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VANCOMYCIN-RESISTANT ENTEROCOCCI IN SONGKLANAGARIND HOSPITAL: MOLECULAR EPIDEMIOLOGY AND INFECTION CONTROL

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ABSTRACT

Vancomycin-resistant enterococci are important causes of healthcare-associated infections that have recently become the major nosocomial pathogen exhibiting resistance to many antimicrobials especially to vancomycin with increasing frequency. However, the epidemiology data of VRE in Thailand is limited. This study aimed at determining the prevalence, antibiotic resistance patterns, and molecularly characterize of VRE among patients admitted in a tertiary care hospital, in southern part of Thailand. We collected VRE isolates from various clinical samples of a 7-year period (March 2011 to April 2018) in Songklanagarind Hospital. VRE isolates were identified by Kirby Bauer disc diffusion method with Clinical and Laboratory Standards Institute guidelines. Vancomycin-resistance genes and virulence genes were detected by multiplex PCR and the sequence verified by the blast. The highest rate of VRE infection was in 2014 (39.4%). Most VRE isolates originated from the patient's digestive tract or urogenital tract that was positive for 17 (24.3%), followed by body fluid 14 (20.0%). The highest incidence of VRE infection was in Medical wards 29 (40.8%) and followed by surgical ward 16 (22.5%), operation theatres 7 (9.9%), Intensive Care Units (ICUs) 5 (7.0%), and emergency room 6 (8.5%). All the VRE isolates exhibited multidrug resistance, with the rates of resistance to ampicillin, imipenem, gentamicin and ceftazidime reaching high levels. It also found that 5.6% of *E. faecium* isolates were resistant to colistin. Most of these VRE isolates carried the *vanA* gene possed the *esp* genes (enterococcal surface protein), where rates were 100% and 74.6%, respectively. The study showed a high prevalence of diverse VRE strains with threatening resistance phenotypes in clinical sections among different medical wards in Songklanagarind hospitals. These findings indicated that the molecular detection of various glycopeptide resistance genes among VRE isolates are important information that useful to prevent and control of VRE infections.

Keywords: Antibiotic resistance, *enterococci*, multiplex PCR, *van* genes, virulence determinants

1. INTRODUCTION

Enterococci are a leading cause of nosocomial infections. The spread of vancomycin-resistant enterococci (VRE) is an important clinical concern, and VRE has been recognized as a global emerging problem in hospitals (Reyes et al. 2016). *E. faecalis* and *E. faecium* are two main species that cause problems.

Enterococci are most common cause of urinary tract infections, intra-abdominal and intra-pelvic abscesses or post-surgery wound infections and blood stream infections (O'Driscoll and Crank 2015). *Enterococci* are resistant to the common antibiotics especially aminoglycoside and other antibiotics that affect the cells wall, such as ampicillin and vancomycin which are drugs for the treatment of *Enterococcal* infections (Arias et al. 2010; Howden et al. 2013). Additional, people hospitalized previously treated with the antibiotics for long periods of time, and with medical devices that stay in for some time are at increased risk becoming infected with VRE (Billington et al. 2014; Puchter et al. 2018).

The mechanisms of antibiotic resistance in *Enterococci* are mutation and gene transfer horizontally by plasmids and transposons, and mediated by different van gene clusters (Courvalin

2006). Van cluster consisted of nine types containing *A*, *B*, *C*, *D*, *E*, *G*, *L*, *M*, and *vanN* that affected difference response with vancomycin and teicoplanin resistance phenotype (Protonotariou et al. 2010) and treatment options are limited.

Nowadays, biochemical tests have been used to identify *Enterococcus* species, which is time-consuming and costly (Jackson et al. 2004; Fang et al. 2012). Therefore, molecular technique which is fast, high throughput and emphasized the need for laboratories to be able to detect vancomycin resistance genes associated with drug susceptible has been developed and widely used for VRE detection and identification.

Educational insights for molecular identification and detection of vancomycin-resistant genes from VRE infection has not reported from any research study at Songklanagarind Hospital and lack of continuity of information. This present study aimed to describe recent epidemiological features of VRE clinical isolates and detected VRE infection and vancomycin resistant genes using multiplex PCR.

2.METHODOLOGY

2.1 Sample collection

Seventy one of VRE isolates were collected from patients admitted in Songklanagarind Hospital, Thailand, from March 2011 to April 2018. Ethical approval was accepted from the Ethics Committees at Faculty of Medicine, Prince of Songkla University (REC60-234-04-7).

2.2 Bacterial identification and antimicrobial susceptibility testing

All bacterial strains isolates were grown on Tryptic Soy Agar plates at 37°C overnight in order to identify species using routine biochemical test. Antimicrobial susceptibility tests were performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute (CLSI). 2015). Both bacteria characterization and antibiotic susceptibility test were carried out at Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University.

2.3 DNA Extraction

Chromosomal DNA was extracted from 71 *Enterococcus* species that were resistant to vancomycin with the GF-1 bacterial DNA extraction kit (Vivantis) according to the manufacture's procedure. The DNA was measured by spectrophotometry at 260 nm and the ratio of the absorbance at 260 and 280 nm (A260/A280) was used to assess the purity of DNA. The quality of DNA was evaluated using agarose gel electrophoresis.

2.4 Species identification

Confirmation of species identification the *ddl* genes (*E. faecium* and *E. faecalis*) were amplified with the primers listed in Table 1 (Rahimi et al. 2007) with the condition as follows: initial denaturation at 95°C for 10 min, denaturation at 95°C for 45 sec, annealing at 54°C for 1 min and finally extension at 72°C for 1 min (35 cycles).

2.5 Detection glycopeptide resistance gene and virulence factor genes by multiplex-PCR

All resistant to vancomycin isolates were subjected to a multiplex PCR analysis for the detection of the presence of vancomycin-resistant genes and virulence genes using specific primer (Table 1) (Elsner et al. 2000; Vankerckhoven et al. 2004; Bourgogne et al. 2007; Bhatt et al. 2015). PCR amplification was carried out with the following thermal cycling profile: initial denaturation for 3 min at 94°C, 35 cycles of amplification consisting of 1 min at 94°C, 1 min at 54°C, 1 min at 72°C, and final extension for 5 min at 72°C.

2.6.Amplicon detection

DNA fragments were analyzed by electrophoresis in 0.5×Tris-borate-EDTA on a 1% agarose gel stained with ethidium bromide and then visualized under UV transillumination. The PCR product was sent for sequencing and data analysis (BLAST).

2.7 Statistical analysis

Clinical data were collected from database case records of all patients enrolled in the study. Data were presented as percentages unless otherwise stated.

Table 1: List of oligonucleotide primers used in the genetic profiling of resistance genes among the isolates in this study

Primer	Sequence (5′→3′)	Gene	Product size (bp)	Reference
E. faecalis	ATCAAGTACAGTTAGTCT ACGATTCAAAGCTAACTG	*ddl_E. faecalis*	941	((Rahimi et al. 2007)
E. faecium	TTGAGGCAGACCAGATTGACG TATGACAGCGACTCCGATTCC	*ddl_E. faecium*	658	
vanA+ vanA-	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	*vanA*	732	(Bhatt et al. 2015)
vanB+ vanB-	ACGGAATGGGAAGCCGA TGCACCCGATTTCGTTC	*vanB*	647	
vanC+ vanC-	ATGGATTGGTAYTKGTAT TAGCGGGAGTGMCYMGTAA	*van C1/2*	815/827	
ASA 11 ASA 12	GCACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	*asa1*	375	(Vankerckhoven et al. 2004)
GEL 11 GEL 12	TATGACAATGCTTTTTGGGAT AGATGCACCCGAAATAATATA	*gelE*	213	
CYT I CYT IIb	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	*cylA*	688	
ESP 14F ESP 12R	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	*esp*	510	
HYL n1 HYL n2	ACAGAAGAGCTGCAGGAAATG GACTGACGTCCAAGTTTCCAA	*hyl*	276	
cpd-F cpd-R	TGGTGGGTTATTTTTCAATTC TACGGCTCTGGCTTACTA	*cpd*	782	(Elsner et al. 2000)
ebpA-F ebpA-R	AAAAATGATTCGGCTCCAGAA TGCCAGATTCGCTCTCAAAG	*ebpA*	101	(Bourgogne et al. 2007)

3. RESULTS AND DISCUSSION

a. Prevalence of VRE

The incidence rate of VRE infection (Figure 1A) were increased from 2011 and reached the highest rate in 2014 (39.4%). From 2015-2018, the incidence rates were maintained lower than 10%. Among the various clinical specimens presented in Figure 1B, VRE infection was highly found in urine (18, 25.4%), follow by rectal (17, 23.9%) and body fluid (14, 19.7%), respectively.

Moreover, VRE infection was mostly detected in hospitalized patients from medical ward (29, 40.8%), followed by surgical wards (16, 22.5%) and gynecology ward (7, 9.9%) as demonstrated in Figure 1C. According to antimicrobial susceptibility testes (Figure 1D), results showed that the maximum level of resistance was observed with ampicillin (47, 66.2%), follow by imipenem (39, 54.9%) and ceftazidime (12, 16.9%). In contrast, the highest sensitivity was found in meropenem (9, 13.0%), followed by gentamicin (7, 10.0%). The multidrug resistance of VRE infection has 92.96% (66/71) promoted to the problem (data not shown).

The average incidence of VRE isolates (8.0%) in the present study was higher than the incidence reported from India (7.0%) (Yadav et al. 2017) and Egypt (6.3%) (El-Ghazawy 2016). This demonstrates that VRE risks associated with geographic variability. Moreover, lacking of surveillance policies especially in developing countries, transferring of VRE from hospital to patients during VRE outbreak and managing VRE patients affected by use of clinical guidelines are part of factors increasing rate of VRE incidence (Furtado et al. 2005; Fossi Djembi et al. 2017).

Enterococci are major cause of UTI infections, surgical wounds, and catheter infections. Additionally, *Enterococci* infected in bladder, prostate and kidney, particularly in patients with disorders of the urinary tract or tools. Therefore, urine was reported as a main clinical source of VRE isolates which is consistent with VRE infection reported in Brazil (Soares et al. 2014). The high rate resistance to ampicilin reported in this study (>60%) was higher than those found in the community setting in Portugal (50%) (Freitas et al. 2018). The dominance of VRE infection in the present study may be owing to enlarging vancomycin selection pressure.

b. Molecular characterization of VRE

Seventy one VRE isolates were identified for the species level by multiplex PCR. The results indicated that all VRE isolates were *E. faecium* containing *vanA* gene (Figure 2), which is similar to the reports of VRE epidemiology in India (Tripathi et al. 2016) and Turkey (Gozalan et al. 2015).

All *E. faecium* carrying *vanA* gene isolates were further examined for *esp* virulence gene associated with biofilm formation and antibiotic resistance in *Enterococcus* species. Fifty three isolates of *E. faecium* (74.6%) presented *esp* gene. Previous studies on VRE infection found that the incidence of *esp* in *E. faecium* were varied, such as in Southwest Iran (57.5%) (Arshadi et al. 2018), North west of Iran (71.5%) (Sharifi et al. 2012) and China (90%) (Yang et al. 2015). From the results showed that *vanA* gene normally found in every *E. faecium* isolates, but not every *E. faecium* isolates contained *esp* gene.

Therefore, multiplex PCR technique can be applied for characterization and epidemiology study of VRE infection, which is important data for VRE control in the hospital.

4. CONCLUSIONS

We reported that among 71 VRE isolates were more prevalent of *vanA* gene and *esp* gene, which indicated a high resistance level of vancomycin and strongly associated with hospital outbreaks. This highlights the limited gene spreading and transferring of resistant genes within the hospital. Therefore, the multiplex PCR technique is a validated method for determining antibiotic resistance genes. Our knowledge also help explain the role of the hospital in the transmission of pathogens resistant to antibiotics for human populations.

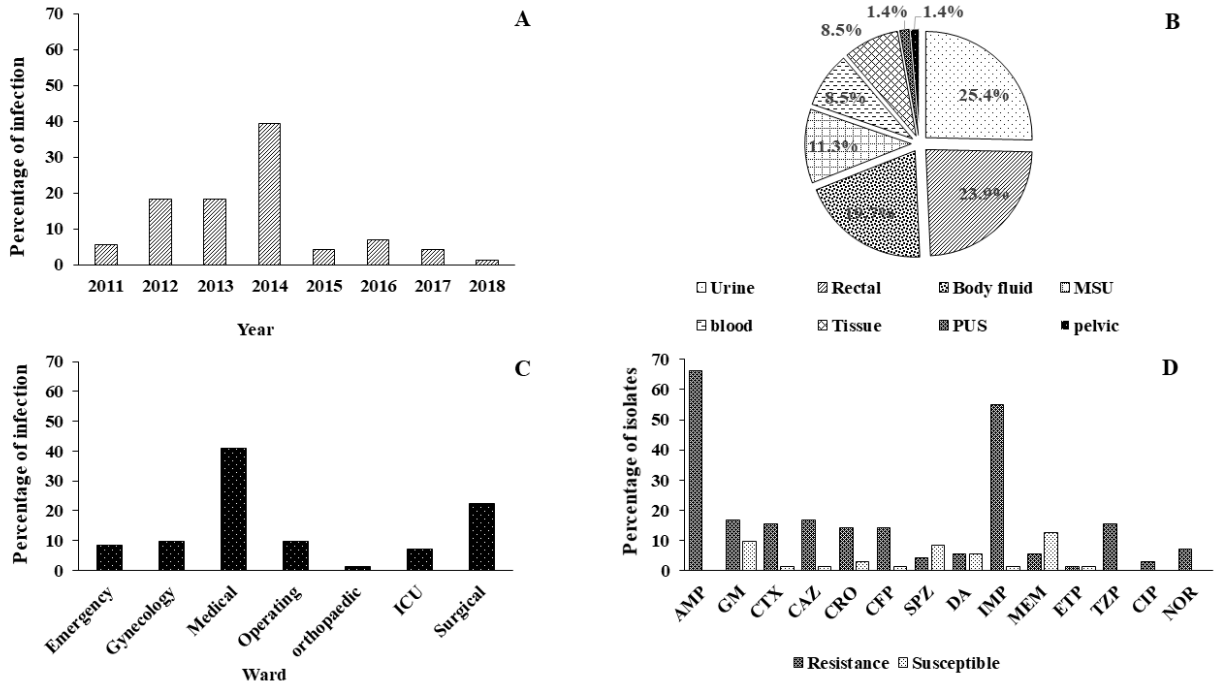

Figure 1: The prevalence of VRE isolates from hospitalized patients between March 2011 to April 2018 from Songklanagarind Hospital: incidence rate of VRE infection (A), isolation rates of VRE infection from various clinical specimens (B), source of VRE isolates among patients (C), and percentage of antibiotic resistance of clinical VRE isolates to various antibiotics (D)

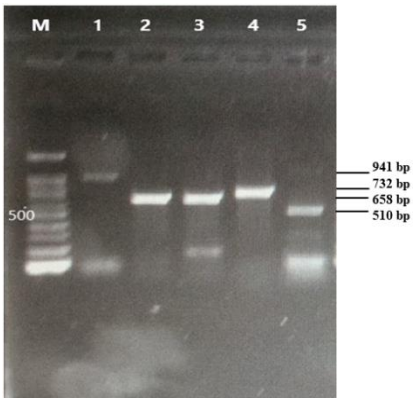

Figure 2: Multiplex PCR based genotypic characterization of vancomycin resistant enterococci isolates recovered from clinical. Lane: M -100 bp molecular size marker, lane 1: positive control (*E. faecalis* ATCC 29212), lane 2 : positive control (*E. faecium* ULCA192) , lane 3 -4 : clinical VRE isolates showing band at 658 bp denoting *ddl_E. faecium* and *vanA* gene, band at 732 bp, respectively, and lane 5: *E. faecium* isolates exhibiting *esp* gene, band at 510 bp.

5. ACKNOWLEDGMENTS

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