European Journal of Clinical Microbiology & Infectious Diseases (2020) 39:1349–1356
https://doi.org/10.1007/s10096-020-03853-4

ORIGINAL ARTICLE

Emergence of vancomycin-resistant *Enterococcus faecium* ST1421 lacking the *pstS* gene in Korea

Hye Mee Kim [1,2] · Doo Ryeon Chung [1,2,3] · Sun Young Cho [1,3] · Kyungmin Huh [1] · Cheol-In Kang [1] · Kyong Ran Peck [1]

Received: 1 December 2019 / Accepted: 16 February 2020 / Published online: 3 March 2020

Abstract

Although multilocus sequence typing (MLST) has been used to study molecular epidemiology and to explore the population structure of *Enterococcus faecium*, vancomycin-resistant *E. faecium* (VREF) strains lacking the *pstS* gene that were non-typable using conventional MLST methods were reported recently. We found nationwide emergence of VREF isolates lacking *pstS* in Korea and hereby report the molecular characteristics of these isolates. Forty-six VREF isolates lacking the *pstS* gene were identified among 300 VREF rectal isolates collected from hospitalized patients between 2014 and 2015. MLST was performed and clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE). Four VREF ST1421 isolates were whole-genome sequenced. Among the VREF rectal isolates lacking *pstS*, 98% were classified as ST1421, which has identical allelic profiles to ST17 for all housekeeping genes except *pstS*. PFGE pattern analyses revealed 32 pulsotypes. All isolates harbored Tn*1546* components with various transposase and insertion sequences. The whole-genome sequencing of four VREF ST1421 isolates showed that the *pstS* gene region was deleted at various locations with considerable inversion. The *pstS* gene was also depleted in 12.1% of 33 VREF clinical isolates in 2006–2007 and in 11.8% of 59 clinical isolates in 2012–2013. VREF ST1421 strains lacking the *pstS* gene have emerged in Korea. The emergence and spread of *pstS*-deleted VREF strains pose a serious challenge for epidemiological investigation. Alternative molecular typing methods to MLST will be increasingly necessary.

Keywords Housekeeping gene · *pstS* · Multilocus sequence typing · Whole-genome sequencing · Gene deletion

Introduction

Vancomycin-resistant *Enterococcus faecium* (VREF) is a major pathogen causing healthcare-associated infections in many countries and has been particularly associated with outbreaks in hospitals. Multilocus sequence typing (MLST) has been successfully used to study the molecular epidemiology and population structure of *E. faecium* [1]. However, the genomic plasticity and propensity for recombination seen in the enterococci may be limiting factors for epidemiological investigations by MLST [1]. In particular, four MLST loci (*atpA*, *gyd*, *pstS*, and *ddl*) are located in recombination hotspots [2]. Recently, VREF strains lacking *pstS* have been reported in Australia [3], the UK [2], Scotland [4], and Denmark [5].

We have identified the nationwide emergence of VREF isolates lacking the *pstS* gene in Korea and hereby report the molecular characteristics of these isolates.

Materials and methods

Bacterial isolates and microbiological characteristics

This study was conducted at Samsung Medical Center (Seoul, Korea), a large tertiary referral hospital where more than 70% of hospitalized patients came from other parts of the country other than Seoul. A total of 300 VREF isolates were collected using rectal swab surveillance cultures from newly admitted patients between April 2014 and March 2015 according to the infection prevention policy

✉ Doo Ryeon Chung
iddrchung@gmail.com

[1] Division of Infectious Diseases, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea

[2] Asia Pacific Foundation for Infectious Diseases (APFID), Seoul, South Korea

[3] Center for Infection Prevention and Control, Samsung Medical Center, Seoul, South Korea

of the hospital. In particular, screening tests were performed on patients transferred from other hospitals. In 46 (15.3%) of these isolates, typing by MLST was not possible because the *pstS* gene was not detected. MLST was conducted using PCR and sequencing of seven housekeeping genes (*adk*, *atpA*, *ddl*, *gdh*, *gyd*, *purK*, and *pstS*). Allelic profiles and sequence types (STs) were assigned according to the database available on the MLST website (https://pubmlst.org/efaecium/). Species identification and antimicrobial susceptibility testing were performed using the VITEK 2 system (bioMeriéux, Marcy-l'Etoile, France). The presence of genes encoding glycopeptide resistance (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, and *vanG*) was determined by PCR using primers previously described [6]. Virulence genes including enterococcal surface protein (*esp*) and hyaluronidase (*hyl*) were detected by PCR and confirmed by sequencing [7]. To identify the *pstS2* gene used for MLST in the place of *pstS* [3], we performed PCR using the following specific primers; pstS2-Forward (5′-CCT TGC CAA TCA CGT TCG AC-3′) and pstS2-Reverse (5′-ATC CAT TAC GGC TGT TGG CT-3′) that amplified a 755 bp internal fragment of the gene. For pulsed-field gel electrophoresis (PFGE), bacterial DNA was digested with the *Sma*I restriction enzyme (TaKaRa Bio Inc., Shiga, Japan) and separated by electrophoresis using the CHEF DR II system (Bio-Rad Laboratories, Hercules, CA, USA). Potential clonal relatedness was determined at the ≥ 80% level of similarity.

Whole-genome sequencing

Four VREF strains (2014-VREF-041, 2014-VREF-063, 2014-VREF-114, and 2014-VREF-268) that could not be typed by MLST were randomly selected for whole-genome sequencing (WGS) based on the allelic profiles of six housekeeping genes and the presence of *esp* and *hyl* virulence genes. High-quality, high-molecular-weight genomic DNA (gDNA) was extracted using the G-spin™ Genomic DNA Extraction Kit (iNtRON, Korea) according to the manufacturer's instructions. The gDNA was used to prepare approximately 20 kb size-selected SMRTbell templates. SMRT libraries were prepared by ligating hairpin adaptors to both ends using the PacBio DNA Template Prep Kit 1.0 (for 3–10 kb) for SMRT sequencing with C4 chemistry. Data (240 min movies) were collected using the PacBio RS II instrument (Pacific Biosciences, Menlo Park, CA, USA). Sequencing raw data was analyzed using the SMRT Analysis software (v2.3). The SMRT Portal allowed the execution of all HGAP steps in the web-based GUI.

Bioinformatic analyses

An overall comparison of the architecture of the isolates was performed with the Mauve genome alignment viewer (Biomatters Ltd., New Zealand) [8] and the BLAST Ring Image Generator (BRIG) [9], as previously described. Specific plasmid genes containing Tn*1546* were further

Table 1 Summary report of the de novo-assembled vancomycin-resistant *E. faecium* ST1421 strains from this study

Strain	NCBI BioSample no.	Type	GenBank accession no.	Genome size (bp)
2014-VREF-041	SAMN06186500	Chromosome	NZ_CP019208	3,009,007
		Plasmid	NZ_CP019209	215,998
		Plasmid	NZ_CP019210	45,501
		Plasmid	NZ_CP019211	5850
		Plasmid	NZ_CP019212	4376
2014-VREF-063	SAMN06472829	Chromosome	NZ_CP019988	2,907,074
		Plasmid	NZ_CP019989	287,502
		Plasmid	NZ_CP019990	25,791
		Plasmid	NZ_CP019991	18,099
2014-VREF-114	SAMN06463381	Chromosome	NZ_CP019970	2,804,968
		Plasmid	NZ_CP019971	129,683
		Plasmid	NZ_CP019972	128,951
		Plasmid	NZ_CP019973	56,285
		Plasmid	NZ_CP019974	55,063
		Plasmid	NZ_CP019975	47,460
		Plasmid	NZ_CP019976	32,787
2014-VREF-268	SAMN06472830	Chromosome	NZ_CP019992	2,990,310
		Plasmid	NZ_CP019993	243,818
		Plasmid	NZ_CP019994	99,829
		Plasmid	NZ_CP019995	58,211

examined with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Bacterial Pan-Genome Analysis (BPGA) was performed for the core genomes and dispensable/accessory genomes among the strains [10]. Each core genome was extracted by BPGA using the USEARCH clustering algorithm, and multiple sequences were aligned using MUSCLE. All alignments were concatenated, and a neighbor-joining phylogenetic tree was constructed [10]. The phylogenetic tree was generated using FigTree v1.4.3 (http://tree.bio.ed.ac.uk /software/figtree/). A bootstrap consensus tree with 1000 replicates was constructed by the maximum likelihood method based on the JTT matrix-based model [11].

Supplementary experiments using clinical isolates from nationwide surveillance collection

We determined the prevalence of VREF isolates lacking the *pstS* gene among the collection of VREF clinical isolates. These isolates came from the Asian Bacterial Bank (the Asia Pacific Foundation for Infectious Diseases, Seoul, Korea) and had been collected through Korean national surveillance studies of bacteremia and urinary tract infection during 2006–2007 (9 university hospitals participating) and 2012–2013 (16 university hospitals) funded by the Korea Centers for Disease Control and Prevention [12, 13].

Nucleotide sequence accession numbers

Genome read sequences for the four VREF ST1421 strains and annotation data of the isolates have been deposited in National Center for Biotechnology Information (NCBI) under project accession number (PRJNA358851) as shown in Table 1.

Results

Among the 46 isolates that lacked the *pstS* gene and were nontypable at the beginning of this study, 45 could be typed as ST1421 and one as ST1424 using a new PubMLST designation scheme. In this new scheme, the missing housekeeping gene allele, *pstS*, was set to 0 and ST17-related strains and

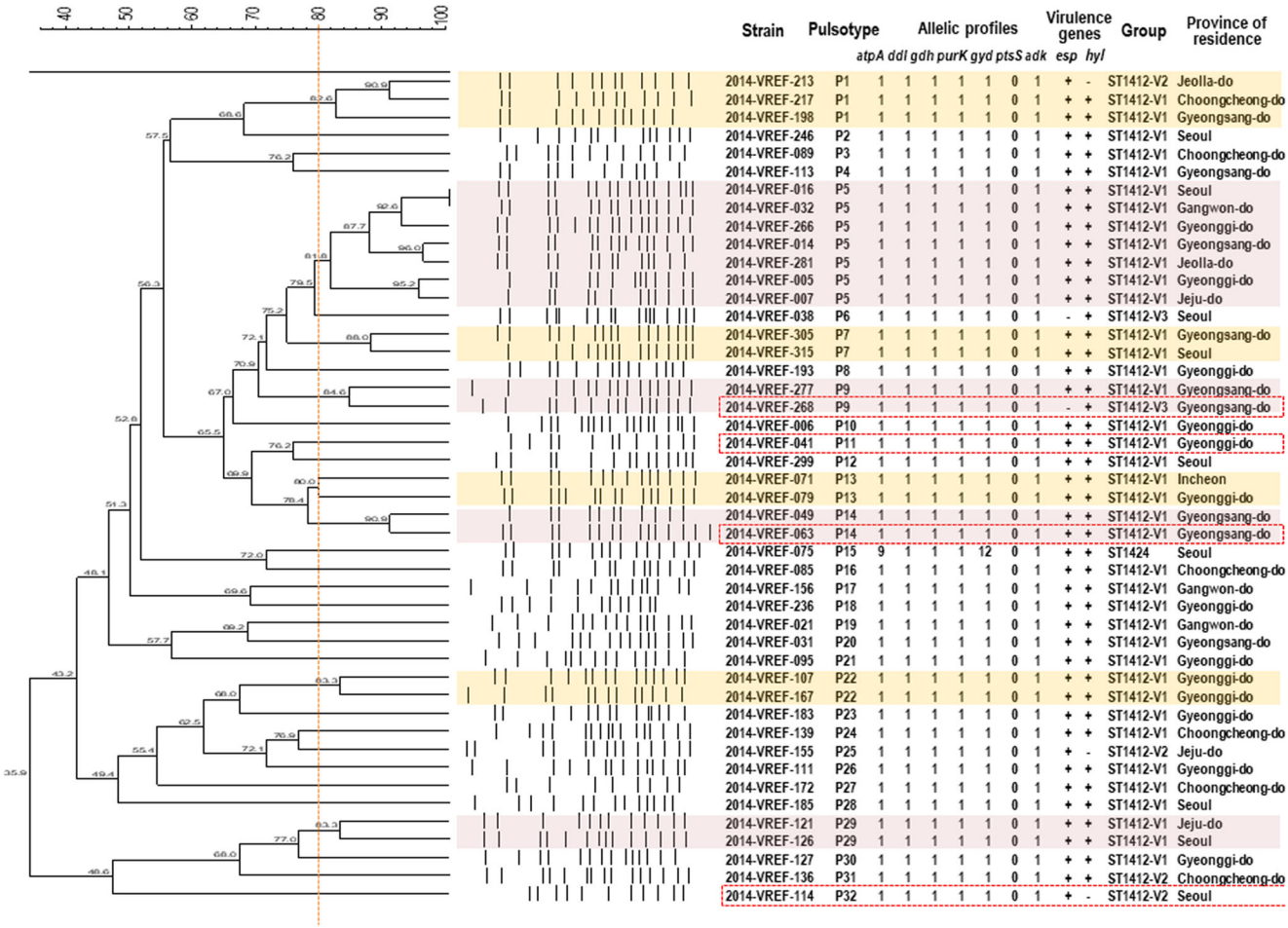

Fig. 1 Dendrogram of the PFGE patterns of 46 vancomycin-resistant *Enterococcus faecium* (VREF) strains with a *pstS* gene deletion. The boxes in dashed lines indicate the strains on which whole-genome sequencing was performed

ST80-related strains were assigned as ST1421 and ST1424, respectively. All these strains were isolated at the time of admission from patients who were referred from eight different provinces in Korea. The ST1421 strains were further subdivided into three groups according to the presence of the *esp* and *hyl* virulence genes. Thirty-nine ST1421 strains (84.7%) containing both *esp* and *hyl* were classified into ST1421-V1 (Fig. 1). ST1421 strains carrying only either *esp* or *hyl* were grouped into ST1421-V2 (8.7%) and ST1421-V3 (4.4%), respectively. All isolates contained the *vanA* gene, and analysis of the PFGE patterns revealed 32 pulsotypes (Fig. 1). All strains belonging to the predominant pulsotype P5 were ST1421-V1, and province of residence of the patients with these isolates was diverse (6 provinces).

The genome sizes of the four ST1421 strains ranged from 3,255,197 to 3,401,730 bp. Transposon Tn*1546* was found within the plasmids of each strain, but the structure of Tn*1546* varied among strains. The structures of the plasmids were analyzed. All isolates harbored Tn*1546* components (*vanR*, *vanS*, *vanHAX*, *vanY*, and *vanZ*) with various transposase and insertion sequences (Fig. 2). Only the original structure (*vanRS*, *vanHAX*, and *vanYZ*) was found in 2014-VREF-041 and 2014-VREF-114. However, additional cassettes with a *vanYZ* deletion in the original structure were detected in 2014-VREF-063 and 2014-VREF-268. We confirmed that the additional cassette was due to two copies in 2014-VREF-063. 2014-VREF-041 and 2014-VREF-268 carried the *aph(3')-IIIa* gene mediating aminoglycoside resistance and the *erm(B)* gene causing macrolide resistance. 2014-VREF-063 had one resistance gene, *aph(2")-Ia*, while 2014-VREF-114 had no resistance genes.

The architecture of the chromosomes of the four ST1421 strains relative to a reference strain (*E. faecium* Aus0085) was studied using the Mauve tool (Fig. 3a). Mauve alignment of the four ST1421 chromosomes identified 25 locally collinear blocks (LCBs) with a variety of inversions. A loss of one or more LCBs compared with Aus0085 was identified in all strains. To identify a genetic deletion in the *pstS* gene, we compared the positions containing the *pstS* gene. All ST1421 isolates showed deletion of the *pstS* gene and hypothetical proteins (Fig. 3b). All ST1421 isolates except one (2014-VREF-114) showed an insertion of prophage ps2 probable integrase (DNA recombination and DNA integration function genes) in front of the 50S ribosomal protein L19 (*rplS* gene), the first gene of LCB containing *pstS* gene (Fig. 3b). One strain (2014-VREF-114) was found to contain a putative transposase (*InsK*) for the insertion sequence element IS*150* inserted in the *pstS* gene position.

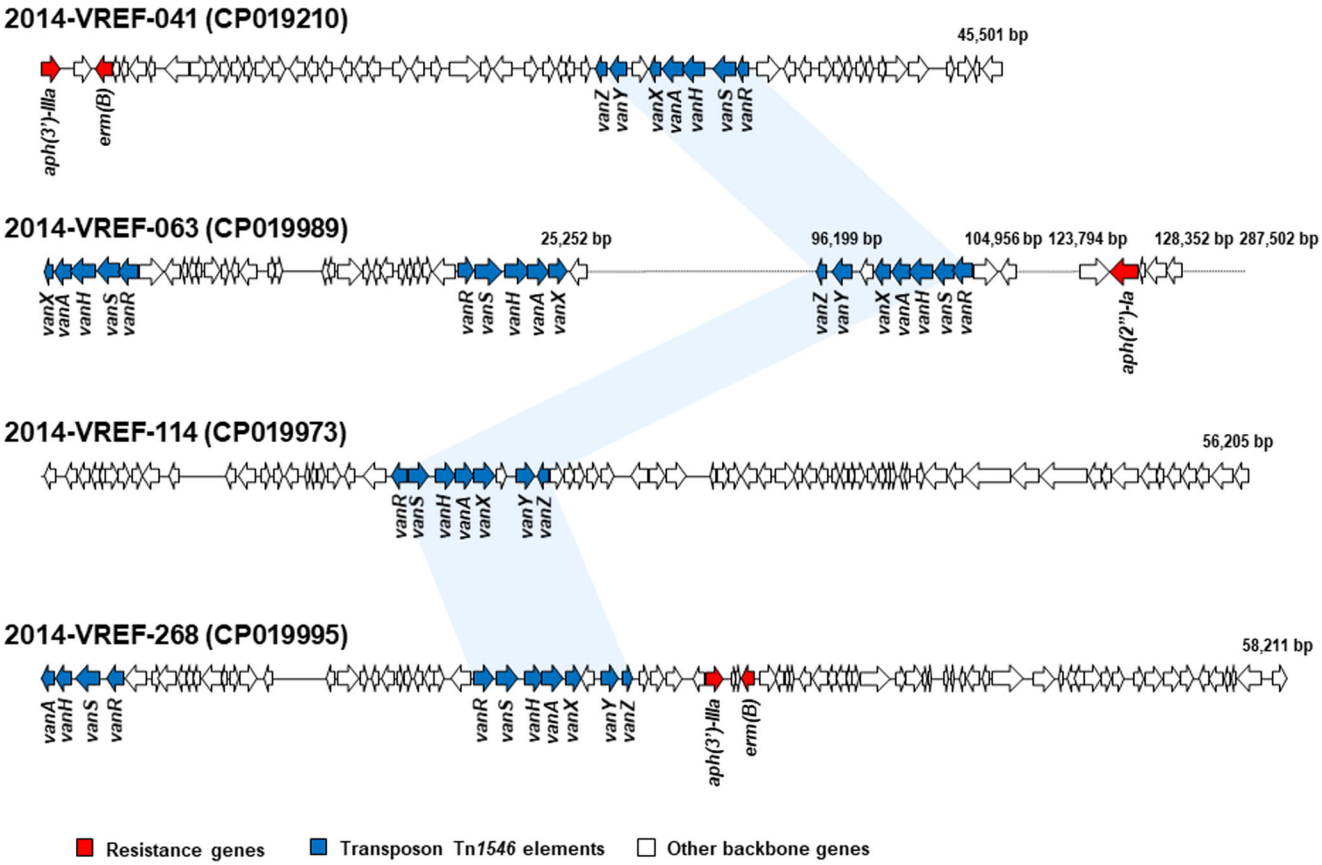

Fig. 2 Genetic environment of Tn*1546* transposon components in four VREF ST1421 isolates. Red and blue arrows indicate antibiotic resistance genes and transposon Tn*1546* components, respectively

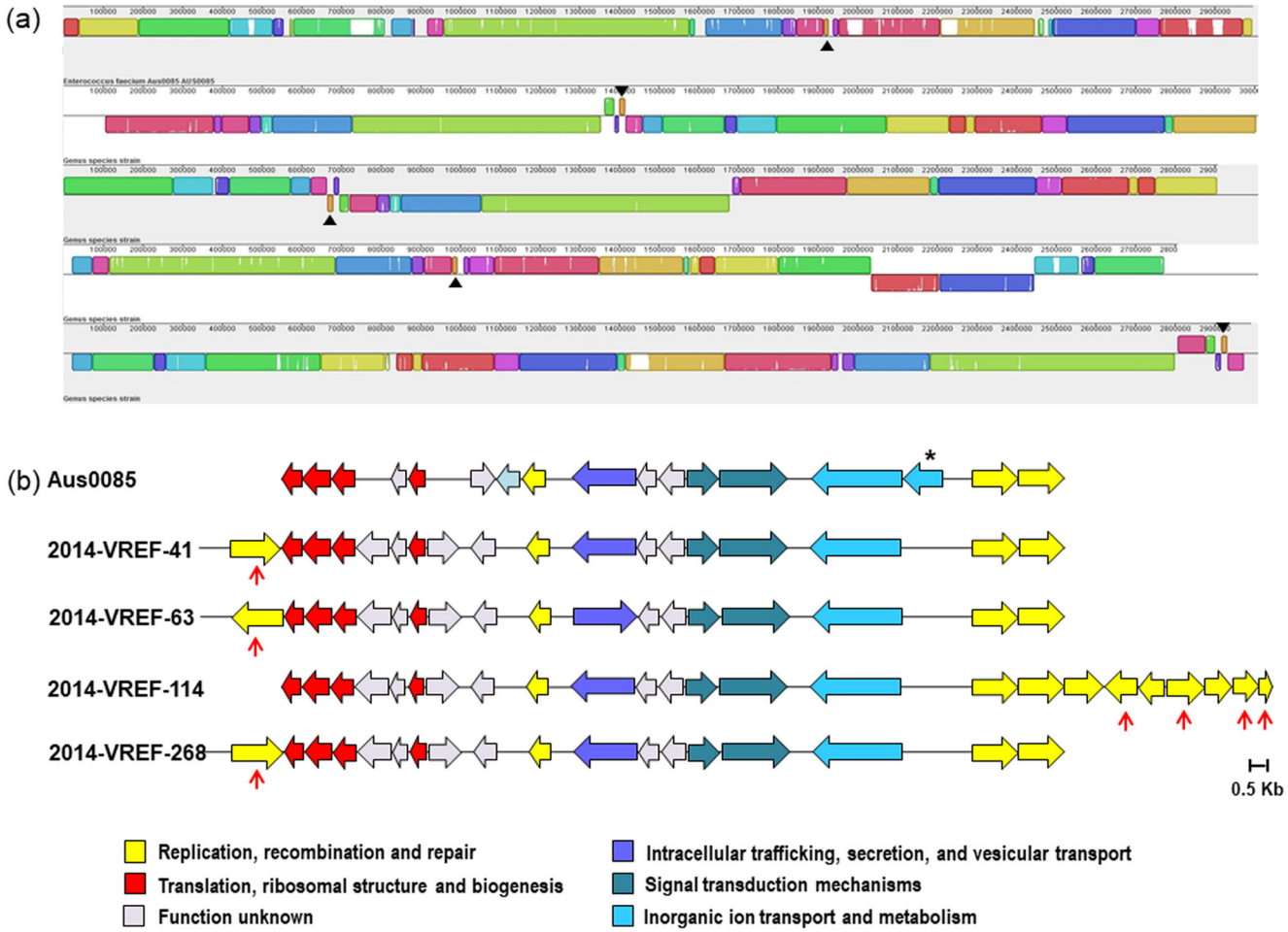

Fig. 3 Comparative analysis of the chromosome of four VREF ST1421 strains relative to the reference strain *E. faecium* Aus0085. **a** Sequences were aligned using the Mauve genome alignment viewer. Each genome panel is composed of different color blocks that represent locally collinear blocks (LCBs) that are aligned to the parts of other genomes. White areas between the LCBs contain sequence elements that are specific to the indicated genome and that are not aligned. LCBs containing the *pstS* gene are indicated with a dark triangle. **b** Genetic organization of the LCBs containing the *pstS* gene is shown diagrammatically for the strains used in this study and reference strain. Genes are color-coded according to predicted function of the corresponding protein product, with associated Cluster of Orthologous Groups (COGs) domains indicated. The inserted genes compared with the reference strain are shown with a red arrow, and an asterisk (*) represents the position of the *pstS* gene that was deleted in our four VREF ST1421 strains. The scale represents DNA length in 0.5-kb pairs

Chromosome structures of the four ST1421 isolates were visually compared with that of Aus0085 using BRIG (Fig.4). The locations of the seven housekeeping genes and the *pstS2* gene, which had been reported in Australia [14], are shown. Compared with the reference strain, four strains in this study were missing several gene segments in common. The 41 VREF ST1421 isolates and one VREF ST1424 isolate that were not sequenced were also determined to contain a chromosomal *pstS2* gene.

We selected 25 *E. faecium* strains with a query coverage percentage of over 80% and an identity greater than 99% compared with the genomes of our four ST1421 strains using BLAST. By BPGA, we selected 1506 conserved core gene clusters from the 25 *E. faecium* reference strains, the four ST1421 strains of this study, and an Australian strain (DMG1500501) showing a *pstS* deletion. The phylogenetic tree generated using FigTree showed that only one ST1421 strain (2014-VREF-114) grouped with the reference strains, whereas the other three ST1421 strains and DMG1500501 (highlighted in blue) were not related to the reference strains (Fig. 5).

A supplementary investigation with VREF clinical isolates collected through Korean national surveillance studies of bacteremia and urinary infection revealed that VREF strains with a deleted *pstS* gene accounted for 12.1% of 33 isolates in 2006–2007 and accounted for 11.8% of 59 isolates in 2012–2013. Among these eleven isolates, eight were ST1421 with *esp* and *hyl* genes belonging to group ST1421-V1, although the hospitals where the strains were collected varied. Of those, seven strains were isolated during 2012–2013 (Table 2).

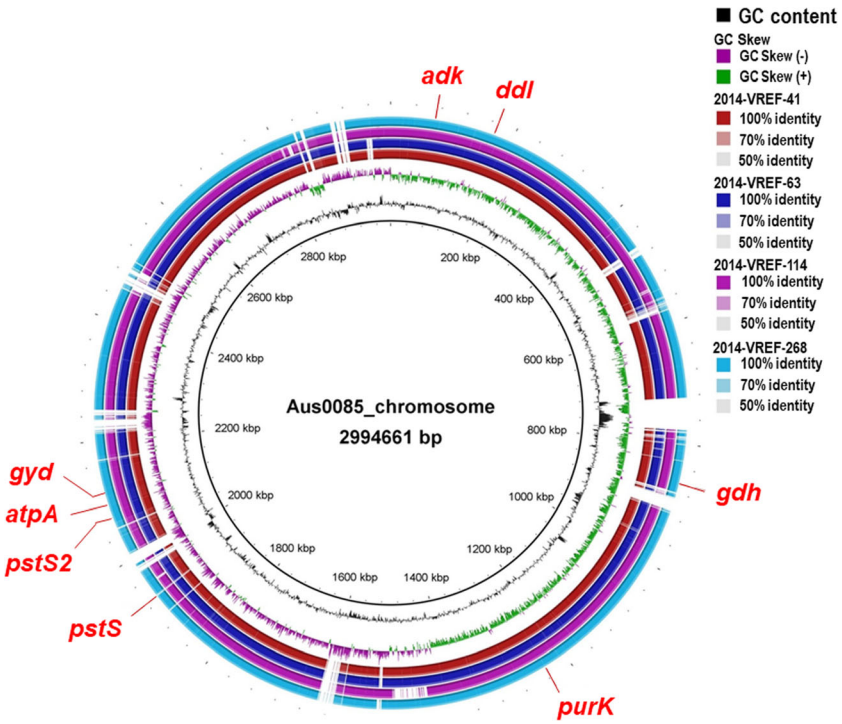

Fig. 4 Comparison of chromosome analysis using the BRIG bacterial pan-genome analysis pipeline. Each ring corresponds to one of the four VREF ST1421 chromosomes (indicated at the right of the figure together with the color code). The *E. faecium* Aus0085 chromosome (inner black ring) was used as a reference. Genes highlighted in red indicate the seven housekeeping genes in *E. faecium*

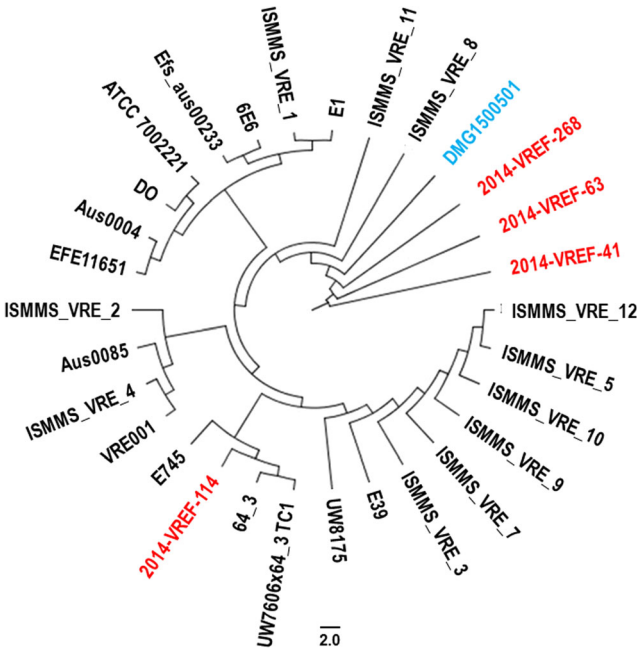

Fig. 5 Maximum likelihood, circular phylogenetic tree of 30 *Enterococcus faecium* strains based on the JTT matrix-based model. Initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BIONJ algorithms to a matrix of pairwise distances estimated using a JTT model and then selecting the topology with superior log likelihood value. The scale bar (2.0) indicates the number of nucleotide substitutions per site. The four VREF ST1421 isolates in this study are highlighted in red, whereas the Australian strain, DMG1500501, that showed deletion of *pstS* gene is highlighted in blue

Discussion

In our study, 46 (15.3%) of 300 VREF rectal isolates collected from 2014 to 2015 were ST1421 or ST1424 strains lacking the *pstS* gene. The failure in MLST genotyping due to the *pstS* gene deletion in a substantial proportion of VREF isolates coincides with a previous report from Australia [3]. WGS in the four ST1421 strains in this study determined that DNA recombination and transposition genes were inserted, resulting in considerable inversion and loss of the *pstS* gene. The same insertion sequence was identified in front of the *pstS* gene region of three VREF ST1421 strains (2014-VREF-041, -063 and -268), whereas a putative transposase was inserted into the *pstS* gene position in one strain (2014-VREF-114) with a deletion of the *pstS* gene. Thus, significant recombination in the chromosome and considerable inversions identified in our strains might have affected the housekeeping gene region. *E. faecium* is known to have a high rate of recombination [14]. Our results support the suggestion that WGS should be used in tracing the epidemiology of *E. faecium* nosocomial infections [15]. The majority VREF strain lacking the *pstS* gene in our study was ST1421, which has an identical allelic profile to ST17 for six housekeeping genes, all except *pstS*. Considering that ST17 is the predominant VREF genotype isolated from healthcare-associated infections in Korea [16], these strains can be assumed to be derived from ST17. There are 16 single-locus variants (SLVs) that have the same allelic profile as ST17 except for the *pstS* gene according to the

Table 2 Characteristics of 11 VREF clinical isolates lacking the *pstS* gene

Strain	Specimen	Year	Hospital	Location of hospital	Housekeeping gene allele								*vanA*	Virulence genes				
					aptA	*ddl*	*gdh*	*purK*	*gyd*	*pstS*	*adk*	ST		*esp*	*hyl*	*asa1*	*gelE*	*ace*	*cylA*
KCDC_EFM_05	Urine	2007	A	Seoul	15	1	1	1	1	0	1	1489	+	+	+	–	–	–	–
KCDC_EFM_12	Urine	2006	B	Gyeongsang-do	15	1	1	1	12	0	1	1494	+	+	+	–	–	–	–
KCDC_EFM_13	Urine	2007	B	Gyeongsang-do	15	1	1	1	1	0	1	1489	+	+	+	–	–	–	–
KCDC_EFM_19	Urine	2006	F	Choongcheong-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_39	Urine	2013	A	Seoul	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_42	Urine	2012	D	Gyeongsang-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_51	Blood	2012	A	Seoul	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_66	Blood	2013	C	Gyeongsang-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_67	Blood	2013	C	Gyeongsang-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_69	Blood	2012	D	Gyeongsang-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–
KCDC_EFM_93	Blood	2013	E	Gyeongsang-do	1	1	1	1	1	0	1	1421	+	+	+	–	–	–	–

MLST web site (ST180, ST181, ST182, ST202, ST233, ST386, ST472, ST485, ST538, ST723, ST803, ST1021, ST1061, ST1335, ST1394, and ST1471). Therefore, another possibility is that the ST1421 strains originated from the SLVs of ST17. In fact, analysis of the PFGE patterns revealed considerable heterogeneity among the 45 VREF ST1421 strains. The finding that very diverse ST1421 strain pulsotypes were found in different geographical regions in the country suggests that deletion of the *pstS* gene occurred sporadically in diverse clones.

Phylogenetic analysis showed that *pstS* gene-deleted strains emerged into new clones with distinct phylogenetic patterns and suggested that VREF ST1421 strains emerged and spread in a non-clonal manner. The mutation in which the *pstS* gene is deleted from VREF appears to have occurred nationwide in Korea. The results of our supplementary experiments using VREF isolates collected nationwide for the surveillance of bacteremia and urinary tract infections support this. Notably, these results indicate that VREF with the *pstS* deletion had already emerged nationwide in Korea in 2006, much earlier than when similar strains were found in Australia [3].

Multiple copies of the *vanA* gene were identified in our isolates through the analysis of Tn*1546*. The copy number of some resistance genes has been reported to affect the minimum inhibitory concentrations (MICs) of corresponding antibiotics [17]. In our study, however, no correlation between *vanA* gene copy number and vancomycin MICs was observed, and all isolates represented high-level resistance to vancomycin (MIC > 32 mg/l). It is suggested that our ST1421 strains acquired a plasmid-mediated *vanA* gene with multiple copies in clones in which the *pstS* gene is missing.

There are some limitations in our study. First, WGS was performed in only four ST1421 strains. In the other 42 isolates (ST1421 and ST1424) that failed to amplify the *pstS* gene, details about the genome structures near the *pstS* region were not determined. Second, rectal bacterial isolates were collected from a single center; however, the strains were isolated from patients who were referred from various provinces in Korea. Additionally, clinical isolates from bacteremia and urinary tract infections were collected from multiple centers through a national surveillance. Therefore, our results could be interpreted as representing the country.

In conclusion, VREF ST1421 strains lacking the *pstS* gene have emerged in Korea and account for a substantial portion among rectal and clinical isolates. Although isolates with deletion of the *pstS* gene have been assigned as new sequence types, the emergence and spread of *pstS*-deleted VREF strains pose a serious challenge for epidemiological investigation. Alternative molecular typing methods to MLST will be increasingly necessary.

Funding information This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Government-wide R&D Fund Project for infectious disease research, HG18C0062) and the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science & ICT (NRF-2016M3A9B6919187).

Compliance with ethical standards

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

Ethical approval Rectal swab surveillance for VREF and collection of bacterial isolates were approved by the Infection Control Committee of the Samsung Medical Center. Collection of VREF blood isolates was approved by the Institutional Review Board of the Samsung Medical Center.

References

1. de Been M, Pinholt M, Top J, Bletz S, Mellmann A, van Schaik W, Brouwer E, Rogers M, Kraat Y, Bonten M, Corander J, Westh H, Harmsen D, Willems RJ (2015) Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. J Clin Microbiol 53(12):3788–3797
2. Raven KE, Reuter S, Reynolds R, Brodrick HJ, Russell JE, Torok ME, Parkhill J, Peacock SJ (2016) A decade of genomic history for healthcare-associated *Enterococcus faecium* in the United Kingdom and Ireland. Genome Res 26(10):1388–1396
3. Carter GP, Buultjens AH, Ballard SA, Baines SL, Tomita T, Strachan J, Johnson PD, Ferguson JK, Seemann T, Stinear TP, Howden BP (2016) Emergence of endemic MLST non-typeable vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother 71(12):3367–3371
4. Lemonidis K, Salih TS, Dancer SJ, Hunter IS, Tucker NP (2019) Emergence of an Australian-like *pstS*-null vancomycin resistant *Enterococcus faecium* clone in Scotland. PLoS One 14(6): e0218185. https://doi.org/10.1371/journal.pone.0218185
5. Hansen TA, Pedersen MS, Nielsen LG, Ma CMG, Soes LM, Worning P, Ostergaard C, Westh H, Pinholt M, Schonning K (2018) Emergence of a vancomycin-variable *Enterococcus faecium* ST1421 strain containing a deletion in vanX. J Antimicrob Chemother 73(11):2936–2940
6. Depardieu F, Perichon B, Courvalin P (2004) Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. J Clin Microbiol 42(12):5857–5860
7. 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(10):4473–4479
8. Darling AC, Mau B, Blattner FR, Perna NT (2004) Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14(7):1394–1403
9. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA (2011) BLAST ring image generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402
10. Chaudhari NM, Gupta VK, Dutta C (2016) BPGA-an ultra-fast pan-genome analysis pipeline. Sci Rep 6:24373
11. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 8(3):275–282
12. Son JS, Song JH, Ko KS, Yeom JS, Ki HK, Kim SW, Chang HH, Ryu SY, Kim YS, Jung SI, Shin SY, Oh HB, Lee YS, Chung DR, Lee NY, Peck KR (2010) Bloodstream infections and clinical significance of healthcare-associated bacteremia: a multicenter surveillance study in Korean hospitals. J Korean Med Sci 25:992–998
13. Chung DR (2014) Antimicrobial resistance and molecular epidemiology of major pathogens isolated from patients with bacteremia and urinary tract infection. Report by Korea Centers for Disease Control and Prevention
14. 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
15. 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(1). https://doi.org/10.1099/mgen.0.000048
16. Kim J, Kwon YI, Lee WG (2017) Comparison of multilocus sequence typing change patterns of vancomycin-resistant *Enterococcus faecium* from 2015 to 2017. Ann Clin Microbiol 20(3):67–73
17. Bertini A, Poirel L, Bernabeu S, Fortini D, Villa L, Nordmann P, Carattoli A (2007) Multicopy blaOXA-58 gene as a source of high-level resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 51(7):2324–2328