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Brief report

Vancomycin-resistant enterococci among haemodialysis patients in Portugal: Prevalence and molecular characterization of resistance, virulence and clonality

Susana Correia^{a,b,c,d}, Pedro Ponce^e, Daniela Jones-Dias^f, Manuela Caniça^f, Gilberto Igrejas^{a,b}, Patrícia Poeta^{c,d,*}

^a Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

^b Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

^c Centre of Studies of Animal and Veterinary Sciences, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

^d Veterinary Science Department, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal

^e Fresenius Medical Care, Nephrocare Lumiar, Rua José da Costa Pedreira n.º 8, 1750-130 Lisboa, Portugal

^f National Reference Laboratory of Antimicrobial Resistances, Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal

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ABSTRACT

Introduction: Vancomycin-resistant enterococci (VRE) among haemodialysis patients has increased rapidly and, to date, there is no report of this incidence in Portugal.

Methods: A total of 121 faecal samples were collected from haemodialysis patients, and then tested for VRE. Antimicrobial resistance, virulence and multilocus sequence typing (MLST) were studied.

Results: VRE prevalence was 3.3%. Three VRE isolates, *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus raffinosus*, were multi-resistant and vanA-positive. *E. faecium* and *E. faecalis* belonged to CC17 and CC2, respectively.

Conclusion: Haemodialysis patients in Portugal are colonized with virulent, multi-resistant enterococci from high-risk clonal complexes, representing a public health concern.

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Enterococos resistentes a la vancomicina en pacientes de hemo-diálisis en Portugal—prevalencia y caracterización molecular de la resistencia, virulencia y clonalidad

RESUMEN

Introducción: La prevalencia de Enterococos Resistentes a la Vancomicina (ERV) en pacientes de hemo-diálisis ha aumentado rápidamente en los últimos años. Hasta la fecha no existe ningún informe sobre esta incidencia en Portugal.

Métodos: Se han tomado 121 muestras fecales de pacientes en hemodiálisis y se han analizado para la presencia de ERV. También se han tomado datos de resistencia a los antimicrobianos, virulencia y MLST. **Resultados:** La prevalencia de ERV fue del 3,3%. Tres aislamientos de ERV: *Enterococcus faecium*, *E. faecalis* y *E. raffinosus*, resultaron ser multirresistentes y vanA-positivos. *E. faecium* y *E. faecalis* fueron adscritos a los CC17 y CC2, respectivamente.

Conclusión: Los pacientes sometidos a hemodiálisis en Portugal son colonizados por enterococos virulentos y multirresistentes de complejos clonales de alto riesgo, lo que representa un problema de salud pública.

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Introduction

One of the first reports of an infection caused by a vancomycin-resistant *Enterococcus* (VRE) came from a chronic haemodialysis unit in England in 1988 and since then, the prevalence of VRE in patients who undergo haemodialysis has increased rapidly.¹

* Corresponding author.

E-mail address: ppoeta@utad.pt (P. Poeta).

Haemodialysis patients have played a major role in the epidemic of vancomycin resistance because this drug is commonly used in this kind of treatment.² Given the rapid growth of the haemodialysis population, the frequency of infection and the impact of antimicrobial resistance on morbidity and mortality rates, it is of great importance to control the spread of antimicrobial-resistant pathogens among these patients.¹ The recent fast spread of VRE is a global threat to public health, not only because of treatment problems, but also because of the potential for vancomycin resistance genes to spread into more virulent pathogens.³ Epidemiological data from the couple last decades have documented the emergence of enterococci as important nosocomial pathogens, exemplified by the expansion of major hospital-adapted polyclonal subclusters and paralleled by the increase in glycopeptide resistance and high-level resistance to aminoglycosides, which represent the remaining therapeutic options since penicillin resistance emerged.⁴ According to the latest report of the European Antimicrobial Resistance Surveillance Network, Portugal was, in 2011, the third country with the highest rate of vancomycin resistant *Enterococcus faecium* (20.2%) and the fourth concerning *Enterococcus faecalis* (3.7%); these are very high rates considering the prevalence of 1.5% for *E. faecium* and 0.1% for *E. faecalis* in the neighbour country Spain and the average vancomycin resistance prevalence of 7.2% for *E. faecium* and 1% for *E. faecalis* in the total of 29 European countries.⁴

To date, there is no report of VRE prevalence among haemodialysis patients from Portugal. Hence, the aim of this study was to determine VRE colonization in faecal samples of haemodialysis patients from Portugal, and also to characterize the isolates regarding resistance to other clinically important antimicrobials, virulence factors and clonality.

Methods

One faecal sample per patient was collected from a total of 121 haemodialysis patients, between September and December 2010, from one of the largest dialysis clinics in Portugal (Nephrocare Lumiar, Lisbon). Ethical approval was obtained from the ethics committee of Nephrocare Lumiar clinic and all participants in the study provided informed consent. Each sample was transported to the laboratory in a Carry-Blair medium, seeded on Slanetz-Bartley agar plates supplemented with 4 mg/mL of vancomycin and incubated for 48 h at 35 °C. Colonies with typical enterococcal morphology were selected and identified to the genus and species level by cultural characteristics, Gram-staining, catalase test, bile-esculin reaction and biochemical tests, using the API 20 Strep system (BioMérieux, La Palme, France). Identification was also confirmed by 16S ribosomal RNA sequence analysis. Antimicrobial susceptibility was tested by disc diffusion for 11 antimicrobials (vancomycin, teicoplanin, ampicillin, gentamicin, streptomycin, kanamycin, chloramphenicol, tetracycline, erythromycin, quinupristin–dalfopristin and ciprofloxacin) following the Clinical and Laboratory Standards Institute (CLSI) criteria.⁵ High-level resistance was considered for aminoglycosides. Vancomycin resistance genes, *vanA*, *vanB*, *vanC1* and *vanC2/3*, were tested by polymerase chain reaction (PCR) in isolates with resistance or reduced susceptibility for glycopeptides.⁶ Macrolide [*erm*(A), *erm*(B), *erm*(C)], streptogramin [*vat*(D) and *vat*(E)], tetracycline [*tet*(M), *tet*(L)] and aminoglycoside [*aph*(3')-IIIa, *aac*(6')-*aph*(2''), *ant*(6)-Ia] resistance genes were screened by PCR in the isolates that showed resistance to these agents.⁶ The presence of virulence genes (*ace*, *gelE*, *fsr*, *cpd*, *esp*, *hyl*, *agg* and *cylL*_{L5ABM}) was also studied.⁶ *E. faecium* and *E. faecalis* isolates were characterized by multilocus sequence typing (MLST).⁶

Results and discussion

Vancomycin-resistant enterococci were detected in four of the 121 analyzed faecal samples (3.3%); this is the first report of VRE colonization rates among haemodialysis patients in Portugal. Nonetheless, VRE prevalence among dialysis patients has been evaluated before elsewhere; Kalocheritis and co-workers⁷ compared several studies from the USA that reported VRE frequencies ranging from 5.8% to 9.5%. In Europe, the VRE prevalence reported in Belgium was 33.2%³ and 13% in Ireland⁸; a much lower prevalence was reported in Greece (3.9%),⁷ which is similar to the results obtained in our study (3.3%). However, the use of enrichment techniques for VRE detection may have influenced the measured prevalence in the different studies.³

Three of the four detected VRE isolates, identified as *E. faecium*, *E. faecalis* and *Enterococcus raffinosus*, exhibited the acquired *vanA* type of resistance; the remaining isolate was identified as a naturally vancomycin resistant *vanC1*-harbouring *Enterococcus gallinarum* that carried important resistance [*tet*(M), *tet*(L), *erm*(B)] and virulence (*hyl*, *cylL*, *cylS*) genes (Table 1). All of these species cause human enterococcal infections; *E. faecalis* causes 80–90% and *E. faecium* causes the majority of the remainder. *E. raffinosus* is a rarely encountered species, which is often misidentified by the widely used identification systems. The presence of the *vanA* gene is of clinical relevance, because it is generally located on mobile genetic elements and already proved its capability to be transferred to other more virulent and potentially pathogenic bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA).⁹ Furthermore, all of these *vanA*-containing isolates showed to be multidrug resistant, exhibiting resistance to three or more structurally unrelated antimicrobial agents of clinical relevance. Recent enterococcal population retrospective analysis suggests that the temporal evolution of the population biology of Enterococci is driven by serial epidemic waves of human specific lineages, *E. faecalis* ST6 and *E. faecium* ST78, that are emerging at a global scale in the last decade.¹⁰ The MLST analysis of the *E. faecalis* isolate (H32) revealed that it belongs to the CC2 (ST6), a high-risk clonal complex globally associated with hospital outbreaks and life-threatening infections.⁹ This major clonal lineage is dominant in the Portuguese nosocomial setting, being established in this country; these are widely disseminated multidrug resistant strains, recovered from Portuguese hospitals since 1996 but also among *vanA* isolates from swine manure and hospital sewage, indicating a community contamination.¹¹ Isolate H32 showed to be highly virulent, presenting the genes coding for gelatinase (*gelE*), hyaluronidase (*hyl*), sex pheromone (*cpd*), extracellular surface proteins (*esp*), and some of the genes of the cytolysin operon (*cylA*, *cylM* and *cylL*). According to recent studies, the diversity of resistance and virulence gene profiles showed by CC2 isolates from Portuguese extra-hospital settings supports that CC2 is particularly proficient at DNA exchange.¹¹ The *E. faecium* isolate (H13) belongs to CC17 (ST78), a globally dispersed clonal lineage that is highly successful in the hospital environment and characterized by resistance to ampicillin and quinolones.⁹ A recent comprehensive multi-hierarchical analysis of VRE isolates from hospitals and aquatic surroundings in Portugal showed that the population structure of VRE comprises main human *E. faecium* lineages, ST18 being more abundant than ST78.¹⁰ Nonetheless, CC17 *vanA*-*E. faecium* are spread among Portuguese human and swine hosts¹² and also among wild animals such as the Iberian Wolf¹³ and common buzzards.⁶ Portugal is one of the European countries with highest rates of VRE associated with nosocomial infections, being *vanA*-*E. faecium* isolates the main responsible.¹⁴ However, in the last decade, the prevalence of VRE *E. faecium* decreased from 46.6% in 2003 to 20.2% in 2011.⁴ Although polyclonality is frequently observed, intra- and inter-hospital dissemination of persisting *E. faecium* and *E. faecalis* clones and specific *vanA* transposon types

Table 1
Characteristics of vancomycin-resistant enterococci isolates recovered from haemodialysis patients in Portugal.

Enterococcus strain	MIC (mg/L)	Vancomycin-resistance genes detected	Resistant phenotype for other antibiotics	Other resistance genes detected	Genes encoding virulence factors	ST (CC)
<i>E. faecium</i> H13	>128	<i>vanA</i>	TET-QD-CIP-AMP-ERY-GEN-KAN	<i>tet(M)</i> + <i>tet(L)</i> + <i>erm(B)</i> + <i>aac(6')</i> – <i>aph(2'')</i>	<i>esp</i> , <i>cylS</i>	ST78 (CC17)
<i>E. faecalis</i> H32	>128	<i>vanA</i>	TET-QD-CIP-ERY-GEN-KAN	<i>tet(M)</i> + <i>erm(B)</i> + <i>aac(6')</i> – <i>aph(2'')</i>	<i>gelE</i> , <i>cpd</i> , <i>esp</i> , <i>hyl</i> , <i>cylA</i> , <i>cylM</i> , <i>cylL</i>	ST6 (CC2)
<i>E. raffinosus</i> H64	>128	<i>vanA</i>	TET-CIP-AMP-ERY-STR-KAN	<i>tet(M)</i> + <i>tet(L)</i> + <i>erm(B)</i> + <i>erm(A)</i>	<i>esp</i> , <i>cylL</i>	ND
<i>E. gallinarum</i> H76	8	<i>vanC1</i>	TET-ERY	<i>tet(M)</i> + <i>tet(L)</i> + <i>erm(B)</i>	<i>hyl</i> , <i>cylL</i> , <i>cylS</i>	ND

MIC, minimal inhibitory concentration; ST, sequence type; CC, clonal complex; ND, not done; VAN, vancomycin; TEI, teicoplanin; TET, tetracycline; QD, quinupristin–dalbafopristin; CIP, ciprofloxacin; AMP, ampicillin; ERY, erythromycin; STR, streptomycin (high-level); GEN, gentamicin (high-level); KAN, kanamycin (high-level).

seemed to have contributed to the rapid and extensive spread of VRE in Portuguese hospitals.¹⁴ Reports of a high proportion of ampicillin-resistant VRE *E. faecium* isolates together with MLST data suggest the wide dissemination of epidemic clones among Portuguese hospitals.¹⁴

In conclusion, the prevalence of VRE colonization in the studied haemodialysis patients was 3.3%. The majority of these enterococci possess the *vanA* gene and also displayed multidrug resistance to the antimicrobials used to treat severe enterococcal infections. Hence, the dissemination of these VRE is a matter of public health concern, particularly among the dialysis population, as these enterococci constitute a reservoir of resistance genes that can be disseminated to other pathogens. This study might contribute to a future understanding on the problem of VRE colonization among this risk population.

Conflicts of interest

The authors have no conflicts of interest to declare.

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