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Linezolid-Resistant Enterococcus faecium Isolated from a Patient Without Prior Exposure to An Oxazolidinone: Case Report from the SENTRY Antimicrobial Surveillance Program

R.N. JONES*, L.V. LEE, P. DELLA-LATTA, D.L. SHINABARGER. The JONES Group/JMI Laboratories, North Liberty, IA; Columbia Presbyterian Medical Center, New York, NY; Pharmacia and Upjohn, Kalamazoo, MI

ABSTRACT

Background: The oxazolidinones have remarkable activity against Gram-positive cocci, and the initial clinical candidate, linezolid (LZD) has proven to be an excellent therapeutic agent. The LZD clinical trials and subsequent published case reports cite very rare resistance (R) emerging in patients receiving prolonged therapy. We report the first isolation of LZD-R *E. faecium*, unassociated with prior therapy.

Methods/Case: A 32 year old diabetic male with end-stage renal disease was admitted for traumatic injuries. Febrile symptoms led to blood cultures on day 1 and 14 that grew *E. faecium*. MRSA was also isolated on 2 occasions from blood and anterior nares on day 16 and 17. The patient was treated with LZD and vancomycin beginning on day 19 until discharge. No LZD therapy prior to the index culture (day 1) could be documented. The strain was discovered by the SENTRY Antimicrobial Surveillance Program and reference broth microdilution tests were performed. Alternative and standardized tests were also used including Etest and disks. Results included the testing of a novel oxazolidinone (AZD2563, AstraZeneca) by MIC and disk diffusion.

Results: Susceptibility testing demonstrated elevated MICs for the oxazolidinones (LZD and AZD2563 at 8 µg/ml) and small zones of inhibition (15 mm; R at ≤ 20 mm). The strain was multi-drug R having a *van B* resistance and co-R to penicillins, carbapenems, chloramphenicol, macrolides, gentamicin (high-level), fluoroquinolones, rifampin, nitrofurantoin and trim/sulfa. Susceptibility to quinupristin/dalfopristin (MIC, 1 - 1.5 µg/ml), doxycycline (1 µg/ml), mupirocin (≤ 4 µg/ml) and streptomycin (high-level) was confirmed. To determine causative mutations, the domain V of 23S rRNA was amplified and PCR products were sequenced revealing a single key mutation, G2576U, described earlier in LZD-R isolates.

Conclusions: We report the isolation of a LZD-R *E. faecium* strain. Uniquely, no evidence of prior LZD therapy could be found, however, patient-to-patient spread must be considered. This contrasts to similar R mutations of the 23S rRNA in enterococci (G2576U) and *S. aureus* (G2576U) that have been associated with long-term exposure/selection by LZD. Laboratories should be aware of the very unusual possibility of these R strains occurring in contemporary practice, and possess in vitro methods capable of detecting oxazolidinone-R.

INTRODUCTION

The oxazolidinones have a remarkable activity against Gram-positive cocci, and the initial clinical candidate, linezolid, has proven to be an excellent therapeutic agent. Several recently completed multi-laboratory surveys have documented essentially complete antimicrobial coverage of staphylococci, streptococci and enterococci at a linezolid MIC of ≤ 4 µg/ml in the United Kingdom, United States and Europe. However, reports from the linezolid clinical trials, and subsequent published case reports of enterococci and staphylococci cite resistances emerging in patients receiving prolonged courses of oxazolidinone therapy. In this communication, we report the isolation of an *Enterococcus faecium* strain from a blood stream infection that was resistant to linezolid, but unassociated with any earlier treatment.

CLINICAL CASE

A 32 year old diabetic male with end-stage renal disease was admitted (April 3, 2001) following a fall. Possible traumatic injuries to the ribcage and left knee were confirmed associated with osteopenic appearance of bones on radiology imaging. Febrile symptoms led to blood cultures on April 4 and 17 that grew *E. faecium* (index strain 15-4011A from April 4, 2001). Also methicillin-resistant S. aureus was isolated on two occasions from blood culture and anterior nares on April 19 and 20, respectively. The patient was treated with linezolid and vancomycin beginning on April 22 until discharge (April 30, 2001), guided by antibiograms of both cultured species. No linezolid therapy prior to the index culture could be documented.

MATERIALS AND METHODS

The strain was forwarded to the SENTRY Antimicrobial Surveillance Program monitor (North Liberty, Iowa) and reference broth microdilution tests were performed. The MIC results were interpreted by National Committee for Clinical Laboratory Standards (NCCLS) criteria. The *E. faecium* identification was confirmed and a Vitek System (bioMerieux, Hazelwood, MO) biotype number of 77767270510 was obtained having a 93% confidence. Alternative and standardized susceptibility tests were also performed including Etest (AB BIODISK, Solna, Sweden) and disk diffusion tests. Table 1 lists those results obtained with the tests that also included the testing of a novel, new oxazolidinone (AZD2563, AstraZeneca, UK) by reference MIC and disk diffusion methods.

To determine causative mutations, a region of the 23S rRNA gene corresponding to bp 2049-2767 was amplified using specific primers 5'-gACggAAAgA-CCCCATgg-3' and 5'-ACACTTAgATgCTTT-3'. PCR products were sequenced directly using an ABI377 fluorescence sequencer (Perkin Elmer Applied Biosystems, Foster City, CA) and the ABI BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with *AmpliTaq* FS DNA polymerase (PE Applied Biosystems, Foster City, CA).

RESULTS

- Susceptibility testing results clearly demonstrated an elevated MIC for the oxazolidinones (linezolid and AZD2563 at 8 µg/ml) and correspondingly smaller zones of inhibition (15 mm; resistance at ≤ 20 mm).
- Etest also indicated resistance with a MIC of 8 µg/ml.
- The strain was multi-drug resistant having a *van B* glycopeptide resistance pattern and co-resistances to penicillins, carbapenems, chloramphenicol, macrolides, gentamicin (high-level), fluoroquinolones, rifampin, nitrofurantoin and trimethoprim/sulfamethoxazole.
- Susceptibility to quinupristin/dalfopristin (MIC, 1 - 1.5 µg/ml), doxycycline (MIC, 1 µg/ml) and streptomycin (high-level, MIC at ≤ 1000 µg/ml) was confirmed by reference microdilution methods. The strain was also susceptible to the topical agent mupirocin (MICs, ≤ 4 and 0.75 µg/ml by microdilution and Etest methods, respectively).
- A single key mutation was identified, G2576U, that has been described earlier in linezolid-resistant isolates from the clinical trials.

Table 1. In vitro susceptibility pattern (21 antimicrobials) for isolate 15-4011A, an oxazolidinone-resistant *E. faecium*[a] strain.

	MIC (µg/ml)		
Antimicrobial agent	Broth microdilution	Etest	Zone diameter (mm)
Linezolid	8	8	15
AZD2563	8		15
Vancomycin	8	12	13
Teicoplanin	0.25[b]	0.75[b]	18[b]
Quinupristin/Dalfopristin	1	1.5	
Ampicillin	>16		
Penicillin	>32		
Imipenem	>8		
Amoxicillin/Clavulanate	>16/8		
Piperacillin/Tazobactam	>64/4		
Gentamicin (high-level)	>1000		
Streptomycin (high-level)	≤1000		
Chloramphenicol	16		
Doxycycline	1		
Ciprofloxacin	>2		
Levofloxacin	>4		
Erythromycin	>8		
Rifampin	>2		
Nitrofurantoin	>32		
Trimethoprim/Sulfamethoxazole	>2/38		

a. Vitek biotype number = 77767270510 (93% confidence).

b. Consistent with a *van B* phenotype.

CONCLUSIONS

- We report the isolation and successful treatment of a *E. faecium* strain resistant to linezolid from a patient with end-stage diabetic renal disease.
- Uniquely no evidence of prior oxazolidinone (linezolid) therapy could be found, but patient-to-patient spread could not be excluded. This contrasts to similar resistant mutations of the 23S rRNA in enterococci (G2576U) and S. aureus (G2576U) that were associated with long-term prior exposure/selection by linezolid.
- This resistance was easily detected using a broth microdilution test producing a reproducible linezolid MIC of 8 µg/ml. These results were confirmed by Etest (AB BIODISK) and disk diffusion methods.
- We agree with the conclusions of Livermore et al. that a susceptible breakpoint of ≤ 4 µg/ml, supported by pharmacodynamic results, for linezolid indicates potential clinical utility, but more importantly a linezolid MIC of ≥ 8 µg/ml would be highly suggestive of mutational resistance.
- Clinical laboratories should be routinely testing linezolid to monitor for emerging resistance (MIC, ≥ 8 µg/ml) among strains isolated from patients on linezolid therapy and for those strains of multi-resistant Gram-positive species where oxazolidinone treatment may be required.

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