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Nosocomial Spread of *Enterococcus faecium* Resistant to Vancomycin and Linezolid in a Tertiary Care Medical Center

Thomas E. Dobbs,[1] Mukesh Patel,[1] Ken B. Waites,[2]* Stephen A. Moser,[2] Alan M. Stamm,[1] and Craig J. Hoesley[1]

Department of Medicine[1] and Department of Pathology,[2] University of Alabama at Birmingham, Birmingham, Alabama 35249-7331

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In May 2004 our institution encountered its first clinical isolate of linezolid-resistant, vancomycin-resistant *Enterococcus faecium* (LRVRE). Between October 2004 and July 2005, 40 patients from whom LRVRE organisms were recovered in clinical specimens were characterized. Epidemiologic investigation and pulsed-field gel electrophoresis patterns indicated a clonal outbreak related to nosocomial spread.

Linezolid, the first drug in the oxazolidinone class, was approved for use in the United States in March 2000 for the treatment of infections due to gram-positive cocci, including vancomycin-resistant enterococci. Despite widespread usage over the past 6 years, surveillance studies have reported rates of linezolid resistance in enterococci of <0.5% (5). In May 2004, our 900-bed tertiary care academic medical center encountered its first case of linezolid-resistant, vancomycin-resistant *Enterococcus faecium* (LRVRE) disease after progressive increases in the utilization of linezolid over the preceding several months. Subsequently, during 2005, 60 unique isolates accounting for 21% of all *E. faecium* isolates recovered from inpatients were intermediately or fully resistant to linezolid. A retrospective investigation was initiated to determine the nature of this outbreak. The study was performed with the approval of the Institutional Review Board.

We examined the medical records of 40 patients from whom LRVRE were isolated from clinical specimens between October 2004 and July 2005 to ascertain factors that may have contributed to acquisition of the organism and the persistence of the outbreak. Demographic data, clinical characteristics, underlying illnesses, prior exposures to antibiotics, corticosteroids, chemotherapy, hyperalimentation, the presence of an indwelling central venous catheter, hospital location, and survival were recorded. Clinical infectious disease versus colonization was defined by the team attending to each case.

Bacteria were identified, and MICs were initially determined using the MicroScan WalkAway (Dade Behring, West Sacramento, CA). Nonsusceptibility to linezolid (MICs of ≥4 μg/ml) was verified by the agar gradient diffusion (Etest) technique (AB Biodisk, Solna, Sweden). LRVRE isolates from 21 of the 40 patients had been stored frozen at −70°C and were tested by pulsed-field gel electrophoresis (PFGE) to determine genetic relatedness. PFGE was performed using techniques described previously with a few minor modifications (6). Banding patterns were analyzed using GelCompar II (Applied Maths, Austin, TX) and interpreted as described by Tenover et al. (11). We performed PFGE on 12 contemporary vancomycin-resistant enterococcus isolates from our institution that were susceptible to linezolid (LSVRE) for comparison. The data management and statistical analysis were performed using Epi Info 3.3.

Etest MICs confirmed linezolid resistance initially identified by the MicroScan for all *E. faecium* isolates, with values ranging from 8 to >256 μg/ml (MIC_{50} = 32 μg/ml, MIC_{90} = 256 μg/ml). All LRVRE isolates were susceptible to tetracycline (MICs of ≤4 μg/ml), and all but one were resistant to ciprofloxacin (MICs of ≥4 μg/ml).

Among the 40 patients whose records were examined, the median age was 54 years (range, 21 to 93 years). Twenty-two (55%) patients had evidence of disease due to LRVRE. The bloodstream (eight patients) and urinary tract (nine patients) were the most common sites of specimen origin. Nine patients overall and six with LRVRE clinical infection died during hospitalization. Serious comorbidities were encountered frequently, with diabetes mellitus and end-stage renal disease being the most common (Table 1). All patients received antibiotics prior to the isolation of LRVRE. Fluoroquinolones and vancomycin were the most common agents administered. Only six (15%) patients received linezolid prior to contracting LRVRE (Table 2).

LRVRE occurred hospital-wide in several different types of units. Twenty-eight (70%) patients had spent time in one or more of nine intensive care units (ICUs). Seventeen (42.5%) were in a specific medical/coronary care ICU prior to acquiring LRVRE. During the first 4 months of the outbreak, 11 of 14 (78.6%) patients spent time in this particular ICU prior to acquiring LRVRE. Many patients were transferred among

TABLE 1. Comorbidities of 40 patients infected with LRVRE

Underlying illness	No. (%) of patients
Diabetes mellitus	15 (37.5)
Renal disease	14 (35)
Postsurgery	11 (27.5)
Malignancy	5 (12.5)
Solid organ transplant	4 (10)
Human immunodeficiency virus infection	3 (7.5)
Trauma or burn	3 (7.5)
Neutropenia	2 (5)
Stem cell transplant	1 (2.5)

* Corresponding author. Mailing address: University of Alabama at Birmingham, 619 South 19th St., Birmingham, AL 35249. Phone: (205) 934-4960. Fax: (205) 975-4468. E-mail: waites@path.uab.edu.

TABLE 2. Prior antibiotic exposure in 40 patients infected with LRVRE

Antibiotic	No. (%) of patients
Fluoroquinolones	34 (85)
Vancomycin	32 (80)
Penicillins	28 (70)
Cephalosporins	21 (52.5)
Aminoglycosides	13 (32.5)
Metronidazole	13 (32,5)
Carbapenems	11 (27.5)
Trimethoprim-sulfamethoxazole	8 (20)
Macrolides	8 (20)
Linezolid	6 (15)
Clindamycin	2 (5)

hospital units several times during the course of their stay (range, one to six moves), but 20 (50%) stayed in only one unit prior to contracting LRVRE.

PFGE patterns of 21 isolates revealed a single dominant clone in 19 cases (Fig. 1). The remaining two isolates were unique from the dominant clone and from each other. All LRVRE isolates were distinct as determined by PFGE from a sample of 12 contemporary LSVRE strains isolated from patients housed in these same hospital locations and from 185 LSVRE strains isolated earlier at our institution (data not shown), indicating that a newly introduced clone was responsible for most of the LRVRE infections we encountered in 2004 and early 2005.

The clinical management of enterococcal infections has been greatly compromised by the emergence of vancomycin-resistant strains over the past two decades (4). Linezolid is one of several new compounds useful for treating these resistant organisms. The identification of linezolid resistance among enterococci further complicates the management of these infections and greatly limits the available treatment options. Risk factors that we identified in patients with LRVRE were typical of what we have observed with LSVRE.

Linezolid resistance in enterococci was recognized soon after its clinical debut, although previously published reports have involved a single patient or a very limited number of cases (1–3, 7, 8, 10). Most linezolid-resistant isolates examined thus far have had the same 23S rRNA mutation in domain V, G2576U (9). A gene-dose effect has been observed that correlates the magnitude of the MIC with the number of loci containing this mutation (9). The wide range of linezolid MICs we encountered also supports this concept.

The rate of development of LRVRE is unknown, but de novo resistance has been documented in several instances when PFGE comparisons were made between susceptible and resistant isolates in the same patient after exposure to linezolid (1, 2, 8). However, as we have demonstrated, prior exposure to

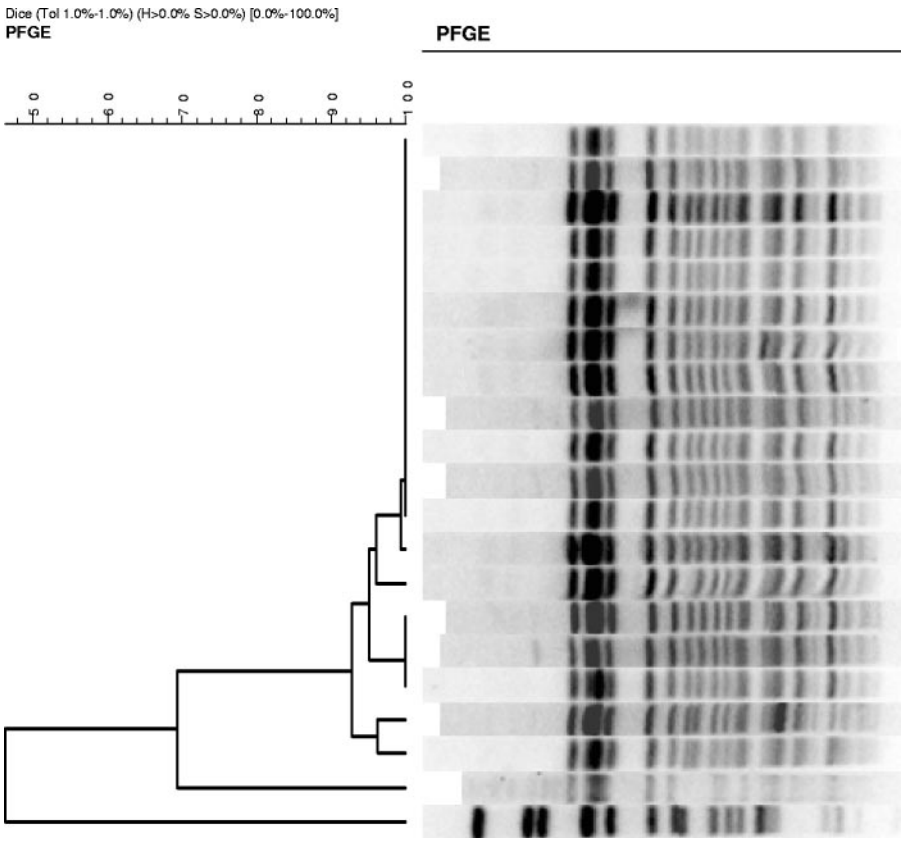

FIG. 1. PFGE characterization of 21 unique linezolid-resistant, vancomycin-resistant *E. faecium* strains demonstrating that 19 isolates were clonally related, with more than 90% similarity in banding patterns, while the remaining two isolates differed from one another and from the dominant clone.

linezolid is not a prerequisite to the development of linezolid resistance. The present report describes the first large outbreak of LRVRE proven to be related to nosocomial spread. Circumstantial evidence implicates a single ICU as the amplifier, if not the original source, of this outbreak. This underscores the critical necessity of effective infection control measures to limit the spread of such resistant pathogens.

The outbreak was controlled by re-education of the medical and coronary care ICU staff in standard and contact precautions, by enhancing adherence to isolation precautions, and by placing the patients and staff into cohorts. No patient acquired LRVRE disease in this ICU from March to June 2005. In July 2005 we began screening by rectal swab culture all patients on admission and weekly thereafter. From July to December 2005, 11 colonized patients were recognized and isolated, four acquired disease due to vancomycin-resistant enterococci, but only one had an LRVRE isolate. During the latter period, LRVRE isolates were recovered from throughout the hospital, seemingly distributed randomly by time, location, or service.

Identification of two other unrelated clones of LRVRE from different patients indicates that the development and spread of resistance was not an isolated event. Our experience suggests that use and exposure to agents other than linezolid, fluoroquinolones in particular, may be important to the emergence of linezolid resistance. Prudent antibiotic utilization practices must also be incorporated into an institutional strategy to limit the burden of resistant pathogens. We are now discouraging the use of fluoroquinolones in the hospital in general and have recommended that alternatives such as daptomycin be used in lieu of linezolid for treating infections due to vancomycin-resistant enterococci.

REFERENCES

1. **Auckland, C., L. Teare, F. Cooke, M. E. Kaufmann, M. Warner, G. Jones, K. Bamford, H. Ayles, and A. P. Johnson.** 2002. Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. J. Antimicrob. Chemother. **50:**743–746.
2. **Bassetti, M., P. A. Farrel, D. A. Callan, J. E. Topal, and L. M. Dembry.** 2003. Emergence of linezolid-resistant *Enterococcus faecium* during treatment of enterococcal infections. Int. J. Antimicrob. Agents **21:**593–594.
3. **Bonora, M. G., M. Ligozzi, A. Luzzani, M. Solbiati, E. Stepan, and R. Fontana.** 2006. Emergence of linezolid resistance in *Enterococcus faecium* not dependent on linezolid treatment. Eur. J. Clin. Microbiol. Infect. Dis. **25:**197–198.
4. **Chavers, L. S., S. A. Moser, W. H. Benjamin, S. E. Banks, J. R. Steinhauer, A. M. Smith, C. N. Johnson, E. Funkhouser, L. P. Chavers, A. M. Stamm, and K. B. Waites.** 2003. Vancomycin-resistant enterococci: 15 years and counting. J. Hosp. Infect. **53:**159–171.
5. **Draghi, D. C., D. J. Sheehan, P. Hogan, and D. F. Sahm.** 2005. In vitro activity of linezolid against key gram-positive organisms isolated in the united states: results of the LEADER 2004 surveillance program. Antimicrob. Agents Chemother. **49:**5024–5032.
6. **Duck, W. M., C. D. Steward, S. N. Banerjee, J. E. McGowan, Jr., and F. C. Tenover.** 2003. Optimization of computer software settings improves accuracy of pulsed-field gel electrophoresis macrorestriction fragment pattern analysis. J. Clin. Microbiol. **41:**3035–3042.
7. **Herrero, I. A., N. C. Issa, and R. Patel.** 2002. Nosocomial spread of linezolid-resistant, vancomycin-resistant *Enterococcus faecium*. N. Engl. J. Med. **346:** 867–869.
8. **Johnson, A. P., L. Tysall, M. V. Stockdale, N. Woodford, M. E. Kaufmann, M. Warner, D. M. Livermore, F. Asboth, and F. J. Allerberger.** 2002. Emerging linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from two Austrian patients in the same intensive care unit. Eur. J. Clin. Microbiol. Infect. Dis. **21:**751–754.
9. **Marshall, S. H., C. J. Donskey, R. Hutton-Thomas, R. A. Salata, and L. B. Rice.** 2002. Gene dosage and linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis*. Antimicrob. Agents Chemother. **46:**3334–3336.
10. **Rahim, S., S. K. Pillai, H. S. Gold, L. Venkataraman, K. Inglima, and R. A. Press.** 2003. Linezolid-resistant, vancomycin-resistant *Enterococcus faecium* infection in patients without prior exposure to linezolid. Clin. Infect. Dis. **36:**E146–E148.
11. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.