Brief report

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

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ARTICLE INFO

Article history:
Received 27 February 2013
Accepted 2 September 2013
Available online 27 October 2013

Keywords:
Haemodialysis
Portugal
Vancomycin-resistant enterococci
vanA gene

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

R E S U M E N

Introducción: La prevalencia de Enterococos Resistentes a la Vancomicina (ERV) en pacientes de hemodiálisis 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. Resuelto: 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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Haemodialysis patients have played a major role in the epidemic of vancomycin resistance because this drug is commonly used in this kind of treatment. 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. 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. 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. 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.

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. 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. Macrolide [erm(A), erm(B), erm(C)], streptogramine [vat(D) and vat(E)], tetracycline [tet(M), tet(L)] and aminoglycoside [aph(3′)-IIa, aac(6′)-aph(2′)], ant(6)-Ia] resistance genes were screened by PCR in the isolates that showed resistance to these agents. The presence of virulence genes (ace, gelE, fsr, cdp, esp, hyl, agg and cyll_L5ABM) was also studied. E. faecium and E. faecalis isolates were characterized by multilocus sequence typing (MLST).
seemed to have contributed to the rapid and extensive spread of VRE in Portuguese hospitals.\textsuperscript{14} Reports of a high proportion of ampicillin-resistant VRE \textit{E. faecium} isolates together with MLST data suggest the wide dissemination of epidemic clones among Portuguese hospitals.\textsuperscript{4}

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.

### Acknowledgements

The authors would like to thank Dr. João Carlos Diniz, Gabriela Filipe and Jesus Salgueiro from Nephrocare Lumiar for their contribution in sample collection and also all the patients who consented for faecal sample recovery.

Susana Correia (SFRH/BD/75160/2010) and Daniela Jones-Dias (SFRH/BD/80001/2011) are supported by PhD fellowships granted by FCT (Fundação para a Ciência e a Tecnologia) and POPH/FSE (Programa Operacional Potencial Humano/Fundo Social Europeu).

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