Molecular characterization of hospital vancomycin-resistant Enterococcus faecalis isolated in Slovakia

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Keywords: Enterococcus, vancomycin, resistance, VRE

Sir,

Vancomycin-resistant enterococci (VRE) represent a reservoir of glycopeptide resistance in hospital patients, especially in high-risk wards. In Slovakia, the incidence of VRE has not yet been published, although Lišková et al. reported the participation of enterococci, with susceptibility to vancomycin over 16 mg/L, in bacteraemias in six Slovak hospitals. Thus, monitoring of glycopeptide resistance in enterococci is crucial because of possible transmission of van genes in the enterococcal population.

This report, to our knowledge, is the first molecular characterization of hospital vancomycin-resistant Enterococcus faecalis strain 605 isolated in Slovakia. The strain was obtained from a patient with chronic nephritis after repeated treatment for Escherichia coli infection with a second-generation cephalosporin and with quinolones (ciprofloxacin and pefloxacin). Subsequently, infection appeared with significant bacteriuria caused by enterococci. The patient was treated with vancomycin (1000 mg per day, serum level measured AxSYM, Abbott Laboratories) for 7 days. After this therapy, the patient was found to be colonized with VRE. Resistance to vancomycin was confirmed by WHO reference laboratories (European Antimicrobial Resistance Surveillance–UK National External Quality Assurance Scheme). The MIC range was 32–48 mg/L.

This vancomycin-resistant Enterococcus was species-identified as E. faecalis by the EN-COCCUS test (Pliva-Lachema, Czech Republic), and was confirmed by PCR with species-specific primers for ddlE, faecalis and ddlE, faecium genes (these genes encode D-Ala–D-Ala ligases). The presence of vanA or vanB operon was studied with PCR assay using primers and conditions described by Kariyama et al. In agreement with other reports on the prevalence of the vanA genotype in VRE in Europe, this genotype was the only one found in our strain (Figure 1). In addition to vancomycin resistance, E. faecalis 605 was resistant to teicoplanin, erythromycin, quinupristin/dalfopristin and high levels of gentamicin (MIC > 500 mg/L) as determined by the disc diffusion method, as well as to other aminoglycosides: tobramycin (MIC 256 mg/L), netilmicin (MIC 32 mg/L), amikacin (MIC 64 mg/L), isepamicin (MIC 32 mg/L) and kanamycin (MIC > 512 mg/L), tested by the agar dilution method. The strain remained susceptible to ampicillin, streptomycin, chloramphenicol, rifampicin, nitrofurantoin and ciprofloxacin. The aminoglycoside-resistance profile suggested the presence of the bifunctional enzyme, aminoglycoside acetyltransferase-6′+aminoglycoside phosphotransferase-2′[AAC(6′)+APH(2′)], which destroys all clinically available aminoglycosides with the exception of streptomycin. Positive PCR detection of the genes encoding the enzyme confirmed that resistance to aminoglycosides is mediated mainly by AAC(6′)+APH(2′). The genes for aminoglycoside resistance are predominantly harboured at mobile elements, so vancomycin-resistant E. faecalis 605 was conjugated with standard recipient strain Enterococcus faecium BM 4105 rifr. The frequency of transfer was relatively high (5.0 × 10−3). Transconjugants possessed a resistance profile similar to the donor strain, with the exception of resistance to quinupristin/dalfopristin, which is, however, located only chromosomally. The potency to transfer resistance determinants was also proved by isolation of a plasmid (~60 kb) from the donor strain as well as from transconjugants. Moreover, E. faecium BM 4105 transconjugants acquired vanA genes (Figure 1), confirming the movement not only of resistance to erythromycin and aminoglycosides, but also of vancomycin and teicoplanin.

Figure 1. Detection of vanA genes in donor strain E. faecalis 605 and its transconjugant E. faecium 4105. Lane 1, lambda restricted with enzyme PstI; lane 2, clinical isolate E. faecalis 605; lane 3, transconjugant E. faecium 4105.

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In summary, molecular study of vancomycin-resistant E. faecalis 605 describes the mechanisms of resistance and the power of this hospital isolate to disseminate resistance markers including van genes. Analysis such as this is important for the creation of antibiotic policy and our understanding of the epidemiology of resistance markers in hospital wards.

Acknowledgements
This research was supported by grant numbers 1/8221/01 and 1/7239/20 from the Slovak Research Grant Agency.

References

**Journal of Antimicrobial Chemotherapy**
DOI: 10.1093/jac/dkh086
Advance Access publication 16 January 2004

**Genetic analysis of 17 genes in Staphylococcus aureus with reduced susceptibility to vancomycin (VISA) and heteroVISA**

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Sir,
A proteome comparison of the vancomycin-intermediate *Staphylococcus aureus* isolates Mu50 and the vancomycin-susceptible *S. aureus* (VISA), N315, concluded that 17 ORFs: *murA*, *metB*, *yibM*, *atl*, *opuD*, *lysP*, *mutS*, *uhpT*, *modA*, *gltT*, *odhA*, *sdhB*, *rnr*, *prfC*, *mrp*-homologue, SA2486 and *ribH*, appeared to be disrupted in the published Mu50 genome (NC002758) and were therefore potentially implicated in the VISA phenotype.1 The aim of this study was to determine whether mutations in one or more of these 17 genes were consistently found in VISA isolates, so providing evidence for the molecular basis of the VISA phenotype. We studied the 17 genes in 10 clinical VISA, including Mu50, and 11 heteroVISA, including Mu3; 11 VISA strains were sequenced. These sequences were analysed comparatively against the published genomes of Mu50 and the VSSAs N315 and MW2. The strains included related strain sets (PC1-heteroVISA/PC3-VISA;2 LIM1-heteroVISA/LIM3-VISA;3 LLA-VSSA/ILLE-heteroVISA), which may indicate whether the heteroVISA phenotype is a genetic precursor to VISA. The vancomycin resistance status of the strains were confirmed by MIC determination (BSAC methodology) and the population analysis profile–area under the curve4 method. DNA was extracted and used to perform PCR with sets of specifically designed primers and then sequencing was performed on each PCR product. The gene sequences were manipulated using a reverse-complement tool (http://www.r9corporation.fsnet.co.uk/bioinformatics_tools/reversecomplement.htm), and aligned alongside the N315/MW2 sequence for the corresponding gene, using the CLUSTALW alignment tool (http://www.ebi.ac.uk/clustalw/). Any observed base substitutions, deletions and insertions were confirmed using the corresponding sequence chromatogram, and then compared with the equivalent sequences in the published Mu50 sequences.

Only the sequences of *gltT*, *uhpT*, SA2486 and the *mrp*-homologue were confirmed to be disrupted in Mu50 and Mu3. The genes *gltT* and *uhpT* were disrupted by a base substitution at G1064A and C718T, respectively, both resulting in the formation of a stop codon. The genes SA2486 and *mrp*-homologue were disrupted by deletion at A814 and A5917, respectively. For the remaining 13 genes, sequences from Mu50 and Mu3 were identical, or encoded predicted