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Enterococcus gallinarum carrying the *vanA* gene cluster: first report in Brazil

I.L.B.C. Camargo[1], A.L. Barth[2], K. Pilger[2], B.G.S. Seligman[2], A.R.L. Machado[2] and A.L.C. Darini[1]

[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
[2]Unidade de Pesquisa Biomédica, Serviço de Patologia Clínica, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil

Abstract

In 2000, *Enterococcus faecalis* resistant to vancomycin was first reported at a tertiary hospital in Porto Alegre, southern Brazil. The resistance spread to other hospitals and surveillance programs were established by hospital infection committees to prevent the spread of vancomycin-resistant enterococci. In February 2002, an isolate initially identified at the genus level as *Enterococcus* was obtained by surveillance culture (rectal swab) from a patient admitted to a hospital for treatment of septic arthritis in the shoulder. The isolate proved to be resistant to vancomycin by the disc diffusion method and confirmed by an E-test resulting in a minimal inhibitory concentration of ≥256 µg/ml. This isolate was sent to a reference laboratory (Laboratório Especial de Bacteriologia e Epidemiologia Molecular, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP) for further study and proved to be an *E. gallinarum* by the polymerase chain reaction (PCR) using specific primers for the species. Due to the phenotype of unusually high vancomycin resistance, the isolate presumably had the resistance genes (*vanA* and *vanB*) and this was confirmed by PCR, which indicated the presence of the *vanA* gene. A 10.8-kb Tn*1546*-related transposon was also identified by long-PCR. Interspecies transfer of the vancomycin-resistance gene from the donor *E. gallinarum* was performed in a successful conjugation experiment *in vitro*, using *E. faecium* GE-1 and *E. faecalis* JH22 as receptors. This is the first report of the detection of a *vanA* determinant naturally acquired by *E. gallinarum* in Brazil, indicating the importance of characterizing VRE by both phenotype and genotype methods.

Key words
- *Enterococcus gallinarum*
- VanA phenotype
- Vancomycin
- Resistance

Correspondence
A.L.C. Darini
Departamento de Análises Clínicas Toxicológicas e Bromatológicas
Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP
Av. do Café, s/n
14040-903 Ribeirão Preto, SP
Brasil
E-mail: aldarini@fcfrp.usp.br

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Enterococci have emerged as increasingly important nosocomial and community-acquired pathogens. First described in 1988 in France and England (1,2), glycopeptide-resistant enterococci have been isolated in Brazil since 1996, when the first case of vancomycin-resistant enterococci (VRE) due to a *Enterococcus faecium* $VanD_4$ was reported in Curitiba (Paraná State) (3,4). Further VRE isolates (*E. faecium* with the *van*A gene) were reported in São Paulo State (5,6).

Glycopeptide resistance in enterococci is associated with a variety of phenotypes and genotypes. VanA and VanB are the two major genetically distinct forms of acquired resistance. The VanA phenotype displays a high level of resistance to vancomycin and teicoplanin but the VanB phenotype displays

a high level of resistance only to vancomycin. However, both have been found primarily in *E. faecalis* and *E. faecium* (7). In contrast, *E. gallinarum*, *E. casseliflavus* and *E. flavescens* have an intrinsic low-level resistance to vancomycin due to the VanC phenotype (7). The *vanC-1* gene is specific for *E. gallinarum*, and *vanC-2/3* is specific for *E. casseliflavus* and *E. flavescens*. Nevertheless, a few cases of *vanA* and *vanB* genes acquired by *E. gallinarum* have been reported: in Switzerland (8), Australia (9), Italy (10), Belgium (11), and Taiwan (12).

In 2000, vancomycin-resistant *E. faecalis* was first described at a tertiary hospital in Porto Alegre, southern Brazil. The resistance spread to other hospitals and surveillance programs were established by the hospital infection committees in order to prevent the spreading of VRE. In February 2002, an isolate initially identified at the genus level as *Enterococcus* by a few basic tests (Gram, bile esculin, catalase, growth on 6.5% NaCl and L-pyrrolidonyl-ß-naphthylamide, PYR) was obtained at Hospital de Clínicas de Porto Alegre by surveillance culture (rectal swab) from a patient admitted to the hospital due to septic arthritis in the shoulder. The isolate proved to be resistant to vancomycin by the disc diffusion method carried out according to NCCLS (13) and this was confirmed by the E-test (AB Biodisk, Sweden) resulting in a minimal inhibitory concentration of ≥256 µg/ml.

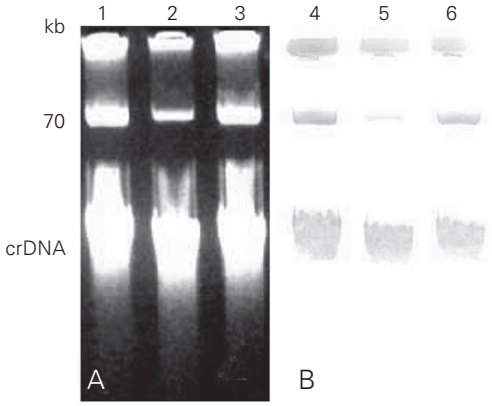

Figure 1. Conjugation experiments. *A*, Plasmid extraction product in 1% agarose gel; *B*, *Southern blotting* hybridization of *vanA* probes in a nylon membrane. *Lane 1*, *E. gallinarum* ET193; *lane 2*, transconjugant of *E. faecium* GE-1; *lane 3*, transconjugant of *E. faecalis* JH22. crDNA = chromosomal DNA.

This isolate was sent to a reference laboratory (Laboratório Especial de Bacteriologia e Epidemiologia Molecular, Faculdade de Ciências Farmacêuticas de Ribeirão Preto) for further study and proved to be an *E. gallinarum* (termed ET193) by the polymerase chain reaction (PCR) using specific primers for the species (11). Due to the phenotype of unusually high level of vancomycin resistance, the isolate was tested for the presence of specific resistance genes (*vanA* and *vanB*) by PCR, which indicated the presence of the *vanA* gene (14,15).

The high level of vancomycin resistance among enterococcus isolates is often mediated by a self-transferable plasmid that has acquired the Tn*1546*-related transposons, which carry the *vanA* gene cluster (16). We demonstrated a Tn*1546*-related 10.8-kb transposon which was found to be identical to the Tn*1546* prototype in the *E. gallinarum* ET193 isolate by long-PCR (17).

Interspecies transfer of the vancomycin resistance determinant from the *E. gallinarum* ET193 donor was successfully carried out by a conjugation experiment *in vitro* using *E. faecium* GE-1 and *E. faecalis* JH22 as receptors (18). After successful conjugation, several transconjugants were obtained by selection on plates containing 4 µg/ml vancomycin, 200 µg/ml rifampicin, and 100 µg/ml fusidic acid. Plasmid extraction was performed and a plasmid of about 70 kb was found in the transconjugants and in the *E. gallinarum* ET193 donor isolate. Eventually, the electrophoretic profile was analyzed by Southern blot and hybridization using a digoxigenin-labeled *vanA* gene probe in order to locate the presence of the gene. The gene-specific probe was bound to both plasmids and chromosomal DNA of the several transconjugants and donor *E. gallinarum* ET193 (Figure 1).

This is the first case of *E. gallinarum* carrying the *vanA* gene cluster isolated in Brazil. As the patient was also colonized by a *vanA* genotype vancomycin-resistant *E. faecalis*, it is reasonable to speculate that this gene cluster was transferred from *E. faecalis* to *E. gallinarum in vivo*. This was, most probably, a natural acquisition of a peculiar high level resistance gene by the *E. gallinarum* species and indicates the importance of characterizing VRE by both phenotype and genotype methods.

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