Diversity of plasmids and Tn1546-type transposons among VanA Enterococcus faecium in Poland

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Abstract The objective of this study was to investigate the antimicrobial resistance, Tn1546 transposon variability and plasmid diversity among Polish vancomycin-resistant Enterococcus faecium (VREfm) isolates of VanA phenotype in the context of their clonal structure. Two hundred sixteen clinical VREfm isolates collected between 1997 and 2010 were studied by antimicrobial susceptibility testing, MLST, MLVA and detection of IS16, espEfm, pilA, intA and plasmid-specific genes by PCR. Tn1546 structure was revealed by overlapping PCR and sequencing. Selected isolates were subjected to PFGE-S1 and Southern hybridization analyses. The vast majority of the isolates (95.8 %) belonged to lineages 17/18 (during the whole study period 1997–2010) and 78 (mostly in 2006–2010) of hospital-adapted meroclone of E. faecium. All isolates displayed a multi-drug resistance phenotype. Twenty-eight Tn1546 types (including 26 novel ones) were associated with eight different ISs (IS1216, IS1251, ISEfa4, ISEfa5, ISEfim2, ISEf1, IS3-like, ISEfim1-like). The vanA-determinant was typically located on plasmids, which most commonly carried rep2pRE25, rep17pRUM, rep18, rep1pIF51, ω-ε-ζ, and axe-xe genes. VanA isolates from 1997–2005 to 2006–2010 differed in clonal composition, prevalence of gentamicin- and tetracycline-resistance and plasmidome. Our analysis revealed high complexity of Tn1546-type transposons and vanA-plasmids, and suggested that diverse genetic events, such as conjugation transfer, recombination, chromosomal integration and DNA mutations shaped the structure of these elements among Polish VREfm.

Introduction

In the past 20 years, vancomycin-resistant enterococci (VRE) have emerged as nosocomial pathogens worldwide. In Poland, the first VRE outbreak due to Enterococcus faecium (VREfm) of VanA phenotype started in December 1996 in the Gdansk Medical University [1]. The vast majority of VREfm observed worldwide belongs to a specific hospital meroclone, initially described as clonal complex 17 (CC17), later divided into three distinct lineages 17, 18 and 78 based on multilocus sequence typing (MLST) analyses [2, 3]. Recently, the approach called Bayesian Analysis of Population Structure (BAPS), applied to the E. faecium MLST data delimited two groups within the hospital meroclone, 2–1 and 3–3, corresponding to lineages 78 and 17/18, respectively [4]. Strains belonging to the hospital merocline are ciprofloxacin- and ampicillin-resistant, enriched in putative virulence traits, and show a distinct genetic repertoire, including cell surface protein genes (fms), regulatory genes, putative pathogenicity islands, plasmids, insertion sequences (IS) and integrated phages, which promote their adaptation [5–7]. The presence of IS16 and the E. faecium-specific esp gene (espEfm), carried on the integrative conjugative element ICEEfm1, together with the intA integrase gene, are
proven molecular markers of hospital-associated *E. faecium* [8–10].

Several glycopeptide-resistance phenotypes have been described so far, with VanA and VanB being the most common in enterococci isolated from hospital infections [11]. The vanA gene cluster is carried on Tn1546-type transposons [12], which show a significant degree of heterogeneity, associated with presence of point mutations, deletions and presence of various ISs [13, 14]. A few studies demonstrated the location of Tn1546 on Inc18, pRUM-like, pMG1-like, and pLG1 plasmids [15, 16]; however, the knowledge of vanA-plasmids and their epidemiology is still far from being satisfactory and may differ significantly among countries.

In Poland, hospital VRE isolates are continuously submitted for confirmation and further analyses to the National Reference Centre for Susceptibility Testing (NRCST), located at the National Medicines Institute in Warsaw. The aim of this study was to characterize *E. faecium* VanA isolates collected by the NRCST since 1997 until the end of 2010, focusing on the Tn1546 transposon variability and vanA-plasmid diversity in the context of the clonal structure of VRE*fm* isolates to provide the country-wide picture of these important hospital pathogens.

Materials and methods

**Bacterial isolates and susceptibility testing**

The study comprised 216 consecutive, non-repetitive (1 isolate per patient) VRE*fm* VanA isolates received by the NRCST from 42 hospitals in 24 cities in Poland over the period 1997–2010. Part of the isolates analyzed in this work correspond to strains partially tested in previous surveillance studies, including: 108 VanA representatives of the VRE*fm* collection from 1997 to 2005 [17] and 20 representative isolates of a *E. faecium* VanA outbreak in 2009 [18]. The majority of isolates (*n* = 137) were derived from 11 VanA outbreaks and the remaining 79 isolates were reported as single isolations. Of the 216 isolates, 211 (97.7 %) were from hospitalized patients and five (2.3 %) were from the hospital environment. Among the isolates from hospitalized patients, a total of 37 isolates (17.5 %) were from invasive infections (31 isolates from blood and 6 from other sources); 52 isolates (24.6 %) were from non-invasive infections (21 from urine, 18 from wounds, and 13 from other sources) and 122 (57.8 %) represented faecal carriage. Antimicrobial susceptibility of 88 isolates, not investigated previously, was tested by the Etest method (bioMérieux, Marcy l’Etoile, France) for daptomycin, teicoplanin and vancomycin and by a broth microdilution method [19] for the remaining compounds (Table 1). Multidrug-resistant (MDR) isolates were defined as recommended [20]. Vancomycin-resistance determinants were detected by PCR as described previously [21] with the *E. faecium* BM4147 and *E. faecalis* V583 strains as positive *vanA* and *vanB* controls, respectively.

**DNA isolation and genotyping of isolates**

Total DNA of isolates was extracted using Genomic DNA Prep Plus kit (A&A Biotechnology, Gdansk, Poland). Multilocus VNTR analysis (MLVA), MLST, and detection of 19 *rep* families and the unique *rep*MG1 gene were performed as described [22–24]. Sequence types (STs) were grouped to CCs by the comparative eBURST analysis performed against the whole *E. faecium* MLST database. PCR detection of IS16, *esp*Efms, *fms*21 (*piLA*), *rep*pLG1, plasmid addiction systems, relaxase genes, and *intA*ICE*Efms1* was performed as described (Supplementary Table 1 and references therein). DNA of enterococcal isolates from our laboratory collection [17, 18, 25] served as positive controls.

**Plasmid profiling, hybridization analyses, Tn1546 typing and statistical analysis**

DNA in agarose plugs was obtained as described [21], treated with SI nuclease (Takara Bio, Japan) and separated by PFGE with Lambda Ladder PFG marker (New England Biolabs, Beverly, MA) [26] followed by blotting onto the Hybond membrane (GE Healthcare, Buckinghamshire, UK) by capillary transfer. Hybridization was carried out using the Amersham ECL Random-Prime Labelling and Detection System (GE Healthcare, Buckinghamshire, UK). Tn1546 transposon was investigated by PCR mapping and sequencing (Supplementary Table 1 and references therein) of selected regions encompassing 7571 bp out of 10851 bp (i.e., ~70 % of the transposon, Fig. 1). The Tn1546 sequence of *E. faecium* BM4147 (GenBank acc. no.: M97297) [12] was used as a reference. The nomenclature of Tn1546-type transposons in the present study was based on the following alphanumeric code: the ‘A’ types (A1-A6) referred to transposon variants of the wild type (wt) Tn1546 structure (A1) not interrupted by insertion sequences; the ‘B’ types contained 1–3 copies of IS1216 (B, BB, BBB types); the C, D, E, F, G, H and I types carried IS1251, *ISEfa5*, *ISEfa4*, *ISEfm2*, *ISEfj1*, *ISEfm1*-like and IS3-like elements, respectively. Transposons with more than one IS type were described by a two-, three- or four-letter code (e.g., ‘BC’ with both IS1216 and IS1251). The Arabic numerals indicated differences in the presence of particular point mutations as well as the orientation of ISs and the localization of their insertion sites (e.g., B1–B4). The novel *ISEfm1*-like sequence was submitted to GenBank
Chi-square test was used to assess the differences of distributions, with $p \leq 0.05$ considered significant.

**Results**

**Susceptibility to antimicrobial agents**

All isolates were resistant to vancomycin and teicoplanin (Table 1) and carried \textit{vanA}. Resistance to ampicillin, ciprofloxacin, tetracycline, chloramphenicol, gentamicin and streptomycin (high level) was prevalent or highly prevalent and all isolates showed the MDR phenotype. A significant decline in the prevalence of both tetracycline-resistance (from 68.9 to 52.3 %, $p = 0.01$) and high-level gentamicin resistance (from 92.1 to 64.4 %, $p < 0.0001$) was found between the 1995–2005 and 2006–2010 periods. A single isolate was resistant to linezolid and two isolates to quinupristin/dalfopristin. All isolates were susceptible to tigecycline and daptomycin.

**MLVA, MLST, IS16 and virulence markers detection**

MLVA was performed for 196 isolates and these results were analysed together with data obtained earlier for 20 isolates from the 2009 outbreak [18]. Among 216 isolates, 37 different MLVA types (MTs) and three incomplete profiles (due to lack of VNTR7 amplification) were observed, that included 207 and nine isolates, respectively (Supplementary Table 2). MT1, MT159, MT25 and MT13 were most prevalent, with 36, 34, 26 and 20 isolates, respectively. All MT159 isolates except one were isolated in 2006–2010 ($p \leq 0.0001$), in contrast to isolates of MT25, which all except one were isolated in 1997–2005 ($p = 0.0001$). The MT1 isolates showed a similar frequency over the whole study period (13.2 % vs 21.6 %, $p = 0.07$).

**Table 1**

| Compound/phenotype | 1997–2010 N = 216 | 1997–2005 N = 128 | 2006–2010 N = 88 | MIC breakpoints/ECOFF (μg/ml) |
|--------------------|------------------|------------------|-----------------|-----------------------------|
|                    | Number (%) non-susceptible | Number (%) non-susceptible | MIC 50 (mg/l) | MIC 90 (mg/l) | Number (%) non-susceptible | MIC 50 (mg/l) | MIC 90 (mg/l) | S ≤ | R > |
| Vancomycin\(^a\)  | 216 (100) | 128 (100) | 512 >512 | 88 (100) | >256 >256 | 4 | 4 |
| Teicoplanin\(^a\) | 216 (100) | 128 (100) | 64 128 | 88 (100) | 48 >256 | 2 | 2 |
| Ampicillin\(^a\)  | 215 (99.5) | 128 (100) | >128 >256 | 87 (98.8) | 128 >256 | 4 | 8 |
| HLRG\(^a\)        | 172 (79.6) | 118 (92.2) | >1024 >1024 | 54 (64.4) | >1024 >1024 | 128 128 |
| HLSR\(^a\)        | 167 (77.3) | 112 (87.5) | >1024 >2048 | 55 (62.5) | >1024 >2048 | 512 512 |
| HLAR\(^28\)       | 147 (68.1) | 110 (86) | >1024 >1024 | 37 (42) | >1024 >2048 | - - |
| Quinupristin/ dalfopristin\(^a\) | 2 (0.9) | 1 (0.8) | 1 2 | 1 (1.1) | 1 1.5 | 1 4 |
| Linezolid\(^d\)   | 1 (0.5) | 0 (0) | 1 4 | 1 (1.1) | 1 2 | 4 4 |
| Tigecycline\(^e\) | 0 (0) | 0 (0) | 0.06 0.19 | 0 (0) | 0.06 0.25 | 0.25 0.5 |
| Tetracycline\(^b\) | 135 (62.2) | 89 (68.9) | 64 128 | 46 (52.3) | 8 128 | 4 4 |
| Ciprofloxacin\(^b\) | 215 (99.5) | 127 (99.2) | 128 >256 | 88 (100) | 128 256 | 4 4 |
| Daptomycin\(^b\)  | 0 (0) | 0 (0) | 2 3 | 0 (0) | 2 3 | 4 4 |
| Chloramphenicol\(^c\) | 53 (24.4) | 30 (23.2) | 8 16 | 23(26.1) | 8 16 | 8 4 |
| MDR\(^28\)        | 216 (100) | 128 (100) | nc nc | 88 (100) | nc nc | - - |

\(\text{nc not calculated}, n \text{ number of isolates}\)

The results were interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-approved breakpoints [53] and the Ecological Cut-Off (ECOFF) values for compounds without defined breakpoints (http://mic.eucast.org/Eucast2/, last accessed 20th July 2015). For chloramphenicol the Clinical and Laboratory Standards Institute (CLSI) breakpoints were used [19]\(^a\) Interpretation according to the EUCAST clinical breakpoint value \(^b\) Interpretation according to the EUCAST Ecological Cut-off (ECOFF) value \(^c\) Interpretation according to CLSI breakpoint value

(KT719407).
Fig. 1 Diversity of Tn1546 transposon types among *E. faecium* VanA isolates. Position of primers used in PCR mapping and sequencing indicated by arrows with primer names; open rectangles, transposon genes; stars, positions of point mutations; analyzed areas of the transposon shadowed; dashed lines, deletions in the left arm of the transposon; filled rectangles, deletions within the transposon; vertical arrow, triangles with arrows, the IS positions; single-nucleotide insertion in *vanY*
Structural diversity of Tn1546 transposons

The structure of Tn1546-type transposons was determined for 187 isolates while for 20 isolates the structure of the transposon (representing types A1 and G, Fig. 1) had been published earlier [18]. In the case of nine isolates the structure of the transposon could not be determined in spite of repeated attempts due to lack of PCR products for some parts of the transposon, which might have been caused by sequence polymorphism(s) within PCR primers annealing sites, and discrepancies of sequencing results in certain regions, likely associated with the presence of

Fig. 1 continued.
more than one transposon in a single isolate. Twenty-eight transposon types, including 26 new ones, were discerned in the analyzed group (Fig. 1). The most predominant types, including C1 (40 isolates), B2 \((n = 38)\), A3 \((n = 36)\), G \((n = 25)\), E \((n = 14)\), A1 \((n = 13)\) and D \((n = 7)\), were associated with several STs and typically showed a multicenter distribution. Eight different ISs were detected within Tn1546, including IS1216, IS1251, ISEfa4, ISEfa5, ISEfm2, ISEf1, IS3-like and ISEfm1-like. The most common IS1216 was present in all 16 B-type transposons, both in the direct and reversed orientations, with five different types of 8-bp direct repeats. These B-type transposons were found in Gdańsk and Warsaw, as well as in 14 other cities. IS1251 was associated with seven Tn1546 types (C1-C2, BC1-BC5) and present in 53 isolates (25.6 %), which mainly originated from Kraków and Warsaw. In these isolates, IS1251 was always inserted in the vanS-vanH intergenic region of the transposon at the position 5813. The D, E, F and G types of transposon were characterized by the presence of ISEfa5, ISEfa4, ISEfm2 and ISEf1 in the vanX-vanY intergenic region, respectively. These types were generally limited to one or two centres, with the exception of the G type, which apart from the outbreak in two Warsaw hospitals [18].
occurred in seven other centres. An insertion of an IS in 98% identical to IS\textsubscript{Efm1} (GenBank no. AF138282) in the \textit{vanX-vanY} intergenic region resulted in the BH-type of transposon.

The variability of Tn\textsubscript{1546} was additionally associated with the presence of deletions, insertions and point mutations. In the A4 type, a 44 nt deletion in the \textit{vanS-vanH} intergenic region (nt 5896–5939) was observed. Other deletions, located in \textit{orf2}, \textit{vanX} and the \textit{vanX-vanY} intergenic region coincided with the presence of ISs (six types: BB1, BB2, BC4, BC5, BBI, BBBI2). Seven different point mutations were detected, including four known previously (T7658C, G7747T, G8234T, C9692T) [18, 27, 28] and three new ones (G5603A, A8138G and G9063T). The G5603A mutation resulted in the A80T change in VanS and the G9063T mutation in the L4F change in VanY. The B4 type, found in two independent isolates, demonstrated the presence of a novel single-nucleotide T insertion between nt 9063–9064 within the \textit{vanY} gene, resulting in translational frameshift and a truncated VanY. Nevertheless, these two isolates showed high MIC values for vancomycin (>512 mg/L in both cases) and teicoplanin (64 and >128 mg/L).
Plasmid gene content among VREfm and diversity of vanA-plasmids

PCR-based typing of plasmid replication initiator genes (rep) was performed for 196 isolates and these results were combined with the data for 20 isolates, published previously [18]. Altogether, ten rep-types were observed among VREfm-VanA (Fig. 2). Isolates carried from one up to seven different rep genes, with 4.7 rep genes per isolate on average. Isolates positive for rep1pIP501, rep7pT181 and rep5pMG1 appeared mainly in the period 1997–2005, while rep11pEF1071 gene was typical for isolates obtained in 2006–2010. The plasmid stabilization systems axe-txe and rep17pRUM were present in the majority of isolates. Another system, ω-ε-ζ, was also quite common, predominantly among rep2pRE25-carrying strains, and occurred mainly in the period of 2006–2010. Two additional systems, mazEF and relBE, were observed only in 2003 for six and two isolates, respectively. The rep4c222 and relpEF1 relaxase genes were prevalent, and additionally relpHTB and reppRE25 were detected. The majority of reppRE25-positive isolates (n=22, 91.7%) were also rep2pRE25-positive, however, most of 169 rep2pRE25-positive isolates lacked this relaxase. Isolates carrying reppHTB dominated in 1997–2005 (40 out of 45 reppHTB-positive isolates) and the majority of reppHTB-positive isolates also harboured reppMG1 (n=43, 95.5%).

Fifty-two isolates, obtained from 24 medical centres over the whole study period and representing 26 different STs and 21 Tn1546 types were selected for PFGE of S1-digested DNA and hybridization analyses. Additionally, the results obtained previously for three isolates from the 2009 outbreak [18] were included for comparative purposes. Investigated isolates showed the presence from one up to 11 plasmid bands per isolate in PFGE-S1 analyses. Subsequent hybridization with the vanA probe revealed the presence of 86 vanA-plasmids with up to four such plasmids in an isolate, and two cases of chromosomal localization of vanA (Table 2). Further hybridization studies showed the co-localization of vanA determinants with all six tested rep types, including rep2pRE25, rep17pRUM, rep18pEF418, rep1pIP501, rep5pMG1 and rep1pBM1 that accounted for 40.7% (n=35), 40.7% (n=35), 24.4% (n=21), 19.8% (n=17), 5.8% (n=5) and 1.2% (n=1) of vanA-plasmids, respectively. The vanA-plasmids with rep1pIP501 were limited to isolates from 1997 to 2005, circulating in two hospitals in Poznań. These plasmids differed by size (from ca. 30 to ca. 265 kb) and presence of other rep and toxin-antitoxin genes, and carried four different types of Tn1546, with A3 being predominant (8 out of 13 isolates harbouring vanA-plasmids with rep1pIP501). The vanA-plasmids with rep2pRE25, rep17pRUM and rep18pEF418 genes showed a multicentre distribution and occurred during the whole study period. In total, 37 (43%) vanA-plasmids were associated with more than a single rep type and 21 vanA-plasmids (24.4%), present in 11 isolates, did not hybridize with any of the tested rep genes. With a single exception, these latter plasmids were obtained during 2006–2010 (p = 0.001). Five of the isolates with these unknown replicons concomitantly carried three vanA-plasmids, ca. 30, 160 and 380 kb in size, which did not hybridize with any probes of toxin-antitoxin and relaxase genes tested. All these isolates carried B2 transposons, but belonged to diverse STs and MTs, and originated from four different medical centres over 2006–2010. Two toxin-antitoxin systems, ω-ε-ζ and axe-txe

![Fig. 2 Plasmid-associated gene distribution among Polish VREfm VanA. Number of isolates with a particular gene given above the graph bars](Image)
were commonly carried by vanA-plasmids (35 and 32 plasmids, respectively). The ω-ε-ζ system was characteristic for rep2_{PRBS} plasmids and axe-txe for rep17_{PRUM} plasmids (71.4 and 62.9 % of the respective vanA-plasmids). The gene specifying pEF1-relaxase was located on 11 vanA-plasmids (12.8 %), with various rep types. Some of the presumed genetic events, that could be inferred on the basis of these analyses, include examples of transposon evolution within an enterococcal strain, Tn1546 transposition among plasmids, conjugative transfer of plasmids, and their changes such as recombination or chromosomal integration as proposed in Table 2.

**Discussion**

Currently, VREfm play an increasingly important role in nosocomial infections and are considered alert pathogens [29], with vanA as a main determinant of this phenotype within many countries [30]. In Poland VRE remain less prevalent than in the United States or some European countries, e.g. our recent study revealed 7 % vancomycin-resistance among invasive *E. faecium* collected during 2010–2011 [31]. Although we observed an increasing prevalence of VanB *E. faecium* [17], VanA is still most frequent among Polish VREfm ([31] and NRCST unpublished observations). In the present study we aimed at the characterization of clonality of VanA-VREfm and genetic elements associated with this resistance determinant. Numerous reports show that in the case of human nosocomial infections vancomycin resistance is almost exclusively acquired by the hospital-adapted merocline of *E. faecium*, now widespread all over the world [2, 3] and prevalent among invasive *E. faecium* in Polish hospitals [31]. In this study, the vanA determinant was carried by representatives of this merocline with only a few exceptions limited to the 1997–2005 period. These isolates might represent intermediates, by which glycopeptide resistance determinants were introduced into hospitals. All isolates belonging to hospital merocline, as expected, were resistant to both ampicillin and ciprofloxacin, and enriched in putative virulence traits / markers such as IS16, *esp*Efm, *intA*Efm and pili genes. The population structure determined for Polish VREfm VanA closely resembled these of hospital-associated *E. faecium* in other countries. High diversity of STs/MTs is consistent with the presence of polyclonal hospital population of *E. faecium* that subsequently acquires vancomycin resistance determinants [13, 14]. The vast majority of isolates grouped into hospital lineage 17/18, mostly represented by STs 17, 117, 18, 132, 202 and lineage 78, which included STs 78, 192 and 412. In contrast to several other countries, where ST203 and ST16 constituted a significant proportion of hospital *E. faecium* [3, 14, 32, 33], in our population only one representative of ST16 was found and ST203 was completely absent. The characteristic change in the proportion of isolates belonging to lineage 17/18 and lineage 78 was observed since the year 2005 when lineage 78 started to be significantly more frequent in Poland. Our results are in agreement with observations made in other studies, suggesting waves of successful *E. faecium*, first from lineage 17/18 and followed by lineage 78 strains [4, 34]. This population shift, apparent in MLST and MLVA, was additionally associated with a change in plasmidome composition and observed decreased resistance levels to tetracycline and aminoglycosides.

Diversity of Tn1546 in VREfm is typical for this transposon, as reported by others [13, 14]. Nevertheless, in the present study we observed several new variants of Tn1546. VanA transposons indistinguishable from the Tn1546 A1 prototype [12] were frequently encountered in Europe, especially in the late 1990s and 2000s [14, 27]. This type and its mutational derivatives (A2-A6) were ubiquitous among early VREfm in our study. Single-nucleotide T insertion between nt 9063–9064 in the vanY gene of B4 type of transposon resulted in a translational frameshift and a truncated translation product. This change, however, did not abolish the glycopeptide resistance. VanY is a membrane-associated D,D-carboxypeptidase that hydrolyses the C-terminal D-Ala or D-Lac residue of peptidoglycan precursors but lacks transpeptidase activity. VanY, together with VanZ, represent accessory proteins, which are not required for the expression of glycopeptide resistance but increase its level [35]. Isolates with a deletion of vanY gene showed lower resistance levels to teicoplanin, likely due to the diminished transcription of vanZ while point mutations in vanY, observed so far were not associated with a loss of protein function [27, 36].

Activity of various ISs represented a very important factor, contributing to the formation of several novel transposon types. IS1216, the most common IS in our study, characteristic for B-types, was detected at various positions of the transposon, and its insertion often resulted in deletions of adjacent sequences in ORF2, vanX and the vanX-vanY intergenic region, as observed by others [13, 27]. The BI, BBI, BBBI types, apart from IS1216, exhibited the concomitant presence of a IS1216V-IS3-like element, originally reported in 1995 [37]. Since then, this element was described in several studies, which reported intact as well as a 5′-truncated IS3-like sequence [27, 38], both of which were also detected in our study. The C-type, harbouring IS1251, was relatively frequent and the integration sites of this IS were identical to those published by others [14, 37, 39]. Sequencing analysis allowed discerning the C1-type, specific for Krakow hospitals and the C2-type, found in other cities. The D type transposon, containing ISEfa5, to our knowledge, represents the first example of this variant outside of South America [34]. Type E represents the first insertion of ISEfa4 in the vanX-vanY intergenic region. This IS was described
| Strain ID/ year of isolation | Code of medical centre | Tn/1546 type | MLST type (lineage) | Number of VanA plasmid bands | Hypothetical genetic event | VanA plasmid replicon types and stabilization systems (approximate size in kb) |
|-----------------------------|-----------------------|--------------|---------------------|-------------------------------|--------------------------|--------------------------------------------------------------------------------|
| 1639/1997                  | Gd-a                  | BBB11        | 407 (17/18)         | 1                             | Chromosomal integration of 50-kb plasmid | rep17 TA1 (45) rep17 rep18 TA1 (50) rep2 rep17 rep18 TA1 (chr) |
| 1641/1997                  | Gd-a                  | BBB12        | 408 (17/18)         | 1                             | Chromosomal integration of 50-kb plasmid | rep2 rep17 TA1 (35) rep2 TA2 (40) rep2 TA2 (270) |
| 3132/1998                  | Gd-p                  | A1           | 18 (17/18)          | 2                             | Transfer of 40-kb plasmid between ST18 and ST411 strains, followed by plasmid recombination or transposition of A1 | rep2 rep17 TA1 (35) rep17 TA1 (170) rep17 TA1 (320) |
| 3136/1998                  | Gd-p                  | A1 (singleton) | 411                | 2                             | Chromosomal integration of 40-kb plasmid | rep2 (30) rep2 TA1 TA2 (40) rep1 rep2 TA1 TA2 (chr) |
| 7952/1999                  | Gd-p                  | nt           | 381 (17/18)         | 3                             | Transposition of A3 or plasmid recombination in ST385 strain | rep17 TA2 (40) rep1 TA2 repEF1 (140) rep1 reppLG1 TA2 repEF1 (265) |
| 2509/2000                  | Po-1                  | A3           | 386 (17/18)         | 2                             | Derivative of A3 in ST385 strain, with concomitant change of plasmid backbone | rep1 (265) rep1 rep18 TA1 TA2 (265) |
| 2524/2000                  | Po-1                  | A3           | 382 (17/18)         | 2                             | Clonal spread of ST117 with 265-kb plasmid, followed by transposition of A3 among plasmids or plasmid recombination | rep1 (265) rep2 rep17 TA1 TA2 (255,310,360) |
| 3136/1998                  | Po-1                  | A3           | 385 (17/18)         | 3                             | Transposition of E or plasmid recombination in ST117 strain | rep1 rep18 reppLG1 TA1 TA2 (165) rep1 reppLG1 (165) rep1 TA2 (30) |
| 1409/2002                  | Po-4                  | A3           | 385 (17/18)         | 1                             | Clonal spread of ST117 strain and concomitant change of plasmid size by a | rep1 rep17 TA1 (155) rep1 (145) rep1 rep18 rep17 TA1 TA2 (40) |
| 291/2002                   | Po-2                  | A3           | 385 (17/18)         | 1                             | Transposition of E or plasmid recombination in ST117 strain | rep1 rep18 reppLG1 TA1 TA2 (165) rep1 reppLG1 (165) rep1 TA2 (30) |
| 1156/2002                  | Po-2                  | A3           | 192 (78)            | 1                             | Clonal spread of ST117 strain and concomitant change of plasmid size by a | rep1 rep18 rep17 rep18 TA2 TA2 repEF1 (70) |
| 2127/2004                  | Po-2                  | E            | 117 (17/18)         | 1                             | Clonal spread of ST117 strain and concomitant change of | rep1 rep18 rep17 rep18 TA2 repEF1 (70) |
| 714/2003                   | Kr-1                  | C1           | 117 (17/18)         | 1                             | Clonal spread of ST117 strain and concomitant change of | rep1 rep18 rep17 rep18 TA2 repEF1 (70) |
| 756/2003                   | Kr-1                  | C1           | 117 (17/18)         | 1                             | Clonal spread of ST117 strain and concomitant change of | rep1 rep18 rep17 rep18 TA2 repEF1 (70) |
| 1679/2003                  | Kr-1                  | C1           | 18 (17/18)          | 1                             | Clonal spread of ST117 strain and concomitant change of | rep1 rep18 rep17 rep18 TA2 repEF1 (70) |
| 3779/2004                  | Kr-4                  | C1           | 18 (17/18)          | 1                             | Clonal spread of ST117 strain and concomitant change of | rep1 rep18 rep17 rep18 TA2 repEF1 (70) |
Table 2 (continued)

| Strain ID/ year of isolation | Code of medical centre | Tn1546 type | MLST type (lineage) | Number of VanA plasmid bands | Hypothetical genetic event | VanA plasmid replicon types and stabilization systems (approximate size in kb) |
|-----------------------------|------------------------|-------------|---------------------|-----------------------------|---------------------------|--------------------------------------------------------------------------|
| 4002/2005                  | Kr-3                   | C1          | 387(17/18)          | 1                          | presumable deletion (loss of repEF1)                                  | rep2 rep18 TA1 TA2 (65) rep2 rep18 TA1 TA2 repEF1 (55) rep17 TA1 (45) |
| 1332/2003                  | Kr-1                   | C1          | 132(17/18)          | 1                          | Clonal spread of ST132 strain with 45-kb plasmid harbouring C1 transposon | rep17 TA1 (45) rep2 rep17 rep18 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 1336/2003                  | Kr-3                   | C1          | 132(17/18)          | 1                          | Evolution of C1 transposon within the same ST132 strain and 45-kb plasmid backbone | rep17 TA1 (45) rep2 rep17 rep18 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 2981/2003                  | Mi                     | nd          | 132(17/18)          | 1                          | Clonal spread of ST132 strain with 45-kb plasmid harbouring C1 transposon | rep17 TA1 (45) rep2 rep17 rep18 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 2216/2005                  | Kr-1                   | C1          | 388(17/18)          | 1                          | Evolution of C1 transposon within the same ST132 strain and 45-kb plasmid backbone | rep17 TA1 (45) rep2 rep17 rep18 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 84/2010                    | Gdy                    | C2          | 17(17/18)           | 1                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 3552/2009f                 | Wa-10                  | A1          | 18(17/18)           | 2                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 3240/2006                  | Po-5                   | B2          | 17(17/18)           | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 1930/2007                  | Wa-1                   | B2          | 64(17/18)           | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 4285/2008                  | Wa-1                   | B2          | 192(78)            | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 5151/2008                  | Osw                    | B2          | 18(17/18)           | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 1767/2010                  | Wa-3                   | B2          | 780(17/18)          | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 5009/2009                  | Wa-2                   | B2          | 230(78)            | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 3238/2006                  | Sk                     | B2          | 279(17/18)          | 2                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 2546/2008                  | Gr                     | BH          | 202(17/18)          | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 8744/2010                  | Wa-2                   | B2          | 561(17/18)          | 1                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
| 484/2010                   | Ke                     | B2          | 17(17/18)           | 3                          | Concomitant transfer of three ∼30-, 160- and 380-kb plasmids with unknown rep-type(s), carrying B2 transposon, into diverse clonal backgrounds | rep2 rep17 TA1 TA2 repEF1 (75) rep2 rep17 TA1 TA2 (50) |
### Table 2 (continued)

| Strain ID/year of isolation | Code of medical centre | Tn1546 type | MLST type (lineage) | Number of VanA plasmid bands | Hypothetical genetic event | VanA plasmid replicon types and stabilization systems (approximate size in kb) |
|----------------------------|-----------------------|-------------|---------------------|-------------------------------|---------------------------|-----------------------------------------------------------------------------|
| 9363/2010 Sw               | B2                    | 877(17/18)  | 1                   | Transfer of <30-kb plasmid among strains of ST877 and ST17 | rep2 rep17 rep18 TA1 (<30) |
| 3856/2005 Wa-1             | B4                    | 78(78)     | 1                   |                               | rep2 TA2 (50)               |
| 991/2009a Wa-4             | G                     | 18(17/18)  | 1                   | Recombination events or transposition of G among rep17 plasmids. | rep17 (100) rep17 (50) |
| 3554/2009a Wa-10           | G                     | 192(78)    | 1                   |                               | rep2 rep17 TA2 (35) rep17 relpEF1 (115) |
| 2944/2009 Ko               | G                     | 18(17/18)  | 1                   |                               | rep2 rep17 TA2 (35) rep17 relpEF1 (45) |
| 3392/2009 Ost              | G                     | 78(78)     | 1                   |                               | rep2 rep17 TA2 (35) rep17 relpEF1 (65) |
| 726/2010 In                | G                     | 17(17/18)  | 1                   |                               | rep2 rep17 TA2 (35) rep17 relpEF1 (100) |
| 3322/2007 Wa-2             | BC1                   | 412(78)    | 1                   | Evolution of BC transposons within the same ST412 strain in the ~35-kb plasmid backbone | rep2 rep17 TA2 (35) rep17 rep18 TA2 (50) |
| 107/2005 Wa-2              | BC5                   | 412(78)    | 1                   |                               | rep2 rep17 TA2 (35) rep17 rep18 TA2 (50) |
| 3948/2010a Wa-2            | BC4                   | 412(78)    | 1                   |                               | rep2 rep17 TA2 (35) rep17 rep18 TA2 (50) |
| 1901/2005 Lo               | F                     | 279(17/18) | 1                   |                               | rep2 rep17 TA1 TA2 relpEF1 (50) |
| 8034/2010 Kr-5             | B3                    | 341(78)    | 2                   |                               | rep2 rep17 TA2 relpEF1 (65) |
| 8628/2010 Ka               | BBI                   | 202(17/18) | 1                   |                               |                                                                 |

*nt non-typeable

a By Bydgoszcz, Gd-a Gdańsk, adult hematology ward; Gd-p Gdańsk, paediatric haematology ward; Gd-y Gdynia, Gr Grodzisk Mazowiecki, In Inowroclaw, Ka Katowice, Ke Kętryzn, Ko Konin, Koś Kościerzyna, Kr Kraków, Lo Łódź, Mi Mielec, Op Opole, Os Ostrów Mazowiecki, Osł Ostrów Wielkopolski, Ost Ostrzeszów, Ot Otwock, Pł Pisz, Płock, Poz Poznań, Rz Rzeszów, Sk Skieniewice, Sw Świdnica, Wł Warszawa, Wr Wróclaw, Zi Zielona Góra; the city abbreviation is followed by the centre number.
b Shadowed boxes indicate presumable associations among isolates.
c *rep1, rep2, rep7, rep18* – plasmid replicon families according to Jensen et al., 2010 [24]; TA1, TA2-axe-tce and ω-ε-ζ stabilization systems specific for pRUM and pRE25, respectively.
d Presumably C1 transposon type, however no amplification of the region containing IS1251 could be obtained, in spite of several attempts.
e Results from Wardal et al., 2014 [18].
earlier in orf2-vanR and vanS-vanR intergenic regions [40, 41], as well as within IS1542 [42]. The F type harboured ISEfm2, representative of the IS256 family. Thus far, in enterococci this IS has been solely observed inserted between orf13 and tetS within CTn6000 transposon [43]. In our study, we report for the first time the insertion of ISEfm2 into Tn1546. The G type transposon with ISEf1, reported earlier for the VREfm outbreak in two neighbouring Warsaw medical centres in 2009 [18] was additionally detected in isolates from 2009 to 2010 derived from seven different cities, which may indicate its multicenter spread. Integration of the ISEfm1-like element, belonging to the IS982 family in the BH type, represents yet another novel insertion event in Tn1546. The presence of ISEfm1 was described previously in the vanX-vanY intergenic region [44], as well as within the vanD operon [45]. Complex analysis of transposon structures described in our study revealed the potential scheme of their hypothetical evolution among Polish VREfm. In this scenario, we propose the A1 type as presumable ancestor variant, with remaining types being its direct or indirect derivatives (Fig. 3).

The vanA determinants are almost exclusively located on plasmids and these elements play a very important role in the spread of glycopeptides resistance [46]. Our results show that the Polish VREfm population is enriched in plasmid replicons of different families including megaplasmids, Inc18-, pRUM- and pMG1/ pHT-like plasmids, encountered in VanA-VREfm in other countries [16, 46, 47]. Size variation of vanA-plasmids, even within the same family indicates their flexibility, and identification of multiple rep types in a single plasmid suggests a common presence of plasmid cointegrates. We observed an interesting change in the VanA-associated plasmidome between early (1997–2005) and more recent (2006–2010) isolates. In particular, rep1-vanA replicons were quite abundant among early isolates and typically located on plasmids over 140 bp in size. Together with rep2-vanA replicons they represent the Inc18 family, associated with Tn1546 elements among clinical E. faecium in Europe [16, 47]. Another shift in plasmidome composition between early and recent isolates was shown for pMG1 replicons, present exclusively among early VREfm. High prevalence of pMG1-like elements was observed among VREfm in the United States and Japan, where they contributed to the spread of both aminoglycoside and glycopeptide resistance [46]. Apart from Inc18 plasmids, the

Fig. 3 Hypothetical evolution of Tn1546 structures among Polish VREfm VanA. Type A1, found in different cities, is a presumable ancestor variant with remaining types being its direct or indirect derivatives. Types A2, A3 and A5 developed by point mutations in wt type. A4 developed from A3 through single deletion events in the vanS-vanH intergenic region. A6 is an A3 derivative that lacks ca. 1900 bps in the S' end. The E type transposon, a third potential derivative of A3, arose through acquisition of ISEf1 between vanX and vanY. A3 and its derivatives were typical for Poznań (Po) medical centres. B3 and G variants presumably developed from A5 after insertion events of IS1216 and ISEf1 within vanX-vanY intergenic region in Kraków (Kr) and Warsaw (Wa), respectively. The ubiquitous B2 type, typical for Warsaw, probably emerged from a single insertion event of IS1216 within the A1 type with a concomitant deletion of the S' end of the transposon. The B4 (additional single nucleotide insertion within vanY) and BH (ISEfm1-like insertion between vanX and vanY) types represent possible derivatives of B2. The B1, D and F transposon types are potential derivatives of A1 formed by IS1216, ISEf5 and ISEfm2 insertions, respectively. Another group of transposon variants, encompassing types B1, BBI, BBH1, BBH2, BB1 and BB2 emerged through complex insertion and deletion events in different regions of wt transposon promoted mostly by IS1216 elements. This group was detected mainly in Gdańsk (Gd). The activity of another insertion sequence, IS1251, followed by IS1216 insertions and several point mutations resulted in the formation of C- and BC-types in Kraków and Warsaw, respectively.
pRUM derivatives constitute the second main carrier of vancomycin resistance among the contemporary E. faecium isolates [16, 46]. Plasmids with the pRUM-like rep can be divided into two groups, one with axe-txe genes and mob regions from the staphylococcal pC223 plasmid and the other with relaxase from pEF1 and lacking axe-txe [15, 16, 46]. Our results indicate that representatives of both these groups are present among Polish VREfm. Additionally, we observed plasmids with unknown rep types among isolates obtained since 2006, which suggests the appearance of a new vanA-plasmid type(s), not included in the available classification scheme [24] and which will be a subject to further studies.

Finally, the analysis of PFGE-S1 hybridization results in the context of epidemiological information, determined Tn1546 types and the clonal background of the isolates, which revealed a high complexity of genetic events involving VREfm with VanA phenotype and resulting in the dissemination of this type of resistance. As these 52 isolates were pre-selected for a maximal representation of the collection diversity, only a few examples of clonal spread were observed, such as dissemination of ST132 strain with a 45-kb plasmid harbouring C1 transposon in a Krakow hospital (Table 2). The role of VREfm clonal spread in Poland, however, had been demonstrated before in our outbreak studies [18, 39, 48]. Particular types of transposons in the analysed group were frequently associated with various plasmid vectors. This situation may have resulted from transposition of Tn1546 among plasmids [12], promoted by integration ‘hot-spots’ [49] and from the recombination processes among enterococcal plasmids [15, 50]. The present study also provided examples of involvement of plasmids as vectors of vancomycin resistance, by demonstrating the presence of plasmids of the same size and with the same transposon types and plasmid-specific genes in different strains, as found in other studies [15, 51]. Occasionally, vanA-plasmids appeared to be integrated into bacterial chromosome, in agreement with other observations [52]. Further detailed studies employing extensive sequencing are indispensable to fully elucidate the events involving genetic elements engaged in the dissemination of the vanA gene cluster in the population of Polish VREfm.

In conclusion, the VREfm of the VanA phenotype collected in our country over the period 1997–2010 represent a highly variable group in the respect of their clonal composition, plasmid content and structures of Tn1546, a direct carrier of vanA genes. High genetic plasticity of these organisms, together with a rapid global spread of successful hospital-adapted enterococcal clones constitute a significant and continuously increasing epidemiological threat for human health. Thus, both epidemiological situation concerning VREfm as well as genetic elements and strains associated with VanA vancomycin resistance warrant further studies.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent Isolates were obtained in a part of routine activity of the NRCSF and were analysed anonymously in a retrospective manner. Ethical approval and informed consent were thus not required.

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References

1. Hryniewicz W, Szczypa K, Bronk M, Samet A, Hellmann A, Trzciński K (1999) First report of vancomycin-resistant Enterococcus faecium isolated in Poland. Clin Microbiol Infect 5(8):503–505
2. Willems RJ, Top J, van Santen M, Robinson DA, Coque TM, Baquero F, Grundmann H, Bonten MJ (2005) Global spread of vancomycin-resistant Enterococcus faecium from distinct nosocomial genetic complex. Emerg Infect Dis 11(6):821–828. doi:10.3201/eid1106.041204
3. Willems RJ, Hanage WP, Bessen DE, Feil EJ (2011) Population biology of gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS Microbiol Rev 35(5):872–900. doi:10.1111/j.1574-6976.2011.00284.x
4. Willems RJ, Hanage WP, Bessen DE, Feil EJ (2011) Population biology of gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS Microbiol Rev 35(5):872–900. doi:10.1111/j.1574-6976.2011.00284.x
5. Hendrickx AP, Bonten MJ, van Luit-Asbroek M, Schapendonk CM, Kragten AH, Willems RJ (2008) Expression of two distinct types of pili by a hospital-acquired Enterococcus faecium isolate. Microbiology 154(Pt 10):3212–3223. doi:10.1099/mic.0.2008/020891-0
6. Heikens E, van Schaik W, Leavis HL, Bonten M, Siren J, Hanage WP, Corander J (2012) Restricted gene flow among hospital subpopulations of Enterococcus faecium. MBio 3(4):e00151-00112. doi:10.1128/mBio.00151-12
7. Hendrickx AP, Bonten MJ, van Luit-Asbroek M, Schapendonk CM, Kragten AH, Willems RJ (2008) Expression of two distinct types of pili by a hospital-acquired Enterococcus faecium isolate. Microbiology 154(Pt 10):3212–3223. doi:10.1099/mic.0.2008 /020891-0
8. Heikens E, van Schaik W, Leavis HL, Bonten M, Willems RJ (2008) Identification of a novel genomic island specific to hospital-acquired clonal complex 17 enterococcus faecium isolates. Appl Environ Microbiol 74(22):7094–7097. doi:10.1128/AEM.01378-08
9. 7. Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, Zeng Q,
Wortman J, Birren B, Willems RJ, Earl AM, Gilmore MS (2013) Emergence of epidemic multidrug-resistant Enterococcus faecium from animal and commensal strains. MBio 4(4). doi:10.1128/mBio.00534-13

Top J, Sinnige JC, Majoer EA, Bonten MJ, Willems RJ, van Schaik W (2011) The recombinase InIA is required for excision of esp-containing ICEE1m1 in Enterococcus faecium. J Bacteriol 193(4):1003–1006. doi:10.1128/JB.00952-10

Willems RJL, Homan W, Top J, van Santen-Verheuvel M, Tribbe D, Manziorszo X, Gaillard C, Vandenbroucke-Grauls CMJE, Mascini EM, van Kregten E, van Embden JDA, Bonten MJM (2001) Variant esp gene as a marker of a distinct genetic lineage of vancomycinresistant Enterococcus faecium spreading in hospitals. Lancet 357(9259):853–855. doi:10.1016/S0140-6736(01)05205-7

Werner G, Fleige C, Geringer U, van Schaik W, Klare I, Witte W (2011) IS element IS16 as a molecular screening tool to identify hospital-associated strains of Enterococcus faecium. BMC Infect Dis 11:80. doi:10.1186/1471-2334-11-80

Courvalin P (2005) Genetics of glycopeptide resistance in gram-positive pathogens. Int J Med Microbiol 294(8):479–486. doi:10.1016/j.ijmm.2005.04.004

Arthor M, Molinas C, Depardieu F, Courvalin P (1993) Characterization of Tn5146, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in Enterococcus faecium BM4147. J Bacteriol 175(1):117–127

Talebi M, Poursahef MR, Katouli M, Molby R (2008) Molecular structure and transferability of Tn5146-like elements in Enterococcus faecium isolates from clinical, sewage, and surface water samples in Iran. Appl Environ Microbiol 74(5):1530–1536. doi:10.1128/AEM.02254-07

Werner G, Klare I, Fleige C, Witte W (2008) Increasing rates of vancomycin resistance among Enterococcus faecium isolated from German hospitals between 2004 and 2006 are due to wide clonal dissemination of vancomycin-resistant enterococci and horizontal spread of vanA clusters. Int J Med Microbiol 298(5–6):515–527. doi:10.1016/j.ijmm.2007.05.008

Freitas AR, Novais C, Tedim AP, Francia MV, Baquero F, Peixe L, Coque TM (2013) Microevolutionary events involving narrow host plasmids influences local fixation of vancomycin-resistance in Enterococcus faecalis populations. PLoS One 8(3):e60589. doi:10.1371/journal.pone.0060589

Rosvoll TC, Pedersen T, Sletvold H, Johnsen PJ, Sollid JE, Freitas AR, Novais C, Tedim AP, Francia MV, Baquero F, Peixe L, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Sadowy E (2013) Characterization of Tn5146-like elements in Enterococcus faecium strains reveals widely distributed prRE25–prUML, pLP501- and pHTbeta-related replicons associated with glycopeptide resistance and stabilizing toxin-antitoxin systems. FEMS Immunol Med Microbiol 58(2):254–268. doi:10.1111/j.1574-695X.2009.00633.x

Sadowy E, Siemko A, Gawryszewska I, Bojarska A, Malinowska K, Hryniewicz W (2013) High abundance and diversity of antimicrobial resistance determinants among early vancomycin-resistant Enterococcus faecium in Poland. Eur J Clin Microbiol Infect Dis 32(9):1193–1203. doi:10.1007/s10096-013-1868-y

Wardal E, Gawryszewska I, Hryniewicz W, Sadowy E (2013) Emergence and spread of vancomycin resistance among Enterococcus faecium isolates from animal and commensal strains reveals widely distributed prRE25, prUML, pLP501 and pHTbeta-related replicons associated with glycopeptide resistance and stabilizing toxin-antitoxin systems. FEMS Immunol Med Microbiol 58(2):254–268. doi:10.1111/j.1574-695X.2009.00633.x

Johnson PD, Ballard SA, Grabsch EA, Stinear TP, Seemann T, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH, Lillie 2004) A sustained hospital outbreak of vancomycin-resistant Enterococcus faecium bacteremia. J Infect Dis 190(12):1878–1886. doi:10.1086/456319

Zheng B, Tomita H, Xiao YH, Wang S, Li Y, Ike Y (2007) Molecular characterization of vancomycin-resistant enterococcal faecium isolates from mainland China. J Clin Microbiol 45(9):2813–2818. doi:10.1128/JCM.00457-07

Khan MA, Northwood JB, Loor RG, Tholen AT, Riera E, Falcon M, Paraguayan Antimicrobial N, van Belkum A, van Westreem M, Hays JP (2010) High prevalence of ST-78 infection-associated vancomycin-resistant Enterococcus faecium from hospitals in
