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Research Article

Vancomycin Resistant Enterococci in a Tertiary Care Hospital of Dhaka, Bangladesh

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Abstract

Vancomycin resistant Enterococci (VRE) are multidrug resistant emerging pathogens that can cause various hospital and community acquired infections with increased mortality and morbidity. VRE is now becoming a global threat due to limited treatment options. So, this cross-sectional study was undertaken to detect VRE in a tertiary care hospital of Bangladesh by both phenotypic and genotypic method. A total of 87 *Enterococcus* spp. isolated from different clinical samples in the laboratory of Department of Microbiology and Immunology of Bangladesh Medical University (BMU) were studied from March 2019-February 2020. Enterococci of this study comprises *E. faecalis* (65), *E. faecium* (20) and *E. raffinosus* (2). According to this study, 67.8%, 60.9%, 56.3%, 47.1% and 43.7% of Enterococci were resistant to ciprofloxacin, gentamicin, nitrofurantoin, ampicillin and cotrimoxazole respectively. VRE was detected phenotypically by determination of minimal inhibitory concentration (MIC) of vancomycin by agar dilution method and genotypically by detection of *vanA* and *vanB* gene by conventional PCR. The prevalence of VRE was 3.4% (3 out of 87). The highest MIC value of vancomycin 256 µg/ml and 128 µg/ml was observed in two and one VRE isolates respectively. The *vanA* gene was detected in all VRE isolates. Two (66.67%) VRE were isolated from urine and 1 (33.33%) from blood sample. All VRE isolates were *E. faecium* and only susceptible to Linezolid and Fosfomycin. Detection of VRE in this study place warrants strict infection control and prevention policy to combat this difficulty to treat pathogen. It also provides important data regarding susceptibility of *Enterococcus* spp. and VRE.

Keywords

VRE, Enterococci, Prevalence, MIC, *vanA*, *vanB*, PCR

1. Introduction

Multidrug resistant Enterococci are important pathogen that can cause several infections like urinary tract infection, sepsis, surgical site infection, prosthetic valve endocarditis [1]. Vancomycin is the drug of choice for the treatment of multi-drug resistant enterococcal infection. In the last two decades, Vancomycin resistant Enterococci (VRE) has emerged as an important cause of nosocomial infections all over the world. VRE are responsible for causing various mild to life threat-

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ening infections [2]. The mortality and morbidity rates are also high in VRE infection [3]. In 2017, World Health Organization (WHO) included VRE as one of the most important resistant bacteria in their "Global Priority list of antibiotic-resistant bacteria" [4]. Approximately 54,500 infections and 5,400 estimated deaths due to VRE in hospitalized patients of USA was reported by the Centers for Disease Control and Prevention (CDC) in 2017 [5]. The extensive use of extended-spectrum cephalosporins and vancomycin in enterococcal infections is the leading cause of worldwide spread of VRE [6]. The antibiotic resistance in Enterococci is acquired by both mutation and horizontal gene transfer which is mediated via plasmids and transposons [7]. Vancomycin resistance is mediated by Vancomycin resistance (Van) gene operons which include vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM and vanN genes. Among them, the most common is vanA gene followed by vanB and vanC gene. VanA and VanB phenotypes of vancomycin resistance are inducible in nature and VanC resistance phenotype is constitutive, non-inducible intrinsic low-level resistance which is mainly seen in E. gallinarum and E. casseliflavus. The inducing factors includes previous use of vancomycin in patients or the use of drugs like avoparcin and ristocetin in poultry [2]. VanA phenotype is associated with high level resistance to both vancomycin (MIC≥64 μg/ml) and teicoplanin (MIC≥16 μg/ml) whereas VanB phenotype is associated with varying levels of resistance to vancomycin (MIC 4 -1000 μg/ml) with sensitivity to teicoplanin [8]. The vanA and the vanB gene are located on the transposable elements Tn1546 and Tn1549 and can be transferred between interspecies and intraspecies through conjugative transposons and plasmid [9, 10]. Enterococci has the capacity to transfer its vancomycin-resistant genes to methicillin-resistant S. aureus, that makes it a public health threat [11]. The emerging multidrug resistant VRE, an important cause of nosocomial infections, has seriously limited the available choices to clinicians for treating these serious infections. VRE is associated with treatment failure and responsible for transfer of antibiotic resistance gene to other bacteria. Detection of VRE is important for controlling the spread of it and for epidemiological purposes. So, this study was undertaken to detect vancomycin resistance in Enterococcus species by both phenotypic and genotypic methods.

2. Materials and Methods

2.1. Isolation of Enterococci

This cross-sectional study was conducted at the Department of Microbiology and Immunology, BMU (previous BSMMU) over a period of 1 year from March 2019 to February 2020. Enterococci isolated from different clinical samples (urine, blood, wound swab, pus and bile) during the study period were included in this study. These samples were sent from different departments of BMU hospital to the Laboratory of Microbiology and Immunology Department of BMU for culture and sensitivity test. Enterococci were identified based on colony morphology on Chromogenic and blood agar media, Gram staining method, standard biochemical test (catalase test, salt tolerance test, bile esculin test). [12] Then identification of *Enterococcus* spp. was done by fermentation of mannitol, sorbitol, raffinose, arabinose, utilization of pyruvate and arginine decarboxylase test [13].

2.2. Antibiotic Susceptibility Testing by Disc Diffusion

The modified Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing of the isolated Enterococci. For disc diffusion test, Mueller-Hinton agar media and commercially available antibiotic discs (purchased from BioMaxima, Poland) were used. Antibiotic discs and their concentrations were selected as recommended by the Clinical Laboratory Standards Institute guideline (CLSI, 2019) for *Enterococcus* spp. [14]. *S. aureus* ATCC 25923 was used as a quality control strain.

2.3. Determination of MIC of Vancomycin

MIC of vancomycin for all Enterococi isolates were done by agar dilution method according to CLSI guideline M07-A9 [15]. According to the guideline, different concentrations of vancomycin ranging from 2 μg/ml to 256 μg/ml were prepared and impregnated in Mueller-Hinton agar media. Bacterial suspension of 0.5 McFarland turbidity standard containing 1.5×10^8 CFU/ml was prepared which was further diluted 10 times (1 ml test inoculum compared to turbidity standard added with 9 ml of normal saline) to achieve 1.5×10^7 CFU/ml. To obtain 1.5×10^4 CFU/ml on the agar surface, 1 μl of 10 times diluted inoculum was placed on the Mueller-Hinton agar plate. The plate was then incubated aerobically at 37 ℃ overnight. The lowest concentration of antibiotic-impregnated Mueller-Hinton agar showing no visible growth on agar media was considered MIC of that drug for that test isolates [15]. MIC of vancomycin was interpreted according to MIC breakpoints determined by CLSI 2019 guideline: ≤ 4 μg/ml as susceptible and ≥32 μg/ml as resistant [14].

2.4. Molecular Detection of *vanA* and *vanB* Gene by Conventional PCR

DNA was extracted by boiling method from the overnight grown culture of *Enterococcus* [16]. Primer sets used for the detection of *vanA* and *vanB* genes were listed in (Table 1).

Table 1. *Primer sequences and properties of vanA and vanB genes used in conventional PCR.*

Primer	Sequence	Tm	GC Ratio	Base pair	Reference
vanA	P1: GGGAAAACGACAATTGC	50.0	47.06	732	[16]
	P2: GTACAATGCGGCCGTTA	52.4	52.94		
vanB	P1: CATCGCCGTCCCCGAATTTCAAA	64.7	52.17	297	[16]
	P2: GATGCGGAAGATACCGTGGCT	63.3	57.14		

A 25 µl of reaction volume containing 15 µl of master mix (HELINI, India), 0.15 µlTaq polymerase (Solis BioDyne Germany), 1 µl of forward and reverse primer of each (10 pmol/µl), 2.85 µl of nuclease free water and 5 µl of undiluted extracted DNA was used for amplification. The amplified products were electrophoresed on 1.5% agarose gel. Amplified products of the *vanA* gene by conventional PCR were shown in (Figure 1).

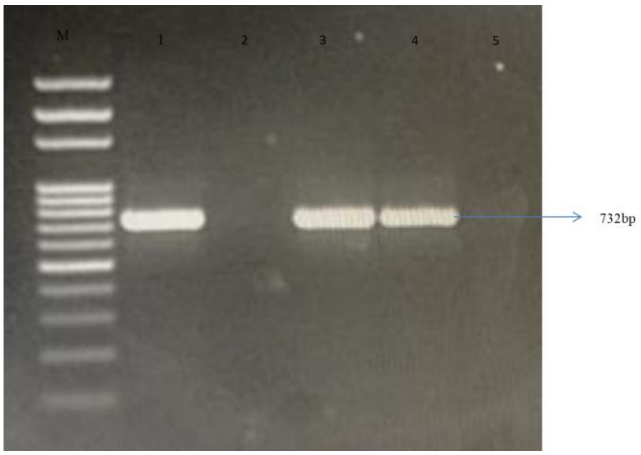

Figure 1. *Agarose gel electrophoresis analysis showed amplified DNA product of van A (732 bp).*

Note: Lane M: 100 bp DNA ladder; Lane 1: Positive control of *vanA* gene (732 bp) of Enterococci. Lane 2: Test sample negative for *vanA* gene. Lane 3, 4: Test samples positive for *vanA* gene. Lane 5: Negative control

Statistical analysis: Statistical analysis was performed using the SPSS™ software, version 23.0 (IBM Crop. New York, NY). The qualitative data were expressed as numbers and percentages.

3. Results

A total of 87 Enterococci were isolated from different clinical samples during the study period which comprises *E. faecalis* (65), *E. faecium* (20) and *E. raffinosus* (2).

3.1. Source and Antibiogram of *Enterococcus* spp.

Eighty-three (95.4%) out of 87 *Enterococcus* spp. were isolated from urine samples. The distribution of other sources and antibiotic susceptibility patterns of Enterococci isolates were shown in Table 2 and Table 3 respectively.

Table 2. *Distribution of Enterococcus spp. according to source of samples for culture (N = 87).*

Sample type	Number (n)	Percentage (%)
Urine	83	95.40
Blood	1	1.15
Pus	1	1.15
Wound swab	1	1.15
Bile	1	1.15
Total	87	100.00

Table 3. *Antimicrobial susceptibility pattern of Enterococcus spp. (N = 87).*

Antimicrobial agents (µg)	Susceptible n (%)	Resistant n (%)
Ampicillin (10)	46 (52.87)	41 (47.13)
Cotrimoxazole (1.25/23.75)	49 (56.32)	38 (43.68)

Antimicrobial agents (μg)	Susceptible n (%)	Resistant n (%)
Ciprofloxacin (5)	28 (32.18)	59 (67.82)
Nitrofurantoin (300)	38 (43.68)	49 (56.32)
Gentamicin (120)	34 (39.08)	53 (60.92)
Vancomycin (30)	84 (96.55)	3 (3.45)
Linezolid (30)	87 (100.00)	0 (0.00)
Teicoplanin (30)	84 (96.55)	3 (3.45)
Fosfomycin (200)	87 (100.00)	0 (0.00)
*Quinopristin- Dalfopristin- (15), n=22	10 (45.45)	12 (54.55)

Note: *E. faecalis*is intrinsically resistant to Quinopristin - dalfopristin.

3.2. VRE by Vancomycin Agar Dilution

Three out of 87 Enterococci isolates (3.4%) exhibited MIC value of ≥ 32 μg/ml which were considered as VRE. MIC value of vancomycin 256 μg/ml was observed in 2 VRE isolates and 128 μg/ml in one isolate. Two (66.67%) VRE were isolated from urine and 1 (33.33%) from blood sample. All VRE (3) isolates were *E. faecium* and only susceptible to Linezolid and Fosfomycin.

3.3. Vancomycin Encoding Genes in VRE Isolates

All (3) VRE isolates were positive for *vanA* gene by conventional PCR. The *vanB* gene was not detected in any of the isolates.

4. Discussion

Enterococci are opportunistic pathogens and cause various health care associated infections. Among different species of Enterococci, *E. faecalis* and *E. faecium* are responsible for 80%-90% of enterococcal infections [17]. The interest in enterococcal infection is increasing because of the severity of the infections and development of resistance to different antibiotics [18]. VRE have emerged as a leading cause of nosocomial infections [19]. VRE was first detected in England and France in 1986 followed by detection of VRE in the USA one year later. Today VRE has been spread throughout the world [20]. In the last twenty years, VRE have risen by 20-folds specially in the intensive care unit, with the reported rate of VRE varying between (0 - 35) % internationally [21]. A pooled prevalence of VRE was reported 8.10% in Asia. The highest prevalence of VRE with a pooled prevalence of 11.40% was seen in Western Asia followed by South Asia (7.70%), East Asia (3.10%) and South-East Asia (1.80%) [22]. The prevalence of VRE has increased in India in last two decades. Between 1% and 8.7% have been reported as VRE prevalence in India between 1999 and 2021 [23].

The majority (95.4%) of the *Enterococcus* spp. were isolated from urine followed by 1.15% (1) from pus, wound swab, blood and bile sample of each. Similarly, a study of Bangladesh reported 97.3%, 1.8% and 0.9% of Enterococci were isolated from urine, pus and wound swab respectively [24]. According to antimicrobial susceptibility test, 67.8%, 60.9%, 56.3%, 47.1% and 43.7% of Enterococci were resistant to ciprofloxacin, gentamicin, nitrofurantoin, ampicillin and cotrimoxazole respectively which is supported by the result of another study of India where 76.8%, 59.61%, 59.17% Enterococci were resistant to ciprofloxacin, gentamicin, ampicillin respectively [25]. Overall percentage of VRE in this study was 3.4% which is lower than a study done in Bangladesh where it was 6.2% [24]. All VRE were also resistant to teicoplanin. VRE isolates were only susceptible to linezolid and fosfomycin. A study done in Saudi Arabia also found that VRE were susceptible to linezolid which supports our study [26].

Among VRE isolates, 67.0% were isolated from urine and 33.0% were isolated from blood samples. Several studies reported that VRE is most found in urine and blood samples [27, 28]. All the 3 VRE isolates of this study were *E. faecium*. All VRE isolates as *E. faecium* were also reported by other study of Turkey [29]. Among 3 VRE isolates of this study, two had MIC equal to 256 μg/ml and one had MIC equal to 128 μg/ml. VRE isolates with vancomycin MIC equal to 256 μg/ml and 128 μg/ml were also found in different studies conducted in tertiary care hospital of Northern and Southern Thailand [27, 28]. In this study, all VRE isolates were found to be positive for *vanA* gene which was supported by other studies [27, 28, 30]. On the contrary, approximately 17.5% of VRE containing *vanB* gene was observed in a study of Australia while none of them was positive for *vanA* gene. The reason explained in that study was that *vanB* gene has been endemic in VRE in that region [31]. It may also be *vanA* gene is associated with clinical strains while food contamination and epidemics is associated with *vanB* gene [32].

5. Conclusion

The prevalence of VRE in this study is 3.4%. All VRE are resistant to teicoplanin but susceptible to Linezolid and Fosfomycin. VRE identification in this specialized healthcare facility is a public health threat for the health care settings of Bangladesh due to its nature of transmission.

Abbreviations

CDC	Centers for Disease Control and Prevention
CLSI	Clinical Laboratory Standards Institute Guideline
MIC	Minimal Inhibitory Concentration
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

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Author Contributions

Tahani Momotaz: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft

Rehana Razzak Khan: Formal Analysis, Software, Supervision, Writing – review & editing

Fatima Afroz: Formal Analysis, Methodology, Software, Writing – review & editing

Abu Naser Ibne Sattar: Conceptualization, Formal Analysis, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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