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Difficulties in detection and identification of *Enterococcus faecium* with low-level inducible resistance to vancomycin, during a hospital outbreak

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ABSTRACT

Between June and November 2004, a vancomycin-resistant *Enterococcus faecium* (VRE) strain was isolated from 13 patients in the haematology/bone marrow transplant unit. There were difficulties in identifying the organism, which had low-level, inducible vancomycin resistance, and standard screening methods did not reveal carriage in patients or their contacts. These technical failures led to spread of VRE and delays in providing appropriate management, which might otherwise have been avoided. Therefore, we reviewed our laboratory methods and compared three identification systems to determine which would best identify this VRE strain. The VITEK 2 (BioMerieux) correctly identified, as *E. faecium*, only two of 16 isolates, whereas API Rapid ID 32 Strep (BioMerieux) and Phoenix 100 (Becton Dickinson and Co.) correctly identified 13 of 15 and 12 of 13 isolates tested, respectively. Isolates from urine, tested by the CLSI disk diffusion method, were apparently susceptible or of intermediate susceptibility to vancomycin, upon primary testing. VITEK 2 and Phoenix 100 identified all isolates as vancomycin-resistant, although the MICs, measured by Etest, were in the susceptible range for three of 16 isolates. Reducing the vancomycin concentration in screening media substantially increased the sensitivity for detection of VRE. Isolates were characterized as genotype *van*B2/3 by PCR and were indistinguishable from each other by pulsed-field gel electrophoresis. VRE with low-level inducible resistance can be missed by routine screening methods. Better identification and screening methods for detection of low-level vancomycin resistance are needed to improve surveillance and prevent transmission of VRE.

Keywords enterococci, *Enterococcus faecium*, inducible vancomycin resistance, outbreak, *van* B, VRE

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INTRODUCTION

Enterococci are important nosocomial pathogens, and can cause significant morbidity and mortality in hospitalized patients [1]. Nosocomial transmission of vancomycin-resistant *Enterococcus faecium* (VRE) with moderate- to high-level resistance to vancomycin in haematology patients is well described [2–4]. *E. faecium* with low-level inducible vancomycin resistance has also been described as causing hospital outbreaks, but infrequently [5]. In Australia, VRE isolates were first detected in 1994 [6], and since then several sporadic and outbreak-related strains have been reported in patients from most Australian states [7–10]. The majority have been of the *van*B genotype with moderate - to high-level resistance to vancomycin (64–256 mg/L), but *E. faecium* carrying *van*A has also been reported [11,12]. VRE strains with the *van*B phenotype were originally described as having inducible low-level resistance to vancomycin but not teicoplanin [13]. However, glycopeptide-resistant enterococci carrying *van*B are considered to be phenotypically diverse, and typically show a wide range of vancomycin MICs, usually with moderate- to high-level resistance (64–1024 mg/L) [14]. Although clinical laboratories can reliably detect high-level resistance to vancomycin, there are reports of poor proficiency in the detection of low-level inducible resistance to vancomycin [15–17]. Several problems associated with screening,

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identification and susceptibility testing of a strain of *E. faecium* with low-level inducible vancomycin resistance and modifications to overcome them are described here.

METHODS

Patients and bacterial isolates

Westmead Hospital is a tertiary-referral hospital, providing care for patients in the greater western Sydney area; it includes a 23-bed haematology/bone marrow transplant (BMT) unit. In June–July 2004, vancomycin-resistant Gram-positive cocci, identified as *E. faecium*, were isolated from blood cultures of two patients in the haematology/BMT unit. Both patients had been treated with vancomycin for apparently susceptible enterococcal urinary tract infections immediately prior to developing bloodstream infections with VRE. This led to initiation of screening of all patients in the unit upon admission and weekly thereafter.

Identification of enterococci in blood and urine

Isolates obtained from blood cultures were identified to genus level by Gram staining, L-pyrrolindonyl-β-naphthylamide reaction, catalase reaction, and streptococcal grouping (Phadebact Strep D Test, Boule Diagnostics AB, Huddinge, Sweden). At the time of this outbreak, identification to species level and susceptibility testing were performed using the automated VITEK 2 system (BioMérieux VITEK Inc., Hazelwood, MI, USA). If this failed to provide definite identification, the API Rapid ID 32 Strep system (BioMérieux, Marcy l'Etoile, France) was used, according to the manufacturer's recommendations.

Isolates from urine were presumptively identified using chromogenic agar [18] (CHROMagar Orientation, Paris, France). Gram-positive cocci, which appeared as dark blue colonies on chromogenic agar, were reported as 'group D streptococci' if they were susceptible to ampicillin and vancomycin. They were identified to species level, as for blood culture isolates, if they were ampicillin-resistant or not fully susceptible to vancomycin.

Susceptibility testing

Susceptibilities to ampicillin, erythromycin, tetracycline, ciprofloxacin, high-level gentamicin, vancomycin and teicoplanin were determined using the VITEK 2 Gram-positive susceptibility card. For isolates that were not fully susceptible to vancomycin, the MICs of vancomycin and teicoplanin were determined by Etest (AB Biodisk, Solna, Sweden), using the high-inoculum method on Mueller–Hinton agar, according to the manufacturer's recommendations [19,20]. For urine isolates, antibiotic susceptibility testing was performed by disk diffusion, as recommended by the CLSI [21], and if not fully susceptible to vancomycin, MICs were determined by Etest.

Screening of patients for intestinal carriage of VRE

Perianal swabs were collected from all patients in the haematology/BMT unit, and inoculated into Todd–Hewitt broth and chromogenic agar, both supplemented with gentamicin and vancomycin. After 24 h of incubation, the Todd–Hewitt broths were subcultured onto non-selective chromogenic agar to assist in the detection of enterococci. The concentration of vancomycin used, initially, was 5.4 mg/L, or slightly less than the concentration (6 mg/L) recommended by the CLSI, based on local experience. Subsequently, the vancomycin concentration was reduced, when VRE screening of patients with proven VRE infections was persistently negative. The final vancomycin concentration was 4.3 mg/L. This allowed growth of the *E. faecium* strain involved in this outbreak, while preventing excessive overgrowth of other bacteria.

Further comparative testing of stored isolates

All bloodstream isolates are routinely stored in our laboratory, and those from the outbreak were retrieved for further testing. Urine isolates from the first two patients involved in this outbreak and one of the perianal isolates were not available for retesting. Thirteen stored VRE isolates were available for retesting. Species identification was attempted, using three methods: VITEK 2, the API Rapid ID 32 Strep system and Phoenix 100 (BD Phoenix Automated Microbiology System, Becton Dickinson and Co., Sparks, MD, USA), which was under evaluation in our laboratory at the time. They were also tested for susceptibility to vancomycin using VITEK 2, Phoenix 100 and Etest as described above.

Susceptibility to ampicillin, erythromycin, tetracycline, ciprofloxacin, gentamicin (high-level resistance) and teicoplanin was tested by VITEK 2 on all stored isolates.

Where multiple isolates were obtained from a single episode of infection or from repeated perianal swabs, only one was fully characterized. If isolates were obtained from different sites, then one isolate from each site was fully characterized.

Molecular testing

Vancomycin resistance genotypes were identified using the LightCycler VRE detection kit according to the manufacturer's instructions (Roche Diagnostics, Penzberg, Germany). This kit provides the primers and hybridization probes for the amplification and detection of *van*A, *van*B and *van*B2/3. Briefly, a 232-bp fragment of *van*A and a 187-bp fragment of *van*B were amplified by PCR from DNA extracted from colonies grown on horse blood agar. Each test run included a water blank as negative control and the positive control from the kit, which contained specific sequences of the *van*A and *van*B gene targets. All reactions included an internal control of plasmid DNA that acts as an extraction and inhibition control. Confirmation of the resistance genotype was determined by melt curve analysis using the Roche LightCycler. *Van*A and *van*B genotypes display characteristic melting (T_m) peaks at 67 ± 2.5°C and 60 ± 2.5°C, respectively.

Pulsed-field gel electrophoresis was performed using the CHEF-Mapper instrument (Bio-Rad Laboratories, Hercules, CA, USA), following digestion of genomic DNA with the restriction endonuclease *Sma*1 (New England Biolabs, Ipswich, MA, USA). The digested DNA underwent electrophoresis for 20 h at 14°C and 6 V/cm. Switch times were ramped from 0.47 to 21.79 s over the length of the run. Gel analysis was carried out using BIONUMERICS software (Applied-Maths, Kortrijk, Belgium). Gel comparison settings were fixed on the Dice coefficient to produce an unweighted pair group method using