Linezolid-resistant enterococci: report of the first isolates in the United Kingdom

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Linezolid, the first oxazolidinone antibacterial agent to be developed for clinical use, was licensed in the UK in early 2001. We report the first three examples of resistant enterococci (two isolates of Enterococcus faecium and one Enterococcus faecalis) isolated in the UK, which were obtained from patients who had received linezolid. The linezolid MICs for the resistant isolates were 64 mg/L. Pulsed-field gel electrophoresis (PFGE) analysis of the linezolid-susceptible and -resistant isolates from two of the patients, combined with sequence analysis of rRNA, indicated that resistance developed in previously susceptible strains, most probably via a point mutation in the 23S rRNA.

Keywords: linezolid, resistance, enterococci

Introduction

Linezolid is the first member of a new class of synthetic antimicrobials, the oxazolidinones, to be licensed for clinical use in the UK. Oxazolidinones are protein synthesis inhibitors that bind to the 50S ribosomal subunit and prevent the formation of the initiation complex.1 They are active against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), with a primarily bacteriostatic mode of action. Although laboratory studies suggested that resistance would be slow to develop,2 there have been a number of reports of linezolid-resistant enterococci and one report of a linezolid-resistant S. aureus.3–8 We report the first cases of linezolid-resistant enterococci from patients in three geographically distinct UK hospitals.

Materials and methods

Susceptibility testing

MICs were determined on Diagnostic Sensitivity Test agar (Oxoid, Basingstoke, UK) containing 5% lysed horse blood (TCS Microbiology, Buckingham, UK). The inoculum comprised 10⁴–10⁵ cfu/spot, delivered with a multipoint inoculator, and incubation was for 18 h at 37°C in air. Susceptibility was categorized using the BSAC breakpoint.

Pulsed-field gel electrophoresis (PFGE)

Genomic DNA was extracted, digested with SmaI and analysed by PFGE as described previously, using GelCompar software (Applied Maths, Kortrijk, Belgium).9

Results

Case 1

A 30-year-old, previously fit man sustained 85% burns following a motorcycle accident. He had been wearing a crash helmet and his head and neck were the only parts of his body spared from burning. The patient was admitted to the Regional Burns Unit, where he was intubated and ventilated. The day after admission the patient was taken to theatre where he underwent near total burn wound excision and

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initial grafting with integra. By day 6 he was widely colonized with enterococci and a multi-resistant *Acinetobacter* sp., susceptible only to meropenem and colistin. The management of this patient over the following weeks was complex, and courses of antimicrobials were kept to a minimum in an attempt to limit the emergence of resistance. Nonetheless, the patient received courses of meropenem, ceftazidime and vancomycin. Linezolid [600 mg intravenously (iv) twice daily] was given for 5 days (from day 16 to day 20) for the treatment of glycopeptide-resistant *Enterococcus faecium*, and for a further 6 days (day 26–31) for the treatment of glycopeptide-susceptible *Enterococcus faecalis* in blood cultures. On day 30, *Candida parapsilosis* was isolated from blood cultures and liposomal amphotericin was commenced.

On day 39, swabs of back tissue yielded isolates of glycopeptide-resistant *E. faecium* that were resistant to linezolid (MIC 64 mg/L). Linezolid-resistant enterococci were isolated again on day 73 from the scrotal area and the left upper arm. Other sites continued to grow the glycopeptide- and linezolid-susceptible *E. faecalis*.

On day 83, a multi-resistant *Acinetobacter* spp. and *E. faecium* susceptible to vancomycin and linezolid were isolated from blood culture. The enterococcus was susceptible to quinupristin/dalfopristin with which the patient was treated. However, the patient remained bacteraemic, and linezolid, 600 mg iv twice daily, was re-started on day 87 for 3 days, being replaced with vancomycin on day 90. On day 101, further blood cultures grew *E. faecium* that was resistant to vancomycin and linezolid. The patient was treated with quinupristin/dalfopristin, but by this time had multi-organ failure and died 6 days later.

**Case 2**

Oral linezolid, 600 mg twice daily, was given as prophylaxis to a 65-year-old woman with a 15-year history of severe recurrent cellulitis secondary to gross bilateral leg lymphoedema, from which *S. aureus* and Group A streptococci had originally been isolated. She had required in excess of 140 in-patient hospital days each year for the previous 5 years, and many different prophylactic regimens had been tried with variable success. She had an indwelling central venous catheter. Linezolid was effective in limiting her admissions for exacerbations of cellulitis, but was discontinued after 4 months following the development of a rash. Subsequently the patient was admitted with signs of septicemia, and blood culture yielded *E. faecalis* that was resistant to linezolid (MIC 64 mg/L). The isolate was susceptible to ampicillin and glycopeptides, with low-level resistance to gentamicin. The patient was successfully treated with iv vancomycin with conservation of the central line. No relapses have occurred in over 8 months of follow up.

**Case 3**

A 66-year-old woman with cholangiocarcinoma underwent a left hepatectomy and right hepaticojejunostomy with radiofrequency ablation to the remaining tumour. She received cefuroxime and metronidazole, and initially made a good post-operative recovery. However, she deteriorated on day 4 and abdominal imaging revealed an intra-abdominal fluid collection adjacent to an internal biliary stent. One litre of biliary fluid was drained and her antibiotic regimen was changed to piperacillin/tazobactam, gentamicin and metronidazole. *E. faecium* and *C. albicans* were cultured from abdominal drain fluid, and fluconazole was added. Despite this, *C. albicans* was isolated from blood cultures, and VRE were isolated from abdominal drain fluid on day 16. She continued to deteriorate clinically and was started on linezolid 600 mg iv twice daily. Further abdominal imaging revealed a liquefying splenic haematoma extending into the pelvis. VRE was isolated from blood cultures at this time. The hepatic bed haematoma and the biliary stent were implicated as the likely sources of sepsis, but she was too unstable to go to theatre and attempts at endoscopic removal of the stent failed. Blood cultures remained positive with VRE despite linezolid therapy and also yielded a multi-resistant *Klebsiella* spp.

Imipenem was started.

On day 39, after 16 days of treatment with linezolid, multiple blood cultures yielded linezolid-resistant vancomycin-resistant *E. faecium* (linezolid MIC 64 mg/L). The patient was switched to quinupristin/dalfopristin and treated for a total of 14 days. She made steady improvement, with the biliary stent being removed on day 44, when she was discharged to the ward.

**Typing of isolates**

PFGE analysis of the linezolid-susceptible and -resistant isolates of *E. faecium* from patients 1 and 3 was identical in each case (Figure 1). Linezolid-susceptible isolates were not available from patient 2.

**Discussion**

These are the first three known isolates of linezolid-resistant enterococci from patients in the UK. Interestingly, two of the isolates were *E. faecium* and one was *E. faecalis*. The MIC of linezolid for the resistant isolates was 64 mg/L in each case, where the susceptibility breakpoint for linezolid is 4 mg/L. In two of the cases, molecular typing of linezolid-susceptible and -resistant isolates by PFGE showed that they were identical, indicating that resistance had developed in previously susceptible strains. All the resistant isolates had a G2576U mutation at bp 2576 in the peptidyl-transferase region of the 23S rRNA, which is identical to the mutation...
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previously described in several laboratory-derived linezolid-resistant mutants of *E. faecalis.* Although other mutations have been reported under experimental conditions, only this mutation has been seen in clinical isolates found to date. Pre-licensing studies showed low spontaneous mutation rates to linezolid resistance in enterococci and staphylococci (10^{-9} to 10^{-11}), and it was thought that linezolid resistance would be slow to develop. Therefore, the reported cases of linezolid resistance are disappointing.

In at least nine reported cases, linezolid resistance emerged in enterococci during therapy, whereas a further six cases acquired a resistant strain as a result of cross-infection. A further case has been reported in an individual patient without prior exposure to linezolid. Previously reported patient factors that might predispose to the development of linezolid resistance include indwelling intravascular devices, underdosage, immunosuppression after transplantation and long courses of linezolid therapy (20–40 days). Each of our three cases demonstrated at least one such factor. Case 1 was unusual in that only three short courses (5, 6 and 3 days) of linezolid therapy were given prior to the detection of linezolid resistance. He was, however, relatively immunosuppressed with extensive burns, and the bacterial load at sites of infection or colonization may have been very high. Case 2 received in excess of 100 days therapy and had a ‘Hickman’ line in situ, whereas case 3 had 16 days treatment with linezolid and had central venous access.

We recommend susceptibility testing of clinically significant Gram-positive pathogens before starting linezolid therapy and also in cases of treatment failure. To minimize the emergence of linezolid resistance, courses of treatment should be kept as short as possible, and risk factors for resistance development considered before starting.

An additional concern is the risk of nosocomial spread of linezolid-resistant organisms. There is already a report of cross-infection with a linezolid-resistant, vancomycin-resistant *E. faecium* on a transplantation unit. The index patient had been treated with linezolid and despite standard infection control precautions including source isolation, the strain was detected on routine rectal screening swabs of six other patients on the same unit. There is little experience with these infections, and specific infection control advice has yet to be formulated. We suggest that the issues which need to be considered include the use of isolation precautions and screening of contacts.

References


Figure 1. PFGE of SmaI digests of linezolid-susceptible and -resistant isolates of *E. faecium.* (a) Isolates from patient 1: linezolid-susceptible isolates are shown in lanes 1, 2, 4 and 7 and linezolid-resistant isolates are shown in lanes 3, 5, 8 and 9. Lane 6, molecular size markers. (b) Isolates from patient 3: linezolid-susceptible isolates are shown in lanes 2 and 3, and linezolid-resistant isolates are shown in lanes 4–7. Lanes 1 and 8, molecular size markers.
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