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.

Abbreviations: MRSA, meticillin-resistant *Staphylococcus aureus*; RS, rectal swab; VLREfs, vancomycin- and linezolid-resistant *Enterococcus faecalis*.

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 patient's thoracic drainage catheter was removed, and culture of its tip showed a strain of *E. faecalis*. An antimicrobial susceptibility test performed by the automated Vitek 2 system,

software version 4.01 (bioMérieux), showed that the strain was resistant to vancomycin and teicoplanin (MIC $\geqslant$32 μg ml^{-1} suggestive of a VanA phenotype), linezolid (MIC $\geqslant$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 Enterococcosel 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 procedure), however, it was necessary to pick and test several colonies. We tried to increase the sensitivity of the technique by plating a dense suspension (2 McFarland) of H_2S positive colonies grown on Enterococcosel agar on Mueller–Hinton agar with 4 μg linezolid ml^{-1} (Lin-screen); after 48 h of incubation at 36 °C, the plates were examined for the presence of growth. Vancomycin- and linezolid-resistant *E. faecium* SM 944 or SM 941 (Bonora *et al.*, 2006b) were used as positive controls. Resistant strains (Table 1, RS 1 Lin-screen) form colonies that emerge from the layer of sensitive strains, which remain alive due to the bacteriostatic activity of linezolid.

Two months after the isolation of VLREfs, another RS was taken to check for resistant strains, but only vancomycin- and linezolid-susceptible *E. faecalis* were isolated (Table 1, RS 2), both by normal culture method and by Lin-screen. The patient recovered and was discharged from the hospital.

E. faecalis strains isolated from the catheter tip (E 970) and from surveillance RSs, by the classical method (E 981, E 982) and by Lin-screen (E 985, E 986, E 990), were 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.

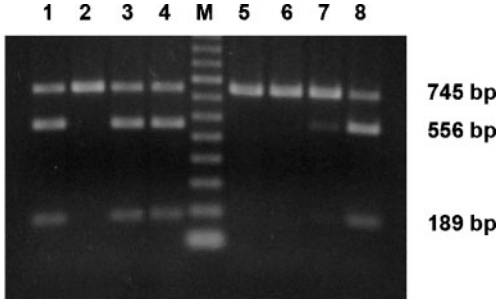

Fig. 1. *Nhe*I digestion of 745 bp PCR products amplified from domain V of the 23S rRNA gene. Lanes 1–6: E 970 [linezolid resistant (LinR)], E 981 [linezolid susceptible (LinS)], E 982 (LinR), E 985 (LinR), E 986 (LinS), E 990 (LinS). MICs are reported in Table 1. Lanes 7–8: SM 941 and SM 944 [positive controls (*E. faecium*), linezolid MIC 8 and 64 μg ml^{-1}, respectively (Bonora *et al.*, 2006b)]. M, 100 bp DNA size marker (the 100 and 800 bp bands are the more intense bands).

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 rRNA gene (Bonora *et al.*, 2006b). The amplification products (745 bp) were subsequently digested with *Nhe*I (New England BioLabs), which recognizes a site generated as a consequence of the G2576T mutation (Bonora *et al.*, 2006b). The *Nhe*I 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 B*st*UI 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 *Nhe*I one.

The clonality among the strains was tested by PFGE. DNA was prepared as described by Seifert *et al.* (2005) and digested with *Sma*I (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 patterns 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)

The Clinical and Laboratory Standards Institute guidelines and MIC interpretative standards for *Enterococcus* spp. were followed (CLSI, 2007). MIC (μg ml^{-1}): S ⩽2, I 4, R ⩾8. Disc diffusion Ø (mm): S ⩾23, I 21–22, R ⩽20. R, Resistant; I, intermediate; S, susceptible.

Isolate	Source	Identification	Antibiotic susceptibility test (Vitek) MIC (μg ml^{-1})			Disc diffusion Ø for LNZ (mm)	Etest MIC for LNZ (μg ml^{-1})	PFGE pattern
			VA	TEC	LNZ			
E 970	Thoracic catheter	*E. faecalis*	R ⩾32	R ⩾32	R ⩾32	R 9	R 128	A
E 981	RS 1 D	*E. faecalis*	S ⩽1	S ⩽0.5	I 4	S 25	S 1.5	B
E 982	RS 1 D	*E. faecalis*	R ⩾32	R ⩾32	R ⩾8	R 18	R 64	A
E 985	RS 1 LS	*E. faecalis*	R ⩾32	R ⩾32	R ⩾8	R 6	R 128	A
E 986	RS 1 LS	*E. faecalis*	S ⩽1	S ⩽0.5	S 2	S 25	S 1.5	B
E 990	RS 2 LS	*E. faecalis*	S 2	S ⩽0.5	S 2	S 23	S 2	C

pre-existing MRSA infection and long-term hospitalization (Kainer *et al.*, 2007).

The resistant strain, which was at first isolated from a catheter tip, carried the G2576T mutation and was then detected in surveillance RSs of the same patient. PFGE profiling demonstrated that it was not related to the susceptible strains that were present in the RSs as well.

Possible explanations of the presence of VLREfs in the patient could be the transfer of a resistant strain via health-care workers or objects that were in contact with people undergoing linezolid therapy, or the occurrence of a casual mutation in the 23S rRNA gene. Transfer of resistant strains has already been demonstrated with enterococci (Duckro *et al.*, 2005; Dobbs *et al.*, 2006). At the same time as the present case, another patient receiving a short linezolid therapy was concomitantly admitted in the same ward. The latter patient had a MRSA infection, but no surveillance swabs were taken so we cannot exclude a possible colonization and subsequent spreading of VLREfs. The hypothesis of a casual mutation is also plausible, but is weakened by the fact that a susceptible strain, related to E 970, could not be isolated.

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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