European Journal of Clinical Microbiology & Infectious Diseases (2018) 37:927–936
https://doi.org/10.1007/s10096-018-3209-7

ORIGINAL ARTICLE

The changing epidemiology of VanB *Enterococcus faecium* in Poland

Ewa Sadowy[1] · Iwona Gawryszewska[1] · Alicja Kuch[2] · Dorota Żabicka[2] · Waleria Hryniewicz[2]

Received: 23 November 2017 / Accepted: 1 February 2018 / Published online: 13 February 2018

Abstract

Increasing prevalence of VanB *Enterococcus faecium* in Polish hospitals reported to National Reference Centre for Susceptibility Testing (NRCST) prompted us to investigate the basis of this phenomenon. Two-hundred seventy-eight *E. faecium* isolates of VanB phenotype from the period 1999 to 2010 obtained by NRCST were investigated by multilocus sequence typing (MLST) and multilocus VNTR analysis (MLVA). Localization, transferability, and partial structure of the *vanB*-carrying Tn*1549* transposon were studied by hybridization, PCR mapping, sequencing, and conjugation. VanB isolates almost exclusively represented hospital-associated *E. faecium*, with a significant shift from representatives of 17/18 lineage to 78 lineage after 2005. The *vanB* determinant, initially located mostly on transferable plasmids of the pRUM-, pLG1-, and pRE25-replicon types, later on was found almost exclusively on the host chromosome. Fifteen different plasmid and chromosomal insertion sites were identified, typically associated with single transposon coupling sequences, mostly not observed before. Our study demonstrates the significant change in the epidemiology of VanB-*E. faecium* in Poland, associated with the introduction and spread of the lineage 78 of the hospital-adapted *E. faecium*. These data point to the importance of the lineage 78 for the spread of vancomycin-resistance, determined by the *vanB* gene cluster, resulting in an increasing VRE prevalence in hospitals. This study also supports the scenario, in which representatives of the hospital-associated *E. faecium* independently acquire the *vanB* determinant de novo and spread within and among hospitals, concomitantly undergoing differentiation.

Keywords Epidemic lineage · Population shift · Transposon · Plasmid · Diversity

Introduction

The importance of enterococci as etiologic agents of hospital-acquired infections (HAIs) is currently increasing [1], and common glycopeptide resistance among these bacteria is especially alarming [2]. Among two most ubiquitous *van* gene clusters, responsible for this phenotype, *vanA* confers resistance to both vancomycin and teicoplanin, and *vanB* typically determines resistance only to vancomycin [3]. The *vanB* cluster is predominantly associated with the Tn*1549*-type transposons [4], which may reside either on plasmids or on the bacterial chromosome [4–9]. During the initial steps of conjugative transfer of transposon, the staggered cleavage by the Int recombinase results in the formation of a circular intermediate, joined by a 5- to 6-bp sequence originating from the donor genome, termed a coupling sequence, which, after transposition, is found adjacent to the transposon termini in the recipient [7].

Among the two clinically most important enterococcal species, i.e., *Enterococcus faecalis* and *Enterococcus faecium*, the latter is particularly prone to the acquisition of antimicrobial resistance determinants, including *vanA* and *vanB* clusters (vancomycin-resistant *E. faecium*, VR*Efm*), resulting in increasing proportion of VR*Efm* among hospital *E. faecium* [10]. Concomitantly, an increase in the incidence of HAIs caused by *E. faecium* is observed [10, 11], likely due to the selection and worldwide dissemination of successful hospital-adapted clonal complex 17 (CC17) [12] that combines resistance to several antimicrobials with the enrichment in pathogenicity factors and increased epidemic potential. The

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-018-3209-7) contains supplementary material, which is available to authorized users.

✉ Ewa Sadowy
ewasadowy@cls.edu.pl

[1] Department of Molecular Microbiology, National Medicines Institute, ul. Chełmska 30/34, 00-725 Warsaw, Poland

[2] Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Chełmska 30/34, 00-725 Warsaw, Poland

Bayesian Analysis of Population Structure (BAPS) of the data obtained by multilocus sequence typing (MLST) demonstrated that CC17 may be divided into two subgroups corresponding to major lineages 17/18 and 78 [13].

The *vanB* gene was identified in *E. faecalis* at the beginning of the 1990s [14]. In Poland, the first VR*Efm* with *vanB2* was isolated in 1999 [15] followed by a growing VanB prevalence in 1999–2005 [16]. A further increase in VanB-VR*Efm* after 2005, noticed by the National Reference Centre for Antimicrobial Resistance and Surveillance (NRCARS), prompted us to investigate these important pathogens to better understand the factors underlying the spread of VanB-*E. faecium* in Poland.

Materials and methods

Bacterial isolates and antimicrobial susceptibility testing

Altogether, 278 non-repetitive isolates with the VanB phenotype received by the NRCARS during 1999–2010 from 36 centers in 22 cities were investigated. Fifty-eight VanB isolates from 1999 to 2005 were partly characterized previously [16]; of these, 56 were available and 222 isolates were received in 2006–2010. Twenty-seven and 48 isolates were obtained from invasive and non-invasive infections, respectively, and 201 from carriage; for two remaining isolates, the source was not reported. Antimicrobial susceptibility was tested using the broth microdilution method [17] and the Etest method for vancomycin, teicoplanin, and daptomycin (bioMerieux, Marcy l'Etoile, France). Results were interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-approved breakpoints [18] and the Epidemiological Cut-Offs (ECOFFs) (http://mic.eucast.org/Eucast2/, 6th November 2017, date last accessed).

Detection of *vanB*, IS*16* and *esp*, and molecular typing

DNA was purified using the Genomic DNA Prep Plus kit (A&A Biotechnology, Gdynia, Poland) and *vanB*; IS*16* and *esp* were detected by PCR [19–21]. Multilocus VNTR (variable-number tandem repeat) analysis (MLVA) and MLST were performed as described [22, 23]; sequence types (STs) were assigned using the MLST database http://pubmlst.org/efaecium/ (6th November 2017, date last accessed). On the basis of eBURST analysis [24] of the whole MLST database (as of the 21st of April 2015), STs were included into CCs and lineages [13, 25].

Analysis of Tn*1549*, insertion sites, and coupling sequences

The presence of int_{Tn1549} and $ORF1_{Tn1549}$ was confirmed by PCR, and the *vanY-vanX* sequence in Tn*1549* was established using overlapping PCR and sequencing. The Tn*1549* insertion sites were identified by inverse-PCR (iPCR) [26] with *Bsp*143I (Fermentas, Lithuania). Primers targeting sequences adjacent to Tn*1549* were designed based on iPCR results. Sequences were analyzed with the Lasergene package (DNASTAR, MD, USA). Primer sequences are available upon request.

Plasmid gene detection, S1 profiles, hybridization, and conjugation

Plasmid *rep* ($rep1_{pIP501}$, $rep2_{pRE25}$, $rep8_{pAM373}$, $rep9_{pAD1}$, $rep17_{pRUM}$, rep_{pMG1}, rep_{pLG1}) and toxin-antitoxin systems (TAS) *axe-txe* and ω-ε-ζ were detected by PCR [26–29] with controls from our collection [28, 30]. For profiling of plasmids, DNA in agarose plugs was treated with S1 nuclease (Takara Bio, Japan), separated by pulsed-gel electrophoresis (PFGE) [31] and blotted onto Hybond-N+ (GE Healthcare, Buckinghamshire, UK). Hybridization was carried out using the Amersham ECL System (GE Healthcare). Transferability of vancomycin resistance was examined as described [32] with the recipient *E. faecium* strain 64/3.

Statistics

The differences in distributions were evaluated by the chi-squared test, with a p value ≤ 0.05 considered significant.

GenBank accession numbers

New sequences of the *vanY-vanX* region: A1-A6 (KC489780-KC489785), A9-A20 (KT003969-KT003980), B1 (KC489787), B2 (KT003981), D (KC489790), and E (KT003982); $rep17_{pRUM}$ (KM014782), rep_{pLG1}-1 (KM014783), and rep_{pLG1}-2 (KM014784) were submitted to GenBank.

Results

Antimicrobial susceptibility phenotypes and clonal relationships of VanB-VR*Efm* in Poland

All isolates were analyzed by MLVA, yielding 23 different MTs; 13 non-typable isolates repeatedly yielded incomplete MLVA profiles (Table 1). The most prevalent MT159 (186 isolates, 83.0%) was observed solely since 2006. Eighty isolates from 2006 to 2010, representing all hospitals providing

Table 1 Epidemiological and typing data, *vanY-vanX* region structure and Tn*1549* localization among VanB *E. faecium* in Poland, 1999–2010

A. Plasmid localization of Tn*1549*

Variant name[a]	Centre (*n*)[a]	Year	ST (*n*)[b]	MT (*n*)[b]	Line age	*vanY-vanX* (*n*)[b]	*vanB*-plasmid representatives approximate size in kb; {plasmid-specific genes}; (*n*)[b]
CS_P1a	KRA2 (4)	2000	381 (2)	264 (2)	17/18	A3	- 100 {*rep*2}
		2000	384 (2)	326 (2)	17/18		- 160 {−}; 320 {*rep*2, rep_{pLG1}, *axe-txe*}
							- 150 {rep_{pLG1}} (2)
CS_P1b	WAW1 (4)	1999–2000	382 (2)	325 (2)	17/18	B1	- 100 {*rep*2, *axe-txe*}
		1999–2000	383 (2)	325 (2)	17/18		- 80 {*rep*2, rep_{pLG1}, *axe-txe*}
							- 100 {*rep*2}; 240 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*}
							- 60 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*}; 90 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*}
CS_P2	SZC2 (9)	2002	386	4	17/18	C (10)	- 220 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*}
		2005	562	375 (4)	17/18		- 220 {*rep*17, rep_{pLG1}, *axe-txe*}
		2005	260 (2)	13 (2)	17/18		- 220 {*rep*17, rep_{pLG1}} (2)
		2005	920	231	17/18		- 80 {*rep*17, rep_{pLG1}}; 220 {*rep*17, rep_{pLG1}}
		2005	74	*nt*	S		- 240 {rep_{pLG1}}
							- 300 {*rep*17, rep_{pLG1}, *axe-txe*}
							- 220 {−}
							- 160 {rep_{pLG1}}
CS_P3	POZ1 (1)	2003	202	1	17/18	A4	- 200 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*}
	WAW3 (1)	2005	18	1	17/18	A4	- 150 {−}
	WRO (3)	2005	440	7	17/18	A4	- 150 {−}
	ZGO (1)	2006	17	4	17/18	A9	- 150 {−}
	ZAB1 (1)	2007	279	3	17/18	A12	*nd*
CS_P4	WAW1 (11)	2005	17	4 (5)	17/18	D (11)	- 150 {−}
		2005	387 (4)	50 (4)	17/18		- 150 {−}
		2005	384	325	17/18		- 150 {−}
		2005	384	326	17/18		- 150 {−}
CS_P5	WAW5 (1)	2005	387	376	17/18	A2	- 120 {*rep*2}
CS_P6a1	KSZ (2)	2010	78 (2)	159 (2)	78	A20	- 250 {*rep*2, *rep*17, rep_{pLG1}} (2)
	WAW2 (1)	2010	17	11	17/18	A19	[c]HMW
CS_P6a2	KSZ (1)	2010	78	159	78	*nd*	- 250 {*rep*2, *rep*17, rep_{pLG1}}
CS_P6b	POZ7 (6)	2008	78 (2)	159 (6)	78	A13, A14	- 250 {*rep*2, *rep*17, rep_{pLG1}} (5)
CS_P7	LUB (3)	2009–2010	78 (3)	159 (3)	78	A15	- 70 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*} (2)
	POZ1 (2)	2009	192 (2)	159 (2)	78		- < 50 {*rep*2}, 270 {*rep*17, rep_{pLG1}, *axe-txe*}
							- 80 {*rep*2, *rep*17, rep_{pLG1}, *axe-txe*} (2)

B. Chromosomal localization of Tn*1549*

Variant name[a]	Centre (*n*)[a]	Year	ST (*n*)[b]	MT (*n*)[b]	Lineage	*vanY-vanX* (*n*)[b]
CS_C1	KRA1 (1)	2003	387	50	17/18	A1
	KON (13)	2004	387 (13)	50 (3), nt (10)	17/18	A1
	KAL (1)	2004	387	50	17/18	A1
	POZ1 (1)	2004	387	376	17/18	A1
	POZ2 (2)	2005	387 (2)	50 (2)	17/18	A1
	KRA1 (1)	2005	387	50	17/18	A1
	POZ6 (1)	2006	561	50	17/18	A1
	POZ7 (1)	2008	202	1	17/18	*nd*

Table 1 (continued)

CS_C2	WAW1 (1)	2005	279 (1)	152	17/18	A8
CS_C3	KKE (1)	2006	78	12	78	A2
	BYD (2)	2007	78, 856	299, 159	78	A2 (2)
	KSC (1)	2007	78	159	78	A2
	PLO (1)	2007	78	159	78	A2
	GWP (12)	2007–2008	78 (6), 192	159 (12)	78	A2
	KSZ (56)	2007–2010	17, 18, 267	nt, 1 (3), 7	17/18	A1
	POZ1 (20)	2007–2009	78 (2)	159 (51)	78	*nd*
	POZ2 (48)	2007	64, 918 (2)	1, nt (2)	17/18	A2
	POZ3 (5)	2010	78 (3)	159 (15); 334 (2)	78	A2
	POZ4 (7)	2008–2009	64, 267 (4), 857	1 (8), 296	17/18	A2
	POZ5 (2)	2009	78 (7)	159 (35), 250, 293 (3)	78	A2
	POZ6 (5)	2010	78	159 (5)	78	A2
	POZ7 (4)	2007–2008	78	159 (7)	78	A2
	ZGO (17)	2007–2010	78	334 (2)	78	A2
	KIE (1)	2008	78 (2)	159 (5)	78	*nd*
	SZC2	2008	78 (4)	11, 159 (2), 291	78	A11
	WAW3 (1)	2008	382	50	17/18	*nd*
	WAW4 (1)	2008	78 (3)	159 (16)	78	A1
	ZAB2 (1)	2008	78	159	78	A1
	KAL (1)	2010	78 (2)	159, 334	78	A2
	NWS (1)	2010	78	159	78	C
	OBR (1)	2010	78	159	78	E
	OLS (8)	2010	78	159	78	A2
			78	159	78	A17
			78	159	78	A2
			78	159	78	A18
			78 (2), 192	159 (8)	78	F (8)
CS_C4	PRZ (1)	2004	279	231	17/18	A5
	PSZ (1)	2005	561	231	17/18	A6
CS_C5	LUB (1)	2008	78	159	78	F
CS_C6	ZAB1 (1)	2007	18	7	17/18	A10
CS_C7	OLS (2)	2009	78	159 (2)	78	B2, C
CS_C8	SZC1 (1)	2010	192	159	78	A16

[a] *BYD*, Bydgoszcz; *GWP*, Gorzów Wlkp.; *KAL*, Kalisz; *KIE*, Kielce; *KKE*, Kędzierzyn-Koźle; *KON*, Konin; *KRA*, Kraków; *KSC*, Kościerzyna; *KSZ*, Koszalin; *LUB*, Lublin; *NWS*, Nowa Sól; *OBR*, Oborniki; *OLS*, Olsztyn; *PLO*, Płock; *POZ*, Poznań; *PRZ*, Przasnysz; *PSZ*, Pszczyna; *SZC*, Szczecin; *WAW*, Warszawa; *WRO*, Wrocław; *ZAB*, Zabrze; *ZGO*, Zielona Góra; numbers adjacent to these abbreviations indicate hospitals in a city; number of isolates from a hospital given in brackets

[b] Number of isolates given in brackets if different from one

[c] *HMW*, high-molecular weight DNA band, a presumable integration of plasmid into chromosome; *S*, singleton; *nt*, non-typable; *nd*, not determined

isolates and all MTs, were resistant to ciprofloxacin and ampicillin; 88.8 and 88.8% isolates showed high-level resistance to gentamicin (HLGR) and streptomycin (HLSR), respectively; 20.0% of isolates were resistant to tetracycline, which represented a significant decrease ($p = 0.0002$) after 2005 (61.5% [16]). All isolates were susceptible to linezolid, tigecycline, and daptomycin. STs of 26 VanB isolates from the period 1999–2005 were reported previously [16], and additionally 21 isolates from this group were analyzed by MLST, together with 80 representative isolates from the period 2006–2010, mentioned above, yielding altogether 23 STs, characteristic for 127 isolates. Except for ST74, all isolates belonged to lineages 17/18 and 78, and representatives of 78 lineage were frequently associated with MT159 (Table 1). All isolates carried *vanB* and IS*16*; *esp* was present in 98% isolates from 2006 to 2010, similarly to the earlier period [16]. Based on the combined MLVA and MLST results, no representatives of lineage 78 were observed before 2006; the first VanB isolate from lineage 78 occurred in 2006 and since 2007 isolates from this lineage became much more common, representing 89% of isolates from the period 2006–2010 ($p < 0.001$).

Diversity of the *vanY-vanX* region in Tn*1549*

All isolates were positive for $ORF1_{Tn1549}$ and int_{Tn1549}. Sequencing of the *vanY-vanX* region (encompassing genes *vanY*, *vanW*, *vanH*, *vanB*, *vanX*; Fig. 1a) revealed 26 variants among 57 isolates, representing all centers and STs within a center. The most numerous group included A1-A20 variants, differing only by single-nucleotide polymorphisms (SNPs) at 21 nucleotide positions and highly similar to the corresponding region in *Clostridium* spp. and *Eggerthella lenta* (Fig. 1b). The A-type variants were characteristic for 48 of investigated isolates from 31 centers, and associated with 14 STs and 16 MTs. The B variants differed from the A-type by several SNPs and 6-bp insertion between *vanS-vanY*. They were 99% identical to the variant reported for the V583 [33]. An insertion of the IS*Efa11* between *vanS-vanY* in B-type yielded C variants (Fig. 1a). The D-, E-, and F-types represented probable derivatives of an A-type, with a deletion encompassing the nt 12-799 of *vanW*, an 11-bp deletion upstream *vanY*, and insertion of IS*L3* between *vanS* and *vanY*, respectively. All *vanB* genes represented the *vanB2* variant [34].

Analysis of Tn*1549* insertion sites and coupling sequences

To determine Tn*1549* insertion sites, selected isolates were analyzed by iPCR and thus obtained sequences were used to search GenBank and to design primers specific for a genetic neighborhood of Tn*1549*. These primers were used to screen the whole collection, revealing 15 insertion sites and 14 coupling sequences in total (Table 2). For two isolates, the coupling sequence could not be established due to the fact that sequences resulting from iPCR had no homologs in GenBank. Typically coupling sequences were identical in a given insertion site, with an exception of CS_P1a/CS_P1b in *aacA-aphD* and CS_P6a/CS_P6b in *citH*. The most prevalent coupling sequence, CS_C3 (198 isolates from 24 centers in 16 cities) was associated with 16 MTs and 14 STs. The first CS_C3 isolate was observed in 2006 (Table 1B).

Analysis of Tn*1549* localization, plasmidome composition, and *vanB* transferability

Seventy-eight isolates were analyzed by S1/PFGE-hybridization with the *vanB* probe (Table 1 and Supplementary Fig. 1). These isolates represented all observed variants of coupling sequence and hospital centers; additional isolates from the same center were included in the case of isolates showing plasmid localization of *vanB*. In the case of 39 isolates with coupling sequence C1-C8, *vanB* hybridized with a band of high-molecular weight, consistent with transposon insertion within chromosomal sequences and 39 isolates showed hybridization with plasmids from ~ 30 to ~ 310 kb in size; in five isolates, *vanB* was located on two plasmids. These hybridization studies and iPCR/PCR-based analyses of coupling sequences were consistent with the chromosomal localization of Tn*1549* for 227 isolates (81.6%) and plasmid localization for 50 isolates (18.0%); in a single case, a presumable integration of plasmid into chromosome was observed (variant CS_6a1 from WAW2). Isolates with the plasmid localization of *vanB* were much more prevalent among early VR*Efm*, i.e., from 1999 to 2005 (61% of these isolates) compared to the isolates collected from 2006 to 2010 (0.7% of these isolates, $p < 0.001$). Among isolates with the plasmid localization of *vanB*, $rep17_{pRUM}$ was found among 49 isolates, followed by rep_{pLG1}, $rep2_{pRE25}$, $rep1_{pIP501}$, rep_{pMG1}, and $rep9_{pAD1}$ (42, 35, 32, 27, and 5 isolates, respectively). Thirty-one and 12 of these isolates carried *axe-txe* and ω-ε-ζ), respectively. S1-PFGE/hybridization analyses revealed that 29, 23, and 22 plasmids hybridized with the rep_{pLG1}, $rep17_{pRUM}$, and $rep2_{pRE25}$ probes, respectively (Table 1A). In several cases, a single plasmid was associated with two or three *rep* genes. Sixteen *vanB*-plasmids hybridized with the *axe-txe* probe; among them, 13 co-hybridized with rep_{pLG1} and 12 with $rep17_{pRUM}$, respectively. Nine plasmids did not hybridize with any of the four probes used. Sequencing revealed a low variability of *rep* genes within this group that included two, one, three, one, and three variants of $rep1_{pIP501}$, $rep2_{pRE25}$, $rep17_{pRUM}$, rep_{pMG1}, and rep_{pLG1}, respectively. Among 50 isolates with the plasmid localization of *vanB*, 43 isolates (86.0%) were able to transfer vancomycin resistance while conjugation experiments involving 32 representative isolates with various chromosomal localizations of *vanB* were negative in 29 cases.

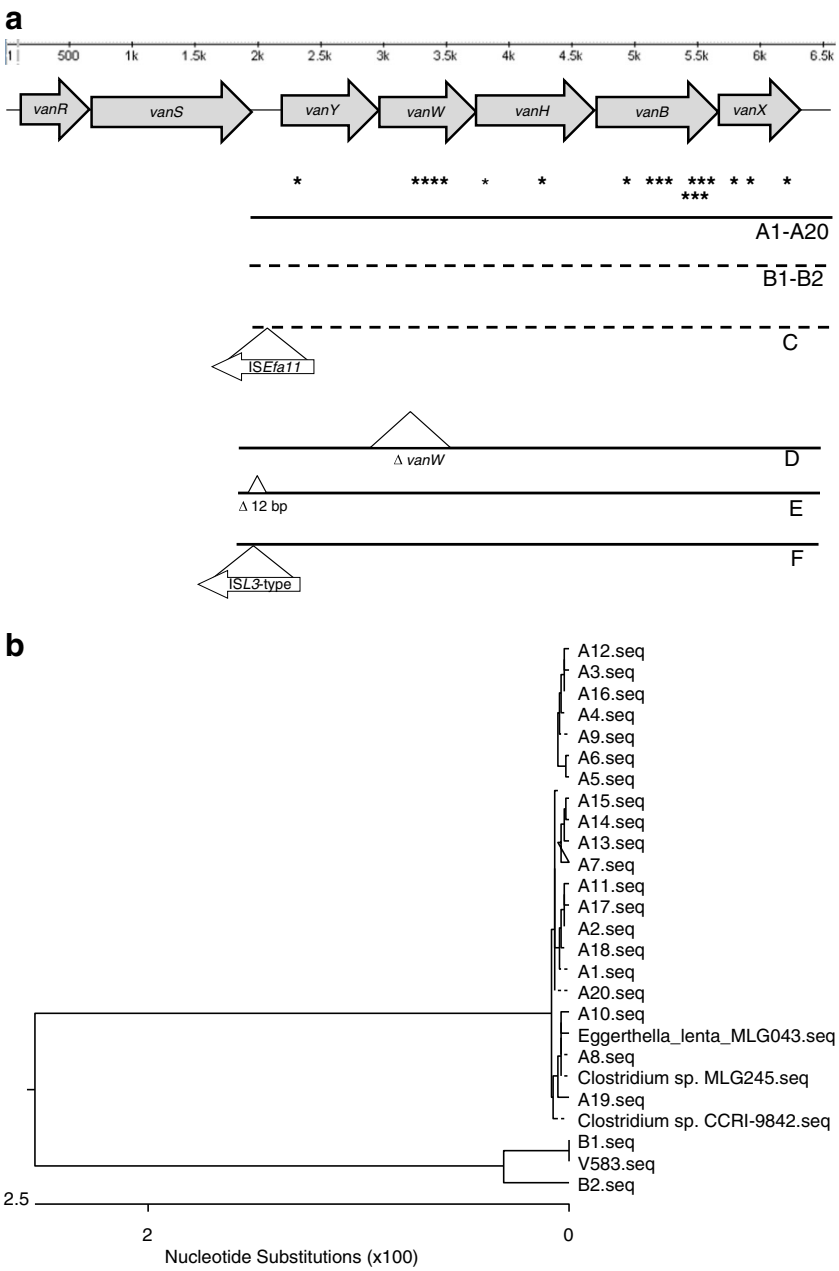

Fig. 1 Diversity of *vanY-vanX* region among VanB-VR*Efm* in Poland, 1998–2010. **a** Structure of the region, distribution of single-nucleotide polymorphisms (marked by asterisks) among A-type variants, and localization of deletions and ISs. **b** Similarity tree of nucleotide sequences of A- and B-type variants and sequences from the V583 strain of *E. faecalis* and isolates of *E. lenta* and *Clostridium* spp

Discussion

The first VanB-VR*Efm* was detected in Poland in 1999 [15], and our study investigated the VanB epidemiology during the following 12 years. Considering a relatively moderate incidence of VR*Efm* in Poland during this period (e.g., in 2010 amounting to 7.8% of invasive infections [http://ecdc.europa.eu/en/publications/Publications/1111_SUR_AMR_data.pdf.pdf; 6th November 2017, date last accessed]), it may be assumed that our collection reasonably well reflected the epidemiological situation in Polish hospitals. Although initially VanA represented the major VR*Efm* phenotype in Poland [16, 35], after 2006, the NRCARS recorded an increasing number of VanB-VR*Efm*, affecting several hospitals. The current global epidemiology of VR*Efm* shows considerable differences, with VanA predominant in Europe and the USA [36], and VanB constituting over 80% of invasive VR*Efm* in Australia [37]. A recent rise of VanB-*E. faecium* has been reported in Germany [8]. Nearly all isolates in our study belonged to the hospital *E. faecium*, since 2006 with the predominant role (89%) of lineage 78. VanB-VR*Efm* belonging to this lineage were responsible for recent outbreaks in Germany, Sweden, and Australia [38], and representatives of lineage 78 played a role in *vanA* dissemination in Polish hospitals [34].

Table 2 Insertion sites and coupling sequences of Tn*1549*-type transposons in VanB *E. faecium* in Poland, 1999–2010

Variant name[a]	Number of isolates	Flanking target sequence (20 bp)[b]	CS	Tn*1549*		CS	Flanking target sequence (20 bp)[b]	Insertion region
				Left end	Right end			
								Plasmid (GenBank hits)
CS_P1a	4	TTAGTACTAAATTTTGTTTT$_{676}$	–	AAAA TTTTAG	ATAT AATTTT	GTTTT	$_{675}$AAAATGTATTCATTATTAAC	*aacA-aphD* (LT598665)
CS_P1b	4	TTAGTACTAAATTTTGTTTT$_{676}$	–	AAAA TTTTAG	ATAT AATTTT	TATAT	$_{675}$AAAATGTATTCATTATTAAC	*aacA-aphD* (LT598665)
CS_P2	10	ATTTATCTTGCTGATTATTT$_{79}$	TTGA GG	AAAA TTTTAG	ATAT AATTTT	–	$_{80}$TTTCTCAAAACCATACTAAA	*cadD* (CP011829)
CS_P3	7	GAGAAAGTCGAATTATTTTT$_{89}$	ATTTGG	AAAA TTTTAG	ATAT AATTTT	–	$_{90}$AACACAAAAATTAGCAGAGG	Ef_aus00233 plasmid 3 ORF (nt 493,131–49,077; LT598665)
CS_P4	11	TTATTAATTATTTTTGATCT	–	AAAA TTTTAG	ATAT AATTTT	GGTAG	AAAAATTAGCTTAACAAATA	Intergenic in p63-1 (CP019989) (AL021_14715-AL021_14720)
CS_P5	1	AATAGCATATTTTTCTGTGC	*nd*	AAAA TTTTAG	ATAT AATTTT	*nd*	CAATCTCAAAATTTCGTTGA	Unknown (no GenBank hits)
CS_P6a1	2	GGGCTAAAATGCTTGGTTTT$_{912}$	GTACAT	AAAA TTTTAG	ATAT AATTTT	–	$_{913}$TATCCCTAAAAATATCGAAA	*citH* Aus0085 plasmid 1 (CP006621)
CS_P6a2	1	GGGCTAAAATGCTTGGTTTT$_{912}$	*GTACAT*	AAAA TTTTAG	ATAT AATTTT	*GTACAT*	$_{919}$TAAAAATATCGAAAAAGGTG	*citH* Aus0085 plasmid 1 (CP006621)
CS_P6b	6	GGGCTAAAATGCTTGGTTTT$_{912}$	TTATGA	AAAA TTTTAG	ATAT AATTTT	–	$_{913}$TATCCCTAAAAATATCGAAA	*citH* Aus0085 plasmid 1 (CP006621)
CS_P7	5	CTGTTGCAAAGTTTTAAATA	–	AAAA TTTTAG	ATAT AATTTT	TTATGA	AAAGAAAAAATCCCTTACGG	intergenic in pTT39_p3 (CP023426) (*repB*_pseudogene-IS*6*)
								Chromosome[c]
CS_C1	21	TTCTAGCAGCTTTTATCGAA	–	AAAA TTTTAG	ATAT AATTTT	CCAA TT	AAAACTTAGCATCAGCGACG	Intergenic (AFK60264-AFK60265)
CS_C2	1	ACTTCATTGCTTTTTAAATC$_{406}$	–	AAAA TTTTAG	ATAT AATTTT	CACTA	$_{405}$ACAACTGATATCCTTATACT	AFK59023
CS_C3	198	CTAGAAAAGGCCCAGCTTTT	TGGCTA	AAAA TTTTAG	ATAT AATTTT	–	$_{842}$TGCATAAAAGTTTGTGCGAG	AFK58314
CS_C4	2	^{843}CCACAAATAGAGTAAATTTT	ATCGT	AAAA TTTTAG	ATAT AATTTT	–	AGAATAAAATTTTAAAAAGG	Intergenic (AFK10635-AFK10636)
CS_C5	1	TGTATAATGAGAAAAATATT$_{677}$	ATAGAA	AAAA TTTTAG	ATAT AATTTT	–	$_{678}$AAAGGAAAATTTTGTCGATT	AFK58216
CS_C6	1	ATAGAGTAAATTTACAAATT	*nd*	AAAA TTTTAG	–	*nd*[d]	*nd*[d]	Unknown (no GenBank hits)
CS_C8	2	GTGGATTTGATGTTATAAAA	–	AAAA TTTTAG	ATAT AATTTT	TTATAT	AAAAATTTCTCATTTTTGGC	Intergenic (IS*6770*_AFK57968)
CS_C9	1	CTTCTAAAAAATTTTCATTT$_{225}$	–	AAAA TTTTAG	ATAT AATTTT	CATTT	$_{227}$AAAAAACAACATCT GCGCAA	AFK58870

Duplicated CS italicized

nd, not determined

[a] *CS*, coupling sequence; *P*, plasmid integration site; *C*, chromosomal integration site

[b] For CS_P5 and CS_C6 sequences adjacent to the transposon termini are provided

[c] Hits corresponding to the DO genome of *E. faecium*

[d] No amplification product in the inverse-PCR

While the structure of Tn*1546*, harboring *vanA* shows a high variability [34, 39], the *vanY-vanX* region in the *vanB* gene cluster appeared to be less divergent. In the studied collection, the A-type showed the highest prevalence, with variants very similar or identical to these found in *E. faecium* in Australia, France, and Taiwan [40–42], and in the pMG2200 plasmid of *E. faecalis* [5]. Importantly, the A variants are also present in gut anaerobes such as *Clostridium* spp. and *E. lenta* [26, 40], a presumable reservoir of Tn*1549*-type transposons. Genomic analyses of VanB-*E. faecium* and concomitantly isolated *vanB*-positive gut anaerobes indicated the epidemiological significance of de novo acquisition of Tn*1549* by hospital-adapted *E. faecium* [7, 41]. The B-type characteristic for the first vancomycin-resistant *E. faecalis* V583 strain [33], to our knowledge, has not been reported in *E. faecium* so far. The presence of ISs targeting the *vanS-vanY* intergenic region (resulting in C- and F-types), was observed also elsewhere [43]. Such variability of *vanB* clusters may be useful in analyses of suspected VRE outbreaks. For example, plasmid-located D-type was found in isolates representing various MTs and STs from the WAW1 hospital (Table 1A). Thus, a spread of a stable ~150 kb conjugative plasmid of undetermined replicon type, harboring this specific variant of the *vanB* cluster was most likely responsible for the outbreak. Similarly, although isolates from SZC2 differed both in the clonal composition and *vanB*-associated plasmidome, C-type was detected in all these isolates (Table 1A), indicating extensive plasmid recombination during an outbreak. Until now, more detailed knowledge of plasmids carrying *vanB* in *E. faecium* remains limited [38]. In our study, *vanB*-plasmids represented mostly the rep_{pLG1}, $rep17_{pRUM}$, and $rep2_{pRE25}$ replicons, similarly to the situation observed for *vanA*-plasmids in Poland [34]. The original pLG1 contained the complete *vanA* gene cluster [44] and plasmids with this *rep* were responsible for an increase of HLGR among *E. faecium* in Norway [45] but, to our knowledge, *vanB*-plasmid of the rep_{pLG1} type has not yet been reported. The second observed *rep* type, $rep17_{pRUM}$ was involved in a multicenter VanB outbreak in Sweden [46] and in the HLGR spread in Norway [45]. The rep_{pLG1} and $rep17_{pRUM}$ genes frequently occurred together and in combination with the *axe-txe*, characteristic for plasmids with these replicons [34, 45, 46]. Plasmids harboring *vanB* were typically transferable by conjugation and during outbreaks (e.g., in KRA2, WAW1 and SZC2) were associated with diverse clonal backgrounds. Such plasmid dissemination was additionally accompanied by presumable recombination/co-integration events, resulting in the observed variability of *vanB*-plasmids. A similar dynamics was observed also for $rep17_{pRUM}$-type *vanA*-plasmids [30]. Recombination/co-integration likely contributed to the association of *vanB* with more than a single *rep*, observed in the current study and characteristic for *E. faecium* plasmids in general [30, 34, 38, 45]. Two plasmid-located genes, *aacA-aphD* and *citH*, showed the integration of Tn*1549* with different coupling sequences and might represent transposon integration hotspots. Such hotspots were indeed observed for *E. faecium* [7].

Isolates with plasmid-borne *vanB* prevailed until 2006, and later this determinant showed usually a chromosomal localization. This change occurred in parallel with the emergence and spread of lineage 78. The predominantly chromosomal localization of the *vanB* cluster in lineage 78 was observed recently also in Germany and Australia [7, 8, 40]. Two variants of coupling sequences, CS_C1 and CS_C3, were associated with two most numerous groups of isolates (Table 1). Twenty-one isolates with the CS_C1 variant, present in 17/18 lineage and A1 type of the *vanY-vanX* region, showed multicenter distribution over 2003–2008. These isolates showed some divergence of their STs/MTs, which may be explained by a transfer of transposon-containing region to a new clonal background [8] and/or exchange of other genomic regions, leading to formation of new STs/MTs [9]. An even more complex epidemiological situation was associated with isolates harboring the CS_C3 variant. This particular group appears to be the main contributor to the increasing proportion of VanB among VR*Efm* and general increase of prevalence of VRE in Poland and was responsible for extensive outbreaks, e.g., in KSZ and POZ2 hospitals. With the exception of a single isolate with CS_C5, which shared a coupling sequence and insertion site with several ST192 isolates from Germany [8], none of the remaining coupling sequences showed identity to coupling sequences described elsewhere [7–9]. This finding is consistent with proposed independent de novo acquisition of Tn*1549* [7].

This study provides an analysis of VanB-*E. faecium*, performed on a country level and over an extensive period of time. We demonstrate a significant change both in the clonal background as well as localization of Tn*1549*-type transposons, carrying *vanB* genes. Our study supports the role of lineage 78 of the hospital-adapted *E. faecium*, presumably acquiring de novo the *vanB* determinant, followed by spread and differentiation of certain strains as a major factor beyond the current increasing prevalence of VanB-VR*Efm* in Polish hospitals.

Acknowledgements We thank all microbiologists who provided isolates to the National Reference Centre for Antimicrobial Resistance and Surveillance in Warsaw and Dr. Kenneth Van Horn for a critical reading of the manuscript. This publication made use of the *Enterococcus faecium* MLST website (http://pubmlst.org/efaecium/) sited at the University of Oxford and funded by the Wellcome Trust.

[Parts of this study were presented at the *Applied Bioinformatics & Public Health Microbiology Conference*, 15-17 May 2013, Cambridge, UK; the *Microbiology after genomic revolution: Genomes 2014 EMBO Conference*, 24-27 June 2014, Paris, France and the *International Conference on the Evolution and Transfer of Antibiotic Resistance*, 24-26 June 2015, Amsterdam, The Netherlands.]

Funding This work was supported by grant N N401588540 from the National Science Centre (NCN), Poland, by the MIKROBANK funding from the Ministry of Science and Higher Education, Poland, and by

statutory funds from the Ministry of Science and Higher Education, Poland.

Compliance with ethical standards

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

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

References

1. Sydnor ER, Perl TM (2011) Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev 24:141–173. https://doi.org/10.1128/CMR.00027-10
2. Arias CA, Murray BE (2008) Emergence and management of drug-resistant enterococcal infections. Exp Rev Anti Infect Ther 6:637–655. https://doi.org/10.1586/14787210.6.5.637
3. Courvalin P (2006) Vancomycin resistance in Gram-positive cocci. Clin Infect Dis 42(Suppl 1):S25–S34. https://doi.org/10.1086/491711
4. Garnier F, Taourit S, Glaser P, Courvalin P, Galimand M (2000) Characterization of transposon Tn*1549*, conferring VanB-type resistance in *Enterococcus* spp. Microbiology 146:1481–1489. https://doi.org/10.1099/00221287-146-6-1481
5. Zheng B, Tomita H, Inoue T, Ike Y (2009) Isolation of VanB-type *Enterococcus faecalis* strains from nosocomial infections: first report of the isolation and identification of the pheromone-responsive plasmids pMG2200, encoding VanB-type vancomycin resistance and a Bac41-type bacteriocin, and pMG2201, encoding erythromycin resistance and cytolysin (Hly/Bac). Antimicrob Agents Chemother 53:735–747. https://doi.org/10.1128/AAC.00754-08
6. Bjørkeng E, Rasmussen G, Sundsfjord A, Sjöberg L, Hegstad K, Söderquist B (2011) Clustering of polyclonal VanB-type vancomycin-resistant *Enterococcus faecium* in a low-endemic area was associated with CC17-genogroup strains harbouring transferable *vanB2*-Tn*5382* and pRUM-like *repA* containing plasmids with *axe-txe* plasmid addiction systems. APMIS 119:247–258. https://doi.org/10.1111/j.1600-0463.2011.02724
7. Howden BP, Holt KE, Lam MM, Seemann T, Ballard S, Coombs GW, Tong SY, Grayson ML, Johnson PD, Stinear TP (2013) Genomic insights to control the emergence of vancomycin-resistant enterococci. MBio 4:e00412–e00413. https://doi.org/10.1128/mBio.00412-13
8. Bender JK, Kalmbach A, Fleige C, Klare I, Fuchs S, Werner G (2016) Population structure and acquisition of the *vanB* resistance determinant in German clinical isolates of *Enterococcus faecium* ST192. Sci Rep 6:21847. https://doi.org/10.1038/srep21847
9. van Hal SJ, Ip CL, Ansari MA, Wilson DJ, Espedido BA, Jensen SO, Bowden R (2016) Evolutionary dynamics of *Enterococcus faecium* reveals complex genomic relationships between isolates with independent emergence of vancomycin resistance. Microb Genom 2. doi: https://doi.org/10.1099/mgen.0.000048
10. Edelsberg J, Weycker D, Barron R, Li X, Wu H, Oster G, Badre S, Langeberg WJ, Weber DJ (2014) Prevalence of antibiotic resistance in US hospitals. Diagn Microbiol Infect Dis 78:255–262. https://doi.org/10.1016/j.diagmicrobio.2013.11.011
11. de Kraker ME, Jarlier V, Monen JC, Heuer OE, van de Sande N, Grundmann H (2013) The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect 19:860–868. https://doi.org/10.1111/1469-0691.12028
12. 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 11:821–828. https://doi.org/10.3201/eid1106.041204
13. Willems RJ, Top J, van Schaik W, Leavis H, Bonten M, Sirén J, Hanage WP, Corander J (2012) Restricted gene flow among hospital subpopulations of *Enterococcus faecium*. MBio 3:e00151–e00112. https://doi.org/10.1128/mBio.00151-12
14. Evers S, Sahm DF, Courvalin P (1993) The *vanB* gene of vancomycin-resistant *Enterococcus faecalis* V583 is structurally related to genes encoding D-Ala:D-Ala ligases and glycopeptide-resistance proteins VanA and VanC. Gene 124:143–144. https://doi.org/10.1016/0378-1119(93)90779-3
15. Kawalec M, Gniadkowski M, Zielińska U, Kłos W, Hryniewicz W (2001) Vancomycin-resistant *Enterococcus faecium* strain carrying the *vanB2* gene variant in a Polish hospital. J Clin Microbiol 39:811–815. https://doi.org/10.1128/JCM.39.2.811-815.2001
16. Sadowy E, Sieńko 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 32:1193–1203. https://doi.org/10.1007/s10096-013-1868-y
17. Clinical and Laboratory Standards Institute (2010) Performance standards for antimicrobial susceptibility testing; 20th informational supplement 2010; Wayne, PA M100-S20
18. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf. 6th November 2017, date last accessed
19. Klare I, Konstabel C, Mueller-Bertling S, Werner G, Strommenger B, Kettlitz C, Borgmann S, Schulte B, Jonas D, Serr A, Fahr AM, Eigner U, Witte W (2005) Spread of ampicillin/vancomycin-resistant *Enterococcus faecium* of the epidemic-virulent clonal complex-17 carrying the genes *esp* and *hyl* in German hospitals. Eur J Clin Microbiol Infect Dis 24:815–825. https://doi.org/10.1007/s10096-005-0056-0
20. Werner G, Fleige C, Geringer U, van Schaik W, Klare I, Witte W (2011) IS element IS*16* as a molecular screening tool to identify hospital-associated strains of *Enterococcus faecium*. BMC Infect Dis 31:80. https://doi.org/10.1186/1471-2334-11-80
21. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H (2004) Development of a multiplex PCR for the detection of *asa1, gelE, cylA, esp* and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. J Clin Microbiol 42:4473–4479. https://doi.org/10.1128/JCM.42.10.4473-4479.2004
22. Top J, Schouls LM, Bonten MJ, Willems RJ (2004) Multiple-locus variable-number tandem repeat analysis, a novel typing scheme to study the genetic relatedness and epidemiology of *Enterococcus faecium* isolates. J Clin Microbiol 42:4305–4511. https://doi.org/10.1128/JCM.42.10.4503-4511.2004

23. Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, Van Embden JD, Willems RJ (2002) Multilocus sequence typing scheme for *Enterococcus faecium*. J Clin Microbiol 40:1963–1971. https://doi.org/10.1128/JCM.40.6.1963-1971.2002
24. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG (2004) eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol 186:1518–1530. https://doi.org/10.1128/JB.186.5.1518-1530.2004
25. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L (2009) Dispersion of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. Appl Environ Microbiol 75:4904–4908. https://doi.org/10.1128/AEM.02945-08
26. Launay A, Ballard SA, Johnson PD, Grayson ML, Lambert T (2006) Transfer of vancomycin resistance transposon Tn*1549* from *Clostridium symbiosum* to *Enterococcus* spp. in the gut of gnotobiotic mice. Antimicrob Agents Chemother 50:1054–1062. https://doi.org/10.1128/AAC.50.3.1054-1062.2006
27. Jensen LB, Garcia-Migura L, Valenzuela AJ, Løhr M, Hasman H, Aarestrup FM (2010) A classification system for plasmids from enterococci and other Gram-positive bacteria. J Microbiol Methods 80:25–43. https://doi.org/10.1016/j.mimet.2009.10.012
28. Wardal E, Gawryszewska I, Hryniewicz W, Sadowy E (2013) Abundance and diversity of plasmid-associated genes among clinical isolates of *Enterococcus faecalis*. Plasmid 70:329–342. https://doi.org/10.1016/j.plasmid.2013.07.003
29. Moritz EM, Hergenrother PJ (2007) Toxin-antitoxin systems are ubiquitous and plasmid-encoded in vancomycin-resistant enterococci. Proc Natl Acad Sci U S A 104:311–316. https://doi.org/10.1073/pnas.0601168104
30. Wardal E, Markowska K, Zabicka D, Wróblewska M, Giemza M, Mik E, Połowniak-Pracka H, Woźniak A, Hryniewicz W, Sadowy E (2014) Molecular analysis of *vanA* outbreak of *Enterococcus faecium* in two Warsaw hospitals: the importance of mobile genetic elements. Biomed Res Int 2014:575367. https://doi.org/10.1155/2014/575367
31. Barton BM, Harding GP, Zuccarelli AJ (1995) A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220
32. Manson JM, Hancock LE, Gilmore MS (2010) Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. Proc Natl Acad Sci U S A 107:12269–12274. https://doi.org/10.1073/pnas.1000139107
33. Paulsen IT, Banerjei L, Myers GS, Nelson KE, Seshadri R, Read TD, Fouts DE, Eisen JA, Gill SR, Heidelberg JF, Tettelin H, Dodson RJ, Umayam L, Brinkac L, Beanan M, Daugherty S, DeBoy RT, Durkin S, Kolonay J, Madupu R, Nelson W, Vamathevan J, Tran B, Upton J, Hansen T, Shetty J, Khouri H, Utterback T, Radune D, Ketchum KA, Dougherty BA, Fraser CM (2003) Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science 299:2071–2074. https://doi.org/10.1126/science.1080613
34. Patel R, Uhl JR, Kohner P, Hopkins MK, Steckelberg JM, Kline B, Cockerill FR 3rd (1998) DNA sequence variation within *vanA*, *vanB*, *vanC*-1, and *vanC*-2/3 genes of clinical *Enterococcus* isolates. Antimicrob Agents Chemother 42:202–205
35. Wardal E, Kuch A, Gawryszewska I, Żabicka D, Hryniewicz W, Sadowy E (2017) Diversity of plasmids and Tn*1546*-type transposons among VanA *Enterococcus faecium* in Poland. Eur J Clin Microbiol Infect Dis 36:313–328. https://doi.org/10.1007/s10096-016-2804-8
36. Deshpande LM, Fritsche TR, Moet GJ, Biedenbach DJ, Jones RN (2007) Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. Diagn Microbiol Infect Dis 58:163–170. https://doi.org/10.1016/j.diagmicrobio.2006.12.022
37. Coombs GW, Daley DA, Thin Lee Y, Pang S, Pearson JC, Robinson JO, Johnson PD, Kotsanas D, Bell JM, Turnidge JD, Australian Group on Antimicrobial Resistance (2016) Australian group on antimicrobial resistance Australian enterococcal sepsis outcome programme annual report, 2014. Commun Dis Intell Q Rep 40:E236–E243
38. Freitas AR, Tedim AP, Francia MV, Jensen LB, Novais C, Peixe L, Sánchez-Valenzuela A, Sundsfjord A, Hegstad K, Werner G, Sadowy E, Hammerum AM, Garcia-Migura L, Willems RJ, Baquero F, Coque TM (2016) Multilevel population genetic analysis of *vanA* and *vanB Enterococcus faecium* causing nosocomial outbreaks in 27 countries (1986–2012). J Antimicrob Chemother 71:3351–3366. https://doi.org/10.1093/jac/dkw312
39. Willems RJ, Top J, van den Braak N, van Belkum A, Mevius DJ, Hendriks G, van Santen-Verheuvel M, van Embden JD (1999) Molecular diversity and evolutionary relationships of Tn*1546*-like elements in enterococci from humans and animals. Antimicrob Agents Chemother 43:483–491
40. Ballard SA, Pertile KK, Lim M, Johnson PD, Grayson ML (2005) Molecular characterization of *vanB* elements in naturally occurring gut anaerobes antimicrob. Agents Chemother 49:1688–1694. https://doi.org/10.1128/AAC.49.5.1688-1694.2005
41. Lam MM, Seemann T, Bulach DM, Gladman SL, Chen H, Haring V, Moore RJ, Ballard S, Grayson ML, Johnson PD, Howden BP, Stinear TP (2012) Comparative analysis of the first complete *Enterococcus faecium* genome. J Bacteriol 194:2334–2341. https://doi.org/10.1128/JB.00259-12
42. Lu JJ, Perng CL, Ho MF, Chiueh TS, Lee WH (2001) High prevalence of VanB2 vancomycin-resistant *Enterococcus faecium* in Taiwan. J Clin Microbiol 39:2140–2145. https://doi.org/10.1128/JCM.39.6.2140-2145.2001
43. Dahl KH, Lundblad EW, Rokenes TP, Olsvik O, Sundsfjord A (2000) Genetic linkage of the *vanB2* gene cluster to Tn*5382* in vancomycin-resistant enterococci and characterization of two novel insertion sequences. Microbiology 146:1469–1479. https://doi.org/10.1099/00221287-146-6-1469
44. Laverde Gomez JA, van Schaik W, Freitas AR, Coque TM, Weaver KE, Francia MV, Witte W, Werner G (2011) A multiresistance megaplasmid pLG1 bearing a hyl_{Efm} genomic island in hospital *Enterococcus faecium* isolates. Int J Med Microbiol 301:165–175. https://doi.org/10.1016/j.ijmm.2010.08.015
45. Rosvoll TC, Lindstad BL, Lunde TM, Hegstad K, Aasnaes B, Hammerum AM, Lester CH, Simonsen GS, Sundsfjord A, Pedersen T (2012) Increased high-level gentamicin resistance in invasive *Enterococcus faecium* is associated with *aac(6')Ie-aph(2")Ia*-encoding transferable megaplasmids hosted by major hospital-adapted lineages. FEMS Immunol Med Microbiol 66:166–176. https://doi.org/10.1111/j.1574-695X.2012.00997.x
46. Sivertsen A, Billström H, Melefors O, Liljequist BO, Wisell KT, Ullberg M, Ozenci V, Sundsfjord A, Hegstad K (2014) A multicentre hospital outbreak in Sweden caused by introduction of a *vanB2* transposon into a stably maintained pRUM-plasmid in an *Enterococcus faecium* ST192 clone. PLoS One 9:e103274. https://doi.org/10.1371/journal.pone.0103274