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The clinical impact of linezolid susceptibility reporting in patients with vancomycin-resistant enterococci

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Abstract

Linezolid remains a mainstay of therapy for vancomycin-resistant enterococci (VREs), but resistance has emerged. We describe a cohort of 20 patients with linezolid-intermediate or resistant VRE (LIRVRE) reported by Etest and disk diffusion testing, 18 of whom demonstrated linezolid susceptibility by agar dilution on further investigation. Patients with reported LIRVRE were matched based on culture site and enterococcal species to patients with linezolid-susceptible VRE (LSVRE) in a 1:3 ratio. Patients with reported LIRVRE developed more nosocomial infections ($P = .04$), had more central lines placed ($P = .04$), and underwent more computed tomography scans related to VRE infection ($P = .02$). Multivariate analysis revealed increased surgical procedures related to VRE infections ($P = .008$), increased linezolid use during hospital stay ($P = .03$), and delayed culture and susceptibility results compared with those with LSVRE ($P = .006$). Therefore, inaccurate detection and reporting of LIRVRE by disk diffusion and Etest is associated with increased patient morbidity and resource use.

Keywords: Vancomycin-resistant enterococci; Linezolid; Susceptibility testing; Antimicrobial resistance

1. Introduction

The incidence of nosocomial infections due to vancomycin-resistant enterococci (VREs) continues to increase (Biedenbach et al., 2004; National Nosocomial Infections Surveillance [NNIS] System Report, 2004). Historically, VREs were resistant to most antimicrobial agents, but the recent introduction of linezolid and quinupristin–dalfopristin has provided therapeutic options for many of these infections (Murray, 2000; Linden et al., 2001; Birmingham et al., 2003). Linezolid is widely used because of its favorable pharmacokinetic distribution, low incidence of adverse effects, and oral bioavailability (Birmingham et al., 2003). Given the above, linezolid use has increased 7-fold at our institution since its approval in 2000, and it is now a 1st-line therapy for vancomycin-resistant *Enterococcus faecium* and vancomycin-resistant *Enterococcus faecalis* infections in patients either resistant to or unable to receive a penicillin antibiotic. Hence, decreased linezolid susceptibility significantly impacts the current treatment of this pathogen.

Clinical resistance has emerged with increased linezolid use and has been attributed to the single-point mutation G2576T within the 23S rRNA gene. Six copies of this gene exist within *E. faecium* and 4 copies within *E. faecalis* (Meka and Gold 2004). Previous studies reveal dose-dependent resistance associated with the number of mutations detected for *E. faecalis* and *E. faecium*. As the number of mutant gene copies increases, the level of linezolid resistance increases (Marshall et al., 2002; Ruggero et al. 2003).

Fortunately, reports of linezolid resistance among VRE are still uncommon (Gonzales et al., 2001; Prystowsky et al.,

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2001; Mutnick et al., 2003; Raad et al., 2004). A multivariate analysis to systematically review risk factors for the occurrence of reduced linezolid susceptibility identified previous linezolid treatment as the only predictive variable associated with decreased susceptibility (Pai et al., 2002).

Over a 6-month period, reports of VRE isolates with decreased linezolid susceptibility increased 4-fold at our institution (incidence, 1.61% in 2003 versus 6.5% from January 2004 to August 2004). During the same period, linezolid usage increased only slightly (8.3 defined daily doses per 1000 patient days and 9.5 defined daily doses per 1000 patient days, respectively), and review of laboratory personnel hiring did not indicate significant employee turnover.

We recently defined the optimal susceptibility methods for detection of linezolid-intermediate or resistant VRE (LIRVRE) (Qi et al., 2006). This investigation revealed that most of the initial clinical reports of decreased linezolid susceptibility could not be confirmed by agar dilution. In the present report, we describe the clinical impact of these inaccurate linezolid susceptibility reports.

2. Materials and methods

2.1. Study setting and design

Northwestern Memorial Hospital (NMH) is a 725-bed academic medical center in Chicago, IL. The clinical microbiology laboratory at NMH offers a full range of services including bacteriology, susceptibility testing, and molecular diagnostics.

To determine the impact of linezolid susceptibility reporting on clinical outcomes, a retrospective matched cohort study was undertaken. Patients were included in this cohort if they had clinical VRE isolates that were reported to have LIRVRE from January 2004 to August 2004. These patients were frequency matched to controls with reported linezolid-susceptible VRE (LSVRE) in a 1:3 ratio based on site of infection and VRE species. Patients with multiple VRE isolates were included only once. All patients with linezolid-intermediate isolates were considered cases from the point of the 1st isolation of LIRVRE from a clinical specimen. Patients were excluded from analysis if they were not admitted to the hospital within 24 h of culture result or if procedures related to VRE infection could not be evaluated because of unavailability of the medical record. The study was reviewed and approved by the Northwestern University Institutional Review Board.

2.2. Microbiologic evaluation

2.2.1. Evaluation of clinical specimens

All clinical isolates of VRE were identified to the species level using the Vitek 2 system (Vitek Systems; bioMerieux, St. Louis, MO). When Vitek 2 was unable to identify the species, identification was performed based on manual biochemical reactions (Facklam and Elliott 1995).

2.2.2. Susceptibility testing

Susceptibility testing for antimicrobial agents other than linezolid was performed by the Vitek 2 system for all isolates. Initial linezolid susceptibility testing was performed in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines by either Kirby–Bauer disk diffusion (BBL Becton Dickinson, Sparks, MD) or Etest methodology (AB Biodisk, Solna, Sweden)(Clinical and Laboratory Standards Institute/NCCLS—Performance Standards for Antimicrobial Susceptibility Testing, 2005). Susceptibility testing was repeated by Etest, Kirby Bauer disk diffusion, and agar dilution for all LIRVRE isolates and for selected LSVRE control isolates to confirm previously described results (Qi et al., 2006). For LIRVRE isolates, only the most linezolid-resistant isolate was selected. Inaccurate linezolid susceptibility tests were defined as isolates that initially were reported as intermediate or resistant by phenotypic testing but failed to reproduce based on agar dilution.

2.2.3. Polymerase chain reaction

Results were confirmed for all LIRVRE isolates by polymerase chain reaction (PCR) for the G2576T mutation as previously described (Qi et al., 2006).

2.2.4. Molecular epidemiology

All LIRVRE isolates underwent molecular typing using pulsed-field gel electrophoresis (PFGE) according to previously published methodologies (Turabelidze et al., 2000) with the addition of 15 U of mutanolysin to the lysozyme/lysostaphin mix. The similarity between isolates was determined by visual comparison of DNA banding patterns using the criteria of Tenover et al. (1995), and a difference of greater than 6 bands was considered genetically distinct.

2.3. Clinical investigation

Inpatient electronic medical records, pharmacy and microbiology databases, and paper charts were reviewed. Patients with reported LIRVRE were matched based on culture site and enterococcal species to patients with LSVRE in a 1:3 ratio. The following independent variables were recorded: age, race, sex, transplant status, Charlson score calculated by *International Classification of Diseases, Ninth Revision*, codes (Charlson et al., 1987; Deyo et al., 1992), VRE species, site of infection, and number of days from admission to positive culture. Outcomes assessed included duration of VRE infection, duration of time until VRE susceptibility was reported to the physician, length of hospital stay, targeted antibiotic use pre- and post-VRE isolation (ampicillin [when susceptible], piperacillin [when susceptible], linezolid, quinupristin–dalfopristin, and daptomycin), directed therapy change after culture results were reported, adverse drug reactions due to VRE therapy, nosocomial infections after VRE isolation, procedures and tests, transfer to intensive care unit (ICU), discharge location, in-hospital mortality, and whether death or discharge