The first report of the vanC\textsubscript{1} gene in Enterococcus faecium isolated from a human clinical specimen

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The vanC\textsubscript{1} gene, which is chromosomally located, confers resistance to vancomycin and serves as a species marker for Enterococcus gallinarum. Enterococcus faecium TJ4031 was isolated from a blood culture and harbours the vanC\textsubscript{1} gene. Polymerase chain reaction (PCR) assays were performed to detect vanXYc\textsubscript{1} and vanTc\textsubscript{1} genes. Only the vanXYc\textsubscript{1} gene was found in the E. faecium TJ4031 isolate. The minimum inhibitory concentrations of vancomycin and teicoplanin were 2 µg/mL and 1 µg/mL, respectively. Real-time reverse transcription-PCR results revealed that the vanC\textsubscript{1} and vanXYc\textsubscript{1} genes were not expressed. Pulsed-field gel electrophoresis and southern hybridisation results showed that the vanC\textsubscript{1} gene was encoded in the chromosome. E. faecalis isolated from animals has been reported to harbour vanC\textsubscript{1} gene. However, this study is the first to report the presence of the vanC\textsubscript{1} gene in E. faecium of human origin. Additionally, our research showed the vanC\textsubscript{1} gene cannot serve as a species-specific gene of E. gallinarum and that it is able to be transferred between bacteria. Although the resistance marker is not expressed in the strain, our results showed that E. faecium could acquire the vanC\textsubscript{1} gene from different species.

Key words: Enterococcus gallinarum - Enterococcus faecium - vanC\textsubscript{1} gene

During the past two decades, glycopeptide-resistant enterococci, in particular Enterococcus faecium, have become increasingly widespread throughout the world and have been identified as multi-resistant opportunistic pathogens in hospitals and in the environment (e.g., in foods and animals) (Lebreton et al. 2011, Nomura et al. 2012). Since the first detection of vancomycin resistance in E. faecium in 1988, nine operons that confer resistance to glycopeptides have been distinguished based on the sequence of the structure for the resistance ligase (Leclercq et al. 1988, Uttley et al. 1988, Lebreton et al. 2011). These operons are classified according to the characteristics of the ligase gene, which can encode either a D-alanly-D-lactate ligase or a D-alanyl-D-serine ligase. Genes that encode D-alanyl-D-lactate ligases include the vanA, vanB, vanD and vanM genes and those that encode D-alanyl-D-serine ligases include the vanC\textsubscript{1}, vanC\textsubscript{2}, vanC\textsubscript{3}, vanX, vanE, vanG, vanL and vanN genes (Arthur et al. 1996, Courvalin 2006, Lebreton et al. 2011, Nomura et al. 2012). Resistance types can be acquired for vanC\textsubscript{1}-type resistance, which is thought to be intrinsic to Enterococcus gallinarum and Enterococcus casseliflavus. The vanC\textsubscript{1} cluster is composed of five genes: vanC\textsubscript{1}, vanXYc, vanTc, vanRe and vanSc. Three of these genes are involved in inducing resistance according to the following mechanism: vanC\textsubscript{1} encodes a ligase that synthesises the dipeptide D-Ala-D-Ser, which is added to the UDP-MurNAc-tripeptide; vanXYc encodes a D,D-dipeptidase-carboxypeptidase that hydrolyses D-Ala-D-Ala and removes D-Ala from UDP-MurNAc-pentapeptide; vanT encodes a membrane-bound serine racemase that provides D-Ser to the synthetic pathway (Arias et al. 2000). The vanC\textsubscript{1} gene is thought to occur only in E. gallinarum and should therefore be useful for species identification (Ramotar et al. 2000). Furthermore, this gene is chromosomally located and has not been found in E. faecium until now. Since the vanC\textsubscript{1} gene was first identified in vancomycin-susceptible Enterococcus faecalis strains isolated from pig manure samples in Germany, vanC\textsubscript{1}-type E. faecalis of animal origin has been reported in Spain and Brazil. This finding emphasises that the chromosomal location of a gene in intrinsically resistant strains does not necessarily prevent gene transfer to another species, which is in contrast to traditional views (Schwaiger et al. 2012, de Garnica et al. 2013, de Moura et al. 2013). So far, there has been one report of the genetic location of the vanC\textsubscript{1} gene isolated from cloacal swabs of broilers and this gene was detected on plasmid (de Moura et al. 2013). We also identified vanC\textsubscript{1}-type E. faecium strain isolated from a blood culture.

In this study, E. faecium TJ4031 was susceptible to both vancomycin and teicoplanin, but harboured the vanC\textsubscript{1} resistance gene. We also presented evidence showing that the vanC\textsubscript{1} gene cluster was incomplete; the vanC\textsubscript{1} and vanXYc genes were not expressed. Additionally, the resistance gene in this clinical isolate was located on the chromosome.

MATERIALS AND METHODS

Strains - E. faecium TJ4031 was isolated in 2012 from a blood culture from an outpatient in our hospital. This isolate was initially identified by Gram staining and bio-
Characterisation of *E. faecium* TJ4031 - The clinical isolate TJ4031, which was isolated from a blood culture, was identified as *E. faecium* using conventional tests and the Vitrek2-Compact system; species identity was confirmed by PCR. *E. gallinarum* was excluded as a possibility by a negative result in an *E. gallinarum* species-specific PCR. TJ4031 was susceptible to vancomycin (MIC = 2 µg/mL) and teicoplanin (MIC = 1 µg/mL). TJ4031 was also susceptible to chloramphenicol, linezolid, ciprofloxacin, levofloxacin and fosfomycin. By comparison, this strain was resistant to penicillin, rifampicin, erythromycin and nitrofurantoin. TJ4031 was positive for the vanC and vanXYc genes, but negative for the vanTc gene. The vanC gene sequence was deposited in GenBank (accession KF849246).

Identification of gene expression by real-time RT-PCR - RT-PCR assays failed to detect the corresponding *vanC* and *vanXYc* gene transcripts in the *vanC* gene positive *E. faecium* TJ4031 strain. The positive and negative controls worked as expected.

**RESULTS**

**Detection of vanC-type *E. faecium***

Figure 1 shows the restriction endonuclease pattern of the *vanC* genotype in the *E. faecium* TJ4031 strain after PFGE with *Smal* was performed. The location of the *vanC* gene was determined using southern blot hybridisation with a *vanC* probe. PFGE hybridisation analysis results showed that the *vanC* probe hybridised with a genomic DNA fragment corresponding to the *vanC* gene. This result indicates that the strain TJ4031 may possess a multi-locus sequence typing (MLST) allele, which is associated with the pathogenicity of *E. faecium*.
The vanC intrinsic resistance genotype is associated with several enterococcal species, including *E. gallinarum* (vanC1), *E. casseliflavus* (vanC1) and *E. flavaesens* (vanC2). These vanC operons are chromosome associated and testing *E. faecium* or other *Enterococcus* species for the presence of vanC1 is considered unnecessary because this gene is thought to be species-specific for *E. gallinarum* (Leclercq et al. 1992, Schwaiger et al. 2012). However, the vanC1 gene has also been detected in *E. faecalis* strains isolated from pig manure samples (Germany), sheep bulk tank milk samples (Spain) and cloacal swabs of broilers (Brazil).

In our study, expected biochemical reactions were observed in the vanC1 genotype-positive *E. faecium* TJ4031. *E. faecium* was also analysed using the Vitek2-Compact system. Moreover, the *Enterococcus* and *E. gallinarum*-specific PCR results of this study are consistent with those of other bacteriological studies (Patel et al. 1998, Arias et al. 2000, Zheng et al. 2007, de Moura et al. 2013). Our study failed to detect the corresponding vanC1 and vanXYc genes in a vanC1 genotype-positive strain with real-time RT-PCR assays and similar results have been previously reported (Schwaiger et al. 2012, de Moura et al. 2013). This result could be attributed to a non-functional vanC1 gene cluster that has been transferred from a bacterial community to our strain or to a failed recombination event that inserted a non-functional gene and removed beneficial DNA (Lawrence et al. 2001, de Moura et al. 2013). In a previous study, the vanC1 gene was found on a plasmid (de Moura et al. 2013). However, our study showed that the vanC1 genotype of the *E. faecium* TJ4031 isolate contained no plasmid; this procedure was repeated in triplicate to verify our initial findings. The possible explanation is that megaplasmids, which cannot be detected with current techniques, or bacteria without plasmids may be present. Furthermore, the vanC1 gene was successfully hybridised to the chromosome band using southern blot, showing that this gene was located in this chromosome.

*E. faecium* TJ4031 may have acquired the vanC1 gene via a horizontal gene transfer from a natural carrier (*E. gallinarum*) or from a carrier of animal origin (*E. faecalis*) (Schwaiger et al. 2012, de Garnica et al. 2013, de Moura et al. 2013) because strains from human-adapted CCs that cause enterococcal infection may be recovered from farm and companion animals and strains from CCs commonly found among animals have also been isolated from humans. Furthermore, other studies have revealed several cases of animal-human transmission of vancomycin-resistant enterococci, resulting in frequent infections of healthy humans that closely interact with animals (Damborg et al. 2009, Freitas et al. 2009b, 2011, Willems & van Schaik 2009, Larsen et al. 2010).

This study was the first to identify the vanC1 gene in a vancomycin-susceptible *E. faecium* strain isolated from the blood culture of a patient in China. Our result is important because the vanC1 gene is often used to identify *E. gallinarum*; without this gene, species may be erroneously identified. This result also emphasises that the chromosomal location of a gene in an intrinsically resistant strain does not necessarily prevent transfer to other species, thereby contributing to species diversity. Furthermore, the vanC1-type vancomycin resistance gene was encoded on the chromosome. Our data indicate that vanC1-type *E. faecium* strains could be detected in humans. Even if the strain was phenotypically susceptible to vancomycin, the fact that *E. faecium* are able to naturally acquire vanC1 from the bacterial community is a cause for concern because the possibility that complete gene clusters and functional genes will be transferred and expressed cannot be ruled out. This study underlines that *E. faecium* are very potent resistance gene collectors and possibly donors (Schwaiger et al. 2012). We should always monitor enterococci in not only human clinical isolates, but also in the commensals from diverse habitats.
REFERENCES

Arias CA, Courvalin P, Revolods PE 2000. vanC cluster of vancomycin-resistant Enterococcus gallinarum BM4174. *Antimicrob Agents Chemother* 44: 1660-1666.

Arthur M, Revolods P, Courvalin P 1996. Glycopeptide resistance in enterococci. *Trends Microbiol* 4: 401-407.

CLSI - Clinical and Laboratory Standards Institute 2012. Performance standards for antimicrobial susceptibility testing. 22nd informational supplement M100-S13, CLSI, Villanova, 188 pp.

Courvalin P 2006. Vancomycin resistance in Gram-positive cocci. *Clin Infect Dis* 42 (Suppl. 1): S25-S34.

Damborg P, Top J, Hendrickx AP, Dawson S, Willems RJ, Guardabassi L 2009. Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. *Appl Environ Microbiol* 75: 2360-2365.

de Garnica ML, Valdezate S, Gonzalo C, Saez-Nieto JA 2013. Presence of the vanC1 gene in a vancomycin-resistant *Enterococcus faecalis* strain isolated from ewe bulk tank milk. *J Med Microbiol* 62: 494-495.

de Moura TM, Cassenego APV, Campos FS, Ribeiro AML, Franco AC, d’Azevedo PA, Frazzon J, Frazzon APG 2013. Detection of vanC, gene transcription in vancomycin-resistant *Enterococcus faecalis*. *Mem Inst Oswaldo Cruz* 108: 453-456.

Facklam RR, Collins MD 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* 27: 731-734.

Freitas AR, Coque TM, Novais C, Hammerum AM, Lester CH, Zervos MJ, Donabedian S, Jensen LB, Francia MV, Baquero F, Peixe L 2011. Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CCS and *Enterococcus faecalis* CC2 clonal clusters harboring Tn1546 on indistinguishable plasmids. *J Clin Microbiol* 49: 925-931.

Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L 2009a. Clonal expansion within clonal complex 2 and spread of vancomycin-resistant plasmids among different genetic lineages of *Enterococcus faecalis* from Portugal. *J Antimicrob Chemother* 63: 1104-1111.

Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L 2009b. Dispersion of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. *Appl Environ Microbiol* 75: 4904-4908.

Jackson CR, Fedorka-Cray PJ, Barrett JB 2004. Use of a genus and species-specific multiplex PCR for identification of enterococci. *J Clin Microbiol* 42: 3558-3565.

Larsen J, Schonheyder HC, Lester CH, Olsen SS, Porso L, Garcia-Migura L, Jensen LB, Bisgaard M, Hammerum AM 2010. Porcine-origin gentamicin-resistant *Enterococcus faecalis* in humans, Denmark. *Emerg Infect Dis* 16: 682-684.

Lawrence JG, Hendrix RW, Casjens S 2001. Where are the pseudogenes in bacterial genomes? *Trends Microbiol* 9: 535-540.

Lebreton F, Depardié F, Bourdon N, Fines-Guyon M, Berger P, Camiade S, Leclercq R, Courvalin P, Cattoir V 2011. D-Ala-d-Ser vanN-type transferable vancomycin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* 55: 4606-4612.

Leclercq R, Derlot E, Duval J, Courvalin P 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* 319: 157-161.

Leclercq R, Dutka-Malen S, Duval J, Courvalin P 1992. Vancomycin resistance gene vanC is specific to *Enterococcus gallinarum*. *Antimicrob Agents Chemother* 36: 2005-2008.

Nomura T, Tanimoto K, Shibayama K, Arakawa Y, Fujimoto S, Ike Y, Tomita H 2012. Identification of vanN-type vancomycin resistance in an *Enterococcus faecium* isolate from chicken meat in Japan. *Antimicrob Agents Chemother* 56: 6389-6392.

Patel R, Piper KE, Rouse MS, Steelenberg JM, Uhl JR, Kohner P, Hopkins MK, Cockerill FR 3rd, Kline BC 1998. Determination of 16S rRNA sequences of enterococci and application to species identification of non-motile *Enterococcus gallinarum* isolates. *J Clin Microbiol* 36: 3399-3407.

Poyart C, Quesnes G, Tricu-Cut P 2000. Sequencing the gene encoding manganese-dependent superoxide dismutase for rapid species identification of enterococci. *J Clin Microbiol* 38: 415-418.

Ramotar K, Woods W, Larocque L, Toye B 2000. Comparison of phenotypic methods to identify enterococci intrinsically resistant to vancomycin (vanC VRE). *Diag Microbiol Infect Dis* 36: 119-124.

Schwaiger K, Bauer J, Hörmansdorfer S, Mölle G, Preikschat P, Perrot A, P_lower_auk J, Bischoff M, Hözel C 2012. Presence of the resistance genes vanC, and pbp5 in phenotypically vancomycin and ampicillin susceptible *Enterococcus faecalis*. *Microb Drug Resist* 18: 434-439.

Uttley AH, Collins CH, Naidoo J, George RC 1988. Vancomycin-resistant enterococci. *Lancet* 1: 57-58.

Willems RJ, van Schaik W 2009. Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. *Future Microbiol* 4: 1125-1135.

Zheng B, Tomita H, Xiao YH, Wang S, Li Y, Ike Y 2007. Molecular characterization of vancomycin-resistant *Enterococcus faecalis* isolates from mainland China. *J Clin Microbiol* 45: 2813-2818.