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**First report of a linezolid-resistant vancomycin-resistant *Enterococcus faecium* strain in Greece**

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Sir,

The emergence of vancomycin-resistant enterococci (VRE) has raised major concerns because of the limited therapeutic options for treating infections resulting from these organisms. In the USA, the National Nosocomial Surveillance System has described a 20-fold rise in VRE recovered from bloodstream infections during the past 10 years. The limitation in therapeutic options has resulted in the development of new drugs such as quinupristin/dalfopristin and linezolid.

Although linezolid has been used in clinical practice for a relatively short period of time, there are already several reports of linezolid-resistant enterococci.1,2 In Greece, this antibiotic is only used for the treatment of infections caused by vancomycin-resistant enterococci and teicoplanin-resistant *Staphylococcus epidermidis* strains, which are circulating in several Greek hospitals (data not shown). To our knowledge, this is the first report of a linezolid-resistant *Enterococcus faecium* strain isolated in a tertiary care Greek hospital.

The isolate was recovered from a blood culture of a 20-year-old male patient, who was admitted to the University Hospital of Larissa after a car accident. He was operated on immediately for a fractured femur. During corrective surgery, a fatty embolism occurred and he was immediately transferred to the intensive care unit (ICU) where he was treated for about a month. The patient was empirically treated with aminoglycosides, teicoplanin and piperacillin plus tazobactam. However, the isolation from blood culture on the 14th day of an oxacillin-resistant *S. epidermidis* strain, resistant to teicoplanin and other antimicrobial agents, was the main cause for the replacement of teicoplanin by linezolid. Four days later, an *E. faecium* isolate, resistant to glycopeptides and linezolid (MIC 16 mg/L) was recovered from blood culture.

The microorganism was identified to the species level by the automated Vitek System (bioMérieux, Hazelwood, MO, USA). Susceptibility testing to penicillin, ampicillin, ciprofloxacin, gentamicin (high-level), streptomycin (high-level), tetracycline, nitrofurantoin and vancomycin was performed using the Vitek System, and susceptibility to erythromycin, rifampicin, linezolid and quinupristin/dalfopristin was tested by Etest strips (AB Biodisk, Solna, Sweden). MICs of vancomycin, teicoplanin and linezolid were determined by the agar dilution method (NCCLS guidelines).3 To examine the stability of linezolid resistance, the isolate was subcultured on drug-free agar once weekly for 2 months and then re-tested to determine the final linezolid MIC. *Enterococcus faecalis* ATCC 29212 was used as a control for the estimation of MICs. PCRs for *erm* (B), vanA/B and esp genes were carried out as described previously.4 To elucidate the mechanism of linezolid resistance, the gene encoding domain V of the 23S rRNA was amplified.5 The G2576T mutation, which is mainly associated with the expression of linezolid resistance in clinical isolates, creates a cutting site for the restriction endonuclease *Nhe*I.6 Thus, PCR-RFLP analysis of the 420 bp amplicons was performed to detect this mutation: 10 µL of the PCR products was digested with 20 U of *Nhe*I for 4 h at 37°C, and the fragments were separated by electrophoresis through a 3.5% Metaphor agarose gel. In addition, in order to detect the presence of mutations other than G2576T, the 420 bp PCR product was sequenced.1,3,7 The epidemiological relationship of the isolate to other isolates of VRE from our hospital, including those from stool samples from patients in the ICU, was assessed by PFGE using *Smal* digests.1

Following repeated subcultures on drug-free agar, the expression of linezolid resistance remained stable. The isolate was also resistant to various other antimicrobial agents, such as penicillin, ampicillin, tetracycline, erythromycin, vancomycin, teicoplanin, streptomycin, gentamicin, nitrofurantoin, rifampicin and ciprofloxacin, but remained susceptible to quinupristin/dalfopristin. PCRs were positive for *vanA* and *erm*(B), but negative for the *esp* gene. The results of *Nhe*I PCR-RFLP analysis of the 420 bp DNA amplicons showed that the isolate was heterozygous: amplicons were cleaved to give undigested products of 420 bp and digested products of 326 bp and 94 bp. *E. faecalis* ATCC 29212, which is linezolid-susceptible, gave only the undigested 420 bp band. Analysis of sequencing data failed to detect the G2576T mutation.

PFGE analysis revealed that the linezolid-resistant *E. faecium* strain belonged in a clone that differed markedly from the endemic *E. faecium* hospital clones (Figure 1). Furthermore, two vancomycin- and linezolid-resistant *E. faecium* strains, isolated from stool cultures—the first from the studied patient (patient 1) and the second from another ICU patient (patient 2)—showed the same *Smal* pattern as the blood strain, suggesting a possible transfer of the strain between the two patients (Figure 1).

This study describes the first isolation of a linezolid-resistant *E. faecium* strain in a Greek hospital, where the use of linezolid is limited. The low-level linezolid resistance (MIC 16 mg/L) correlated with a heterozygous profile of the strain. The *Nhe*I PCR-RFLP assay is a reliable and rapid method for detecting the G2576T mutation, mainly for heterozygous isolates. We have no definitive explanation of how this strain has emerged. It is possible that it was acquired following the prior linezolid exposure. Previous case reports have suggested that emergence of resistance is associated with prolonged administration of the drug.1,2 However, the 4 day administration period of linezolid was significantly shorter in comparison with other reports.1,2 Another possibility is that our patient acquired the linezolid-resistant strain from another patient, who was colonized with a similar strain but had not received linezolid therapy. Finally, it is possible that our patient carried the linezolid-resistant strain prior to his admis-
sion and that the use of antimicrobial drugs selected the resistant strain, which was then transmitted to the second patient. Unfortunately, none of the patients was screened for VRE colonization at the time of ICU admission, and thus we cannot support or exclude the possibility that the patients already carried the isolates before admission.

Emerging linezolid resistance is a fact. Therefore, one could suggest that clinical laboratories should be routinely testing linezolid susceptibility in all clinically significant enterococci. Worldwide surveillance programmes should closely monitor all linezolid resistance reports in order to trace any trends in the development of resistance.

References


Effect of subinhibitory concentrations of azithromycin on adherence of Pseudomonas aeruginosa to bronchial mucins collected from cystic fibrosis patients

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Sir,

Pseudomonas aeruginosa, an opportunistic pathogen, is the major pathogen in the airways of patients with cystic fibrosis (CF) and is currently associated with the morbidity and mortality seen in this disease. Macrolides, such as azithromycin, are normally not included in the anti-pseudomonal therapeutic arsenal because of the absence of bactericidal or bacteriostatic activity. However, several previous investigators have reported that long-term administration of azithromycin is effective in patients with pulmonary P. aeruginosa infections.1 The clinical benefits achieved could include the effects of an anti-inflammatory2 and/or modulation of the production of virulence factors of P. aeruginosa, such as bacterial exoproducts,3 the formation of biofilm,4 or the synthesis of flagella5 and some outer membrane proteins.6 Adherence of P. aeruginosa to respiratory mucins plays an important role in the colonization of the airways of these patients. This adherence process involves flagella and several non-pilus adhesins localized on the outer membrane of P. aeruginosa. Supporting the clinical observations, adherence of this organism to human salivary or airway mucins has been demonstrated in vitro using liquid- or solid-phase adherence assays.6

In order to investigate the action of azithromycin on the pathogenesis of P. aeruginosa, we evaluated—in a solid-phase adherence assay—the action of subinhibitory concentrations of the macroline on the adherence of different bacterial strains to respiratory mucins of CF