Dissemination of vancomycin-resistant enterococci among haemodialysis patients in Athens, Greece

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Objectives: Vancomycin-resistant enterococci (VRE) may colonize haemodialysis patients, but their epidemiology in this population is not well defined. Within the few last years, VRE strains have emerged and are increasingly isolated in the nosocomial environment in Greece, but colonization of dialysis patients has never been evaluated before. This study sought to determine the epidemiology of VRE colonization within this high-risk population and define the risk factors.

Materials and methods: During a 4 month period, rectal swabs or faecal specimens were collected from 334 consecutive outpatients, who were treated at four independent dialysis units located in the same area of Athens and referring patients to the same local hospital. The relatedness of isolates was defined by molecular typing, and demographic and clinical patient data were recorded.

Results: Thirteen multiresistant Enterococcus faecium vanA strains were isolated corresponding to a colonization frequency of 3.9%. They were separated into seven clusters: type A (two strains), type B (six strains) and types C to G (one strain each). Type B strains originated from three units, while a single unit demonstrated four type B and two type A strains. Univariate statistical analysis revealed that prior hospitalization ($P < 0.001$), prior administration of antimicrobials ($P < 0.026$) and male gender ($P = 0.019$) were associated with VRE colonization.

Conclusions: In Greece, haemodialysis patients are colonized with VRE at a low frequency. The predominance of one clone and its isolation from several units strongly indicate interfacility transmission of strains, most probably within a health care environment shared by all patients.

Keywords: epidemiology, colonization, surveillance cultures

Introduction

Enterococcal strains with acquired resistance to vancomycin (vancomycin-resistant enterococci, VRE) were first described in 1986 and, since then, they have been encountered with increasing frequency in many countries.1,2 It has been shown that the incidence of VRE intestinal colonization or infection depends upon geographical parameters.2

In the United States, infections and nosocomial outbreaks due to multiresistant VRE constitute a particularly serious problem, but colonization in the community is rarely, if ever, reported. The emergence and dissemination of VRE in the USA have been associated with a large consumption of vancomycin and other antimicrobials and lack of implementation of appropriate infection control practices.1,2 A different situation prevails in Europe. VRE carriage in the community and VRE isolation from the food chain occur frequently in countries where antimicrobials like avoparcin have been used in animal husbandry, but VRE hospital outbreaks occur infrequently and serious infections are not common.1

In Greece, the first VRE isolate was reported in 1999, and within 2 years a polyclonal nosocomial dissemination of multiresistant vanA Enterococcus faecium strains was described.4 Recently, an ICU outbreak due to a vanA Enterococcus faecalis...
clone was reported, while surveillance cultures of high-risk inpatients in another hospital demonstrated an increase in VRE carriage from 1.2% to 34.9% within 4 years. Thus, in contrast to other European countries, there has been a relatively late emergence of VRE followed by a rapid dissemination, while the occurrence of community carriage has never been assessed.

Several risk factors have been identified for VRE colonization or infection including chronic haemodialysis treatment. In the USA, 32.7% of dialysis units report at least one colonized or infected patient. VRE-colonized haemodialysis patients are not only more likely to develop a VRE infection, but also demonstrate decreased survival. It is important, therefore, to elucidate their mode of VRE acquisition and define colonization risk factors, but only a few studies have examined this high-risk population.

As VRE strains are emerging in Greece, we investigated for the first time their possible detection among haemodialysis patients. We searched for VRE colonization among outpatients treated in four independent dialysis units located in the same district of Athens and referring their patients to the same local hospital. Isolates were characterized phenotypically and genotypically, they were typed by pulsed-field gel electrophoresis (PFGE) and clinical and demographic data were recorded to define colonization risk factors.

Materials and methods

During a 4 month period (September to December 2001), 334 non-replicate swab specimens were obtained from consecutive haemodialysis patients consenting to participate in the study. They were treated at four dialysis units (Units I to IV) located in the same district of Athens and referring their patients to the same local hospital. More than 90% of haemodialysis patients of this district are treated in Unit IV (seven out of 147, 4.8%) and two originated from each of the other three units (Unit I: two out of 60, 3.3%; Unit II: two out of 67, 3.0%; and Unit III: two out of 60, 3.3%). All were resistant to ampicillin, erythromycin, ciprofloxacin and streptomycin and three were also resistant to gentamicin.

DNA fingerprinting allocated the 13 VRE isolates into seven distinct PFGE types designated A to G (Figure 1). Type B was the predominant type being represented by six isolates (46.2%), type A was represented by two isolates (15.4%), while there was one isolate each of types C to G (7.7% each). Two isolates of distinct types were obtained from Unit I (one type E, one type F), Unit II (one type B, one type D) and Unit III (one type B, one type G), while seven isolates representing three types originated from Unit IV (two type A, four type B and one type C).

The characteristics of VRE-colonized and non-colonized patients are depicted in Table 1. VRE-positive patients were more likely to be males (92.3% versus 55.5%, \( P = 0.019, OR 4.199 \)). These patients were more likely to have been hospitalized during the last 6 months (61.5% versus 18.1%, \( P = 0.001, OR 7.255 \)) and to have received any antimicrobial treatment during this period (69.2% versus 34.9%, \( P = 0.026, OR 4.199 \)).

Because of the association between male sex and VRE colonization, a statistical analysis was undertaken to evaluate the corresponding isolates could be epidemiologically related. Statistical analysis was carried out with \( t \)-tests, and \( \chi^2 \) and Fisher exact tests using the SPSS software package.

This study was considered by the hospital scientific committee as adhering to ethical standards and was therefore approved.

Results

Enterococcus species were isolated from 60 patients. By multiplex PCR, 31 (51.7%) and 16 (26.7%) isolates were E. gallinarum and E. casseliflavus, respectively, whilst 13 (21.7%) were E. faecium. All 13 E. faecium strains possessed the vanA gene and were resistant to vancomycin and teicoplanin by the Etest. Hence, the frequency of VRE colonization was 3.9% (13 out of 334 patients).

Seven of the 13 VRE strains were isolated from patients treated in Unit IV (seven out of 147, 4.8%) and two originated from each of the other three units (Unit I: two out of 60, 3.3%; Unit II: two out of 67, 3.0%; and Unit III: two out of 60, 3.3%). All were resistant to ampicillin, erythromycin, ciprofloxacin and streptomycin and three were also resistant to gentamicin.

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Vancomycin-resistant enterococci and haemodialysis

Table 1. Univariate analysis of demographic and clinical data among 334 haemodialysis patients colonized or not colonized with VRE

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>VRE positive (n = 13)</th>
<th>VRE negative (n = 321)</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>12 (92.3%)</td>
<td>178 (55.5%)</td>
<td>0.019</td>
<td>9.64</td>
<td>1.284–200.779</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>60.85 ± 11.92</td>
<td>63.29 ± 14.24</td>
<td>0.485</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean time on haemodialysis (months)</td>
<td>23.54 ± 24.60</td>
<td>38.73 ± 42.07</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital admission a</td>
<td>8 (61.5%)</td>
<td>58 (18.1%)</td>
<td>0.001</td>
<td>7.255</td>
<td>2.056–26.639</td>
</tr>
<tr>
<td>Administration of antimicrobials a</td>
<td>any antimicrobial</td>
<td>9 (69.2%)</td>
<td>112 (34.9%)</td>
<td>0.026</td>
<td>4.199</td>
</tr>
<tr>
<td>vancomycin</td>
<td>5 (38.5%)</td>
<td>60 (18.7%)</td>
<td>0.159</td>
<td>2.719</td>
<td>0.742–9.591</td>
</tr>
<tr>
<td>other than vancomycin</td>
<td>4 (30.8%)</td>
<td>52 (16.2%)</td>
<td>0.318</td>
<td>2.299</td>
<td>0.571–8.589</td>
</tr>
</tbody>
</table>

a During the last 6 months.

In this study, 13 (3.9%) of 334 patients treated at four dialysis units were VRE carriers. Colonization prevalence ranged from 3.0% to 4.8% among units. All VRE strains were multiresistant vanA E. faecium and formed seven PFGE clusters. The most predominant type, B, was represented by six strains and was isolated from three units. In addition, six patients treated in a single unit shared two distinct genetic VRE types: two type A and four type B.

VRE colonization rates among haemodialysis patients have been evaluated before.8–12 High frequencies ranging from 8.1% to 9.5% were demonstrated in three studies from the USA,8–10 while lower numbers were reported by Tokars et al.10 examining patients from seven unrelated units in Maryland and Virginia. In this study, VRE colonization prevalence was 5.8% ranging from 1.0% to 7.9% among units. Clustering was demonstrated in one unit, where among eight VRE isolates, three and two strains, respectively, belonged to distinct genetic types.

Only one European study searched for VRE colonization among haemodialysis patients in Belgium, a country where community carriage is known to occur.11 Patients from 29 units were examined and colonized patients were identified in all except for two, while colonization rates ranged from 5.6% to 23.4%.12 These high rates were partly attributed to the applied methodology, broth enrichment and prolonged incubation time, which increases isolation yields compared with direct plating. Molecular typing of isolates revealed a high genetic variability pointing to their community origin rather than spread among patients.

Finally, a prospective study from Tennessee demonstrated that haemodialysis patients admitted to a hospital with very high VRE isolation rates acquired a VRE strain from an epidemiologically linked patient colonized with an identical genotype.13

This first report from Greece demonstrates a low prevalence of VRE-positive patients (3.9%), consistent with the recent emergence of VRE in this country. Our results strongly indicate that VRE transmission occurred between patients treated at independent units. This evidence of interfacility transmission points to a common source of acquisition, most probably a health care setting visited by all patients. Although the location of previous hospitalizations was not recorded to conclusively prove our hypothesis, this could only be the local hospital, to which patients are referred. VRE strains have been isolated in this hospital, but they were not available for typing. Finally, and similar to the findings of Tokars et al.,10 sharing of two distinct genetic types occurred among patients of a single unit raising concerns about intrafacility VRE transmission.

The analysis of putative risk factors for VRE colonization demonstrated that male gender (P = 0.019), prior hospitalization (P = 0.001) and prior antimicrobial treatment (P = 0.026) were significant factors. VRE colonization has already been linked to prior hospitalization among dialysis patients.8–10 Most probably reflecting VRE acquisition within the hospital and/or being related to the multitude of risk factors operating within this environment. A positive association between VRE colonization of patients and previous antimicrobial administration is established.1,2 Among dialysis patients, the receipt of vancomycin only, or other antimicrobials only, or any antimicrobial during a time period ranging from 90 days to 2 years were significant risk factors for VRE colonization.9–11 A predominance of male sex among colonized patients has never been reported before, and it remains to be shown whether it reflects some other significant association not assessed in our study, such as, for example, the impact of disease severity.11

In conclusion, haemodialysis patients in Greece are colonized with VRE strains at a low frequency and there is strong evidence of interfacility transmission of strains. Our findings supplement previous observations from Greece demonstrating that multiresistant VRE isolates are rapidly spreading within the health care environment. The importance of restricting the use of antimicrobials and adhering strictly to appropriate infection control practices cannot be overemphasized. Further, prospective studies for a more precise elucidation of VRE acquisition modes and definition of risk factors within this high-risk population are in progress.

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References