Presence of the vanC1 gene in a vancomycin-resistant Enterococcus faecalis strain isolated from ewe bulk tank milk

A broad spectrum of antimicrobial resistance, mainly to aminoglycosides, β-lactams and glycopeptides (vancomycin and teicoplanin) is a well-known characteristic of Enterococcus species. Resistance mechanisms can be classified as either acquired or intrinsic and are usually associated with a particular species or a given group of species. Due to the difficulty in eliminating systemic and local infections, especially in patients with an impaired immune system, a synergistic combination of a cell-wall-active antimicrobial (β-lactam or glycopeptide) plus an aminoglycoside constitutes a first-line choice for serious infections (Teixeira & Facklam, 2005). In 1988 vancomycin resistance was described for the first time in enterococcal strains (Leclercq et al., 1988; Uttley et al., 1989) and nowadays the food chain is suspected of being a source of vancomycin-resistant enterococci (VRE) (Robredo et al., 2000; Bonten et al., 2001). At present, one type of non-inducible (VanC) and eight different types of inducible glycopeptide resistance have been described (Teixeira & Facklam, 2005; Boyd et al., 2008; Xu et al., 2010; Lebrerton et al., 2011). The most common phenotypes are VanA, VanB and VanC. VanA and VanB (encoded by the vanA and vanB genes) are linked to Enterococcus faecalis and Enterococcus faecium strains, and are considered the most clinically relevant phenotypes, resulting in high-level resistance to both vancomycin and teicoplanin in the case of VanA strains, and a moderate to high level of vancomycin resistance and sensitivity to teicoplanin in the case of VanB enterococci (Teixeira & Facklam, 2005). The vanC intrinsic resistance genotype is associated with several enterococcal species: E. gallinarum (vanC1), E. casseliflavus (vanC2) and E. flavescens (vanC3). The chromosomal location of VanC genes makes them presumably non-transferable, conferring an intermediate resistance level to vancomycin (MIC 4–32 mg l⁻¹) and sensitivity to teicoplanin (MIC ≤8 mg l⁻¹), according to the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints (CLSI, 2012). In the context of a study on the diversity of Gram-positive, catalase-negative cocci isolated from sheep’s bulk tank milk destined for the fabrication of cheese, a total of 57 strains of E. faecalis was isolated. Laboratory isolation from milk was performed by inoculating modified Edward’s medium (Oxoid) supplemented with 5% defibrinated sheep blood, as per manufacturer’s instructions. Colonies obtained were subcultured overnight on Columbia agar (Oxoid) in a 5% CO₂ atmosphere at 37 °C. DNA from pure cultures was extracted with BackLight Cards (2B Blackbio S.L). Strain identity was confirmed by API Rapid ID 32 Strep (bioMérieux) and by 16S rRNA gene sequencing. Glycopeptide-resistance genotype screening by PCR ( Dutka-Malen et al., 1995) detected the presence of vanC1 in a unique isolate, strain CMN_460/12. This vanC1 amplicon was purified with ExoSAP-IT (Isogen Life Science), sequenced and compared with sequences in the National Center for Biotechnology Information database (Basic Local Alignment search tool, www.ncbi.nlm.nih.gov), which corroborated its similarity with the sequence of the vanC1 gene from E. gallinarum strain e9464 (GenBank accession no. EU151772) and with those of other VanC1 strains. In order to determine phenotypic antimicrobial susceptibilities, the strain was subjected to Microscan PM28 plates (Siemens) and showed susceptibility to ampicillin, macrolides, aminoglycosides and fluoroquinolones, unlike clinical VRE strains. The Etest (AB bioMérieux) confirmed resistance to vancomycin (MIC=12 mg l⁻¹) and susceptibility to teicoplanin (MIC=1 mg l⁻¹), interpreted according to CLSI criteria (CLSI, 2012). The following enterococcal virulence factors were tested by multiplex PCR (Vankerckhoven et al., 2004): aggregation substance (asa1), gelatinase (gelE), cytolysin activator (cylA), enterococcal surface protein (esp) and hyaluronidase (hyl), with positive results for the asa1, gelE and cylA genes. Multi-locus sequence typing analysis was performed as described in http://efaecalis.mlst.net/ and determined that this strain belongs to sequence type (ST)168 (allelic profile: gdh-20, gyd-1, psts-7, gki-23, aroE-23, xpt-2, yqil-2). This infrequent ST is shared by 15 other Spanish strains isolated from vegetables and two clinical isolates from France and the Netherlands. This is the first report, to the best of our knowledge, of a vanC1 gene in E. faecalis ST168 with a vancomycin-resistant phenotype isolated from sheep bulk tank milk intended for dairy products. A previous report on diseased pigs in Germany detected the vanC1 gene in two E. faecalis isolates; however, these strains were susceptible to vancomycin (MIC=1 mg l⁻¹) (Schwaiger et al., 2012). This finding is significant, as the detection of the vanC1 resistance gene is a useful tool for the identification of E. gallinarum (Dutka-Malen et al., 1995; Schwaiger et al., 2012). Because the veterinary use of glycopeptide compounds is not permitted in Spain, we can presume, as in the case of porcine isolates, that the presence of the vanC1 gene in an E. faecalis isolate may be due to the horizontal transfer between E. gallinarum and E. faecalis. This phenomenon may be attributed to the asa1 gene, as it facilitates conjugative exchange (Vankerckhoven et al., 2004; Schwaiger et al., 2012). These two recent occurrences of vanC1 genotypes in E. faecalis animal isolates emphasize the need for screening for the presence of both acquired and intrinsic glycopeptide resistance genes. The important finding of vancomycin-resistant E. faecalis in sheep’s milk intended for manufacturing dairy products is significant, as E. faecalis is the predominant enterococcal species in
Mediterranean cheeses (enterococcal counts range from $10^4$ to $10^7$ c.f.u. g$^{-1}$) (Franz et al., 2003; Nieto-Arribas et al., 2011), which could potentially be transferred to humans. In addition, the ability of this species to acquire and transfer resistance genes suggest that its presence should be carefully monitored throughout the food chain.

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