Large clonal outbreak of multidrug-resistant CC17 ST17 Enterococcus faecium containing Tn5382 in a Spanish hospital

Sylvia Valdezate1,2*, Cristina Labayru3, Ana Navarro1, María A. Mantecón3, María Ortega3, Teresa M. Coque4–6, Moisés García3 and Juan A. Saéz-Nieto1

1Departamento de Bacteriología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; 2Unidad de Alertas y Emergencias, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; 3Servicio de Microbiología, Hospital General Yagüe, Burgos, Spain; 4Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Madrid, Spain; 5CIBER en Epidemiología y Salud Pública (CIBERESP), Spain; 6Unidad de Resistencia a Antibióticos y Virulencia Bacteriana Asociada al Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

Received 24 July 2008; returned 13 August 2008; revised 3 October 2008; accepted 5 October 2008

Objectives: A large clonal outbreak of multidrug-resistant CC17 ST17 Enterococcus faecium containing Tn5382 in a hospital in the north of Spain is described.

Methods: We characterized vancomycin-resistant E. faecium isolates from 10 infected and 40 colonized inpatients from a single hospital by PFGE, multiple-locus variable-number tandem-repeat analysis (MLVA) and multilocus sequence typing (MLST). Genes encoding antibiotic resistance (ampicillin, aminoglycosides, macrolides, quinupristin/dalfopristin, quinolones, tetracycline) and putative virulence traits were analysed.

Results: All isolates showed highly similar PFGE profiles and were assigned to the type MT1 by MLVA and to ST17 (CC17) by MLST. The Tn5382 type identified in all isolates was linked to pbp5 and contained a 5 bp deletion and 10 point mutations within the intergenic vanS–vanY region. Other resistance genes identified were erm(B), mef(E), tet(M), ant(6')-Ia, aph(3')-Ila and aac(6')-Ie-aph(2')-Ia. All isolates carried the unexpressed tet(M) gene. The high level of ciprofloxacin resistance was attributable to the first described Gly-61 and Ile-80 mutations in ParC and the Tyr-83 or Arg-83 mutations in GyrA. All isolates contained esp. The presence of hyl was variable.

Conclusions: A large clonal outbreak caused by multidrug-resistant CC17 E. faecium containing pbp5–Tn5382 is described. The persistence of this clone, which has been recovered from both hospital and community settings since 2005, and the possibility of transferring this Tn5382 to other epidemic ampicillin-resistant clonal types currently circulating in Spain might contribute to increasing the prevalence of vancomycin-resistant enterococci in our area. This study constitutes the first description of mef(E) in E. faecium.

Keywords: genotypes, molecular resistance mechanisms, vanB2, mef(E), tet(M)

Introduction

Vancomycin-resistant enterococci (VRE) in the hospital setting have increasingly been reported worldwide, although prevalence rates vary greatly among different geographical areas. Five genotypes have been described, of which VanA (Tn1546) and VanB (Tn1549/Tn5382) are the most common types detected.1

Most of the VRE causing human infections have been identified as Enterococcus faecium of the clonal complex CC17. This is mainly resistant to ampicillin, macrolides and quinolones and often contains the esp gene coding for a protein involved in colonization and biofilm formation.1 CC17 dissemination seems to have preceded the emergence of vancomycin resistance as reflected by its predominance in hospitals throughout the world.1–4

*Corresponding author. Servicio de Bacteriología Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, 28220 Madrid, Spain. Tel: +34-91-509-7901; Fax: +34-91-509-7966; E-mail: svaldezate@isciii.es

© The Author 2008. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
Spain is one of the European countries with the lowest rate of VRE (<5%), although self-limited hospital clonal outbreaks caused by VanA Enterococcus faecalis or VanA E. faecium have been reported from 1994 to 2006. VanB E. faecium has scarcely been described in Spain since its first description in 2001, although its prevalence in Spanish hospitals might have been underestimated since inter-hospital dissemination of a particular clone has been recently identified. In this report, we describe a clonal outbreak caused by CC17 VanB2 E. faecium in a single Spanish hospital that involved 50 patients. This clonal outbreak is the largest reported in Spain to date and is the second caused by a VanB in our country.4

Materials and methods

Bacterial strains

Fifty vancomycin-resistant E. faecium isolates were collected from June 2006 to June 2008 in Hospital General Yague in Burgos, a 634 bed tertiary care hospital in the north of Spain. They included nine clinical isolates from different infected patients (average length of stay 33 days, range 6–48 days), from post-surgery injury (n = 5), abscess and abdominal drainage (n = 2), urine (n = 1) and ascites (n = 1). When a hospital surveillance programme was implemented, vancomycin-resistant E. faecium was detected from rectal swabs of 38 patients. Patients were located in General Surgery (70.2%), Internal Medicine (19.1%), Medical-ICU (8.5%) and Primary Care Service (2.1%). Identification of the strains was done by the API2032 Strep system (bioMérieux, Marcy l’Étoile, France) and confirmed by sequencing of 16S rDNA. In the following 9 months, vancomycin-resistant E. faecium were not identified. However, the outbreak strain was recovered from the urine of a patient at a long-term care facility and from rectal swabs of two patients located in the Surgery ward in January and June of 2008, respectively.

Antimicrobial susceptibility

MICs of 28 antibiotics were determined by Etest (AB BIODISK, Solna, Sweden) or by the semi-automatic Phoenix system (Becton Dickinson Diagnostic System, Pont de Claix, France) following the manufacturers’ instructions. MICs were interpreted by CLSI criteria.

Virulence-epidemicity genes

The presence of the genes encoding enterococcal surface protein (esp), hyaluronidase (hyl), aggregation substance (asa1), cytolysin (cylA) and gelatinase (gelE) was analysed by a multiplex PCR assay using primers listed in Table S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

Clonal relatedness

Clonal relatedness was established by PFGE,5 multilocus sequence typing (MLST; http://efaecium.mlst.net/) and multiple-locus variable-number tandem-repeat analysis (MLVA; www.mlva.umcutrecht.nl/).

Characterization of genes coding for antibiotic resistance

The presence of genes involved in resistance to glycopeptides (vanA, vanB, vanC1, vanC2/3, vanD), macrolides [erm(A), erm(B), erm(C), mef(A/E)], quinupristin/dalfopristin [vga(D) and vga(E), vgb(A)], tetracyclines [tet(M)], quinolones (gyrA, gyrB, parC, parE) and aminoglycosides [aac(6’)-Ie-aph(2”)-Ia, aac(3’)-Ila, ant(6’)-Ia] was investigated (Table S1). The Tn5382 backbone was characterized by analysing specific sequences corresponding to pbp5–Tn5382, vanS–vanV and vanX–ORFC regions (Table S1).

Results and discussion

Epidemiological background

Eight highly related PFGE types arbitrarily designated with numbers were identified among the 50 vancomycin-resistant E. faecium isolates studied (Figure 1). Three main PFGE types were identified: type 1, the first to be detected, was isolated from three wards for 2 years (n = 37 patients, 74%); type 6 was recovered from five patients located in two wards for 101 days; and type 7 was identified in two patients from the same ward for a 7 day period. PFGE types 3, 4, 5 and 8 were recovered for 105 days. Following the emergence of type 1, the highly related type 2 was detected 30 days later from an outpatient, and all the remaining variants were identified thereafter.

Strains representative of each PFGE type were assigned to a single MLVA type MT1 (5:7:3:2:3) and to the MLST type ST17, which belongs to the clonal complex CC17. ST17 constitutes one of the most common sequence types detected among CC17 isolates worldwide, and it is associated with clonal outbreaks in Spain.2,4 The clonal strain recovered from a recent VanB outbreak in Spain showed a similar PFGE pattern to those of type 1.4

Antimicrobial susceptibilities

All isolates showed a low level of resistance to vancomycin (MIC = 12–16 mg/L), and susceptibility to teicoplanin. They were resistant to: ampicillin (MIC > 256 mg/L); erythromycin, clarithromycin and clindamycin (MIC > 256 mg/L); and ciprofloxacin and levofloxacin (MIC > 32 mg/L); with a high level of resistance to streptomycin (MIC > 1000 mg/L), amikacin and tobramycin (MIC > 256 mg/L). Variable susceptibility ranges were obtained for quinupristin/dalfopristin (MIC = 3–16 mg/L), gentamicin (MIC = 32 to >256 mg/L), moxifloxacin (MIC = 8 to >32 mg/L) and rifampicin (MIC = 4 to >16 mg/L), linked to specific clones. All isolates remained susceptible to tetracycline, tigecycline, trimethoprim/sulfamethoxazole, chloramphenicol and linezolid.

Virulence-epidemicity genes

The esp gene was detected in all isolates, while the hyl gene was sporadically identified (7/50, 14%) in isolates of the PFGE types 1 (n = 4/37), 4, 5 and 8 (one each). esp/hyl sequences were identical with others described previously. The variable presence of esp and hyl among isolates of the same clone has previously been observed in other settings, although an association with a transferable element has been proved only for esp.2,6 The asa1, cylA and gelE genes were not detected.
CC17 vanB2 E. faecium multiresistant outbreak

The analysed sequences of Tn5382 from representative isolates of the eight PFGE types and 22 isolates of type 1 were highly similar to those of variants described previously in Norway, Spain and France that contained a characteristic 5 bp deletion and 10 point mutations within the intergenic vanS\textsubscript{n}–vanY\textsubscript{p} region.\textsuperscript{3,4} However, the vanB2 sequence showed the change Met-151(ATG)→Ile(ATT) identified previously only in the Australian E. faecium strain MLG856-2 (accession no. AY655721.2). In addition, the pbp5 located at the 5’ end of Tn5382 differed in 10 amino acids from the pbp5 prototype (accession no. X84860): Lys-461, Gln-470, Ala-485, Lys-496, Thr-499, Asp-525, Leu-586, Val-629, Gln-632 and Ser-667; for representative isolates of each clone and six isolates of type 1. This sequence showed the maximum identity with that described by Torres et al.\textsuperscript{3} differing only at positions 466', 632 and 667. As observed for Italian and Spanish E. faecium isolates, the pbp5 did not contain a Ser-466' insertion, which seems to contribute greatly to β-lactam resistance in combination with other mutations in the C-terminal site.\textsuperscript{7}

An unexpressed tet(M) gene was detected in all isolates (tetracycline MIC ≤ 0.25 mg/L), showing a sequence identical to that of Staphylococcus aureus (accession no. M21136). The presence of tet(M) in tetracycline-susceptible and tetracycline-resistant isolates is frequent among both streptococci and enterococci harbouring Tn916-like elements.\textsuperscript{8,9} In addition, two macrolide resistance genes were detected in 11 isolates corresponding to different PFGE types, erm(B) (coding for a methylase) and mef(E) (coding for a macrolide-efflux protein), which were identical to others described previously. While erm(B) is widely distributed among enterococci and may be located on different genetic elements, mef(E) has only been associated with enterococci once, this study being the first description of mef(E) in E. faecium.\textsuperscript{9} The mef genes are linked to Tn1207.1 or the Tn2010 mega element of Streptococcus pneumoniae, sometimes located in a Tn916 platform, which often contains erm(B) besides the characteristic tet(M).\textsuperscript{9} The possible association of these genes in a common platform as reported for other Gram-positive organisms cannot be discarded.

The var(D), var(E) and vgb(A) genes were absent in all isolates.

Genes coding for aminoglycoside adenyltransferase ANT(6')-Ia and the phosphotransferase APH(3’)-IIIa were present in all strains, while that encoding AAC(6')-Ie-APH(2')-Ia was only detected in an isolate of type 6 that displayed high-level resistance to gentamicin.

All clones showed double mutations in ParC (Gly-61 and Ile-80). The Tyr-83 or Arg-83 mutations in GyrA were identified in types 1–7 and type 8, respectively. These mutations, with the exception of Gly-61 in ParC, have already been published. The ParC mutation Ser-80→Ile alone or combined with the GyrA mutation Ser-83→Tyr confers high-level ciprofloxacin resistance (MIC = 64 to >128 mg/L). It is noteworthy that the moxifloxacin MIC increased from 12 mg/L in clones containing Tyr-83 GyrA to >32 mg/L for the strain containing Arg-83. This ability of CC17 E. faecium strains to accumulate GyrA/ParC mutations confers a higher level of ciprofloxacin resistance (64 to >128 mg/L).\textsuperscript{10}

To summarize, we describe the second Spanish clonal outbreak caused by CC17 vanB2 E. faecium, which is one of the largest reported in Europe to date. The persistence of this clone, which is still detected in Hospital General Yagüe, and the possibility of transferring this Tn5382 to other ampicillin-resistant clonal types currently circulating in Spain (P. Ruiz-Garbajosa, G. M. Cárdenas, R. Cantón, F. Baquero and T. M. Coque, unpublished results) might contribute to increasing the prevalence of VRE in our country as has recently happened in other EU locations. The findings associated with genes encoding resistance to florquinolones, tetracycline [unexpressed tet(M)] and macrolides [mef(E)], first described in E. faecium, deserve to be further studied.

GenBank accession numbers
The allele variants of vanB2, pbp5, mef(E), tet(M) and parC genes were assigned to accession numbers EU908677–EU908681.

Acknowledgements
Our appreciation to the Biopolymers Unit of the CNM for assistance in sequencing and M. Harper for revising the English language of the manuscript.

Funding
This research was supported by a contract (MPY 1116/07) from the Instituto de Salud Carlos III to A. N. This work was partially
supported by grants from the European Union Sixth Framework Programme (ACE-LSHE-2007-037410) and from the Fondo de Investigaciones Sanitarias (PI06/1141).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


