Analysis of VanA vancomycin-resistant Enterococcus faecium isolates from Saudi Arabian hospitals reveals the presence of clonal cluster 17 and two new Tn1546 lineage types

Mushtaq A. Khan1, Martin van der Wal1, David J. Farrell2, Luke Cossins2, Alex van Belkum1, Alwaleed Alaidan3 and John P. Hays1*

1Department of Medical Microbiology and Infectious Diseases, Erasmus MC, s-Gravendijkwal 230, 3015CE Rotterdam, The Netherlands; 2Department of Molecular Biology, GR Micro Ltd, 7-9 William Road, London NW1 3ER, UK; 3King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh 11211, Kingdom of Saudi Arabia

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Objectives: The aim of this study was to characterize 34 vancomycin-resistant VanA Enterococcus faecium isolates obtained from two hospitals in Saudi Arabia and to assess Tn1546 variation within these isolates.

Methods: PFGE and multilocus sequence typing (MLST) genotypes, antibiotic susceptibility patterns, the presence of enterococcal surface protein (esp) and hyaluronidase (hyl) genes and conjugation frequencies were determined. In addition, Tn1546 elements were characterized.

Results: PFGE and MLST analysis revealed the presence of 31 and 6 different genotypes, respectively. Further, three new ST types were discovered. Ninety-seven percent (33/34) of the isolates were associated with clonal complex 17 (CC17), with all isolates but one being resistant to ampicillin and all isolates being susceptible to linezolid. The esp and hyl genes were found in 44% (15/34) and 53% (18/34) of the isolates, respectively. Tn1546 analysis revealed that the isolates belonged to five different groups, including two new lineages. The IS-element insertions described did not abolish the transfer of VanA resistance.

Conclusions: VanA vancomycin-resistant E. faecium isolates obtained from Saudi Arabian hospitals include CC17 MLST types, a clonal cluster associated with E. faecium nosocomial infection worldwide. Novel E. faecium MLST types are circulating in Saudi Arabia, as well as novel Tn1546 types. It seems likely that CC17 E. faecium isolates may be distributed throughout the Middle East as well as Europe, America, Africa and Australia.

Keywords: transposons, bacterial genotypes, pulsed-field gel electrophoresis, multilocus sequence typing

Introduction

Enterococci and vancomycin-resistant enterococci (VRE) are often found in the human and animal gut and have become increasingly responsible for nosocomial infections, particularly in the USA. In Europe, however, VRE have tended to be associated with community carriage and occasional nosocomial outbreaks, although the incidence of VRE infection may be changing.1 Enterococci often express high-level resistance to glycopeptides and aminoglycosides, with vancomycin resistance having been promoted via the extensive use of vancomycin in hospitals, as well as the animal growth promoter avoparcin.

Six different vancomycin resistance types have so far been described in enterococci, namely VanA, VanB, VanC, VanD, VanE and VanG. The VanA type is characterized by both high-level and inducible resistance to vancomycin (MICs 64–1024 mg/L) and teicoplanin (MICs 16–512 mg/L) and has been shown to be directly facilitated by the carriage of transposon Tn1546, a transposon widely disseminated in humans, animals and the environment.2

*Corresponding author. Tel: +31-10-4632177; Fax: +31-10-4633875; E-mail: j.hays@erasmusmc.nl

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Currently, there is a distinct lack of data regarding the molecular analysis of VanA Enterococcus faecium Tn1546 carriage in VRE isolates originating from the Middle East, including Saudi Arabia. Therefore, the aim of this study was to determine E. faecium genotypic variation and VanA Tn1546 transposon diversity in VRE isolates recovered from the Kingdom of Saudi Arabia.

Materials and methods

Bacterial isolates

Thirty-four vancomycin-resistant E. faecium isolates were cultured from clinical specimens obtained from two large tertiary-care hospitals in the Kingdom of Saudi Arabia, namely the King Faisal Specialist Hospital and Research Centre, Riyadh (KFSH&RC), and the King Fahad National Guard Hospital, King Abdulaziz Medical City, Riyadh (KFNGH). Isolates were collected between 2000 and 2003. Two pairs of isolates (25/29 and 17/32) were isolated from two different patients, but on separate occasions.

PFGE and multilocus sequence typing (MLST)

PFGE was performed using Smal-digested fragments of bacterial chromosomal DNA, with fragment separation achieved in 0.8% agarose. Electrophoresis conditions comprised using a constant voltage of 6 V/cm at 14 °C and pulse times of 3.5–25 s increased linearly over 12 h (block 1), followed by 1–5 s increased over 8 h. Gel patterns were analysed using BioNumerics software (Applied Maths) with the band tolerance set at 1.0%. MLST was performed using internal fragments from seven housekeeping genes. Allele numbers and sequence types were assigned after reference to the online E. faecium MLST database at http://efaecium.mlst.net/.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the VITEK 2 automated identification and susceptibility system (bioMérieux, Marcy l’Étoile, France) according to the manufacturer’s instructions.

PCR characterization of Tn1546 elements and screening for virulence genes

Primer pairs used to characterize Tn1546 were based on those published by Miele et al. In addition, primer walking was utilized to define insertion site-specific sequences. PCR screening for the enterococcal surface protein (esp) and hyaluronidase (hyl) virulence genes used three different primer pairs. For the esp gene, primer pairs esp11 (5′-TTGCTAATGTGATCTCCAGGACC-3′)/esp12 (5′-GCGTCAAACATTGATGGCCGAA-3′) and 14F (5′-AGATTTATCTTGGG3′)/12R (5′-AATTGATCTTTTAGCTTGGA-3′) were used, and for the hyl gene, primer pair HYL n1 (5′-ACAGAAGACTGACGAAAATG-3′)/HYL n2 (5′-GACTGACGTCCAAGTTCACCA-3′). A touchdown PCR protocol was used for all primer pairs, comprising an initial annealing temperature of 65 °C, which was initially reduced by 1 °C per cycle over 15 cycles, and then followed by 20 cycles of amplification using an annealing temperature of 55 °C. All PCRs were performed using Taq DNA polymerase (EP0402, Fermentas).

Conjugation frequency

Conjugation frequency was determined using a standard methodology, with E. faecium GE1 being used as the recipient strain, and antibiotic concentrations of 6 mg/L vancomycin, 64 mg/L rifampicin, and 10 mg/L fusidic acid being used. Conjugation frequencies were calculated with reference to the donor isolate.

Results and discussion

PFGE and MLST genotyping

PFGE identified 18 ‘possibly related’ PFGE genotypes based on a 70% similarity cut-off value (approximately equivalent to four to six PFGE fragment differences), with all isolates being genotypically related at a similarity of 34% (Figure 1). MLST genotyping revealed six ST types, with three new ST genotypes (ST 358, 359 and 360) and 59% (20/34) of the isolates being ST 17 genotypes. Further, the three new ST types (four isolates) and ST 16 (two isolates) were exclusively associated with the KFSH&RC, whereas ST 18 (eight isolates) was exclusively associated with the KFNGH. In contrast, ST 17 was recovered from both hospitals. Ninety-seven percent (33/34) of the isolates belonged to clonal complex 17 (CC17), a cluster of E. faecium MLST genotypes previously associated with global nosocomial infections. CC17 has been previously associated with ampicillin resistance and carriage of the enterococcal surface protein pathogenicity island. In Saudi isolates, ampicillin resistance and esp gene carriage were found in 99% and 45% of the isolates, respectively, indicating that CC17 isolates from Saudi Arabia possess similar characteristics to those obtained from other well-documented regions of the world.

Antibiotic susceptibility patterns

All isolates but one were resistant to vancomycin and ampicillin, and all isolates were susceptible to linezolid (Figure 1). No pattern was observed with respect to the general expression of multiple antibiotic resistances and PFGE genotype.

Characterization of Tn1546 elements

In total, five different Tn1546 types were observed within our 34 E. faecium isolates (Figure 2). In eight isolates (numbers 2, 4, 5, 6, 12, 34, 35, and 41), the PCR amplicon size analysis revealed no major insertions or deletions, compared with theoretical amplicon products generated using reference Tn1546 BM4147 sequence. In isolates where Tn1546 polymorphisms were found, the majority of polymorphic events involved the insertion of IS1216V within the intergenic regions of either vanS and vanH or vanX and vanY genes, along with other less frequent insertions of IS1485 and IS1251. In fact, IS1216V is one of the most common insertion sequences found in Tn1546-bearing vancomycin-resistant enterococci worldwide. Further, IS1251 has previously been demonstrated to be present in Tn1546 transposons present within human clinical E. faecium isolates from Brazil, Korea, the USA, Norway and Ireland. Our Tn1546 results allowed us to add two new lineages to a previously published evolutionary scheme, namely lineage III (IS1485 insertion in orfI) and lineage IV (IS1216V insertion between vanS and vanH).
Using PCR screening, the enterococcal surface protein (esp) gene was found to be present in 44% (15/34) and the hyaluronidase (hyl) gene in 53% (18/34) of the isolates tested. esp-positive isolates made up 46% (10/22) of the isolates from the KFNGH and 41% (5/12) of the isolates from the KFSH&RC. In contrast, hyl-positive isolates made up 41% (9/22) of the isolates from the KFNGH but 75% (9/12) of the isolates from the KFSH&RC. Only four esp-positive isolates were also found to be hyl-positive, whereas 14 hyl-positive isolates were found to be esp-negative. A significant difference in the prevalence of esp and hyl-positive isolates was observed with respect to PFGE clusters (Figure 1), with 71% (10/14) of the esp-positives belonging to the cluster bordered by isolates 19 and 40 (Fisher’s exact test \( P = 0.004 \)). The distribution of the two hospitals between the PFGE clusters defined earlier was not significant (Fisher’s exact test \( P = 0.066 \)). The expression of enterococcal surface protein (Esp) has been associated with both biofilm formation and nosocomial infection in \( E. \) faecium,7 as well as CC17 isolates.8 Further, a recent publication indicated that clinical \( E. \) faecium isolates carrying the esp gene possess an increased conjugation frequency with respect to the ability to acquire VanA vancomycin resistance.9 The prevalence of the esp gene within our 33 CC17 isolates was somewhat lower than previously reported,1 although all isolates were ampicillin-resistant. However, 91% (30/33) of the CC17 isolates possessed either the esp or hyl virulence genes. The hyaluronidase protein (Hyl) is a virulence trait also associated with clinical \( E. \) faecium isolates, with a ratio of 2:1 for esp:hyl-positive isolates reported in a sample of 577 isolates worldwide.10 This compares with a ratio of 1:1.2 in our smaller group.
Conjugation frequency

Conjugation frequencies of representative isolates from each transposon group ranged from $2.2 \times 10^{-4}$ to $1.9 \times 10^{-6}$. These frequencies lie within the range of previously published transformation frequencies observed for other global VanA-resistant, Tn1546-carrying E. faecium isolates.

Conclusions

In this study, we investigated 34 vancomycin-resistant VanA E. faecium isolates cultured from two hospitals situated in Riyadh, Saudi Arabia. The majority of the isolates tested belonged to CC17 and were resistant to ampicillin, suggesting that E. faecium MLST types associated with nosocomial infection are also circulating in the Middle East. The Tn1546 analysis revealed two new lineages, including two previously undescribed IS1485 and IS1216V insertions.

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Transparency declarations

None to declare.

References

Vancomycin-resistant Enterococcus faecium in Saudi Arabia


