

## HIGH-LEVEL, MULTIRESTANT ENTEROCOCCI ASSOCIATED WITH SYSTEMIC DISEASE: A REPORT OF TWO CASES

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We describe two patients who died from enterococcal systemic disease. The organisms were identified as *Enterococcus faecium*. One isolate was resistant to all available antimicrobials and the other was sensitive only to chloramphenicol. The recent emergence of multiple-resistant enterococcal species reflects the critically fragile status of antimicrobial therapeutics. The clinical implications and possible means of their control are discussed.

Although the term "enterocoque" was first used almost 100 years ago to emphasize the intestinal origin of these bacteria,<sup>1</sup> until recently they were classified in the genus *Streptococcus*, mainly because of their morphological resemblance and common biochemical tests. However, in 1984 it was shown by nucleic acid hybridization studies that *S. faecalis* and *S. faecium* were so distantly related to streptococci that they should be transferred to a new genus called *Enterococcus*.<sup>2</sup> These bacteria also differ significantly in their antimicrobial susceptibility patterns from other streptococci. Recently these organisms have drawn much attention, not only because of their increasing role in hospital-acquired infections but because of their increasing resistance to antimicrobial agents. Their intrinsic resistance or relative resistance to  $\beta$ -lactams, clindamycin and aminoglycosides, and ability to acquire resistance to ciprofloxacin, erythromycin and tetracycline is well known.<sup>3-6</sup> The most recent and most disturbing trait to emerge is resistance to vancomycin.<sup>7</sup> Three years ago we had reported the first case of wound infection caused by vancomycin-resistant *Enterococcus*.<sup>7</sup> In this paper we report two cases in which multiresistant enterococci were associated with systemic disease.

### Case 1

A 15-year-old male patient with acute myelogenous leukemia and neutropenia developed fever and was treated with ceftazidime 40 mg/kg q8h intravenously, amikacin 15 mg/kg daily, vancomycin 15 mg/kg q12h and amphotericin B 0.5 mg/kg daily for four weeks. He eventually defervesced,

but no organisms were isolated. He then received a regimen which included busulphan and total body irradiation for refractory relapsed acute leukemia prior to bone marrow transplantation. Although he was afebrile at the time of transplantation, he was profoundly neutropenic with a white count of  $<0.1 \times 10^9/L$ , and was therefore continued on the same broad spectrum antimicrobials. On the sixth day after the bone marrow transplant, he again became febrile; blood cultures taken 14 days post-transplantation grew *Enterococcus*, which was identified as *E. faecium*. Clinical management included catheter and line change, with the administration of imipenem 20 mg/kg q6h intravenously. The patient continued to be unwell, developing peritonitis and right lower lobe pneumonia in the presence of persistent neutropenia. Bone marrow examination showed a hypocellular marrow. The enterococcal isolate was highly resistant to all antimicrobials in clinical practice at this hospital (Table 1), including vancomycin, except chloramphenicol. Despite the administration of colony-stimulating factor, and the addition of intravenous chloramphenicol 500 mg q4h, the patient's course was inexorably downhill. He developed multi-organ failure and terminal cardiac arrest, from which he could not be resuscitated. He died on the 21st post-transplant day.

### Case 2

A 50-year-old male, with chronic hepatitis C virus infection, complicated by esophageal varices, had sustained a recent perforated duodenal ulcer, which was managed surgically with oversewing, vagotomy and pyloroplasty. He was admitted with a three-day history of abdominal pain, fever and jaundice. Examination showed an ill man with a temperature of 37.8°C. His blood pressure was 140/80 mmHg, he had a flapping tremor, with shifting dullness, compatible with the ascites. Investigations revealed a peripheral white cell count of 39,000  $\times 10^9/L$  with 85% polymorphs. Culture of the ascitic fluid and surgical wound swabs grew *E. faecium*. The organism was resistant to all antimicrobials, including vancomycin (Table 1). Despite initial antimicrobial treatment with ceftazidime and flagyl, later changing to imipenem and vancomycin, he deteriorated. He developed disseminated intravascular

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TABLE 1. Antimicrobial resistance in of *E. Faecium* isolates from two patients.

Antimicrobial	Minimum inhibitory concentration (mg/L) (Interpretation)	
	Case # 1	Case # 2
Ampicillin	>32 (Resistant)	>32 (Resistant)
Augmentin	>32 (Resistant)	>32 (Resistant)
Erythromycin	>16 (Resistant)	>16 (Resistant)
Clindamycin	>8.0 (Resistant)	>8.0 (Resistant)
Chloramphenicol	8.0 (Susceptible)	32.0 (Resistant)
Ciprofloxacin	8.0 (Resistant)	8.0 (Resistant)
Trimethoprim-sulfamethoxazole	>16/304 (Resistant)	8/152 (Resistant)
Ceftriaxone	>32 (Resistant)	32 (Resistant)
Ceftazidime	>32 (Resistant)	16 (Resistant)
Gentamicin	>128 (Resistant)	8.0 (Resistant)
Netilmycin	>128 (Resistant)	8.0 (Resistant)
Amikacin	>64 (Resistant)	8.0 (Resistant)
Tetracycline	>32 (Resistant)	32 (Resistant)
Imipenem	8.0 (Resistant)	8.0 (Resistant)
Vancomycin	>128 (Resistant)	64 (Resistant)

coagulation with uncontrolled bleeding, and ultimately multiorgan failure, from which he did not recover, and eventually died.

### Microbiological Studies

The bacteria were isolated from blood of the first patient using BacTAlert system (Organon Teknika, Durham, NC, USA) and from the wound and ascetic fluid of the second patient on 5% sheep blood agar and McConkey agar and were identified as per standard procedure.<sup>9</sup> Bacteria were identified as *E. faecium* by conventional methods (bile-esculin +, 6.5% NaCl tolerance +, arginine +, arabinose + and sorbitol negative) and was confirmed by the GPI (Gram-positive identification) system (Vitek Systems, Inc., Hazelwood, MO, USA). Initial susceptibility to antimicrobial agents was tested by disk-diffusion test according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).<sup>10</sup> Minimum inhibitory concentration (MIC) of *Enterococcus* against vancomycin was determined by Vitek Systems and confirmed by standard broth dilution method.<sup>11</sup> For MIC determination, Mueller-Hinton broth, pH 7.2-7.4, was used as the test medium and inoculated with the turbidity-adjusted suspension of the isolate to achieve a final inoculum

of  $5 \times 10^5$  CFU/mL.<sup>11</sup> *S. faecalis* ATCC 29212 was used as a quality control organism during MIC determinations. Results were found to be within expected ranges. MIC for various antimicrobials for the two patients is shown in Table 1.

### Discussion

Enterococci are normal flora of the human bowel and previously had been regarded as organisms of low virulence, but associated with bacteriuria, intra-abdominal sepsis and endocarditis. Over the last 10-15 years a number of major changes have occurred with regard to these organisms, which include a new genus and emergence of new traits of drug resistance. In some reports from the USA, enterococci are now the second most common nosocomial pathogen, with reporting rates increasing substantially in the last 10-15 years.<sup>4</sup> A number of pathoepidemiological mechanisms have emerged over this period of time, including the ability of the organism to translocate to extragastrintestinal sites such as lymph nodes, competition with and displacement of other bacteria and spread via the hands of hospital staff, which may lead to nosocomial outbreaks. However, the most important change, and cause for great concern, is the escalating phenomenon of antimicrobial resistance. Resistance to antimicrobials in the enterococci is mediated in a number of ways. These include modification of penicillin-binding protein,<sup>3</sup>  $\beta$ -lactamase production,<sup>4</sup> the development of aminoglycoside-modifying enzymes,<sup>3</sup> and vancomycin resistance.<sup>6-8</sup>

Vancomycin was introduced in 1956 and for nearly 30 years enjoyed an enviable status, exemplified by absence of development of resistance to the drug. Isolated cases, however, of resistance in animal species were reported, and in the late 1980s, cases of vancomycin-resistant enterococci emerged from Europe and the USA.<sup>6,7</sup> In our own laboratory, nearly 3000 isolates of enterococci have been screened and no resistance was seen until early 1992.<sup>8</sup>

The epidemiological factors responsible for the emergence of these resistance strains include extension of hospital stay, prolonged duration of antimicrobial administration, including oral vancomycin therapy and association with hemodialysis units (possibly related to the prophylactic use of vancomycin for the insertion of lines and shunts).<sup>12</sup> Efforts at reducing these pressure factors may reduce the incidence of resistance in enterococci. These include restriction of the inappropriate use of vancomycin, particularly its prolonged use, revision of the need for prophylactic vancomycin use, and utilization of metronidazole rather than vancomycin for treating antibiotic-related diarrhea and *Clostridium difficile* diarrhea. Although the first patient reported in this paper had prior treatment with vancomycin for four weeks before developing VRE bacteremia, the second patient had no available history of vancomycin administration. The two strains of VRE were distinct from each other and were not isolated from any other patients or environmental cultures of oncology or surgical

units. We would also like to emphasize that the oncology and surgery units are on two different floors and not in close proximity. Furthermore, VRE infection occurred in the second patient several months after the death of the first patient. Since we do not have an existing problem of VRE in this hospital, we believe that the organisms were distinct strains that were derived from the patient's own endogenous flora.

Although Uttley et al.<sup>17</sup> found that most resistant strains remained sensitive to other drugs such as ampicillin and ciprofloxacin, other reports had suggested the occurrence of multi-resistant organisms. The isolation of truly multi-resistant organisms from two of our patients is a cause for concern. The emergence of multi-resistant enterococci to all approved classes of antimicrobial agents is now an established, but as yet uncommon, phenomenon. However, it is a critical issue, since many of these patients are refractory to antimicrobial treatment and usually die.<sup>13,14</sup> Alternative antimicrobial strategies are limited. Combination antimicrobial therapy might prove useful; for example, amoxicillin with vancomycin, or imipenem with vancomycin. However, in our two patients, rapid downhill course of the illness did not permit us to try the above synergistic regimens. Drug combination therapy has often been used to effect clinical cure in patients with enterococcal disease, but even this is now failing.<sup>15</sup> New antimicrobials such as the fluoroquinolones, ramoplanin, tetracycline analogues, quinupristin/dalfopristin and "Elongation Factor" may prove useful in the future.<sup>16</sup> *In vitro* studies in this institution have shown that both the VRE isolates described in this paper were inhibited by four mg/L of quinupristin/dalfopristin.<sup>19</sup> Recently Mekonen et al.<sup>18</sup> reported the successful treatment of persistent bacteremia due to vancomycin-resistant (MIC >16 mg/L), ampicillin-resistant (MIC >64 mg/L) *E. faecium* in a liver transplant recipient. They used very high doses of continuous infusion ampicillin/subactam, plus gentamicin after the patient remained bacteremic on high-dose ampicillin and gentamicin. Brandt et al.<sup>20</sup> recently described the effective treatment of experimental VRE endocarditis in rabbits with the combination of ampicillin and imipenem, even though the strain of *E. faecium* used had high-level resistance to vancomycin and moderate intrinsic resistance to ampicillin and imipenem. Based on their experiments, they concluded that the combination therapy with cell-wall active agents like ampicillin and imipenem may be effective for "some strains of multi-drug-resistant enterococcal infections."

We concur with the suggestions of the Hospital Infection Control Practices Advisory Committee<sup>21</sup> that prevention and control of the spread of VRE will require coordinated efforts from various hospital departments and can only be achieved by education of hospital staff regarding the problem of vancomycin resistance, prudent use of antimicrobials, early detection and reporting and immediate implementation of appropriate infection control measures. So far, the published

outbreaks of vancomycin-resistant enterococci have been controlled rather easily by strict adherence to barrier applications. However, given the inability of most hospitals to eradicate methicillin-resistant *S. aureus*, and aminoglycoside- and ampicillin-resistant enterococci, there is little reason to become sanguine. In the meantime, assiduous handwashing, use of gloves and cohorting of colonized or infected cases may prove beneficial in reducing nosocomial spread. However, the first step is to discourage clinicians from using vancomycin promiscuously.

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