strains resistant to linezolid have rarely been isolated from clinical specimens; these strains are generally susceptible to vancomycin, with few exceptions (Ruggero et al., 2003; Boo et al., 2003). To our knowledge, no vancomycin and linezolid-resistant E. faecalis has yet been described in Italy.

Case report

A 74-year-old man was admitted to the medical unit of our hospital for recurrent dyspnoea, especially during exercise, over the previous month. He was a woodworker and reported workplace exposure to asbestos. His past medical history was remarkable for arterial hypertension and diabetes mellitus. On admission, physical examination showed he had a normal respiratory rate (18 breaths min⁻¹) at rest, arterial pressure 140/80 mmHg and heart rate (72 beats min⁻¹), and no vesicular breathing over the left pulmonary lower lobe. A chest X-ray revealed a broad pleural effusion on the left side of the chest with derangement of the mediastinum towards the opposite side. Following multiple non-diagnostic thoracocentesis, the patient was moved to the thoracic surgery unit for thoracoscopy. Examination of biopsy specimens resulted in the diagnosis of pleural mesothelioma. In addition, culture of pleural fluid showed meticillin-resistant Staphylococcus aureus (MRSA). At that time, a diagnosis of nosocomial pleural empyema was made, and the patient received a 16 day course of intravenous vancomycin and surveillance cultures of pleural fluid became negative. Twelve days after the end of vancomycin therapy, the patients thoracic drainage catheter was removed, and culture of its tip showed a strain of E. faecalis. An antimicrobial susceptibility test performed by the automated Vitrek 2 system,

Abbreviations: MRSA, meticillin-resistant Staphylococcus aureus; RS, rectal swab; VLRÉf, vancomycin- and linezolid-resistant Enterococcus faecalis.
software version 4.01 (bioMérieux), showed that the strain was resistant to vancomycin and teicoplanin (MIC ≥ 32 μg ml⁻¹ suggestive of a VanA phenotype), linezolid (MIC ≥ 32 μg ml⁻¹), ciprofloxacin and moxifloxacin, tetracycline, and to high concentrations of gentamicin; it was susceptible to ampicillin, piperacillin, penicillin G, imipenem and nitrofurantoin. The patient did not receive antibiotic therapy since he had no signs or symptoms of infection.

After the first isolation, we checked for colonizing vancomycin- and linezolid-resistant *E. faecalis* (VLREfs) in a rectal swab (RS) by streaking on Enterococcus agar (Becton Dickinson). Different *Enterococcus* strains were isolated by this procedure. In order to detect the resistant strain among the susceptible ones (Table 1, RS 1 normal isolation), we subjected to further characterization. Resistance to glycopeptides was tested by the Vitek 2 system. Resistance to vancomycin was confirmed by growth on vancomycin screen agar (Becton Dickinson). Moreover, the vanA gene was detected by PCR amplification (Dutka-Malen et al., 1995) in all the glycopeptide-resistant isolates. Resistance to linezolid was tested by the Vitek 2 system, and confirmed by both disc diffusion and Etest (AB Biodisk).

Antimicrobial susceptibility results are reported in Table 1. All VLREfs had comparable MICs toward the reported antibiotics.

The isolates were analysed for the presence of the G2576T point mutation in the 23S rRNA gene. This mutation, known to be associated with linezolid resistance in clinical isolates, may involve one or more of the 23S rRNA gene copies in the chromosome; the level of linezolid resistance increases with the number of copies carrying the mutation (Marshall et al., 2002; Ruggero et al., 2003). To detect the mutation, DNA was extracted by heat treatment (95°C for 10 min) from three to four bacterial colonies taken from an overnight culture on Luria–Bertani agar, and immediately amplified with primers annealing to the 23S RNA gene (Bonora et al., 2006b). The amplification products (745 bp) were subsequently digested with *NheI* (New England BioLabs), which recognizes a site generated as a consequence of the G2576T mutation (Bonora et al., 2006b). The *NheI* digestion patterns revealed that all the linezolid-resistant strains carried the G2576T mutation (Fig. 1) and that the mutation was present in many but not all the copies of the 23S rRNA genes, as demonstrated by the presence of the uncut band of 745 bp (Fig. 1). This result was confirmed by the permanence of a BstUI recognition site in a fraction of the amplicons (data not shown). This enzyme cuts the wild-type sequence and the site is therefore an alternative to the *NheI* one.

The clonality among the strains was tested by PFGE. DNA was prepared as described by Seifert et al. (2005) and digested with *Smal* (Roche Diagnostics). Fragment separation was performed with a Chef DRIII apparatus (Bio-Rad) using a switch time ranging from 5 to 35 s. Linezolid-resistant isolates recovered from RSs with both techniques had PFGE profiles identical to the one of isolate E 970, so they are clearly clonally related (Table 1, PFGE pattern A). By contrast, PFGE patterns of linezolid-susceptible isolates recovered from RSs differed for more than seven bands (Table 1, PFGE pattern B and C), so they are unrelated to isolate E 970 (Tenover et al., 1995).

**Discussion**

This study describes what is believed to be the first case of an *E. faecalis* isolate resistant to both vancomycin and linezolid, in an Italian patient who had never received linezolid. The patient, however, had some risk factors for the development of linezolid resistance, including a
Table 1. Antibiotic susceptibility test results and PFGE patterns of enterococci isolated from the same patient with the normal procedure (D) or with Lin-screen (LS)


<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Identification</th>
<th>Antibiotic susceptibility test (Vitek) MIC (µg ml⁻¹)</th>
<th>Disc diffusion Ø for LNZ (mm)</th>
<th>Etest MIC for LNZ (µg ml⁻¹)</th>
<th>PFGE pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 970</td>
<td>Thoracic catheter</td>
<td><em>E. faecalis</em></td>
<td>R ≥32, R ≥32, R ≥32</td>
<td>R 9</td>
<td>R 128</td>
<td>A</td>
</tr>
<tr>
<td>E 981</td>
<td>RS 1 D</td>
<td><em>E. faecalis</em></td>
<td>S &lt;1, S &lt;0.5, I 4</td>
<td>S 25</td>
<td>S 1.5</td>
<td>B</td>
</tr>
<tr>
<td>E 982</td>
<td>RS 1 D</td>
<td><em>E. faecalis</em></td>
<td>R ≥32, R ≥32, R ≥8</td>
<td>R 18</td>
<td>R 64</td>
<td>A</td>
</tr>
<tr>
<td>E 985</td>
<td>RS 1 LS</td>
<td><em>E. faecalis</em></td>
<td>R ≥32, R ≥32, R ≥8</td>
<td>R 6</td>
<td>R 128</td>
<td>A</td>
</tr>
<tr>
<td>E 986</td>
<td>RS 1 LS</td>
<td><em>E. faecalis</em></td>
<td>S &lt;1, S &lt;0.5, I 2</td>
<td>S 25</td>
<td>S 1.5</td>
<td>B</td>
</tr>
<tr>
<td>E 990</td>
<td>RS 2 LS</td>
<td><em>E. faecalis</em></td>
<td>S 2, S &lt;0.5, I 2</td>
<td>S 23</td>
<td>S 2</td>
<td>C</td>
</tr>
</tbody>
</table>

The emergence of linezolid resistance in *E. faecalis* strains among patients never treated with this drug, and in a hospital setting where the linezolid consumption was quite low over the past 3 year period (0.1 daily defined dose per 100 person days, linezolid doses of 1200 mg per day are considered as 1 daily defined dose), emphasizes the importance of both surveillance and the introduction of effective infection control procedures in order to avoid their transmission to other patients and the persistence of such a dangerous reservoir, which could remain silent for years before creating clinical manifestations (Mitsogiannis et al., 2007). Surveillance should be recommended, particularly for subjects intended to be given linezolid therapy, at the following times: before the beginning of the treatment, to identify possible pre-existing colonizing resistant strains; during and after treatment, to verify the presence of mutant strains selected by the drug, and to avoid their spreading inside and outside the hospital. A reliable and simple method for checking linezolid-resistant strains is desirable and the Lin-screen seems to fulfil these requisites, as it allows direct selection of the resistant strains, which might be a minority of the colonizing enterococci.

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References


