ORIGINAL ARTICLE

Antimicrobial Susceptibility Testing for Enterococci from Clinical Isolates in Phichit Hospital

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Objective: To determine antimicrobial susceptibility testing of enterococci in Phichit Hospital.

Materials and Methods: A retrospective study was carried out to determine the species distribution and antimicrobial susceptibilities of enterococci isolated from clinical samples between January 2020 and April 2023. The antimicrobial susceptibility testing of the 969 clinical isolates enterococci collected was performed by disk diffusion and VITEK 2 automated system. Eleven antimicrobial agents including ampicillin (AMP), penicillin (PEN), erythromycin (ERY), gentamicin (GEN), levofloxacin (LEV), linezolid (LIN), streptomycin (STR), teicoplanin (TCO), tigecycline (TGC), tetracycline (TET), and vancomycin (VAN) were tested.

Results: In the 969 isolates of enterococci collected, 170 out of 969 (17.5%) were vancomycin-resistant enterococci (VRE). Of the 170 isolates, 161 (94.7%) were *Enterococcus faecium*, eight (4.7%) were *Enterococcus faecalis*, and one (0.6%) was *Enterococcus* species. One hundred seventy patients positive for VRE were female (63.53%) and the age ranged from 3 years to 99 years. VRE isolates mainly came from patients aged older than 50 years, as was the case in 81.76% of the patients. The median age of patients was 71 years with an interquartile range of 49 to 79 years. VRE was isolated from urine in 83.53%, pus in 7.06%, and blood in 5.88%. VRE demonstrated susceptibility to LIN in 98.80% and TGC in 98.82%, but less susceptibility to STR in 71.18%, GEN in 68.24%, TET in 53.53%, TCO in 22.35%, AMP in 4.12%, PEN in 3.33%, LEV in 3.23%, and ERY in 1.18%. For non-VRE, which was 799 isolates, they demonstrated more susceptibility than VRE to most drugs tested such as LIN, TGC, and VAN in 100%, followed by TCO in 99.48%, ERY in 7.99%, AMP in 56.57%, STR in 44.97%, GEN in 43.05%, LEV in 26.19%, PEN in 24.76%, and TET in 15.95%.

Conclusion: These results suggested the importance of monitoring the isolation of VRE and non-VRE at a hospital. The antibiogram of susceptibility testing can help clinicians on empirical treatment.

Keywords: Vancomycin-resistant enterococci; VRE; Non-VRE; Drug resistance

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Vancomycin-resistant enterococci (VRE) is one of the most clinically important bacteria due to multidrug-resistant (MDR) property and the principal cause of opportunistic hospital-acquired infections[1-3]. Enterococci are associated with serious and life-threatening infections in humans such as urinary tract infections, and sepsis as blood stream infections[4]. *Enterococcus faecalis* and *Enterococcus faecium* account for the majority of human enterococcal infections[4]. In Europe, an increased proportions of vancomycin-resistant *E. faecium* in enterococcal isolates from patients with sepsis from 8.1% in 2012 to19% in 2018 was reported[5]. The majority of the isolates originated from elderly patients[5]. MDR of VRE has previously been reported[6]. VRE represents a critical medical and public health concerns due to the association with serious nosocomial infections and a high risk of mortality[7]. Patterns of VRE and non-VRE antimicrobial susceptibility may vary according to country, geographic location, patient age, and infection site.

The aim of the present study was to determine the isolation and rate of drug resistance of VRE and non-VRE isolated from patients at Phichit Hospital, the main public hospital in Phichit Province in Central Thailand. The present study determined patterns of antibiogram profiles to provide guidance on the treatment of VRE and non-VRE disease for clinicians.

Materials and Methods

The study was conducted after ethical approval

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Bacterial isolates and identification procedure

The present study was a retrospective study conducted at Phichit Hospital, the main hospital of Phichit Province in Central Thailand. The research involved the isolation of enterococci from Phichit Hospital only. One hundred seventy VRE and 799 non-VRE isolates were collected between January 1, 2020, and April 30, 2023. All consecutive isolates were used for antimicrobial susceptibility testing. There was no randomization process. However, only one isolate from each patient was collected to prevent duplication of the bacterial strains. If there were multiple clinical isolates from the patient, data from the first isolate only were included in the analysis. VRE and non-VRE were isolated from various clinical specimens and identified based on colony morphology and biochemical tests by using standard microbiological methods[1,2]. In addition, the researchers used the VITEK 2 automated system, which is a common tool in Thailand for species-level identification of *Enterococcus* species. Specifically, it utilized the gram-positive cocci automated identification card to achieve rapid and accurate identification.

Antimicrobial susceptibility testing

All enterococcal strains were tested for antimicrobial susceptibility to 10 µg ampicillin, 15 µg erythromycin, 5µg levofloxacin, 30 µg linezolid, 30 µg teicoplanin, 30 µg tetracycline, 10 units penicillin, and 30 µg vancomycin by the disk diffusion method as described by the Clinical Laboratory Standards Institute (CLSI)[8]. The bacterial inoculum was prepared by the *Enterococcus* colony suspension method in which colonies from an overnight culture, which is 20 to 24 hours, on blood agar were used. The turbidity of inoculum was adjusted to the 0.5 McFarland standard. The medium was Mueller Hinton agar (Oxoid, UK), which was incubated at 35°C in ambient incubator for 16 to 18 hours. *Staphylococcus aureus* ATCC 25923 was used as quality control. The criteria for interpretation as susceptible, intermediate, and resistant were carried out according to the CLSI's recommendation[8].

Bacterial susceptibility to gentamicin, streptomycin and tigecycline were evaluated for minimal inhibitory concentration (MIC) in µg/mL by using the VITEK 2 automated system (VITEK 2 system, BioMérieux Inc, Durham, NC, USA) according to the manufacturer's instructions. The following reference strains for internal quality control of the VITEK 2 system were *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *Enterococcus casseliflavus* ATCC 700327, and *Enterococcus saccharolyticus* ATCC 43076. The MIC values of 0.25 µg/mL or less and 0.5 µg/mL or more for tigecycline were considered susceptible and resistant, respectively, according to the EUCAST 2020[9]. The high level of gentamicin resistance with a MIC of 500 µg/mL or more and high level of streptomycin resistance with a MIC of 1,000 µg/mL or more were considered resistant, respectively, according to the VITEK 2 automated system.

Table 1. Isolation and source of specimens of vancomycin resistant enterococci (VRE)

	No. of patients (n=170); n (%)
Age groups (years)	
<1 to 20	4 (2.35)
21 to 50	27 (15.88)
>50	139 (81.77)
Specimens	
Urine	142 (83.53)
Pus	12 (7.06)
Blood	10 (5.88)
Sputum	2 (1.18)
Other*	4 (2.35)

* Ascetic fluid, buttock, peritoneal dialysis, broncho-alveolar lavage

Data analysis

Data was entered using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA). Data was analyzed for descriptive statistics. The results are expressed as frequency, percentage for discrete variables and mean, standard deviation for continuous variables.

Results

For VRE isolates, 108 patients (63.53%) were female and 62 (36.47%) were male. Hence, the female to male ratio was 1.74:1. The age ranged from 3 years to 99 years old. In 81.77% of the cases, VRE came from patients aged older than 50 years. The median age of the patients was 71 years with an interquartile range of 49 to 79 years. The three most common clinical specimens were urine in 83.53%, pus in 7.06%, and blood in 5.88% (Table 1).

There were 969 isolates of enterococci and 170 out of 969 (17.5%) were VRE. Of the 170 isolates,

161 (94.7%) were *E. faecium*, eight (4.7%) were *E. faecalis*, and one (0.6%) was *Enterococcus* species. Therefore, *E. faecium* was the most frequently isolated pathogen, and 83.53% of all VRE were isolated from urine specimen (Table 1).

VRE isolates were susceptible to linezolid in 98.80%, tigecycline in 98.82%, streptomycin in 71.18% and gentamicin in 68.24%. They were less susceptible to ampicillin, penicillin, erythromycin, levofloxacin, tetracycline and teicoplanin at a range of 1.18% to 53.53% (Table 2).

The data on tigecycline susceptibility testing for MIC from the VITEK 2 automated system were in µg/mL. Tigecycline MIC range, MIC_{50} and MIC_{90} value were 0.12 or less to 0.25, 0.25 and 0.25, respectively.

For non-VRE isolates, 426 patients (53.32%) were female and 373 (46.68%) were male. Hence, the female to male ratio was 1.14:1. The age ranged from two months to 100 years old. In 82.98%, non-VRE came from patients aged older than 50 years. The median age of patients was 69 years with an interquartile range of 57 to 79 years. The species of non-VRE are shown in Table 3, and the two most common were *E. faecalis* in 53.57% and *E. faecium* in 41.68%. Non-VRE isolates were susceptible to linezolid in 100%, tigecycline in 100%, and teicoplanin in 99.48%. They were less susceptible to ampicillin, penicillin, erythromycin, gentamicin, levofloxacin, streptomycin, and tetracycline at a range of 7.99% to 56.57% (Table 4).

Discussion

Data from the National Antimicrobial Resistant Surveillance Center, Thailand for *E. faecalis*, which had 14,078 isolates, *E. faecium* with 8,529 isolates, and *Enterococcus* species with 1,349 isolates, for vancomycin resistance were 1.9%, 13.9%, and 8.9% from all specimens in 68 hospitals, between January and December 2022. Therefore, the authors' enterococcal isolates had higher resistance at 17.5% to vancomycin when compared to NARST data[10].

In the present study, more VRE positive patients were female. The study was similar to a report from Nigeria in 2021 that showed the prevalence of vancomycin resistant *E. faecium* and vancomycin resistant *E. faecalis* in three hospitals in Nigeria was higher in females than in males[3].

The authors reported that most VRE positive patients came mainly from patients older than 50 years of age. The ages of patients susceptible to VRE infection are unpredictable and vary according

Table 2. Antimicrobial susceptibility of vancomycin resistant enterococci (VRE) by the disk diffusion method and the VITEK 2 automated system*

Antimicrobial agents	No. of isolates; n (%)		
	Susceptible	Intermediate	Resistant
Ampicillin	7 (4.12)	-	163 (95.88)
Penicillin	1 (3.33)	-	29 (96.67)
Erythromycin	2 (1.18)	2 (1.18)	166 (97.64)
Gentamicin (high level)*[a]	116 (68.24)	-	54 (31.76)
Levofloxacin	1 (3.23)	-	30 (96.77)
Linezolid	165 (98.80)	-	2 (1.20)
Streptomycin (high level)*[b]	121 (71.18)	1 (0.59)	48 (28.23)
Teicoplanin	38 (22.35)	22 (12.94)	110 (64.71)
Tigecycline*	167 (98.82)	-	2 (1.18)
Tetracycline	91 (53.53)	1 (0.59)	78 (45.88)
Vancomycin	-	-	170 (100)

* VITEK 2, minimal inhibitory concentration (MIC) report was generated
(a) High level gentamycin resistance (MIC ≥500 µg/mL), (b) High level streptomycin resistance (MIC ≥1,000 µg/mL)

Table 3. Species of non-vancomycin resistant enterococci (non-VRE)

Species	No. of isolates; n (%)
Enterococcus faecalis	428 (53.57)
Enterococcus faecium	333 (41.68)
Enterococcus avium	9 (1.13)
Enterococcus raffinosus	9 (1.13)
Enterococcus casseliflavus	5 (0.63)
Enterococcus cecorum	5 (0.62)
Enterococcus gallinarum	5 (0.62)
Enterococcus hirae	5 (0.62)
Total	799 (100)

Table 4. Antimicrobial susceptibility of non-vancomycin resistant enterococci (non-VRE) by the disk diffusion method and the VITEK 2 automated system*

Antimicrobial agents	No. of isolates; n (%)		
	Susceptible	Intermediate	Resistant
Ampicillin	452 (56.57)	-	347 (43.43)
Penicillin	156 (24.76)	-	474 (75.24)
Erythromycin	62 (7.99)	186 (23.97)	528 (68.04)
Gentamicin (high level)*[a]	344 (43.05)	4 (0.50)	451 (56.45)
Levofloxacin	176 (26.19)	-	496 (73.81)
Linezolid	766 (100)	-	-
Streptomycin (high level)*[b]	353 (44.97)	-	432 (55.03)
Teicoplanin	772 (99.48)	4 (0.52)	-
Tigecycline*	738 (100)	-	-
Tetracycline	126 (15.95)	-	644 (84.05)
Vancomycin	799 (100)	-	-

* VITEK 2, minimal inhibitory concentration (MIC) report was generated
(a) High level gentamycin resistance (MIC ≥500 µg/mL), (b) High level streptomycin resistance (MIC ≥1,000 µg/mL)

to geographical locations but are more common in adults[3,5]. The Netherlands and Germany reported high VRE prevalences in high-risk wards such as geriatric and haemato-oncology wards[11]. A previous report in a hospital in southern Thailand was 48.9% of VRE positive patients were 65 years old or older[12].

During the present study period, clinical specimens of VRE were highest in urine at 83.53%. This data confirmed the result of other studies, indicating that the urinary tract is the most common site of infection caused by this group[1,2,12-15]. The present study results showed that the predominant species of VRE was *E. faecium* followed by *E. faecalis*, similar to other reports from across the world[3,14].

In the antimicrobial susceptibility test, the authors found that 95.88% of VRE were resistant to ampicillin, 97.64% resistant to erythromycin, and 64.71% resistant to teicoplanin (Table 2). However, patterns of antimicrobial susceptibility for VRE may vary according to country and geography. VRE isolates in Nigeria[3] and Egypt[7] were 100% and 65.5% resistant to ampicillin, respectively. VRE isolates in Southern Brazil were 100% resistant to ampicillin and teicoplanin[15]. The reason that all VRE isolates in the present study were resistant to vancomycin, but only 4.12% were susceptible to ampicillin was due to different mechanisms of resistance within the bacteria. Vancomycin resistance is caused by alterations in the cell wall structure encoded by many genes such as *vanA*, *vanB*, *vanC*, *vanD*, and *vanE*[6,13,16], while ampicillin resistance, is due to beta-lactamases or changes in penicillin-binding proteins[13]. More than 50% of VRE isolates in the present study were susceptible to aminoglycosides, thus correct identification in clinical laboratory and administration of these drugs can result in decreased usage of drugs such as vancomycin or linezolid and help to reduce the emergence of resistance to these drugs. All VRE isolates in some studies were MDR[3,17]. An isolate is MDR if resistant to three or more classes of drugs. This poses a great risk, as infections resulting from these organisms can cause healthcare-associated infections and increase both length of stay and in-hospital mortality[18]. Prolonged hospitalization, prior exposure to antimicrobial agents, and use of foreign medical devices such as catheters, are risk factors for VRE infection[17].

Currently, enterococci are commensal bacteria and part of the normal enteric microbiota. To date, over 50 different enterococcal species have been described[4]. The concentration of enterococci in human stools is approximately 10^4 to 10^7 CFU/g[19]. The World Health Organization in 2017 listed VRE among those with high priority for research[14]. VRE constitutes a major cause of healthcare-associated infections, with extensive resistance to multiple antimicrobial agents and the capacity to transfer resistance to other pathogens through plasmids[1,2]. Multiple resistance mechanisms in VRE have led to limitations in available treatment options as increased vancomycin resistance in enterococci restricts the choice of vancomycin as a treatment for enterococcal infections[2,6]. The main mechanism of vancomycin resistance involves the alteration of the peptidoglycan synthesis pathway by many genes such as *vanA*, *vanB*, *vanC*, *vanD*, and *vanE*[6,13,16]. However, resistance to vancomycin occurs mainly by acquiring the *vanA* gene and less frequently by the *vanB* gene, already described in detail in *E. faecium*[3].

VRE and non-VRE isolates in the present study were susceptible to linezolid and tigecycline. However, both drugs are bacteriostatic in nature. Linezolid is an oxazolidinone-class antibacterial agent[20], whereas tigecycline is a tetracycline class[21]. Serious enterococcal infections, such as endocarditis may need treatment with combination of bactericidal drugs that should include a beta lactam such as ampicillin, to which *Enterococcus* isolate is susceptible and an aminoglycos side such as gentamicin or streptomycin, which *Enterococcus* isolate does not exhibit high-level resistance. Therefore, high level aminoglycoside resistance of enterococci can predict resistance to this combination therapy[20].

The findings in the present study contribute to the local epidemiology in understanding the population structure regarding the characteristics of the circulating VRE and non-VRE isolates in Phichit Hospital, a secondary care hospital, as well as the emergence and spread of antimicrobial resistance in enterococci. There were limitations to the present study. First, data on isolates from a single site of study was presented for antibiogram profile. Second, the authors did not collect clinical data of patients but collected them from microbiological database. Due to the lack of clinical information in the study, the authors did not correlate the result of the susceptibility test in vitro and clinical outcome in the same individual. Third, only a limited number of VRE and non-VRE isolates were studied.

Conclusion

The results from the present study suggest that tigecycline and linezolid, which showed susceptibility of 98.82% and 98.80%, are effective drugs for VRE treatment. The present data should support ongoing studies to evaluate current trends and increase surveillance of drug resistance so that further development of drug resistance is minimized. Furthermore, antibiogram profiles are necessary to avoid ineffective empirical drug treatment.

What is already known about this topic?

Enterococcus is a bacterium that commonly lives in the intestinal tract of most people and does not cause illness. This is called colonization. Sometimes these bacteria could cause infection if they got into an area of the body where they are not normally found such as wounds, urinary tract, and bloodstream. VRE are enterococci that are resistant to vancomycin. *E. faecium* is the most common species of VRE. There are only a few antimicrobial agents that are able to treat VRE infections. According to the previous studies, antimicrobial resistance of VRE is a problem worldwide. Therefore, study of VRE and non-VRE isolation in Thai patients and antimicrobial resistance should be investigated.

What does this study add?

In Phichit Hospital, unduplicated VRE isolates from 170 patients were isolated from urine in 83.53%, pus in 7.06%, and blood in 5.88%. The predominant species of VRE were *E. faecium* in 94.71%, followed by *E. faecalis* in 4.71%. VRE demonstrated susceptibility at 98.80% to linezolid and at 98.82% to tigecycline, but less susceptibility to streptomycin at 71.18%, gentamicin at 68.24%, tetracycline at 53.53%, and other drugs tested such as teicoplanin, ampicillin, penicillin, levofloxacin, and erythromycin, which ranged from 1.13% to 22.35%. Non-VRE isolates demonstrated more susceptibility than VRE to most drugs tested. This study demonstrated antimicrobial susceptibility results of enterococci to provide guidance on the treatment of both VRE and non-VRE infection.

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Conflicts of interest

The authors declare no personal or professional conflicts of interest, and no financial support.

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