Clonal dissemination of *Enterococcus faecalis* ST201 and *Enterococcus faecium* CC17–ST64 containing Tn*5382*–*vanB2* among 16 hospitals in Chile

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Abstract

We report the clonal dissemination of ST201 *Enterococcus faecalis* carrying Tn*5382*–*vanB2* and of CC17–ST64 *Enterococcus faecium* carrying Tn*5382*–*vanB2*–ISEnfa*110* among 16 hospitals in four geographically distant regions in Chile. This is the first epidemiological characterization of vancomycin resistance in Chile, and also the first report of interhospital dissemination of enterococcal *vanB2* in South America.

Keywords: Clonal dispersion, conjugation, *Enterococcus*, hospital dissemination, MLST, *vanB2* resistance

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Vancomycin-resistant enterococci (VRE) have emerged among the major nosocomial pathogens in many parts of the world. Although several glycopeptide resistance mechanisms have been described in enterococci, the acquired genotypes *vanA* and *vanB* are the most common. VanB-type resistance is encoded by the *vanB* gene cluster, of which three subtypes exist—*vanB1*, *vanB2*, and *vanB3*—that are distinguished on the basis of sequence variations in the ligase-encoding gene *vanB*. The *vanB2* subtype has been found in human and animal enterococcal isolates, carrying transposons Tn*5382* and Tn*1549*, from the USA and different countries in Europe [1–7].

A total of 70 VRE isolates (ten *Enterococcus faecalis* and 60 *Enterococcus faecium*) were submitted to the National Reference Institute in Santiago, Chile (http://www.ispch.cl), from 16 hospitals in four geographically distant regions of Chile: Santiago (12 hospitals, 59 isolates), Concepción (two hospitals, six isolates), Talcahuano (one hospital, four isolates), and Temuco (one hospital, one isolate). VRE isolates were recovered from non-simultaneously hospitalized patients between 2003 and 2005; the samples included 34 faecal swabs, 15 urine samplese, six wound exudates, three blood cultures, two peritoneal liquids, two gastric liquids, and eight samples of undetermined origin. Susceptibility to different antibiotics was tested using the agar dilution method following the CLSI recommendations. Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested DNA was performed to analyse the genetic relatedness of the VRE isolates, using the CHEF DR-III apparatus (BioRad, Birmingham, UK). The electrophoresis conditions were as follows: 6 V/cm^2; 22 h; initial switch time 5 s; and final switch time 35 s. One isolate per clone was further typed by multilocus sequence typing (MLST) (http://www.mlst.net). The presence of the *vanB2* gene, as well as the arrangement of the *vanB* cluster and the surrounding regions ($vanS_B$–$vanY_B$, Tn*5382*, $vanX_B$–ORFC, *pbp*5–Tn*5382*, ISEnfa*110*, and ISEnfa*200*), was determined by PCR and sequencing [2]. Carriage of virulence factors (*esp*, *hyl*, $cyl_{LS-M-A-B}$ *gelE*–fsr_{A-C}) and antibiotic resistance genes (*ermB*, *tetM*, *tetL*, *ant6*, *aph3′*-IIIa, and *aac*(*6′*)–*aph*(*2″*)) was investigated in the different clones by PCR with specific primers [2]. Transferability of vancomycin resistance determinants was tested by conjugation using the filter method with both *E. faecalis* JH2-2 and *E. faecium* GE-1 as recipients (rifampin/fusidic acid-resistant, vancomycin-susceptible) using a donor/recipient ratio of 1 : 10 [8]. All donor strains were susceptible to rifampicin and fusidic acid, and transconjugants were selected on BHI agar plates supplemented with rifampin (100 mg/L), fusidic acid (20 mg/L), and vancomycin (16 mg/L).

All 70 VRE isolates harboured the *vanB2* gene (GeneBank accession number: AF173641) and were resolved into seven PFGE types (two for *E. faecalis* and for five *E. faecium*), using the previously described PFGE typing criteria [9]. Co-resistance to erythromycin, tetracycline, ciprofloxacin, and penicillins (only in *E. faecium* clones), and high-level resistance to aminoglycosides, was observed in all PFGE types, although three isolates of PFGE pattern A remained susceptible to high levels of gentamicin (Table 1). Antibiotic resistance

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TABLE 1. Characteristics of the 70 vancomycin-resistant *Enterococcus* isolates studied

PFGE type (no. of isolates)	Region (no. of hospitals)	Antibiotic resistance pattern	*van*B-associated elements	Other PCR-amplified genes
ST64 *E. faecium*				
A (8)	Santiago (3)	Pen, Amp, HLR-Ags[a], Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*, ISEnfa*110*	*aac(6′)–aph(2″)*, *aph3*, *ant6*, *erm*(B), *esp*
B (49)	Concepcion (1), Santiago (7), Talcahuano (1)	Pen, Amp, HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*, ISEnfa*110*	*aac(6′)–aph(2″)*, *aph3*, *ant6*, *tetM*, *erm*(B), *esp*
D (1)	Santiago (1)	Pen, Amp, HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*, ISEnfa*110*	*aac(6′)–aph(2″)*, *aph3*, *ant6*, *tetM*, *erm*(B), *esp*
E (1)	Santiago (1)	Pen, Amp, HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*, ISEnfa*110*	*aac(6′)–aph(2″)*, *aph3′*, *ant6*, *tetM*, *erm*(B), *esp*
F (1)	Santiago (1)	Pen, Amp, HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*, ISEnfa*110*	*aac(6′)–aph(2″)*, *aph3′*, *ant6*, *tetM*, *erm*(B)
ST201 *E. faecalis*				
C (9)	Concepcion (2), Santiago (4), Talcahuano (1), Temuco (1)	HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*	*aac(6′)–aph(2″)*, *aph3*, *tetM*, *erm*(B), *esp*, *hyl*, *gelE*–$fsr_{A–C}$, $cyl_{LS–M–A–B}$
E (1)	Concepcion (1)	HLR-Ags, Van, Lev, Tet, Ery, SxT	*vanB2*, Tn*5382*	*aac(6′)–aph(2″)*, *aph3*, *tetM*, *erm*(B), *esp*, *hyl*, *gelE*–$fsr_{A–C}$, $cyl_{LS–M–A–B}$

Pen, penicillin resistance; Amp, ampicillin resistance; HLR-Ags, high-level resistance to aminoglycosides; Van, vancomycin resistance; Lev, levofloxacin resistance; Tet, tetracycline resistance; Ery, erythromycin resistance; SUT, sulphamethoxazole resistance; PFGE, pulsed-field gel electrophoresis.
[a]Three isolates remained susceptible to high levels of gentamicin, and the *aac(6′)–aph(2″)* gene was not detected.

genes detected by PCR corroborated the resistance phenotype, except in one tetracycline-resistant *E. faecium* isolate, for which the mechanism responsible for tetracycline resistance was not detected. The nucleotide sequence of the *pbp*5 gene of one ampicillin-resistant *E. faecium* isolate of clone B revealed seven amino acid changes (H470Q, M485A, N496K, A499T, E525D, E629V, and P667S), and also a Ser466′ insertion. Both types of modification have been previously associated with overproduction of penicillin-binding protein 5 [10–12]. No *vanB2* transconjugants were obtained using either the *E. faecalis* or the *E. faecium* recipients at a limiting transfer frequency of 10^{-9}.

All five *E. faecium* PFGE clones were of the same MLST type and corresponded to the hospital-adapted clonal complex CC17–ST64 (7-1-1-1-1-1-1) [13,14]. The *E. faecalis* PFGE clones were also identical according to MLST, and were included in a new singleton, ST201 (1-7-1-7-25-1-4), that has been registered at the MLST website (http://efaecalis.mlst.net/). Nucleotide analysis of the glycopeptide resistance element in all different clones confirmed the association of the *vanB2* cluster with Tn*5382*. All *E. faecium* clones contained ISEnfa*110* between the IR_L and the $vanR_B$ gene, whereas the *E. faecalis* clones did not carry ISEnfa*110* or ISEnfa*200*. Amplicons of $vanX_B$–ORFC were obtained from the seven clones, and corresponded to the AF203412 allele previously deposited in GeneBank. Virulence factor genes were detected in *E. faecalis* clones, and the *esp* gene was found in four of the five *E. faecium* clones (Table 1).

The *vanB2* cluster can be transferred because of its association with the Tn*5382* element containing ISEnfa*110*, and transfer of this element frequently mobilizes a large chromosomal element containing a mutated *pbp*5 gene, which confers ampicillin resistance to *E. faecium* isolates. In contrast, isolates of the five ampicillin-resistant *E. faecium* CC17 PFGE types and of the two *E. faecalis* PFGE types described here were unable to transfer the vancomycin resistance determinant.

The ST64 *E. faecium* Tn*5382*–*vanB2*–ISEnfa*110*-containing clones and the ST201 *E. faecalis* Tn*5382*–*vanB2*-containing clones have been found in 16 hospitals in four regions in Chile. This is the first epidemiological characterization of the VanB phenotype in clinical isolates from South America, showing that *E. faecium* CC17 is distributed worldwide. Moreover, a new singleton named ST201 *E. faecalis* has been found. The apparent absence of *vanB2* transfer by conjugation in the strains studied here indicates that clonal dissemination of the VanB enterococcal strains was critical in the spread of vancomycin resistance among the 16 hospitals.

Transparency Declaration

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Clonal dissemination of two clusters of *Acinetobacter baumannii* producing OXA-23 or OXA-58 in Rome, Italy

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Abstract

Thirty consecutive *Acinetobacter baumannii* isolates producing carbapenem-hydrolysing oxacillinases, OXA-23 or OXA-58, were recovered from patients hospitalized in Rome, Italy, between January and November 2007. Among these isolates, two clones not associated with the European clones I or II were observed. The oxacillinase-encoding genes were plasmid- or chromosome-borne. This study reports the dissemination of carbapenem-resistant *A. baumannii* belonging to two clones among several units in a single hospital and emphasizes the ability of *A. baumannii* to cause epidemic/endemic outbreaks and also to acquire various resistance genes circulating in the hospital environment.

Keywords: *Acinetobacter baumannii*, carbapenem resistance, Italy, OXA-23, OXA-58

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Acinetobacter baumannii is an opportunistic pathogen present in healthcare environments, due to its ability to survive in human skin and dry surfaces to a greater extent than other hospital-acquired Gram-negative bacilli [1]. This microorganism produces chromosomally encoded class C and OXA-51/69-like β-lactamases, with their expression varying according to the presence of IS*Aba* [2–4]. This genetic background

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