Substantially increased faecal carriage of vancomycin-resistant enterococci in a tertiary Greek hospital after a 4 year time interval

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Received 27 February 2004; returned 14 April 2004; revised 19 April 2004; accepted 21 April 2004

Objectives: In a tertiary Greek hospital with no documented vancomycin-resistant enterococci (VRE) infections, a cross-sectional study was conducted in order to determine the degree of VRE faecal carriage among adult patients hospitalized in high-risk units.

Methods: Specimens for the surveillance were collected from separate patients in two periods (January–May 1999 and January–May 2003); 258 specimens were submitted during the first period and 149 during the second period.

Results: Three patients (1.2%) were colonized with VRE during the first period, whereas 52 (34.9%) were colonized during the second period. Two VRE isolates of the first period were Enterococcus faecalis and one Enterococcus faecium, whereas those of the second period were E. faecium except for three E. faecalis and two Enterococcus gallinarum. All VRE isolates apart from the two E. gallinarum isolates were positive for the vanA gene. The 48 vancomycin-resistant E. faecium were classified into eight clonal types, one of those predominating with 29 isolates; the remaining included one to nine isolates. The five vancomycin-resistant E. faecalis formed four distinct clonal types.

Conclusions: The study reports a substantially higher prevalence of VRE carriage when the surveillance was repeated after a 4 year time interval. Urgent infection control measures are needed to prevent emergence of VRE outbreaks in our hospital setting.

Keywords: VRE, surveillance, genotyping

Introduction

In recent years, enterococcal infections have become a major therapeutic challenge because of their increased incidence and the spread of strains that have acquired resistance to several antimicrobial classes. Strains of vancomycin-resistant enterococci (VRE), causing infections or colonizing hospitalized patients, were first detected in Europe in 1986 and in a short period have become common, mainly in the United States. The emergence of nosocomial outbreaks due to VRE has raised serious concerns, and in response, recommendations for preventing the spread of VRE have been developed. Furthermore, in hospitals where VRE have not yet been recovered from clinical infections, periodic culture surveys of stools or rectal swabs of patients at high risk for VRE infection or colonization are indicated.

In Greek hospitals, unlike others in the United States and Europe, VRE causing clinical infections have only recently been recognized and outbreaks of vancomycin-resistant Enterococcus faecium and Enterococcus faecalis have been reported from hospitals in the region of Athens. However, no systematic study has been done to estimate the prevalence of VRE colonization in hospitalized patients. In that respect, a cross-sectional survey was conducted in a tertiary Greek hospital with no documented VRE infections, in order to determine the degree of VRE colonization among hospitalized patients.

Materials and methods

Study design

The study was conducted at Hippokration University Hospital, Thessaloniki, Greece. This is the largest tertiary referral hospital in Northern Greece having 1012 beds (51 with acute facilities) and more than 55,000 admissions each year. Specimens for the study...
were collected in two periods. The first period was from January through May 1999 and the second period from January through May 2003. In the study were included adult patients randomly chosen from those hospitalized for more than 48 h and less than 5 days in eight high-risk units (renal, transplant, oncology and intensive care units).

**Culture and identification**

Faecal samples were diluted with sterile saline and plated onto enterococcal agar (BBL Microbiology Systems, Cockeysville, MD, USA) with and without 6 mg/L of vancomycin. Plates were incubated at 35°C and read after 24 and 48 h of incubation. From each sample, colonies showing macroscopically morphological differences and whose colony morphology was consistent with that of enterococci were subcultured and characterized as enterococci by additional tests (salt tolerance, growth on bile-aesculin agar, catalase activity). Identification to species level was carried out with the automated Vitek system (bioMérieux, Marcy l’Étoile, France). Identification of VRE was also confirmed by PCR analysis using specific primers described by Dutka-Malen et al.\(^7\)

**Susceptibility testing**

For all distinct enterococcal isolates that grew on the screening agar supplemented with 6 mg/L of vancomycin, MICs of vancomycin and teicoplanin were determined by an agar dilution method.\(^8\) Interpretative criteria for susceptibility status were those of the NCCLS.\(^8\) The Vitek system was used in addition to determine susceptibility to a range of antimicrobials (ampicillin, ciprofloxacin, erythromycin, gentamicin, streptomycin and tetracycline). *E. faecalis* ATCC 29212 was used for quality control.

**Genotyping testing**

Isolates for which the vancomycin MIC was >4 mg/L were analysed for the presence of the *vanA*, *vanB*, *vanC1*, *vanC2* and *vanE* gene by PCR using primers and conditions that were described previously.\(^7,9\) Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA was carried out with a contour-clamped homogeneous electric field apparatus (CHEF DRIII apparatus; Bio-Rad Laboratories, Hemel Hempstead, UK) and banding patterns of the strains were compared visually.\(^10\) Ten glycopeptide-sensitive *E. faecium* isolates, which were recovered during the study periods, were also chosen at random and used as controls.

**Results**

A total of 258 specimens were submitted from separate patients during the first period and a total of 149 specimens during the second period of the surveillance. At least one enterococcal isolate was obtained from 219 (84.9%) patients during the first period and 132 (88.6%) patients in the second period; from 12 patients, two distinct enterococcal isolates were recovered on the basis that they belonged to different species or had different biochemical or antibiotic resistance profiles. Five different species were identified of which *E. faecalis* was the most prevalent, accounting for 244 (67.2%) of the isolates whereas *E. faecium* was found in 101 instances (27.8%), the remaining isolates comprising other enterococcal species.

Three of the 258 patients (1.2%) were colonized with VRE during the first period, whereas 52 (34.9%) were colonized during the second period. Two VRE isolates of the first period were identified as *E. faecalis* and one as *E. faecium* whereas

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Unit of isolation</th>
<th>Resistance phenotype</th>
<th>PFGE type</th>
<th>Period of isolation</th>
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<td>VIII</td>
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\(^a\)AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; GEN, high-level gentamicin resistance; STR, high-level streptomycin resistance; TEC, teicoplanin; TET, tetracycline; VAN, vancomycin.

\(^b\)A, January–May 1999; B, January–May 2003.
47 VRE that were recovered during the second period were identified as *E. faecium* isolates; of the remaining isolates, three were *E. faecalis* and two were *Enterococcus gallinarum*. In the selective medium containing vancomycin, all VRE isolates were recovered and in addition 13 enterococcal isolates that were found intermediate (MIC 8–16 mg/L) or susceptible (MIC <8 mg/L) to vancomycin by the agar dilution method. The MICs of vancomycin for the VRE ranged from 32 to >256 mg/L and those of teicoplanin ranged from 4 to 128 mg/L. Most of the vancomycin-resistant *E. faecium* exhibited resistance to ampicillin (95.8%) and high levels of streptomycin (87.5%) and gentamicin (60.4%) (Table 1). In addition, seven enterococci that were identified as *E. gallinarum* (six isolates) or *Enterococcus casseliflavus* (one isolate) were vancomycin intermediate.

PCR analysis revealed that all VRE isolates except the two *E. gallinarum* isolates were positive for the vanA gene and negative for the remaining van genes. It confirmed also that all vancomycin-resistant or vancomycin-intermediate *E. gallinarum* isolates had the vanC1 gene whereas the vancomycin-intermediate *E. casseliflavus* isolate had the vanC2 gene. According to their macrorestriction profiles, the 48 vancomycin-resistant *E. faecium* were classified into eight distinct clonal types whereas the five vancomycin-resistant *E. faecalis* formed four distinct clonal types. Of the vancomycin-resistant *E. faecium* isolates, 29 exhibited a common profile whereas the remaining patterns were more sporadic (one to nine isolates each) (Figure 1). Macrorestriction profiles of the 10 vancomycin-susceptible *E. faecium* isolates yielded totally different profiles from those of the vancomycin-resistant *E. faecium* isolates.

**Discussion**

Active surveillance for VRE colonization in high-risk patients may result in lower VRE infection rates and a more polyclonal population by identifying a need for stricter infection control. This study documents in two periods of time the prevalence of intestinal colonization among high-risk patients from a Greek tertiary hospital with no documented VRE infections. The patients included in the study were at high risk of VRE carriage due to underlying disease, neutropenia, length of hospital stay and prolonged antimicrobial treatment. To our knowledge, this is the first study to be carried out in two different periods of time and reports an almost 30-fold higher prevalence of VRE after a 4 year time interval. This surprisingly increased frequency was due largely to the spread of multi-resistant vanA-positive *E. faecium* clonal strains. This spread could have been facilitated by the extensive use of glycopeptides for treating methicillin-resistant *Staphylococcus aureus* (MRSA) which is commonly isolated in our hospital. It has been stated that the ratio of VRE-infected to VRE-colonized patients is dependent on the specific patient population. It is of interest that in this study, whereas no hospitalized patient developed infection due to VRE during the first period, eight vancomycin-resistant *E. faecium* were recovered from clinical infections of separate patients during the second period; three of them belonged to the predominant PFGE subtype of this study (data not shown).

The prevalence of VRE during the second period of the study seems closest to the levels that were observed recently among high-risk patients in the United States but higher than that reported among patients admitted to at-risk wards of European hospitals. However, comparison of the data is very difficult and should be done cautiously since the populations studied differ not only in age and sex but also in many clinical parameters. The design of a study (only specimens from high-risk units) may also overestimate the overall prevalence of VRE in the hospital setting. In addition, several reports count as VRE enterococcal isolates that exhibit intermediate-level resistance to vancomycin. It must also be mentioned that detection methods are not the same and in several studies, enrichment methods have been used increasing rates of VRE isolation.

It has been suggested that if VRE are not controlled soon after introduction into a hospital, the first sporadic cases of colonization may evolve into hospital outbreaks, which can be especially difficult to control. Therefore, a reduction in the clinical use of vancomycin as well as other antibiotics (metronidazole, clindamycin and cephalosporins) that can increase rate of VRE carriage has been impressed upon our clinicians. In addition, the observation that several VRE were clonal in PFGE analysis, emphasizes the need for urgent infection control strategies and more extensive surveillance of enterococcal carriage and infections. The use of newer antimicrobials and other strategies to control the emergence of VRE should be considered urgent in the attempt to control the spread of this pathogen.
measures in order to prevent the emergence of clinical infections due to VRE in our hospital. Further studies to determine the presence of putative virulence properties such as the enterococcal surface protein Esp in the identified VRE clones will be important in continuing to monitor this situation.

References