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# Correspondence

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

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R. del Campo[a,b]*, C. Tenorio[a], M. Zarazaga[a], R. Gomez-Lus[c], F. Baquero[b] and C. Torres[a]

[a]*Area de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño;*
[b]*Servicio de Microbiología, Hospital Ramón y Cajal, Ctra Colmenar Viejo Km 9.1, 28034 Madrid;*
[c]*Departamento de Microbiología, Universidad Zaragoza, Zaragoza, Spain*

*Corresponding author. Tel: +34-91-336-8330; Fax: +34-91-336-8809;
E-mail: rcampo@hrc.insalud.es

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.[1] 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.[2] 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.[3] The putative presence of v*anR*, *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.[4] Clonal identity was studied by analysing the genomic DNA of *vanA* isolates digested with *Sma*I by pulsed-field gel electrophoresis (PFGE) as described previously.[5] Isolates were classified as indistinguishable, closely related, possible related or unrelated according to published criteria.[6]

All eight *vanA E. 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 (HLR) to kanamycin (>2000 mg/L) and streptomycin (>1000 mg/L); one (AS237) also showed HLR to gentamicin (>1000 mg/L) and one (CT718) showed HLR only to streptomycin. In *E. faecalis* strains that showed HLR 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 HLR 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 *Eco*RI, transferred to a nylon membrane and hybridized with the *van*A probe.

Five unrelated patterns were found by *Sma*I–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,[7] 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[7]

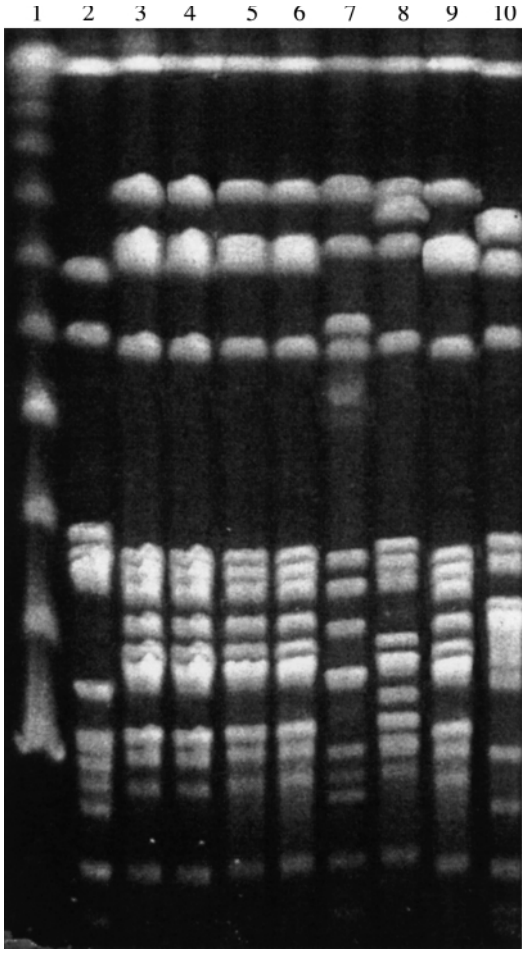


**Figure.** *Sma*I–PFGE of eight *E. faecalis vanA* isolates from various hospitals in separate regions of Spain. Lane 1: PFGE marker; lanes 2–10: *E. faecalis vanA* AS237, AS215, CT716, CT719, LR337, CT715, CT718, AR721 and LRH1.

suggests a single event of *vanA* acquisition, followed by clonal dissemination in different regions.

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