Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* clinical isolates in a tertiary care hospital in southern Thailand: a retrospective study

Phanvasri Saengsuwan[1], Kamonnut Singkhamanan[1], Siribhorn Madla[2], Natnicha Ingviya[3] and Chonticha Romyasamit[4]

[1] Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla, Thailand
[2] School of Pharmacy, Walailak University, Thasala, Nakhon Si Thammarat, Thailand
[3] Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla, Thailand
[4] School of Allied Health Sciences, Walailak University, Thasala, Nakhon Si Thammarat, Thailand

ABSTRACT

Objective. Vancomycin-resistant enterococci are nosocomial pathogens that are responsible for commonly causing healthcare-associated infections, and they exhibit increased resistance to many antimicrobials, particularly to vancomycin. The epidemiological data available on vancomycin-resistant enterococci (VRE) in Thailand are inadequate.

Methods. Using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), this study investigated genes that encode antimicrobial resistance and genetic relatedness to further understand VRE prevalence. Ninety VRE isolates were collected between 2011 and 2019 from a tertiary care hospital in southern Thailand. Antimicrobial susceptibility was determined using the disk diffusion method and E-test methods. Multiplex PCR was performed to detect the *van* gene and virulence genes.

Results. The study showed a high prevalence of diverse multidrug-resistant VRE strains. The prevalence of VRE infection was the highest in 2014 (28 isolates, 39.4%). VRE were mostly found in the urogenital tract (26 isolates, 28.9%), followed by the digestive tract (20%), body fluid, i.e., pancreatic cyst fluid, peritoneal dialysis fluid, Jackson–Pratt (JP) drain (20%), and blood specimens (10%). Patients in medical and surgical wards had 71.1% multi-drug-resistant and 28.9% extensively drug-resistant (XDR) VRE strains, respectively. The most prevalent antibiotic resistance was to ampicillin (74.4%). Susceptibility to gentamicin and meropenem were similar (7% and 10%, respectively). Four isolates (4.4%) were resistant to colistin. Only *vanA* was detected among the strains. The virulence gene test showed that the detection rates of enterococcal surface protein (*esp*) and hyaluronidase (*hyl*) genes were 91.1% and 5.6%, respectively. According to ERIC-PCR analysis, 51 of 90 strains had clonality, with a similarity rate of 95%.

Conclusions. We conclude that there is a need to implement infection control practices and active surveillance. Molecular techniques can effectively detect antibiotic-resistant genes, which would allow monitoring to control VRE infection in hospitals.

INTRODUCTION

Vancomycin-resistant enterococci (VRE) are nosocomial pathogens that are responsible for causing a large number of hospital-acquired infections worldwide. These organisms affect the bloodstream, urinary tract, and surgical wounds and cause intra-abdominal and intra-pelvic abscesses (*Reyes, Bardossy & Zervos, 2016*). Hospitalized patients who have been using certain antibiotics or are on dialysis for a long time are at an increased risk for VRE colonization and infections (*Arias, Contreras & Murray, 2010*; *Howden et al., 2013*). Enterococci VRE are resistant to commonly used antibiotics, particularly aminoglycoside, ampicillin, vancomycin, and others that affect cell walls (*Billington et al., 2014*; *Puchter et al., 2018*).

With the increase in VRE infections among hospitalized patients, the control of VRE prevalence in healthcare units has become a significant concern for public health experts and for our hospital.

Enterococcus faecium and *E. faecalis* are the most common enterococcal species causing nosocomial infections (*Reyes, Bardossy & Zervos, 2016*). According to Thailand's National Antimicrobial Resistance Surveillance Center, the prevalence of *E. faecium* increased from 0.8% to 9.9% between 2012 and 2019, but the occurrence of *E. faecalis* has been stable for 10 years (0.4% in 2018) (*NARST, 2020*).

Using molecular techniques, genes associated with enterococcal virulence can be used for identifying, controlling, and preventing *E. faecium* and *E. faecalis* transmission, as opposed to using biochemical tests that are expensive and time-consuming (*Bhatt et al., 2015*; *Fang et al., 2012*; *Ulu-Kilic et al., 2016*). These virulence factors include *asa* (aggregation substance), *gel* (gelatinase), *cyt* (cytolysin), *esp* (enterococcal surface protein), *hyl* (hyaluronidase), *cpd* (sex pheromone determinant), and *ebp* (endocarditis and biofilm-associated pilus subunit A).

Esp from *E. faecalis* contributes to biofilm formation and colonization (*Pillay, Zishiri & Adeleke, 2018*), and *hyl* influences nasopharynx and lung inflammation (*Jackson, Fedorka-Cray & Barrett, 2004*). The molecular techniques used for the identification of *Enterococcus* species are multilocus sequence typing, pulsed-field gel electrophoresis (PFGE) (*Nasaj et al., 2016*), and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), which is a simplified typing method for hospital-based epidemiology (*Shahi et al., 2020*).

No reports are available on the molecular analysis or epidemiology of *E. faecium* and *E. faecalis*, and there are limited data on the possible relationship between the prevalence of virulence. This study investigates the incidence rate and genetic relationship of *E. faecium* and *E. faecalis*. We analyze the prevalence of resistance genes using clinical samples collected from a tertiary care hospital in southern Thailand during an 8-year period.

MATERIALS & METHODS

Study design and clinical isolates

This retrospective study was conducted at the Songklanagarind Hospital, a 1,000-bed tertiary care hospital in Songkhla province, southern Thailand. The clinical samples used

Table 1 Data on nosocomial infections due to Enterococci for 2011 to 2019 per year from a tertiary care, Songklanagarind Hospital.

Year	Total number (N) of nosocomial enterococcal infections	Number of VRE infections, n (% (n/N), proportion in%)
2011	1196	5 (6, 0.4)
2012	1132	16 (18, 1.4)
2013	839	12 (13, 1.4)
2014	645	28 (31, 4.3)
2015	1252	5 (6, 0.4)
2016	1195	5 (6, 0.4)
2017	1325	3 (3, 0.2)
2018	810	8 (9, 1.0)
2019	879	8 (9, 0.9)
Total	**9273**	**90 (100, 10.5)**

in this study were acquired from patients between February 2011 and February 2019. Nosocomial enterococci and VRE were identified using conventional biochemical tests at the microbiology laboratory in the hospital by referring to guidelines from the Clinical and Laboratory Standards Institute (*CLSI, 2019*). Ninety VRE isolates were included in this study: five isolates (6%) were recovered in 2011; 16 (18%) in 2012; 12 (13%) in 2013; 28 (31%) in 2014; five (6%) in 2015; five (6%) in 2016; three (3%) in 2017; eight (9%) in 2018; and eight (9%) in 2019 (Table 1).

Specimen collection and biochemical identification of enterococci species

Specimen types included blood, body fluid (e.g., pancreatic cyst fluid, peritoneal dialysis fluid, and Jackson–Pratt (JP) drain), urine, midstream urine, pus, and samples from the pelvic region, rectum, and tissue. Before use in experiments, all VRE isolates were preserved in 20% glycerol at $-80\,^{\circ}C$. Sampling of specimen was granted an exemption from requiring ethics approval by the ethical committee of the Faculty of Medicine, Prince of Songkhla University (REC-60-234-04-7).

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed using the disk diffusion method according to CLSI guidelines (*CLSI, 2019*). Each disk (Becton Dickinson, Heidelberg, Germany) contained ampicillin (AM, 10 µg), cefoperazone-sulbactam (sulperazone) (SPZ, 75/30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CRO, 30 µg), ciprofloxacin (CIP, 5 µg), colistin (DA, 10 µg), ertapenem (ETP, 10 µg), gentamicin (GM, 10 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), norfloxacin (NOR, 10 µg), penicillin (P, 10 µg), tazocin (TZP, 100/10 µg), and vancomycin (VA, 30 µg).

Susceptibility testing results were classified as sensitive, intermediate, and resistant according to CLSI breakpoints. Multidrug-resistant (MDR) strains were defined as strains that were resistant to one or more agents in three or more antimicrobial categories

(*Magiorakos et al., 2012*). The minimum inhibitory concentration (MIC) levels for ampicillin-resistant and vancomycin-resistant strains are presented as MIC $\geq$ 16 μg/mL and MIC $\geq$ 8 μg/mL, respectively.

Identification of *Enterococcus faecium* and *E. faecalis* at the species level by multiplex PCR

The genes encoding D-alanine ligases specific for *E. faecium* (*ddl E. faecium*) and *E. faecalis* (*ddl E. faecalis*) and 16S rRNA genes (*rrs*) were detected by a modified multiplex PCR technique using the primers in Table 2 (*Dutka-Malen, Evers & Courvalin, 1995*). The process used was initial denaturation at 95 °C for 10 min, denaturation at 95 ° C for 45 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min (35 cycles) (*Bhatt et al., 2015*). The PCR products were visualized on 1.0% agarose gel.

DNA extraction

Ninety isolates of VRE originating from specimens in a tertiary care teaching hospital (Songkhla, Thailand) were extracted for chromosomal DNA using a GF-1 bacterial DNA extraction kit (Vivantis Technologies Sdn. Bhd., Malaysia) in accordance with the manufacturer's instructions. DNA concentrations were quantified using a spectrophotometer at an absorbance of 260 nm (A_{260}). DNA purity was calculated from the ratio of A_{260} and A_{280}, and DNA quality was evaluated using agarose gel electrophoresis.

Detection of *van* genes and virulence genes

All isolates resistant to vancomycin were subjected to a multiplex PCR analysis to detect vancomycin-resistant and virulence genes using a specific primer as detailed in Table 2 (*Bhatt et al., 2015*; *Bourgogne et al., 2007*; *Elsner et al., 2000*; *Vankerckhoven et al., 2004*). PCR amplification was performed via initial denaturation for 3 min at 94 °C; 35 cycles of amplification consisting of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C; and a final extension for 5 min at 72 °C. A 100-bp DNA ladder (*GeneDireX*, Germany) was used as a molecular-size marker.

ATCC 700221 (for *vanA*) and *E. faecalis* ATCC 51299 (for *vanB*) were used as positive controls. The PCR product was sent for sequencing (1st BASE DNA Sequencing Services, Selangor, Malaysia), and Basic Local Alignment Search Tool search was performed using the National Center for Biotechnology Information database.

Genotyping by enterobacterial repetitive intergenic consensus (ERIC)-PCR

An ERIC-PCR typing was performed on enterococci strains using the protocol described by *Versalovic, Koeuth & Lupski (1991)* with some modifications. ERIC-1 (ATGTAAGCTCCTGGGGATTCAC) and ERIC-2 (AAGTAAGTGACTGGGGTGAGCG) were the primers. Each PCR reaction (25 μL) contained 10$\times$ buffer (2.5 μL), 0.5 mmol/L dNTPs (1 μL), 5 U/μL Taq DNA polymerase (0.2 μL), 100 mmol/L MgCl2 (0.75 μL), 100 mol/L primers (each 0.25 μL), template genomic DNA (2 μL), and distilled water (add to 25 μL). Amplifications were performed using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories) under the following temperature profiles: initial denaturation at 95 ° C

Table 2 List of oligonucleotide primers used in the genetic profiling of resistance genes and virulence genes among the isolates in this study.

Gene	Primer name	Oligonucleotide sequence (5′ to 3′)	Product size (bp)	Reference
E. faecalis	ddl_ E. faecalis	ATCAAGTACAGTTAGTCT ACGATTCAAAGCTAACTG	941	Dutka-Malen, Evers & Courvalin (1995)
E. faecium	ddl_ E. faecium	TTGAGGCAGACCAGATTGACG TATGACAGCGACTCCGATTCC	658	
rrs	16S rRNA _F 16S rRNA_R	GGATTAGATACCCTGGTAGTCC TCGTTGCGGGACTTAACCCAAC	320	
vanA	vanA+ vanA-	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732	Bhatt et al. (2015)
vanB	vanB+ vanB-	ACGGAATGGGAAGCCGA TGCACCCGATTTCGTTC	647	
vanC	vanC+ vanC-	ATGGATTGGTAYTKGTAT TAGCGGGAGTGMCYMGTAA	815/827	
vanD	vanD+ vanD	TGTGGGATGCGATATTCAA TGCAGCCAAGTATCCGGTAA	500	
vanE	vanE+ vanE-	TGTGGGATCGGAGCTGCAG ATAGTTTAGCTGGTAAC	430	
vanG	vanG+ vanG-	CGGCATCCGCTGTTTTTGA GAACGATAGACCAATGCCTT	941	
asa	ASA 11 ASA 12	GCACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	373	Vankerckhoven et al. (2004)
gel	GEL 11 GEL 12	TATGACAATGCTTTTTGGGAT AGATGCACCCGAAATAATATA	213	
cyt	CYT I CYT IIb	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	688	
esp	ESP 14F ESP 12R	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	510	
hyl	HYL n1 HYL n2	ACAGAAGAGCTGCAGGAAATG GACTGACGTCCAAGTTTCCAA	276	
cpd	cpd-F cpd-R	TGGTGGGTTATTTTTCAATTC TACGGCTCTGGCTTACTA	782	Elsner et al. (2000)
ebp	ebpA-F ebpA-R	AAAAATGATTCGGCTCCAGAA TGCCAGATTCGCTCTCAAAG	101	Bourgogne et al. (2007)

for 5 min; 35 cycles of 1 min at 95 °C, 1 min at 48 °C, and 1 min at 72 °C; and a final extension at 72 °C for 10 min. The ERIC-PCR products were separated by electrophoresis in a 1.0% agarose gel with ViSafe green gel stain (0.001%, v/v; Vivantis Technologies Sdn. Bhd., Malaysia) and visualized using the Gel Doc^{TM} XR $^+$ system (Bio-Rad Laboratories, Hercules, CA, US). The images were captured in a TIFF file for further analysis.

ERIC-PCR patterns were analyzed using BioNumerics 7.0 software (Applied Maths, Sint-Martens-Latem, Belgium). A similarity matrix was estimated using Dice's coefficient, and a dendrogram was created based on the unweighted-pair group method with arithmetic averages. Enterococci isolates with a similarity coefficient ≥85% were considered as the same genotype (Said & Abdelmegeed, 2019).

Statistical analysis

Demographic data are presented as percentages (unless otherwise stated) and median values with interquartile ranges (IQR). All statistical analyses were performed using SPSS Statistics version 23 (IBM Corporation, Armonk, New York, US). Qualitative variables were compared using a t-test and Pearson's chi-squared test. A P-value <0.050 was considered statistically significant.

Ethical approval

This study protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkhla University (Date: August 4, 2017; Approval ID: REC-60-234-04-7).

RESULTS

Characteristics of the study population and VRE sources

Of the 90 VRE isolates recovered from patients, 53 (58.9%) were from females and 37 (41.1%) were from males, for a female-to-male ratio of 3:2. Fifty percent of VRE positive patients were ≥65 years old, and the mean age was 64 years (IQR, 43.5–73; range, 3 months to 90 years). Among these isolates, 42 (46.7%) were obtained from the medical ward; 18 (20.0%) from the surgical ward; and 10 (11.1%) from the intensive care unit. Urine samples had the highest percentage of VRE of 28.9% (26), followed by those obtained from the rectal area at 20% (18) and body fluid at 20% (18). The period of collective sample and source of samples showed statistically significant difference with an antimicrobial pattern ($P < 0.05$), but no significant difference was observed in gender, age groups, and hospital units. The pooled prevalence of VRE infections was 9.6% (95% CI [3.948–16.052]). More epidemiological data are presented in Table 3.

Antimicrobial resistance profiles

Each VRE isolate was tested for susceptibility to different classes of antimicrobials (sterile fluid, such as blood, and nonsterile sources, such as pus and surgical sites). Of the total 90 VRE isolates, 67 (74.4%) were tested against ampicillin; 57 (63.3%) against imipenem; 21 (23.3%) against gentamicin; 10–20 (11.1%–12.2%) against tazocin, ceftriaxone, cefotaxime, ceftazidime, cefoperazone-sulbactam, meropenem, penicillin, and vancomycin; and less than 10 (<11.1%), against colistin, norfloxacin, ciprofloxacin, and ertapenem.

On the basis of the diameter of the observed zone of inhibition, the result of antibiotic resistance showed that all the selected VRE isolates were found to be resistant to ampicillin, vancomycin, penicillin, tazocin, norfloxacin, and ciprofloxacin susceptibility. Of the 57 selected VRE isolates, 56 (98.2%) were resistant to imipenem. However, over 50% of selected VRE isolates exhibited resistance when tested for gentamicin, cefoperazone-sulbactam, ceftazidime, cefotaxime, ceftriaxone, colistin, and ertapenem (Table 4).

The determination of MIC levels for vancomycin to VRE isolates showed that all the strains of VRE were resistant to vancomycin with MIC ranging between 0.25 μg/ml and 256 μg/ml. Seventy-three isolates had MIC of ⩾ 256 μg/ml, and seven had MIC of 128 μg/ml. One isolate was in the intermediate range with the MIC ranging between 8 μg/ml and 16 μg/ml (Table 5).

Saengsuwan et al. (2021), *PeerJ*, DOI 10.7717/peerj.11478

6/19

Table 3 Demographic and clinical characteristics of patients with VRE at Songklanagarind Hospital between February 2011 and February 2019.

Variables	MDR-VRE *n* (%)	XDR-VRE *n* (%)	Total (%)	P value
Gender				0.883
Male	26 (40.6)	11 (42.3)	37 (41.1)	
Female	38 (59.4)	15 (57.7)	53 (58.9)	
Age in years				0.669
≤12	3 (4.7)	0	3 (3.3)	
13–24	4 (7.8)	2 (7.7)	6 (6.7)	
25–64	25 (57.8)	12 (46.2)	37 (41.1)	
≥65	32 (60.9)	12 (46.2)	44 (48.9)	
Source of samples				0.007*
Blood	7 (10.7)	2 (7.7)	9 (10.0)	
Body fluid	10 (19.0)	8 (30.8)	18 (20.0)	
Midstream urine	6 (10.7)	3 (11.5)	9 (10.0)	
Pelvic	0	1(3.8)	1 (1.1)	
Pus	0	1(3.8)	1 (1.1)	
Rectal	18 (21.4)	0	18 (20.0)	
Tissue	3 (7.1)	5 (19.2)	8 (8.9)	
Urine	20 (29.8)	6 (23.1)	26 (28.9)	
Hospital unit				0.353
Medical ward	31 (48.4)	11 (42.3)	42 (46.7)	
Gynecology ward	6 (9.4)	2 (7.7)	8 (8.9)	
Intensive care unit	5 (7.8)	5 (19.2)	10 (11.1)	
Surgical ward	15 (23.4)	3 (11.5)	18 (20.0)	
Operating room	4 (6.3)	4 (15.4)	8 (8.9)	
Orthopedic ward	1 (1.6)	1 (3.8)	2 (2.2)	
Pediatric ward	2 (3.1)	0	2 (2.2)	

Notes.

MDR-VRE: multidrug-resistant vancomycin resistant *enterococci*; XDR-VRE: extensively drug-resistant vancomycin resistant *enterococci*.

*$P < 0.05$ is significant.

Detection of van determinants and virulence factor genes in VRE

Ninety VRE strains (100%) harbored the *vanA* gene, whereas the *vanB*, *vanC*, *vanD*, *vane*, and *vanG* genes were not found in any of these isolates. The *esp* and *hyl* genes were found in 82 (91.1%) and 5 (5.6%) isolates, respectively. However, *gel*, *cyt*, *cpd*, and *ebp* genes were absent from all isolates. The amplicons of the VRE isolates and resistance-gene distribution are shown in Fig. 1.

ERIC-PCR analysis

The ERIC-PCR patterns of the 90 VRE isolates are shown in Fig. 2. The number of bands in each varied from one to nine, and the ERIC fragment sizes ranged from 100 bp to 1.5 kb. Genomic typification by ERIC-PCR revealed that 51 VRE isolates (56.7%) were grouped into 14 clusters (A–N) at a similarity level of 95%. The predominant A genotype contained 12 isolates; B genotype, six isolates; C–E genotypes, four isolates; F–H genotypes, three

Saengsuwan et al. (2021), *PeerJ*, DOI 10.7717/peerj.11478

7/19

Table 4   List of antibiotics used in this study.

Mode of action	Class	No. of tested isolates, n (%)	Susceptibility testing results		
			Sensitive	Intermediate	Resistant
Cell membrane					
Colistin	Lipopeptides	8 (8.9)	4	0	4
Cell wall synthesis					
Ampicillin	β-lactams (penicillins)	67 (74.4)	0	0	67
Penicillin G	β-lactams (penicillins)	17 (18.9)	0	0	17
Ertapenem	β-lactams (carbapenem)	2 (1.1)	1	0	1
Imipenem	β-lactams (carbapenem)	57 (63.3)	1	0	56
Meropenem	β-lactams (carbapenem)	15 (16.7)	10	0	5
Cefoperazone	β-lactams (cephalosporins), 3rd generation	14 (15.6)	3	0	11
Cefotaxime	β-lactams (cephalosporins), 3rd generation	13 (14.4)	2	0	11
Ceftazidime	β-lactams (cephalosporins), 3rd generation	14 (15.6)	2	0	12
Ceftriaxone	β-lactams (cephalosporins), 3rd generation	12 (13.3)	2	0	10
Tazocin	Combinations: piperacillin (β-lactams) and tazobactam (β-lactamase inhibitors)	12 (13.3)	0	0	12
Vancomycin	Glycopeptides	20 (22.2)	0	0	20
DNA gyrase					
Ciprofloxacin	Fluoroquinolone	2 (2.2)	0	0	2
Norfloxacin	Fluoroquinolone	5 (5.6)	0	0	5
Protein synthesis (30S ribosomal subunit)					
Gentamicin	Aminoglycosides	21 (23.3)	7	1	13

Table 5   Vancomycin MIC results among 90 VRE isolates.

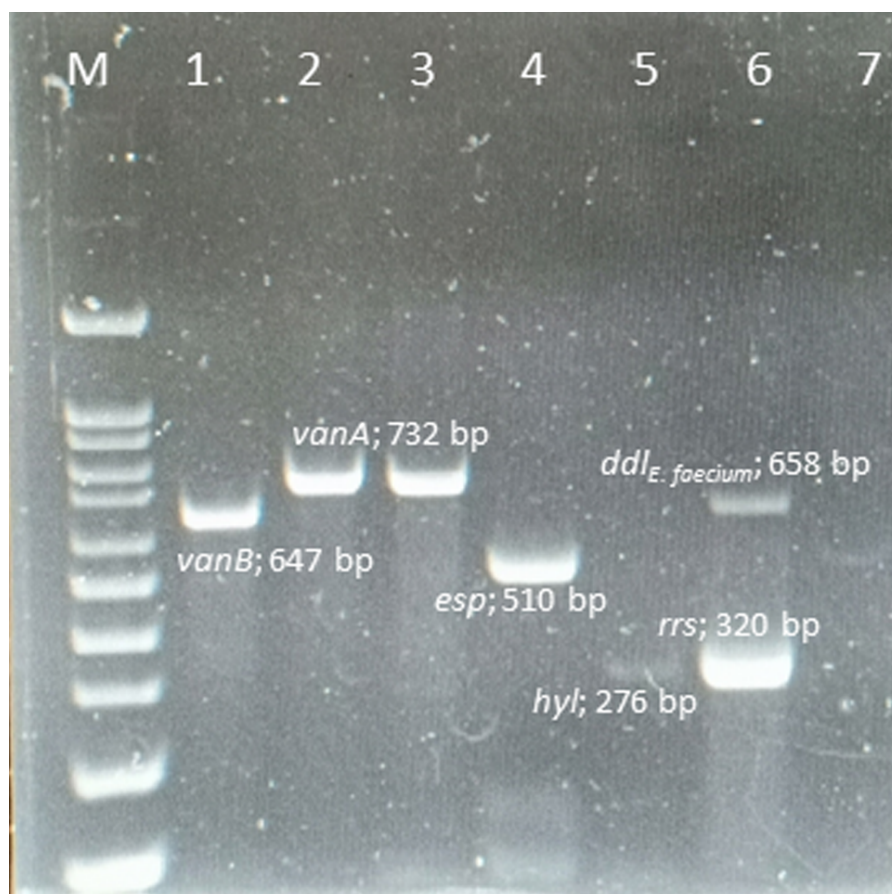| vancomycin (μg/ml)	No. of infection	Percent of infection (%)
0.75	2	2.2
4	2	2.2
16	1	1.1
24	0	0
32	0	0
48	1	1.1
64	3	3.3
96	1	1.1
128	7	7.8
256	53	58.9
>256	20	22.2
Total	90	100

Figure 1 Multiplex PCR positive isolates. Lane M = marker 100-1000 bp; lane 1 = *E. faecalis* ATCC 51299 *vanB* positive control; lane 2 = *E. faecium* ATCC 700221 *vanA* positive control; lane 3 = *vanA* positive from clinical strain *E. faecium* ; lane 4 = *esp* positive from clinical strain *E. faecium*; lane 5 = *hyl* positive from clinical strain *E. faecium*; lane 6 = *rrs* genes and $ddl_{E.faecium}$ from clinical strain; lane 7 = negative control .

Full-size DOI: 10.7717/peerj.11478/fig-1

isolates; and I–N genotypes, two isolates. Thirty-nine isolates were uniquely genotyped. MDR and XDR isolates were distributed among all scattered patterns, except for the three genotypes of the XDR pattern (J, L, and N) (Table 6). The heterogeneity among the isolates obtained from urinary tract infections was stronger than those from other infection sites.

DISCUSSION

Enterococci significantly contribute to nosocomial infections. These infections most often occur in health care settings, particularly in intensive care units (ICU). They can spread by person-to-person contact or from contaminated medical devices (*Olawale, Fadiora & Taiwo, 2011*). Control measures to prevent the spread of enterococci infections are essential. To monitor the spread of enterococci infections at our hospital, samples collected from patients are routinely screened by conventional biochemical tests for nosocomial
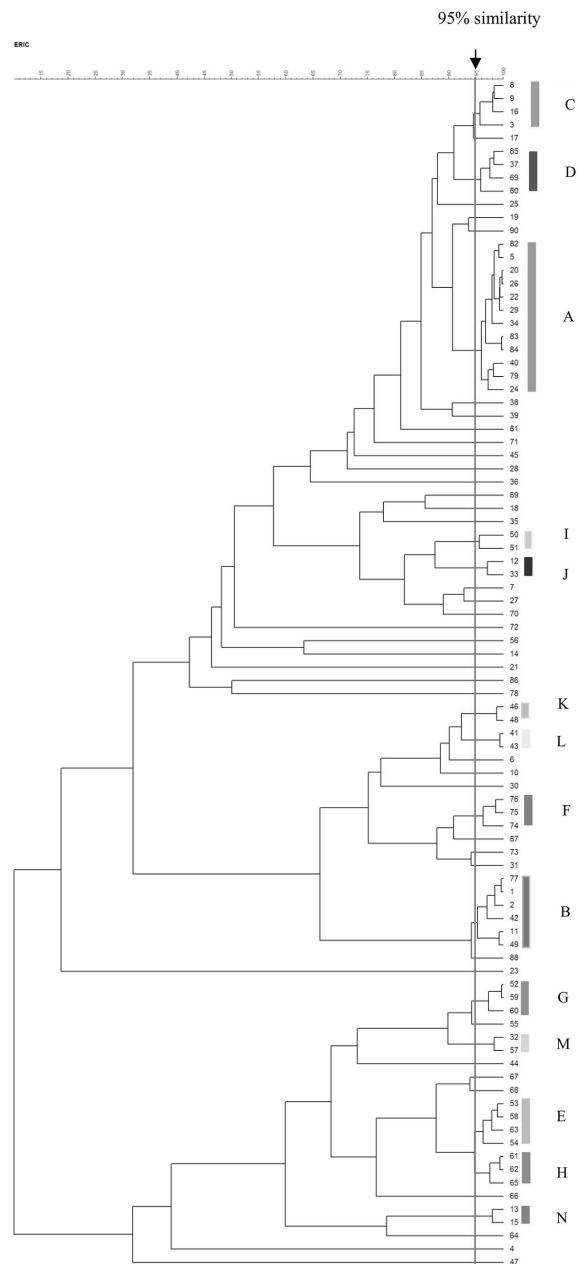
Figure 2 **Dendrogram of ERIC-PCR patterns among 90 VRE isolates.** Dendrogram of ERIC-PCR patterns showing the genetic relationship among 90 VRE isolates collected from clinical specimen in Songklanagarind Hospital, Thailand. Similarity > 95% were considered for clustering of isolates.

Full-size ⊡ DOI: 10.7717/peerj.11478/fig-2

enterococci (resistant or sensitive to vancomycin). The prevalence of VRE among the nosocomial enterococci isolates in our study was 0.2%–4.3%, resulting in an average prevalence of 1.2%.

The prevalence of VRE isolates in our study was relatively low compared with that found in India (7.0%) (*Bhatt et al., 2015*) and in Egypt (6.3%) (*Said & Abdelmegeed, 2019*),

Table 6 ERIC-PCR pattern, source, hospital collection location, antibiogram profile, and isolation date of multidrug-resistant (MDR) and extensively drug-resistant (XDR)-VRE strains from hospitalized patients at Songklanagarind Hospital, southern Thailand.

Code of isolates	ID	source	ward	Male (M) Female (F)	age	Collection date (day/month/year)	Resistance genes	Resistance patterns	ERIC types
5	874-3-PSU	rectal	surgical ward	M	70	05032014	*vanA, esp*	MDR	A
20	1949-PSU	rectal	medical ward	M	60	28072011	*vanA, esp*	MDR	A
22	1114-PSU	blood	surgical ward	F	64	07082014	*vanA, esp*	MDR	A
24	198-PSU	body fluid	surgical ward	M	67	02082014	*vanA, esp*	MDR	A
26	1949-PSU	rectal	medical ward	M	60	13082014	*vanA, esp*	MDR	A
29	1007-PSU	rectal	medical ward	M	87	06082014	*vanA, esp*	MDR	A
34	4129-PSU	tissue	intensive care units	F	53	28022012	*vanA, esp*	XDR	A
40	5056-PSU	urine	medical ward	F	88	29032012	*vanA, esp*	XDR	A
79	5296-12br-PSU	body fluid	medical ward	M	73	24122018	*vanA, esp*	MDR	A
82	2484-1u-PSU	urine	medical ward	F	74	11012019	*vanA, esp*	MDR	A
83	7028-10u-PSU	urine	surgical ward	M	70	30102018	*vanA, esp*	MDR	A
84	2079-4u-PSU	urine	medical ward	F	44	11042018	*vanA, esp*	MDR	A
1	3044-PSU	rectal	medical ward	M	73	17032014	*vanA, esp*	MDR	B
2	3576-PSU	rectal	surgical ward	M	90	20032014	*vanA, esp*	MDR	B
11	3180-8-PSU	urine	medical ward	F	38	19082015	*vanA, esp*	MDR	B
42	696ii-PSU	blood	medical ward	F	63	04122013	*vanA, esp*	MDR	B
49	580-PSU	body fluid	intensive care units	F	21	04022013	*vanA, hyl*	XDR	B
77	5296-PSU	body fluid	medical ward	M	73	24122018	*vanA, esp*	MDR	B
3	3374II-PSU	blood	medical ward	F	23	20022014	*vanA, esp*	MDR	C
8	3384-PSU	rectal	surgical ward	F	71	19032014	*vanA, esp*	MDR	C
9	3576-PSU	rectal	surgical ward	F	90	20032014	*vanA, esp*	MDR	C
16	2725-PSU	tissue	medical ward	M	67	15032016	*vanA, esp*	XDR	C
37	1879-PSU	blood	medical ward	M	86	10032011	*vanA, esp*	XDR	D
69	874-PSU	rectal	surgical ward	M	70	05032014	*vanA, esp*	MDR	D
80	940-1u-PSU	urine	medical ward	F	85	04012019	*vanA, esp*	MDR	D
85	5005-12u	urine	medical ward	F	83	23122018	*vanA, esp*	MDR	D
53	762-PSU	body fluid	medical ward	M	37	05022014	*vanA, esp*	XDR	E
54	176-PSU	blood	gynecology ward	F	25	01112013	*vanA*	MDR	E
58	1243-PSU	MSU	gynecology ward	F	70	08052013	*vanA, esp*	XDR	E
63	135-PSU	body fluid	operating room	F	64	01052012	*vanA*	XDR	E
74	3784-PSU	rectal	medical ward	M	46	21032014	*vanA, esp*	MDR	F
75	32-PSU	urine	medical ward	F	79	01062012	*vanA, esp*	XDR	F
76	5552-1 I-PSU	blood	intensive care units	F	0.25	24012019	*vanA, esp*	MDR	F
52	1134-PSU	rectal	gynecology ward	F	52	07112013	*vanA, esp*	MDR	G
59	3236-PSU	MSU	medical ward	F	50	18102016	*vanA, esp*	XDR	G
60	977-PSU	MSU	medical ward	F	90	6102016	*vanA, hyl*	XDR	G
61	1630-PSU	body fluid	medical ward	F	74	10092013	*vanA, esp*	MDR	H
62	4399-PSU	urine	surgical ward	F	28	29042012	*vanA, esp*	XDR	H

Table 6 (*continued*)

Code of isolates	ID	source	ward	Male (M) Female (F)	age	Collection date (day/month/year)	Resistance genes	Resistance patterns	ERIC types
65	3093-PSU	rectal	gynecology ward	F	52	18112013	*vanA, esp*	MDR	H
50	4215-PSU	urine	medical ward	F	68	22042012	*vanA, esp*	MDR	I
51	5324-PSU	body fluid	surgical ward	M	66	30082012	*vanA, esp*	XDR	I
12	3672-PSU	urine	surgical ward	F	79	20022015	*vanA, esp*	MDR	J
33	1329-PSU	rectal	medical ward	M	74	08082014	*vanA, esp*	MDR	J
46	4006-PSU	urine	medical ward	F	73	24122013	*vanA*	MDR	K
48	3209-PSU	body fluid	medical ward	M	66	19082012	*vanA, esp*	XDR	K
41	910-PSU	MSU	medical ward	F	85	06082012	*vanA, esp*	MDR	L
43	2000-PSU	MSU	gynecology ward	F	42	11112013	*vanA*	MDR	L
32	198-PSU	body fluid	surgical ward	M	63	02082014	*vanA, esp*	MDR	M
57	5245-PSU	pelvic	operating room	F	19	28092016	*vanA, esp*	XDR	M
13	3398-PSU	urine	surgical ward	M	78	18092015	*vanA, esp*	MDR	N
15	4190-PSU	tissue	medical ward	F	38	25042017	*vanA, esp*	MDR	N

Notes.
MSU, mid-stream urine.
BioSample accessions: SAMN18201951, SAMN18201952, SAMN18201953, SAMN18201954, SAMN18201955, SAMN18201956, SAMN18201957, SAMN18201958, SAMN18201959, SAMN18201960.

indicating that risks of VRE infections may be associated with geographic variability. *Blanco et al. (2017)* reported in 2017 that relative humidity and geographical location were factors affecting VRE colonization rates in adult patients admitted to ICUs in the United States. Increasing humidity resulted in a greater VRE colonization rate, and VRE colonization was lower in southern states. In developing countries, hospitals could inadvertently be increasing the risks of VRE colonization and infections because of the lack of oversight procedures for transferring patients from hospitals with VRE outbreaks (*Kang et al., 2014*; *Resende et al., 2014*).

Risk factors for severity of the patient's condition, hemodialysis, and central venous catheter usage and in univariate analysis include male gender, alcohol use, and antibiotics. Hospitalization in certain units of the hospital and lack of isolation measures are primary factors that affect VRE acquisition (*Fossi Djembi et al., 2017*). Our study demonstrated similar results after our 90 patients, whose test samples were positive for VRE, were paired with demographic and clinical data from the patient database. Nearly 50% of VRE carrier patients were ≥65 years old, with a median age of 64 years, and were admitted to the medical ward. In a 2019 study by Mathis et al., older patients had the highest infection during VRE outbreak in 2013–2014 at a teaching hospital in Lyon, France (*Mathis et al., 2019*).

In addition, positive VRE samples were detected in urine specimens (28.9%), followed by those obtained from body fluid (20.0%) and the rectal area (20.0%). These results were similar to a Brazilian study of VRE infection, where the highest VRE concentrations were isolated from patients with urinary tract infections (*Resende et al., 2014*). Vancomycin-resistant *E. faecalis* is a significant cause of nosocomial infections stemming from the

urinary tract, surgical wounds, and catheter use and affecting the bladder, prostate, and kidneys (*Zalipour, Esfahani & Havaei, 2019*).

Because VRE are multidrug-resistant organisms with limited therapeutic alternatives that result in increased morbidity and mortality in patients (*Buetti et al., 2019*; *Zalipour, Esfahani & Havaei, 2019*), we examined the 90 VRE isolates in our study for antimicrobial susceptibility. The findings showed resistance to β-lactam, glycopeptide, and fluoroquinolone antibiotics in most of the tested isolates, with the highest resistance rate to ampicillin (74.4%) and imipenem (62.2%).

Enterococci have intrinsic resistance to several classes of antibiotics that inhibit cell wall synthesis, such as β-lactams and glycopeptides, and they exhibit the ability to acquire new mechanisms of resistance, particularly to penicillin/ampicillin, aminoglycosides (high-level resistance), and glycopeptides (*Cetinkaya, Falk & Mayhall, 2000*; *Gagetti et al., 2019*; *Werner et al., 2008*). Results also showed susceptibility to meropenem in 10 of 15 tested isolates and 7 of the 21 tested isolates were susceptible to gentamicin. Meropenem and gentamicin are β-lactam and aminoglycoside antibiotics, respectively, and combinations of meropenem and aminoglycosides could function as alternative therapies for multi- and VRE infections. Similarly, there is evidence that β-lactams in combination with daptomycin reduced bacterial activity (*Smith et al., 2015*). Moreover, this combination of antibiotic therapy was successful in the treatment of endocarditis/severe MDR enterococcal infection (*Antony et al., 1997*; *El Rafei et al., 2018*).

E. faecium and *E. faecalis* are most frequently detected, and *E. durans*, *E. avium*, *E. casseliflavus*, *E. hirae*, *E. gallinarum*, *E. raffinosus*, and *E. muntdii* are less commonly detected (*Agudelo Higuita & Huycke, 2014*; *Zaheer et al., 2020*). In this study, all the detected VRE isolates were identified as *E. faecium*, a species rarely found in Songklanagarind Hospital. Other studies have found that vancomycin-resistant genetic elements can transfer between enterococcal species (*Murray, 1998*). Most VRE isolates were MDR, and only six VRE isolates were extensively drug-resistant (XDR). All VRE isolates in this study were *vanA E. faecium* strains, whereas *vanB*, *C*, *D*, and *E* were not detected, similar to the findings from a 2014 report from (*Resende et al., 2014*).

Enterococcal virulence that results in pathogenicity is considered a multifactorial process (*Comerlato et al., 2013*). Virulence factors promoting the emergence of hospital-acquired enterococcal infections have been reported in a number of studies. In our study, VRE strains originally isolated from urine specimen expressed a high frequency of the *esp* gene (91.1%), confirming the important role of *esp* in enterococcal colonization and biofilm formation in urinary tract infections (*Comerlato et al., 2013*). This finding was comparable to that of other investigators who reported that the incidence levels of *esp* in *E. faecium* isolates were 71.5% in northwest Iran (*Sharifi et al., 2012*) and 90% in China (*Yang et al., 2015*) but rather low in southwest Iran (57.5%) (*Arshadi et al., 2018*).

Other virulence factors, *hyl*, *asa*, *gel*, and *cyt* genes, were also analyzed. The *hyl* gene was detected in only five (5.6%) VRE isolates in our study, similar to the findings from Haghi et al. who found the *hyl* gene in 3 of 79 (3.8%) VRE isolates in northwest Iran (*Haghi, Lohrasbi & Zeighami, 2019*). We did not detect the *asa*, *gel*, and *cyt* genes, confirming that

esp and *hyl* are primary virulence factors among clinical strains of *vanA E. faecium* and play an important role in pathogenesis.

Molecular techniques for typing bacteria can vary when comparing standardization, cost, reproducibility, discriminatory power, and interpretation. We found that ERIC-PCR is a straightforward procedure for distinguishing organisms that are closely related. This method is less expensive and faster than many other methods, such as PFGE and amplified fragment length polymorphism typing (*Borgmann et al., 2007*).

The ERIC-PCR results identified 14 clusters among the 51 VRE isolates. The majority of the isolates were clustered in the A genotype, mostly from urine samples, and collected from the medical ward, and these were resistant to ampicillin, gentamicin, and carbapenem, similar to a report from Columbia (*Corredor et al., 2019*). We identified 39 unique traits of VRE isolates in our study, implying a circulation of these strains in the Songklanagarind Hospital for a period of time prior to the present study. These findings may suggest a hospital epidemiology stemming from the spread of MDR and vancomycin-resistant *E. faecium* with endemic *vanA* VRE. These bacteria may contribute to antibiotic resistance and virulence through horizontal gene transfer.

CONCLUSIONS

This was the first study conducted in a tertiary care hospital in southern Thailand to show a high prevalence of MDR and XDR-VRE in patients, which could lead to pathogenic effects on public health. The results of this study suggest that overused antibiotics appear to be mediated mainly by the *vanA* gene, which carries one or two virulence genes that create a high resistance level to vancomycin. However, molecular techniques are highly effective in detecting antibiotic-resistant genes, which would allow monitoring to control VRE infection in hospitals.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare there are no competing interests.

Author Contributions
- Phanvasri Saengsuwan conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

- Kamonnut Singkhamanan performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Siribhorn Madla analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Natnicha Ingviya performed the experiments, authored or reviewed drafts of the paper, sample preparation, and approved the final draft.
- Chonticha Romyasamit performed the experiments, authored or reviewed drafts of the paper, did the quality appraisal, data collection, and approved the final draft.

Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The study was approved by the ethical committee of the Faculty of Medicine, Prince of Songkhla University (REC-60-234-04-7).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The sequences are available at GenBank:

BioProject ID: PRJNA707344

BioProject ID: PRJNA707345

BioProject ID: PRJNA707346

BioSample accessions SAMN18201951–SAMN18202001

Data Availability

The following information was supplied regarding data availability:

Our SQL file is available in the Supplementary Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.11478#supplemental-information.

REFERENCES

Agudelo Higuita NI, Huycke MM. 2014. Enterococcal disease, epidemiology, and implications for treatment. In: Gilmore MS, Clewell DB, Clewell DB, Ike Y, Shankar N, eds. *Enterococci: from commensals to leading causes of drug resistant infection.* Boston: Massachusetts Eye and Ear Infirmary.

Antony SJ, Ladner J, Stratton CW, Raudales F, Dummer SJ. 1997. High-level aminoglycoside-resistant enterococcus causing endocarditis successfully treated with a combination of ampicillin, imipenem and vancomycin. *Scandinavian Journal of Infectious Diseases* **29**:628–630 DOI 10.3109/00365549709035908.

Arias CA, Contreras GA, Murray BE. 2010. Management of multidrug-resistant enterococcal infections. *Clinical Microbiology and Infection* **16**:555–562 DOI 10.1111/j.1469-0691.2010.03214.x.

Arshadi M, Mahmoudi M, Motahar MS, Soltani S, Pourmand MR. 2018. Virulence determinants and antimicrobial resistance patterns of vancomycin-resistant enterococcus faecium isolated from different sources in southwest Iran. *Iranian Journal of Public Health* **47**:264–272.

Bhatt P, Sahni A, Praharaj A, Grover N, Kumar M, Chaudhari C, Khajuria A. 2015. Detection of glycopeptide resistance genes in enterococci by multiplex PCR. *Medical Journal Armed Forces India* **71**:43–47 DOI 10.1016/j.mjafi.2014.03.005.

Billington EO, Phang SH, Gregson DB, Pitout JD, Ross T, Church DL, Laupland KB, Parkins MD. 2014. Incidence, risk factors, and outcomes for Enterococcus spp. blood stream infections: a population-based study. *International Journal of Infectious Diseases* **26**:76–82 DOI 10.1016/j.ijid.2014.02.012.

Blanco N, Perencevich E, Li SS, Morgan DJ, Pineles L, Johnson JK, Robinson G, Anderson DJ, Jacob JT, Maragakis LL, Harris AD, Program CDCPE. 2017. Effect of meteorological factors and geographic location on methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci colonization in the US. *PLOS ONE* **12**:e0178254 DOI 10.1371/journal.pone.0178254.

Borgmann S, Schulte B, Wolz C, Gruber H, Werner G, Goerke C, Klare I, Beyser K, Heeg P, Autenrieth IB. 2007. Discrimination between epidemic and non-epidemic glycopeptide-resistant E. faecium in a post-outbreak situation. *Journal of Hospital Infection* **67**:49–55 DOI 10.1016/j.jhin.2007.06.002.

Bourgogne A, Singh KV, Fox KA, Pflughoeft KJ, Murray BE, Garsin DA. 2007. EbpR is important for biofilm formation by activating expression of the endocarditis and biofilm-associated pilus operon (ebpABC) of Enterococcus faecalis OG1RF. *Journal of Bacteriology* **189**:6490–6493 DOI 10.1128/JB.00594-07.

Buetti N, Wassilew N, Rion V, Senn L, Gardiol C, Widmer A, Marschall J. 2019. Emergence of vancomycin-resistant enterococci in Switzerland: a nation-wide survey. *Antimicrobial Resistance and Infection Control* **8**:16 DOI 10.1186/s13756-019-0466-x.

Cetinkaya Y, Falk P, Mayhall CG. 2000. Vancomycin-resistant enterococci. *Clinical Microbiology Reviews* **13**:686–707 DOI 10.1128/cmr.13.4.686-707.2000.

Clinical and Laboratory Standards Institute (CLSI). 2019. *Performance standards for antimicrobial susceptibility testing; Twenty-nineth Informational Supplement M100-S29.* Wayne: CLSI.

Comerlato CB, Resende MCCd, Caierão J, d'Azevedo PA. 2013. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. *Memorias do Instituto Oswaldo Cruz* **108**:590–595 DOI 10.1590/s0074-02762013000500009.

Corredor NC, López C, Aguilera PA, Prieto LM, Rodríguez-Leguizamón G, Leal AL, Ovalle-Guerro MV, Pardo-Oviedo JM, Chica CE, Patarroyo MA. 2019. An epidemiological and molecular study regarding the spread of vancomycin-resistant Enterococcus faecium in a teaching hospital in Bogotá, Colombia 2016. *BMC Infectious Diseases* **19**:258–258 DOI 10.1186/s12879-019-3877-7.

Dutka-Malen S, Evers S, Courvalin P. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *Journal of Clinical Microbiology* **33**:24–27 DOI 10.1128/JCM.33.1.24-27.1995.

El Rafei A, DeSimone DC, Narichania AD, Sohail MR, Vikram HR, Li Z, Steckelberg JM, Wilson WR, Baddour LM. 2018. Comparison of dual β-Lactam therapy to penicillin-aminoglycoside combination in treatment of *Enterococcus faecalis* infective endocarditis. *Journal of Infection* **77**:398–404 DOI 10.1016/j.jinf.2018.06.013.

Elsner H-A, Sobottka I, Mack D, Laufs R, Claussen M, Wirth R. 2000. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. *European Journal of Clinical Microbiology and Infectious Diseases* **19**:39–42 DOI 10.1007/s100960050007.

Fang H, Ohlsson AK, Ullberg M, Ozenci V. 2012. Evaluation of species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the identification of clinical Enterococcus isolates. *European Journal of Clinical Microbiology and Infectious Diseases* **31**:3073–3077 DOI 10.1007/s10096-012-1667-x.

Fossi Djembi L, Hodille E, Chomat-Jaboulay S, Coudrais S, De Santis N, Gardes S, Mauranne CC, Mourey N, Fredenucci I, Girard R. 2017. Factors associated with vancomycin-resistant Enterococcus acquisition during a large outbreak. *Journal of Infection and Public Health* **10**:185–190 DOI 10.1016/j.jiph.2016.04.010.

Gagetti P, Bonofiglio L, García Gabarrot G, Kaufman S, Mollerach M, Vigliarolo L, Von Specht M, Toresani I, Lopardo HA. 2019. Resistance to β-lactams in enterococci. *Revista Argentina de Microbiologia* **51**:179–183 DOI 10.1016/j.ram.2018.01.007.

Haghi F, Lohrasbi V, Zeighami H. 2019. High incidence of virulence determinants, aminoglycoside and vancomycin resistance in enterococci isolated from hospitalized patients in Northwest Iran. *BMC Infectious Diseases* **19**:744–744 DOI 10.1186/s12879-019-4395-3.

Howden BP, Holt KE, Lam MMC, Seemann T, Ballard S, Coombs GW, Tong SYC, Grayson ML, Johnson PDR, Stinear TP. 2013. Genomic insights to control the emergence of vancomycin-resistant Enterococci. *mBio* **4**:e00412–00413 DOI 10.1128/mBio.00412-13.

Jackson CR, Fedorka-Cray PJ, Barrett JB. 2004. Use of a genus- and species-specific multiplex PCR for identification of Enterococci. *Journal of Clinical Microbiology* **42**:3558–3565 DOI 10.1128/jcm.42.8.3558-3565.2004.

Kang M, Xie Y, He C, Chen ZX, Guo L, Yang Q, Liu JY, Du Y, Ou QS, Wang LL. 2014. Molecular characteristics of vancomycin-resistant Enterococcus faecium from a tertiary care hospital in Chengdu, China: molecular characteristics of VRE in China. *European Journal of Clinical Microbiology and Infectious Diseases* **33**:933–939 DOI 10.1007/s10096-013-2029-z.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert

proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* **18**:268–281 DOI 10.1111/j.1469-0691.2011.03570.x.

Mathis B, Haïne M, Girard R, Bonnefoy M. 2019. Risk factors for vancomycin-resistant enterococcus acquisition during a large outbreak in patients aged 65, years and older. *BMC Geriatrics* **19**:377–377 DOI 10.1186/s12877-019-1398-2.

Murray BE. 1998. Diversity among multidrug-resistant enterococci. *Emerging Infectious Diseases* **4**:37–47 DOI 10.3201/eid0401.980106.

Nasaj M, Mousavi SM, Hosseini SM, Arabestani MR. 2016. Prevalence of virulence factors and vancomycin-resistant genes among Enterococcus faecalis and E. faecium Isolated from Clinical Specimens. *Iranian Journal of Public Health* **45**:806–813.

Olawale KO, Fadiora SO, Taiwo SS. 2011. Prevalence of hospital-acquired enterococci infections in two primary-care hospitals in osogbo, southwestern Nigeria. *African Journal of Infectious Diseases* **5**:40–46 DOI 10.4314/ajid.v5i2.66513.

Pillay S, Zishiri OT, Adeleke MA. 2018. Prevalence of virulence genes in Enterococcus species isolated from companion animals and livestock. *Onderstepoort Journal of Veterinary Research* **85**:1–8.

Puchter L, Chaberny IF, Schwab F, Vonberg R-P, Bange F-C, Ebadi E. 2018. Economic burden of nosocomial infections caused by vancomycin-resistant enterococci. *Antimicrobial Resistance and Infection Control* **7**:1 DOI 10.1186/s13756-017-0291-z.

Resende M, Caierao J, Prates JG, Narvaez GA, Dias CA, d'Azevedo PA. 2014. Emergence of vanA vancomycin-resistant Enterococcus faecium in a hospital in Porto Alegre, South Brazil. *Journal of Infection in Developing Countries* **8**:160–167 DOI 10.3855/jidc.4126.

Reyes K, Bardossy AC, Zervos M. 2016. Vancomycin-resistant enterococci: epidemiology, infection prevention, and control. *Infectious Disease Clinics of North America* **30**:953–965 DOI 10.1016/j.idc.2016.07.009.

Said HS, Abdelmegeed ES. 2019. Emergence of multidrug resistance and extensive drug resistance among enterococcal clinical isolates in Egypt. *Infection and Drug Resistance* **12**:1113–1125 DOI 10.2147/IDR.S189341.

Shahi F, Hamidi H, Khoshnood S, Mehdipour G, Dezfouli A, Sheikh A. 2020. Virulence determinants and biofilm formation in clinical isolates of *Enterococcus*: a cross-sectional study. *Journal of Acute Disease* **9**:27–32 DOI 10.4103/2221-6189.276079.

Sharifi Y, Hasani A, Ghotaslou R, Varshochi M, Hasani A, Aghazadeh M, Milani M. 2012. Survey of virulence determinants among vVancomycin resistant Enterococcus faecalis and Enterococcus faecium isolated from clinical specimens of hospitalized patients of North west of Iran. *Open Microbiology Journal* **6**:34–39 DOI 10.2174/1874285801206010034.

Smith JR, Barber KE, Raut A, Aboutaleb M, Sakoulas G, Rybak MJ. 2015. Beta-Lactam combinations with daptomycin provide synergy against vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. *Journal of Antimicrobial Chemotherapy* **70**:1738–1743 DOI 10.1093/jac/dkv007.

National Antimicrobial Resistance Surveillance Thailand. 2020. Situation of antimicrobial resistance during 2000-2019. *Available at* http://narst.dmsc.moph.go.th/data/AMR%202000-2019-12M.pdf (accessed on 09 October 2020).

Ulu-Kilic A, Özhan E, Altun D, Perçin D, Güneş T, Alp E. 2016. Is it worth screening for vancomycin-resistant *Enterococcus faecium* colonization?: financial burden of screening in a developing country. *American Journal of Infection Control* **44**:e45–49 DOI 10.1016/j.ajic.2015.11.008.

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. Journal of Clinical Microbiology* **42**:4473–4479 DOI 10.1128/JCM.42.10.4473-4479.2004.

Versalovic J, Koeuth T, Lupski JR. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* **19**:6823–6831 DOI 10.1093/nar/19.24.6823.

Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH, Lillie M, Novais C, Olsson-Liljequist B, Peixe LV, Sadowy E, Simonsen GS, Top J, Vuopio-Varkila J, Willems RJ, Witte W, Woodford N. 2008. Emergence and spread of vancomycin resistance among enterococci in Europe. *Eurosurveillance* **13(47)**:19046 DOI 10.2807/ese.13.47.19046-en.

Yang JX, Li T, Ning YZ, Shao DH, Liu J, Wang SQ, Liang GW. 2015. Molecular characterization of resistance, virulence and clonality in vancomycin-resistant Enterococcus faecium and Enterococcus faecalis: a hospital-based study in Beijing, China. *Infection, Genetics and Evolution* **33**:253–260 DOI 10.1016/j.meegid.2015.05.012.

Zaheer R, Cook SR, Barbieri R, Goji N, Cameron A, Petkau A, Polo RO, Tymensen L, Stamm C, Song J, Hannon S, Jones T, Church D, Booker CW, Amoako K, Van Domselaar G, Read RR, McAllister TA. 2020. Surveillance of Enterococcus spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. *Scientific Reports* **10**:3937 DOI 10.1038/s41598-020-61002-5.

Zalipour M, Esfahani BN, Havaei SA. 2019. Phenotypic and genotypic characterization of glycopeptide, aminoglycoside and macrolide resistance among clinical isolates of Enterococcus faecalis: a multicenter based study. *BMC Research Notes* **12**:292 DOI 10.1186/s13104-019-4339-4.