Nosocomial superinfections due to linezolid-resistant *Enterococcus faecalis*: evidence for a gene dosage effect on linezolid MICs

Kathleen A. Ruggero\(^a\), Laura K. Schroeder\(^a\), Paul C. Schreckenberger\(^b\), Alexander S. Mankin\(^c\), John P. Quinn\(^{d,*}\)

\(\textit{University of Illinois at Chicago, Department of Internal Medicine, Section of Infectious Disease, 808 S. Wood St., Rm 888, M/C 735, Chicago, IL 60612, USA}\)

\(\textit{bUniversity of Illinois at Chicago, Department of Pathology, 840 S. Wood St., Rm 238, CSB, M/C 750, Chicago, IL 60612, USA}\)

\(\textit{cUniversity of Illinois at Chicago, Center for Pharmaceutical Biotechnology, 900 S. Ashland Ave, Rm 3056, M/C 870, Chicago, IL 60607, USA}\)

\(\textit{dCook County Hospital/Rush University, 1900 W. Polk Street, Rm 1258, Chicago, IL 60612, USA}\)

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Abstract

Resistance to linezolid among *Enterococcus faecium* and *Enterococcus faecalis* isolates has been reported in patients who receive a prolonged course of the drug. We report two cases of linezolid-resistant *Enterococcus faecalis* that occurred in patients who previously received linezolid for infections with vancomycin-resistant *Enterococcus faecium*. Both isolates had the G2576U mutation in the 23S rRNA previously reported in isolates of *Enterococcus faecium*. The number of gene copies mutated in the 23S rRNA correlated with the level of resistance. © 2003 Elsevier Inc. All rights reserved.

1. Introduction

Vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* are important causes of nosocomial infections. Some of these infections may be treated with linezolid (Pharmacia Corp., Kalamazoo, MI), an oxazolidinone antibiotic approved by the FDA in the spring of 2000 for the treatment of Gram-positive infections, including those due to vancomycin-resistant enterococci (VRE). Linezolid resistance in vancomycin-resistant *E. faecium* has been described in multiple reports including one from our institution (Gonzales et al., 2001; Jones et al., 2002; Herrero et al., 1999; Zurenko et al., 1999). Among the first 88 patients treated with linezolid in our institution over the past two years, resistance has emerged in 11 (12.5%) of VRE isolates. The most common risk factor among patients who develop vancomycin-resistant linezolid-resistant *E. faecium* is duration of therapy with linezolid (Pai et al., 2002). We now describe two patients with linezolid-resistant *E. faecalis* infections who had previously received linezolid for linezolid-susceptible *E. faecium* infections. Both were isolated within a one month interval.

2. Materials and methods

Patient 1 was a 74-year-old male who presented to our hospital with a cerebrovascular accident. His hospital course was complicated by acute renal failure, myocardial infarction, pulmonary edema, nosocomial pneumonia and aspiration. He was diagnosed with catheter-related bacteremia due to VRE. This isolate was susceptible to linezolid at 1.0 μg/mL by E-test (AB BIODISK, Piscataway, NJ). He was treated with 10 days of i.v. linezolid 600 milligrams every 12 h. His blood cultures became sterile. Ten days after discontinuing linezolid, the patient became clinically septic, and was placed on empiric antibiotics, including linezolid. He received linezolid for this second time for sixteen days,
even though enterococci were not isolated from blood, sputum, or urine cultures during this time. Subsequently, he developed worsening respiratory status, and cultures performed 32 days after his last positive blood culture for VRE revealed a blood culture positive for *E. faecalis* via Vitek GPI (bioMerieux, Inc., St. Louis, MO) and API 20 Strep (bioMerieux, Inc., St. Louis, MO) identification. This was eight days after linezolid was discontinued. This isolate was susceptible to ampicillin (0.5) and vancomycin (<2) via Microscan (Dade Behring, West Sacramento, CA), but resistant to linezolid at >256 μg/mL via E-test.

Patient 2 was a 53-year-old male who presented with pancreatitis. He subsequently developed multisystem organ failure, requiring intubation. He received vancomycin, imipenem, metronidazole and fluconazole empirically for sepsis syndrome. Surgical debridement was done for necrotizing pancreatitis. Two weeks later, he developed positive blood and urine cultures for vancomycin-resistant, linezolid-susceptible *E. faecalis*. He was given i.v. linezolid for 26 days, at a dose of 600 milligrams every 12 h. His blood cultures and urine cultures became negative. He was off all antibiotics until one month later when a urine culture grew *E. faecalis* sensitive to ampicillin (2), resistant to vancomycin (>16), and resistant to linezolid via E-test at 24 μg/mL. Two and a half months later, he had a pancreatic pseudocyst drained that also grew vancomycin-resistant linezolid-resistant *Enterococcus faecalis*.

Using the polymerase chain reaction (PCR) based protocol previously described (Woodford et al., 2002), we performed restriction fragment length polymorphism (RFLP) studies to see if these isolates shared the characteristic G2576U mutation in domain V of the 23S rRNA previously described in linezolid-resistant *E. faecium* and *E. faecalis* (Prystowsky et al., 2001; Marshall et al., 2002). Enterococcal DNA and RNA were extracted by shaking cells with hot phenol followed by ethanol precipitation. The DNA primers used for amplification were: SfL2100 (5’-CGGT-GAAATTTTAGTACCTGTGAAGATG-3’) and SfL2660-2 (5’-GTCCATCCGGTCTCTCCTCG-3’) designed to amplify a 662 bp segment from domain V of enterococcal 23S rRNA, encompassing the G2576 position. PCR reaction was performed using rTaq DNA polymerase (Fisher Scientific, Hanover Park, IL). Conditions for amplification were: 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. PCR product was purified using a PCR product purification kit (Qiagen, Valencia, CA) and recovered in 40 μL H2O. 5 μL DNA was digested at 37°C for 2 h using 5 or 10 units of *NheI* restriction enzyme (Fermentas, Hanover, MD) under conditions recommended by the enzyme manufacturer. The purified PCR product (5 μL) and products of the restriction digest were analyzed on a 1% (w/v) agarose gel. Both isolates were tested for relatedness by pulsed field gel electrophoresis (PFGE) by previously described technique (Matushek et al., 1996).

3. Results

The G2576U mutation in 23S rRNA gene generates a new restriction site for the enzyme *NheI*. The PCR fragment amplified using DNA from the *E. faecalis* isolate from Patient 1 (linezolid MIC >256 μg/mL) was completely cut by *NheI* to produce the 570 bp band and 100 bp band. This indicates that this isolate contains the G2576U mutation in all four of its 23S rRNA gene copies. The PCR product produced using DNA from the *E. faecalis* isolate from Patient 2 (linezolid MIC = 24 μg/mL) was cut incompletely by *NheI*. Two fold increase in concentration of the restriction enzyme did not increase the extent of cleavage indicating that incomplete restriction digest resulted from heterogeneity of rDNA alleles. The ratio of cut and uncut DNA, as well as allele-specific PCR amplification and RFLP analysis (not shown), indicates that two out of four *E. faecalis* rRNA operons in this isolate carried the G2576U mutation. (See Fig. 1.) PFGE of chromosomal DNA revealed that these strains were unrelated.

4. Discussion

Both of these patients had an infection with vancomycin-resistant *E. faecium*, and were treated with linezolid for a prolonged course. Subsequently, they had cultures positive for linezolid-resistant *E. faecalis*. In in vitro experiments, *E. faecalis* developed resistance to linezolid more quickly than *E. faecium* when exposed to increasing concentrations of linezolid (Prystowsky et al., 2001). In addition, MICs to linezolid were higher for *E. faecalis* than for *E. faecium* (Prystowsky et al., 2001).

Previous investigation showed that the level of resistance
of \textit{E. faecium} to linezolid correlates with the number of 23S rRNA gene copies that carry the G2576U mutation (Marshall et al., 2002). This observation was extended here to isolates of \textit{E. faecalis}. \textit{E. faecalis} contains four copies of rRNA operons in its chromosome (Marshall et al., 2002). The mutation G2576U generates a new site for the restriction enzyme \textit{Nhe}1 in the mutant copies of rRNA operon, while this site is absent in the wild type sequence. All four copies of rRNA genes in the isolate from patient one (referred to as Ent 1 in the figure) carried the G2576U mutation: the PCR fragment encompassing the position 2576 was completely digested with \textit{Nhe}1 (see Fig. 1). In contrast, only a portion of the PCR fragment amplified from DNA of the isolate from patient two (referred to as Ent 2 in the figure) could be digested with \textit{Nhe}1 indicating that some of the rRNA operon did not carry the G2576U mutation. RFLP analysis of individual rRNA alleles in this strain showed that two out of four operons carried the G2576U mutation (data not shown). Our results do not exclude the presence of other mutations in any of the two isolates. However, a clear correlation between the level of resistance and the number of mutated operons indicate that the G2576U mutation is the primary cause of the resistance.

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References


