Detection of a single vanA-containing Enterococcus faecalis clone in hospitals in different regions in Spain

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Sir,

Vancomycin-resistant enterococci constitute an increasing clinical problem in the USA, and clonal dissemination of vancomycin-resistant isolates among hospitals, especially Enterococcus faecium, has been described. In Europe, detection of vanA enterococci in the clinical setting is less frequently reported and few reports exist of clonal dissemination of resistant isolates among different hospitals. This study examines eight vanA-containing Enterococcus faecalis clinical isolates (blood and exudates) from hospitals in four different Spanish regions: Aragón (AR721), Asturias (AS215 and AS237), Cataluña (CT715, CT716, CT718 and CT719) and La Rioja (LR337). Antibiotic resistance phenotype was determined by the agar dilution method of the NCCLS. The putative presence of vanR, vanS, vanH, vanA, vanY, vanX, vanZ, aac(6′)-aph(2′), aph(3′) and erm(B) genes was examined by PCR, using specific primers. Aminoglycoside-modifying enzymes were determined in extracts of resistant Enterococcus isolates obtained by ultrasonic disruption, using the phosphocellulose paper binding assay as described previously. Clonal identity was studied by analysing the genomic DNA of vanA isolates digested with Smal by pulsed-field gel electrophoresis (PFGE) as described previously. Isolates were classified as indistinguishable, closely related, possible related or unrelated according to published criteria.

All eight vanA Enterococcus faecalis isolates were resistant to vancomycin and teicoplanin (MIC ranges 256–512 mg/L and 64–256 mg/L, respectively) as well as to erythromycin (MIC > 128 mg/L), but were susceptible to ampicillin (MIC < 4 mg/L). Seven isolates showed high-level resistance (HRL) to kanamycin (>2000 mg/L) and streptomycin (>1000 mg/L); one (AS237) also showed HRL to gentamicin (>1000 mg/L) and one (CT718) showed HRL only to streptomycin. In E. faecalis strains that showed HRL to kanamycin APH (3′) activity was detected by the radioenzymic assay and the presence of the aph3′-III gene was confirmed by PCR amplification. No streptomycin-modifying enzyme was detected in high-level streptomycin-resistant isolates. APH (2′)-AAC (6′) activity was detected in the E. faecalis strain that showed HRL to gentamicin, and the presence of the aac6-aph2 gene was confirmed by PCR. Positive PCR amplifications were obtained in all eight isolates for all genes of the vanA operon (vanR, vanS, vanH, vanA, vanX, vanY and vanZ) and also for the erm(B) gene.

All E. faecalis isolates carried a high molecular weight plasmid (c. 60 kb) that was transferred to the recipient E. faecalis JH2-2 strain by filter mating. Both donors and transconjugants also showed a positive hybridization pattern with a vanA probe in the chromosome. The same hybridization pattern was obtained when genomic DNA was digested with EcoRI, transferred to a nylon membrane and hybridized with the vanA probe.

Five unrelated patterns were found by Smal–PFGE among the eight E. faecalis isolates. Four vanA-carrying E. faecalis isolates from three geographically distant hospitals showed an indistinguishable PFGE pattern (E. faecalis CT716, CT719, LR337 and AS215) (Figure). These four isolates were also further characterized as bacteriocin producers with a broad spectrum of activity, similar in all four strains. The other four E. faecalis isolates (CT715, CT718, AR721 and AS237) showed unrelated PFGE patterns; from these, one strain was a non-bacteriocin producer, and the other three produced possibly different antibacterial substances.

The genetic data shown in this work indicate that a group of four E. faecalis isolates (CT716, CT719, LR337 and AS215) recovered from clinical samples in hospitals from three geographically distant Spanish regions constitute a single clone. To our knowledge, this is the first time that a single clone of vanA-carrying E. faecalis has been detected in different geographically separate hospitals. An alternative explanation for this event could be the dissemination of a particular vancomycin-susceptible E. faecalis clone among the three Spanish regions that may have independently acquired the vanA operon, erythromycin and aminoglycoside resistance genes. Nevertheless, the unique spectrum of bacteriocin activity of these E. faecalis isolates...
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suggests a single event of vanA acquisition, followed by clonal dissemination in different regions.

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References


