Intensive care unit dissemination of multiple clones of linezolid-resistant Enterococcus faecalis and Enterococcus faecium

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Received 12 January 2012; returned 8 February 2012; revised 24 March 2012; accepted 26 March 2012

Objectives: Outbreaks caused by linezolid-resistant (LR) enterococci remain rare. We report the epidemiological and molecular characteristics of the multiclonal dissemination of LR enterococci in the intensive care unit (ICU) of a Greek hospital.

Methods: All LR enterococcal isolates recovered from patients hospitalised in the ICU of the University Hospital of Larissa, Greece, between January 2007 and October 2008 were included. Isolates were tested by PFGE and PCR followed by sequence analysis of the entire 23S rRNA gene. Patient records were retrieved to access patterns of acquisition and outcome.

Results: Sixteen separate patients were infected and/or colonized by 22 LR enterococcal isolates (17 Enterococcus faecium and 5 Enterococcus faecalis). Linezolid MICs varied from 8 to 16 mg/L; 12 isolates showed cross-resistance to vancomycin. Genotyping revealed as many as seven and three PFGE types among E. faecium and E. faecalis isolates, respectively, indicating multiclonal spread of LR enterococci. Nine patients had received linezolid prior to the recovery of LR enterococci, while the remaining seven patients were not exposed to the drug. All isolates carried the mutation G2576T; the mutated position was heterogeneous in 12 isolates and homogeneous in 10.

Conclusions: The multiclonal composition of LR enterococci indicates that linezolid resistance possibly occurred on several independent occasions. Its acquisition was often not related to linezolid administration; patients might have acquired their LR isolate from another patient that had received linezolid or, alternatively, resistance may have arisen by mutation that occurred independently.

Keywords: 23S rDNA mutations, heterogeneous mutations, linezolid exposure, enterococci, PFGE

Introduction

Infections due to vancomycin-resistant Enterococcus faecalis and Enterococcus faecium (VRE) commonly necessitate the use of linezolid for their treatment.1 Although initial studies suggested that resistance to linezolid would be slow to develop,2 linezolid-resistant (LR) enterococci emerged soon after its clinical introduction.2,3 The linezolid resistance mechanism among clinical enterococci has up to now been mainly attributed to the G2576T mutation in the 23S rRNA gene.2,3

Despite the widespread hospital use of linezolid, LR enterococcal isolates still seem to be very rare, remaining essentially undetectable in large-scale epidemiological surveys,6 including in large VRE collections from Europe.7 Similarly, linezolid resistance remains rare among E. faecalis and E. faecium clinical isolates in Greek hospitals.8 In the literature, the emergence of LR enterococci has been mostly documented from individual cases or small series.3,4,9 Only a few studies have reported outbreaks of clinical infections due to LR enterococci and these either described clonal spread of the isolates10,11 or did not analyse their clonal relationship.12

In our hospital, an LR E. faecium clinical isolate was first recovered from blood cultures of a patient hospitalized in the intensive care unit (ICU) during 2004.13 Preliminary susceptibility data in our hospital indicate that LR enterococci have been increasingly isolated in our ICU since late 2006, when we started monitoring linezolid resistance. We report herein the multiclonal spread of LR E. faecium and E. faecalis in our ICU during 2007–08.

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Materials and methods

Hospital setting and bacterial collection

The study included LR *E. faecalis* and *E. faecium* isolates (linezolid MICs ≥8 mg/L)\(^{14}\) recovered from clinical infection samples of patients hospitalized from January 2007 to October 2008 in the ICU of the University Hospital of Larissa, Greece, which has 600 beds and an ICU with a 12 bed general ward and an 8 bed cardiothoracic surgical ward. LR enterococci were also recovered from rectal samples collected during the routine surveillance of ICU patients for VRE. The isolates were stored at −80°C before testing.

Phenotypic testing

Identification of isolates to the species level was confirmed using conventional biochemical tests\(^ {13}\) and API 20 Strep (bioMérieux, Marcy l’Étoile, France). Susceptibility testing was performed by disc diffusion,\(^ {13}\) and MICs of linezolid, teicoplanin, quinupristin/dalfopristin and vancomycin by Etest (AB Biodisk, Solna, Sweden). Linezolid MICs were additionally determined by agar dilution,\(^ {15}\) with 2 mg/L increments for concentrations 2–32 mg/L and using a final inoculum of 10° cfu/ml. *E. faecalis* ATCC 29212 was used as the control in phenotypic assays.

PCR assays and DNA sequencing

Mutations conferring linezolid resistance were sought by PCR, using four overlapping primers designed for this study to amplify the entire 23S rRNA gene: (i) 1-Fw, 5′-TAAGGGCCAGGTGTGAT-3′ and 1-Rev, 5′-GGTGATACATGATTGGTGGG-3′ (nt position 12–731); (ii) 2-Fw, 5′-TGGCTTTTGTGAGAAAGG-3′ and 2-Rev, 5′-ATTCTAGCTTTCGGC GTC-3′ (597–1486); (iii) 3-Fw, 5′-AGAATCTGTTCACCACGTAG-3′ and 3-Rev, 5′-CGGCCTCTACCTCTACGTG-3′ (1319–2143); and (iv) 4-Fw, 5′-GTAACGATTTGGCACTGTC-3′ and 4-Rev, 5′-CGATTAGTATTGACCGTC-3′ (1988–2896). The primers were designed using Oligo Explorer 1.2 (Gene Link, Hawthorne, NY, USA) and had 100% identity with the 23S rDNA of *E. faecalis* V583 and *E. faecium* V583, respectively. Both strands of the amplicons were sequenced.

Macrogen restriction analysis

PFGE of the LR *E. faecium* and *E. faecalis* isolates was performed with CHEF-DRIII (Bio-Rad, Hemel Hempstead, UK), with a running time of 18 h and pulse times of 5–35 s. PFGE patterns were compared visually and by using Fingerprinting II Software version 3.0 (Bio-Rad Laboratories, Inc., USA).

Review of patients’ data

Medical records of patients that harboured LR enterococci were examined to ascertain factors that may have influenced resistance development and the spread of isolates. The hospital location of patients, anonymized demographic data, clinical characteristics and prior exposure to linezolid for ≥3 days were abstracted.

Results

LR enterococcal isolates and patients’ characteristics

During the study period, 17 LR *E. faecium* and 5 LR *E. faecalis* isolates were recovered from 16 separate patients hospitalized in the ICU wards. The characteristics of the patients and their isolates are presented in Table 1. Isolates were recovered from clinical infection samples of nine patients and rectal samples of seven patients, while two patients (Patients 9 and 11; Table 1) yielded LR *E. faecium* isolates from both clinical and rectal samples. Clinical specimens included blood (seven isolates), pus (three isolates), and one isolate each from vascular catheter and trauma. A single LR isolate was recovered from 11 patients, two LR isolates from 4 patients and three LR isolates from 1 patient. One patient yielded LR *E. faecium* from a rectal sample and soon thereafter LR *E. faecalis* from blood cultures (Patient 6; Table 1).

Nine of the 16 study patients (56.3%) had evidence of clinical infection due to LR enterococci. The crude mortality rate was 18.8%. The patients had acquired the LR enterococci during hospitalization, since all were hospitalized for ≥10 days prior to the recovery of the organism; most of them had significant co-morbidities (Table 1). One patient, who yielded the LR isolate belonging to PFGE type VI, was hospitalized in a neighbouring hospital before being transferred to our ICU, 12 days prior to the LR isolation. Most patients received multiple antimicrobials prior to the recovery of LR isolates. Only nine patients had received linezolid for ≥3 days prior to the isolation of an LR enterococcal isolate.

Antimicrobial susceptibility profiles

Agar dilution MICs of linezolid were 8–16 mg/L. The MICS of vancomycin are shown in Table 1. The 22 LR isolates exhibited cross-resistance to drugs from several other antimicrobial classes, including β-lactams, fluoroquinolones, carbapenems and aminoglycosides.

PFGE typing

PFGE analysis of the 17 *E. faecium* isolates showed seven unrelated genotypes (types I–VII), while three unrelated genotypes (types VIII–X) were identified among the 5 *E. faecalis* isolates (Table 1 and Figure 1). PFGE type I and VIII contained two subtypes each. PFGE type I was the most common, being detected in seven *E. faecium* from four separate patients (Table 1). LR *E. faecium* isolates of PFGE subtype Ia were recovered from two patients with prolonged linezolid administration prior to the LR isolation and from one patient not exposed to linezolid, while subtype Ib was recovered from a patient not given linezolid. *E. faecalis* of subtype VIIIa was recovered from a patient that had received linezolid for 15 days, in contrast to subtype VIIIb, which was recovered from two patients not exposed to linezolid. The LR *E. faecium* isolates that were isolated from both clinical and surveillance samples from the same patient were indistinguishable.

Sequence analysis

Sequencing of the overlapping PCR products showed G2576T as the sole mutation across the entire 23S rRNA gene in all isolates. A careful examination of the sequencing traces revealed both mutated and non-mutated 23S rRNA gene copies in 12 isolates, with the mutated T being the predominant base called by the sequencer (Figure S1, available as Supplementary data at JAC Online). In 10 isolates the mutated trace was homogeneous.
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<th>Comorbidities</th>
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VAN, vancomycin; LZD, linezolid; COPD, chronic obstructive pulmonary disease.
Linezolid resistance is usually selected by prolonged in vivo treatment with linezolid, to help prevent treatment failure. Determination of the presence of linezolid-resistant strains should be performed before commencing treatment with linezolid, to help prevent treatment failure.

Discussion

LR enterococci remain uncommon. The present study describes the spread of several clones of LR E. faecalis and LR E. faecium during a 2 year period in our ICU. In the literature, studies reporting the dissemination of LR enterococci are limited and document clonally related isolates in contrast with our study which detected multiple clones. In particular, one report described a clonal outbreak of LR E. faecium in a US medical centre that affected 40 hospitalized patients; it is of note that only 15% of the patients received linezolid before yielding LR isolates. Also, a clonal outbreak involved 12 indistinguishable LR E. faecalis isolates in the ICU and reanimation unit of a Spanish hospital. In Greece, where multidrug resistance remains rare and there is only one study reporting five LR enterococci colonizing patients in a single ward, with four of the isolates being clonally related. The present survey reports 22 LR enterococci that disseminated in the ICU of our hospital. The multiclonal composition of the LR isolates indicates that linezolid resistance emerged on several independent occasions and patient-to-patient transmission was not the main route of dissemination.

The majority of our patients had multiple risk factors for colonization and/or infection with multidrug-resistant bacteria, and prior exposure to antibiotics. Several of them had not received linezolid; they possibly acquired the LR isolate from another patient or, alternatively, the mutation may have occurred independently. It should be noted that the increased isolation of LR enterococci in the ICU led to the reinforcement of infection control measures and more rational use of linezolid, after which the recovery of such isolates became very uncommon.

Linezolid resistance is usually selected in vivo by prolonged drug treatment or inappropriate linezolid dosage. Most of the reported clinical resistant mutants carry the G2576T mutation. As most bacteria contain four to six 23S rRNA gene copies, multiple copies must be mutated to confer resistance, which possibly accounts for the rarity of resistance. In our study, all isolates bore only the G2576T mutation, despite having sequenced the entire 23S rRNA gene. In 10 isolates the mutated sequencing trace at nt position 2576 was homogeneous, indicating that all 23S rRNA gene copies were mutated, while in the remaining 12 heterogeneous isolates, the trace corresponding to the mutated T was predominant relative to the non-mutated trace.

The emergence of linezolid resistance amongst enterococci, be it as a result of patient-to-patient transmission or selective pressure, is of concern. Tracking or restricting linezolid use according to therapeutic indications might reduce the emergence of LR enterococci. Also, risk factors, such as prior antibiotic administration or underlying diseases, should be taken into consideration and susceptibility testing of clinically significant Gram-positive pathogens should be performed before commencing treatment with linezolid, to help prevent treatment failure.

Funding

This work was supported by a European Society of Clinical Microbiology and Infectious Diseases (ESCMID) research grant (S. P.).

Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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