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Original Article

Microbiology Section

Vancomycin Resistant Enterococcal Urinary Tract Infection: A Potential Threat

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ABSTRACT

Introduction: Enterococci are considered less virulent organisms, but have incurred recognition for being notorious for their acquisition and transfer of resistance. The greatest potential threat posed by enterococci is vancomycin resistance. The transfer of enterococcal vancomycin resistance to *Staphylococcus aureus* has been achieved making scientists apprehensive of its consequences.

Aim: To find the prevalence of Vancomycin-Resistant Enterococci (VRE) and to determine the antimicrobial resistance pattern in enterococcal urinary isolates.

Materials and Methods: A cross-sectional study was carried out on all urinary samples suspected of Urinary Tract Infection (UTI) received for duration of one year from April 2021 to March 2022 in the Department of Microbiology at Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh. Enterococci were isolated and identified with a VITEK-2® COMPACT (bioMérieux) automated system. The antibiotic susceptibility pattern was determined by Kirby-Bauer disc diffusion method. Further, confirmation of VRE was done by Minimum Inhibitory Concentration (MIC) E-test.

Results: A total of 128 urinary enterococcal isolates were identified with the male-to-female ratio 1.37:1 and mean age of patients was 37.18±22.64 years. Out of total, 71.87% were identified as *Enterococcus faecalis* followed by 24.21% *Enterococcus faecium* and the rare species (4%) including *E.durans, E.hirae, E.raffinosus*. The prevalence of VRE was found to be 8.6%. Maximum resistance by isolates has been shown against ampicillin, erythromycin, ciprofloxacin, and doxycycline. All isolates were sensitive to linezolid. Nitrofurantoin resistance was observed in 4.34% and 25.80% of *E.faecalis* and *E.faecium* isolates respectively.

Conclusion: In this study, it was revealed that the emergence of VRE in urinary isolates with antimicrobial resistance was higher among *E. faecium*. All this puts pressure on strict compliance with a multidimensional approach with collaboration of antibiotic stewardship, educational and surveillance programs.

Keywords: Bladder infection, Gram-positive cocci, Prevalence

INTRODUCTION

Enterococcus is one of the pathogens forming the acronym ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) group, which accounts for the majority of nosocomial infections worldwide and has also been considered a pathogen of high priority by WHO in its global list of significant antibiotic-resistant bacteria [1]. Enterococcus was previously classified as group D *Streptococci* but with the help of molecular analysis, they have been placed in a separate genus, *Enterococcus* [2]. They are gram-positive cocci which are indigenous inhabitants of the gastrointestinal tract, oral cavity, and genital tract of humans [3]. Genus *Enterococcus*, includes a variety of species, but only two species are considered to cause the majority of infections, these are *Enterococcus faecalis* (85-90%) and *Enterococcus faecium* (5-15%) [2]. Enterococci are one of the dominant uropathogens. Besides infecting the urinary tract, they are the second prominent cause of hospital-acquired urinary infections and also cause other infections like bacteraemia, wound infections, intra-abdominal and pelvic infections, urinary catheter-associated infections, and infective endocarditis [4]. They rarely cause meningitis or respiratory tract infections [5]. The inception of urinary VRE has emerged as a dreadful infection in immunocompromised patients [6]. Patients who have enterococcal UTIs, either exhibit minimal or no symptoms. Symptoms are mostly related to catheterisation and instrumentation [7]. Complications and recurrences associated with UTIs are due to pre-existing urinary tract abnormalities [8].

Enterococci are considered less virulent organisms but have incurred recognition for being notorious for their resistance traits [9]. The world has seen an increase in drug-resistant bacteria at a remarkable rate and enterococcus is one amongst them. The greatest potential threat posed by enterococci is vancomycin resistance [6]. The empirical use of antibiotics gives bacteria an edge of selective pressure to acquire resistance [10]. Enterococci are intrinsically resistant to antimicrobials used frequently and also acquire resistance by mutation or in conjugation with the transfer of genetic material [11]. The transfer of enterococcal vancomycin resistance to *Staphylococcus aureus* has been achieved and scientists are apprehensive that it may emerge in nature as a new pandemic with devastating consequences [12,13].

Past reports of VRE emerged in the late 1980s and since then it has become of paramount concern for researchers [10]. The prevalence of VRE in Europe is demonstrated to vary between 1% to 30%, whereas in the United States it is estimated to be about 30%, which is considered to be hospital-acquired [2].

Amongst the phenotypic vancomycin resistance, most common in the United States are Van A and Van B, about 70% and 25%, respectively [6]. Van A enterococci are resistant to both vancomycin and teicoplanin, whereas Van B is resistant only to vancomycin and preserve susceptibility to teicoplanin [11]. The assessment of resistance varies extensively in different geographical latitudes, depending on their antimicrobial stewardship programs, empirical practice patterns, and prevalence of habitant-resistant enterococcal species [5].

Patients colonised with VRE remain a threat for hospital outbreaks as they serve as a reservoir of VRE and subsequently spread infection from patient to patient [10,14].

Animals are said to be reservoirs, from which VRE transmits to man via the food chain [11]. Hospitalised patients, hospital equipment, environmental surfaces, and VRE patient areas, may also serve as a reservoir for VRE [14].

UTIs caused by VRE bear serious health and socio-economic burden including the cost of hospitalisation [2]. Categorising VRE associated urinary colonisation, asymptomatic bacteriuria, and UTIs to determine optimal treatment options, duration of therapy, and eventually decreasing mortality and healthcare costs [15].

The global dissemination of recalcitrant VRE showing an increasing trend is a critical situation strikingly the most important is their ability to acquire resistance to the presently available antimicrobial agents, along with the dearth of new agents, with very few in development [6,11,16]. Therefore, the present study was undertaken to know the prevalence and antimicrobial resistance pattern of enterococcal urinary isolates and to determine the phenotypes of VRE.

MATERIALS AND METHODS

A prospective cross-sectional study was performed on urinary samples of patients suspected of UTI for routine culture and sensitivity. The samples were received from both outpatient and inpatient areas in the Department of Microbiology at Uttar Pradesh University of Medical Sciences, Saifai, a Tertiary care hospital Etawah, Uttar Pradesh, India for a period of one year from April 2021 to March 2022.

Inclusion criteria: Urine samples from both outpatient and inpatients of our hospital, of all age groups and both sexes complaining of urinary symptoms were included in the study.

Exclusion criteria: Body fluids, sputum, blood, and specimens other than urine were excluded from the study. Additionally, urine specimens with bacterial growth other than enterococci were also excluded.

Procedure

Using the semiquantitative method, urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED), reading the plates after 24 hours of aerobic incubation at 37°C. Colony counts yielding bacterial growth of $>10^5$ CFU/mL were considered significant bacteriuria. Presumptive identification of the genus *Enterococcus* was done based on using colony morphology, gram-staining, hydrolysis by bile esculin and catalase. Species identification was done separately by using the VITEK-2® COMPACT (bioMérieux, Marcy l'Etoile, France) automated system [17].

Antibiotic susceptibility testing was done for all the enterococcal isolates by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar and the results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Muller-Hinton agar plates were surface seeded by preparing a uniform suspension of culture with turbidity adjusted to 0.5 McFarland standard. The plates were read with the naked eye using transmitted light for appreciating the presence or absence of a zone of inhibition around each antibiotic disc. Quality control was done by using the ATCC strain of *Enterococcus faecalis,* 29212 which was procured from HiMedia Laboratories Pvt., Ltd., Mumbai, Maharastra, India.

The following commonly used antibiotic discs and their disc potency that was used for susceptibility testing of enterococcal isolates is as follows: Ampicillin 10 µg, Erythromycin 15 µg, Tetracycline 30 µg, Doxycycline 30µg, Ciprofloxacin 5 µg, Vancomycin 30µg, Teicoplanin 30 µg, High Level Gentamycin (HLG) 120 µg, Linezolid 30 µg, Nitrofurantoin 300 µg.

All enterococcal isolates with an inhibition zone diameter of ≤14 mm or any growth within the zone of inhibition were considered vancomycin-resistant and isolates with a zone diameter of ≤10mm were considered resistant to teicoplanin by disc diffusion method [18]. VRE isolates by disc diffusion method were further confirmed by E-test strips (MIC range: 0.016 to 256 µg/mL) and read according to the manufacturer's recommendations. Enterococci that had MIC >32 µg/mL were noted as resistant to both vancomycin and teicoplanin according to CLSI guidelines [18]. Phenotypic characterisation of VRE was classified as Van A and Van B, with Van A strains showing resistance to both vancomycin as well as teicoplanin, whereas Van B phenotypes remain susceptible to teicoplanin. Culture media, antiobiotic discs and E-test strips used in the study were secured from HiMedia Laboratories Pvt., Ltd., Mumbai, Maharastra, India.

STATISTICAL ANALYSIS

Data was segregated, coded, and recorded in the Microsoft® Excel spreadsheet program. Descriptive percentages and frequencies were represented by constructing tables. Mean, standard deviation, and ratios were summarised for continuous variables, whereas frequencies and proportions were for categorical variables. All statistical analysis was performed with statistical software using SPSS version 24.0 by IBM USA.

RESULTS

In the present study, 1996 urine samples received in the department were screened for UTIs. Out of which 300 isolates were culture positive. A total of 128 (42.66%) isolates were identified as *Enterococcal* spp. and 172 other bacteria.

Of 128 enterococcal isolates, 74 (57.81%) were males and 54 (42.18%) were females. The male-to-female ratio was 1.37:1. The mean age of patients was 37.18±22.64 years (range: 7 days to 92 years). The highest isolates 43 (33.6%) were from the age group 21-40 years, followed by 38 (29.68%) from age up to 20 years, 26 (20.31%) aged 41-60 years, 18 (14.06%) were aged 61-80 years and 3 (2.34%) were >80 years. Inpatients were 87.5% and outpatients were 12.5%. Among the inpatients, 37 (33%) isolates were obtained from the General Surgery ward followed by 28 (25%) from the Urology ward [Table/Fig-1].

Variables	Enterococcal isolates n=128(%)
Age (in years)	
≤20 years	38 (29.68)
21-40	43 (33.60)
41-60	26 (20.31)
61-80	18 (14.06)
>80 years	3 (2.34)
Gender	
Male	74 (57.81)
Female	54 (42.18)
Outpatient	16 (12.50)
Inpatient	112 (87.50)
Wards	
Obstetrics and Gynaecology	11 (9.82%)
General surgery	37 (33%)
Medicine	19 (16.96%)
Paediatrics	3 (2.67%)
Urology	28 (25%)
Orthopedics	4 (3.57%)
Adult intensive care units	10 (8.92%)

[Table/Fig-1]: Basic demographic data of patients with enterococcal UTI.

Five different species of enterococci were identified as *Enterococcus faecalis* 92 (71.87%), *Enterococcus faecium* 31 (24.21%), and rare species *E. durans* 3 (2.34%), *E. hirae* 1 (0.78%), *E. raffinosus* 1 (0.78%) [Table/Fig-2].

Isolates	No. of Isolates	Percentage (%)
E. faecalis	92	71.87
E. faecium	31	24.21
E. durans	3	2.34
E. hirae	1	0.78
E. raffinosus	1	0.78

[Table/Fig-2]: Distribution of Enterococcal spp. (n=128).

The antimicrobial resistance pattern by the Kirby-Bauer disc diffusion method is depicted in [Table/Fig-3]. The resistance pattern of *E. faecalis* to commonly used drugs was erythromycin (86.95%), ampicillin (84.78%), ciprofloxacin (82.60%), doxycycline (81.52%). *E. faecium* showed resistance mostly (90.32%) to ampicillin, ciprofloxacin, (88%) to erythromycin, and HLG, (83.87%) to doxycycline. All five isolates were 100% sensitive to linezolid. Resistance toward nitrofurantoin was found (4.34%) in *E. faecalis* and (25.80%) in *E. faecium*. *E. raffinosus* was resistant to none of the antibiotics tested [Table/Fig-3].

Antibiotics	*E. faecalis* n=92 (%)	*E. faecium* n=31 (%)	*E. durans* n=3 (%)	*E. hirae* n=1 (%)	*E. raffinosus* n=1 (%)
Ampicillin	78 (84.78)	28 (90.32)	1 (33.33)	1 (100)	0
Erythromycin	80 (86.95)	27 (88)	2 (66.66)	1 (100)	0
Tetracycline	49 (53.26)	23 (74.19)	0	0	0
Ciprofloxacin	76 (82.60)	28 (90.32)	1 (33.33)	1 (100)	0
Doxycycline	75 (81.52)	26 (83.87)	1 (33.33)	0	0
Linezolid	0	0	0	0	0
Nitrofurantoin	4 (4.34)	8 (25.80)	0	0	0
High Level Gentamycin (HLG)	66 (71.73)	27 (88)	0	0	0
Vancomycin	2 (2.17)	9 (29)	0	0	0
Teicoplanin	0	7 (22.58)	0	0	0

[Table/Fig-3]: Antibiotic resistance pattern among enterococcal urinary isolates by disc diffusion method.

[Table/Fig-4] depicts antibiotic resistance pattern of VREs compared to Vancomycin Sensitive Enterococcal (VSE) isolates. All the VRE strains were resistant (100%) to erythromycin, ciprofloxacin and HLG. Around 90% VRE were also resistant to ampicillin and doxycycline. Among VSE strains; 82 (70.08%) were HLG resistant. VSE strains showed highest resistance to erythromycin, ampicillin and ciprofloxacin. All VSE strains were sensitive to teicoplanin.

Antibiotics	VRE n=11 (%)	VSE n=117 (%)
Ampicillin	10 (90.90)	98 (83.76)
Erythromycin	11 (100)	99 (84.61)
Tetracycline	8 (72.72)	64 (54.70)
Ciprofloxacin	11 (100)	95 (81.19)
Doxycycline	10 (90.90)	92 (78.63)
Linezolid	0	0
Nitrofurantoin	6 (54.54)	6 (5.12)
High Level Gentamycin (HLG)	11(100)	82 (70.08)
Teicoplanin	7 (63.63)	0

[Table/Fig-4]: Comparison of resistance pattern between Vancomycin Resistant (VRE) and Vancomycin Sensitive (VSE) Enterococcal urinary isolates.

Out of 128 enterococcal isolates by disc diffusion method, 11 were resistant to vancomycin as such the prevalence of VRE was found to be 8.6%, of which 9 were *E. faecium* and 2 were *E. faecalis*. Vancomycin resistance was not found in other species. Further confirmation of vancomycin-resistant isolates by E-test showed no false susceptibility, as MICs of all 11 isolates were >32 µg/ml. One of the two vancomycin-resistant *E. faecalis* and 8 vancomycin-resistant *E. faecium* isolates had MIC values >256 µg/ml for vancomycin [Table/Fig-5]. Three out of seven *E. faecium* had MIC values >256 µg/ml for teicoplanin [Table/Fig-6]. Seven isolates of *E. faecium* showed Van A phenotype. Four isolates showed Van B phenotype and were distributed among both species equally [Table/Fig-7].

[Table/Fig-5]: Vancomycin MIC >256 µg/ml determined by E-test for Enterococcal isolate. **[Table/Fig-6]:** Teicoplanin MIC >256 µg/ml determined by E-test for enterococcal isolate. (Images from left to right)
MIC: Minimum inhibitory concentration

Species	Vancomycin MIC >32 µg/mL	Teicoplanin MIC >32 µg/mL	Total VRE n=11 Van A[a]	Van B[b]
E. faecalis	2	0	0	2
E. faecium	9	7	7	2

[Table/Fig-7]: Phenotypic speciation of VRE with respect to antibiotic sensitivity of vancomycin and teicoplanin.
[a]Van A phenotype = resistant to both vancomycin and teicoplanin
[b]Van B phenotype = resistant to vancomycin, sensitive to teicoplanin

DISCUSSION

A survey done by the Centers for Disease Control (CDC) and prevention found a twenty-fold increase in VRE cases in a short period [16]. This shows the vulnerability of VRE acquisition and dissemination at a significant rate in healthcare centers, which is a matter of great concern. In our study, the prevalence of VRE was found to be 8.6%, which is similar to the study done from South India by Fernandes SC and Dhanashree B [3]. Although there are studies from India and other countries that showed the prevalence of VRE as low as 0.43% and others as high as 42.9% [Table/Fig-8]

Previous studies	Year of publication	Place of study	Type of samples	Species isolated	VRE n (%)	Genotype[a] and Phenotype[b]	Vancomycin MIC value (µg/mL)
Olawale KO et al., [19]	2011	Nigeria	Blood, urine, sputum, stool, wound swab	*E. faecalis E. faecium*	3 (42.9)	-	-
Sreeja S et al., [20]	2012	Bangalore, India	Urine, pus, tissue, blood, body fluids	*E. faecalis E. faecium*	0	-	-
Praharaj I et al., [4]	2013	Puducherry, India	Blood, pus, CSF, urine, pleural fluid, peritoneal fluid, wound swab	*E. faecalis E. gallinarum E. mundtii*	32 (8.7)	vanA=31 vanC=1 VanA=29 VanB =2 VanC=1	> 8
Jia W et al., [21]	2014	China	Various clinical specimen	*E. faecalis E. faecium E. gallinarum E. casseliflavus E. avium E. raffinosus* and other species	5 (0.43)	VanA=5	-
Goel V et al., [22]	2016	New Delhi, India	Urine	*E. faecalis E. faecium E. gallinarum E. casseliflavus E. avium E. dispar E. pseudoavium*	13 (11.3)	VanA=12 VanB=1	>32
Yadav G et al., [23]	2017	Uttar Pradesh, India	Urine, pus, blood, genital swab, others	*E. faecalis E. faecium E. casseliflavus*	14 (7)	VanA=11 VanB=3	≥ 32

Haghi F et al., [24]	2019	Iran	Urine	*E. faecalis E. faecium* and other species	21 (21)	vanA=10	≥ 32
Saengsuwan P et al., [25]	2021	Thailand	Blood, urine, pus, tissues, body fluid, drain fluid	*E. faecium*	90 (9.6)	vanA=90	>8
Present study	2023	Uttar Pradesh, India	Urine	*E. faecalis E. faecium E. durans E. hirae E. raffinosus*	11 (8.6)	VanA=7 VanB=4	≥ 32

[Table/Fig-8]: Comparison of VRE isolates with other studies.
[a]Genotype=van; [b]Phenotype=Van; CSF: Cerebrospinal fluid

[4,19-25]. Among the enterococcal isolates, *E. faecalis* and *E. faecium* were commonly isolated, with the predominance of the former species. Other rare species such as *E. durans*, *E.hirae*, *and E. raffinosus* were also isolated which corroborates with the findings of other studies done in India and China [21,26]. In a study done from Bangalore by Sreeja S et al., *E. faecalis* and *E. faecium* were found to be the only enterococcal species from various clinical specimens as identification by conventional methods by them may not be able to identify strains with atypical phenotypes [5,20]. This can also be related to different strains circulating in different geographical regions, even study settings, methodologies used, and presentation of cases [2,11,13].

More isolates were from inpatients 112 (87.5%), which seemed to be evidently in excess as compared to outpatients 16 (12.5%), this being in concordance with a study done in Ethiopia, to show hospital-acquired enterococcal infection at a rise [5]. As such, eminent importance should be given to the infection control measures in the hospitals, to recede the disseminating infection [6,14].

In the current study, young patients were found to suffer from enterococcal UTIs. The highest prevalence of 33.6% was seen in the age group 21-40 years, with similar findings recorded in a study done in Nigeria [19]. Our study also showed a male predominance of UTIs which can be related to a major contribution from the urology ward, male-dominated and long-term use of indwelling catheters in these wards leads to the formation of biofilm resulting in persistent infection by drug-resistant microorganisms [2,26].

In the present study, *E. faecium* was found to be more drug-resistant than *E. faecalis* as was evidenced by other study [22]. Among 11 VRE isolated in this study, *E. faecium* 9 (81.81%) was the dominant species, whereas our results were not in congruence with a study from Uttar Pradesh by Yadav G *et al.*, who identified *E. faecalis* to be the only vancomycin-resistant isolate [23]. Vancomycin resistance is less in other enterococcal species [27].

Maximum resistance by enterococcal isolates has been shown against ampicillin, erythromycin, ciprofloxacin, and doxycycline. HLG resistance was seen more in *E. faecium* 27 (88%) than *E. faecalis* 66 (71.73%), with a similar resistance pattern reported from Mumbai by Karmarkar MG *et al.,* [9]. The resistance offered to HLG restricts the use of aminoglycosides for resistant enterococci, thus leaving the therapeutic realm with very few drug options.

Both isolates *E. faecalis* and *E. faecium* displayed low resistance to nitrofurantoin (4.34%) and (25.80%) respectively. This makes nitrofurantoin to be a pertinent option for treating VRE UTI, as it attains good concentration levels in urine, which is in agreement with the recommendations by other researchers [23,28]. None of the *Enterococcal* spp. in our study, showed resistance to linezolid, which is incomparable with the study from North-East India reporting a resistance of 4.5% [29]. Linezolid can be preserved for use in VRE UTI in intricate cases such as complicated renal diseases and urosepsis [15]. While we compared the resistance profile of VRE with vancomycin-sensitive enterococci and it was noteworthy to find that all VRE (100%) were resistant to erythromycin, ciprofloxacin and HLG, which may be due to unusual ability of enterococci to acquire resistance [6]. Other authors from Bihar have reported maximum resistance to pencillin (100%), Ampicillin (91.65) and piperacillin (75.0%) among VRE isolates [13].

Incompatible antibiotic resistance patterns could be due to compliance with infection control measures and monitoring techniques for the detection of VRE, surveillance for colonisation, and hand-washing practice [10]. This is also impacted by regional antimicrobial practice and screening patterns, and isolation policies of the healthcare facilities. Among the 11 VRE, 9 isolates in our study showed a high level of resistance to vancomycin (MIC >256 µg/ml) which can be compared to the study done in Kuwait [30]. This study shows that 7 (63.63%) of VRE isolates had Van A phenotype, with similarly high rates of Van A phenotype reported from South India by Praharaj I *et al.,* [4]. The reliability of methodologies put into use in different laboratories as per their resources, ease, and testing frequency indicate a need for further testing before establishing results, as such, the findings of our study must be inspected with caution and therefore are not generalisable.

Limitation(s)

There are a few limitations to our study. Firstly, in this study, the molecular approach for definitive speciation was not done due to limited resources. Secondly, enterococcal isolates from samples other than urine might have provided more pertinent data. However, these presumptive results can help clinicians in the empirical choice of drugs for treating VRE.

CONCLUSION(S)

This study reveals an 8.6% prevalence of VRE UTI however, which can be a subtle signal for the emergence of VRE demanding VRE surveillance in healthcare facilities. Moreover, *Enterococcal* spp. with its apt ability to acquire and transmit antimicrobial resistance along with insufficient treatment options is a threat to the ecosystem. We reported increased drug resistance by Enterococcal species especially by *E. faecium*. All this puts pressure on strict compliance of multidimensional approach with collaboration of antibiotic stewardship, educational and surveillance programs. Aggressive and active infection control measures need to be proposed to contain the spread of VRE. Nitrofurantoin may be considered a choice for treating uncomplicated infection guided by the regional patterns of drug resistance.

Nevertheless, further studies should be done using molecular diagnostics together with phenotypic modalities which can facilitate inaccurate and definite detection of VRE, also identifying highly heterogeneous strains. Adequate measures must be taken to contain, resistant enterococcal strains from dissemination, to prevent outbreak like situation in hospitals worldwide.

REFERENCES

[1] Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol. 2019;10:539.
[2] Giannakopoulos X, Sakkas H, Ragos V, Tsiambas E, Bozidis P, Evangelou AM, et al. Impact of enterococcal urinary tract infections in immunocompromised-neoplastic patients. J BUON. 2019;24(5):1768-75.
[3] Fernandes SC, Dhanashree B. Drug resistance and virulence determinants in clinical isolatesof Enterococcus species. Indian J Med Res. 2013;137(5):981-85.
[4] Praharaj I, Sujatha S, Parija SC. Phenotypic and genotypic characterisation of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J Med Res. 2013;138(4):549-56.
[5] Ferede ZT, Tullu KD, Derese SG, Yeshanew AG. Prevalence and antimicrobial susceptibility pattern of Enterococcus species isolated from different clinical samples at Black Lion Specialized Teaching Hospital, Addis Ababa, Ethiopia. BMC Res Notes. 2018;11(1):793.
[6] Moellering RC. Vancomycin-resistant enterococci. Clin Infect Dis. 1998;26(5):1196-99.

[7] Barros M, Martinelli R, Rocha H. Enterococcal urinary tract infections in a university hospital: clinical studies. Braz J Infect Dis. 2009;13(4):294-96.
[8] Bitsori M, Maraki S, Raissaki M, Bakantaki A, Galanakis E. Community-acquired enterococcal urinary tract infections. Pediatr Nephrol. 2005;20(11):1583-86.
[9] Karmarkar MG, Gershom ES, Mehta PR. Enterococcal infections with special reference to phenotypic characterisation and drug resistance. Indian J Med Res. 2004;119:22-25.
[10] Gold HS. Vancomycin-resistant enterococci: mechanisms and clinical observations. Clin Infect Dis. 2001;33(2):210-19.
[11] Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000;13(4):686-707.
[12] Zhu W, Clark N, Patel JB. pSK41-like plasmid is necessary for Inc18-like vanA plasmid transfer from Enterococcus faecalis to Staphylococcus aureus in vitro. Antimicrob Agents Chemother. 2013;57(1):212-19.
[13] Biswas PP, Dey S, Adhikari L, Sen A. Detection of vancomycin resistance in enterococcus species isolated from clinical samples and feces of colonised patients by phenotypic and genotypic methods. Indian J Pathol Microbiol. 2016;59(2):188-93.
[14] Ostrowsky BE, Trick WE, Sohn AH, Quirk SB, Holt S, Carson LA, et al. Control of vancomycin-resistant enterococcus in healthcare facilities in a region. N Engl J Med. 2001;344(19):1427-33.
[15] Toner L, Papa N, Aliyu SH, Dev H, Lawrentschuk N, Al-Hayek S. Vancomycin resistant enterococci in urine cultures: Antibiotic susceptibility trends over a decade at a tertiary hospital in the United Kingdom. Investig Clin Urol. 2016;57(2):129-34.
[16] Centers for Disease Control and Prevention (CDC). Nosocomial enterococci resistant to vancomycin--United States, 1989-1993. MMWR Morb Mortal Wkly Rep. 1993;42(30):597-99.
[17] Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J Clin Microbiol. 2002;40(5):1681-86.
[18] Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility* Testing, 30th ed. CLSI supplement M100. Wayne, USA: CLSI; 2020.
[19] Olawale KO, Fadiora SO, Taiwo SS. Prevalence of hospital-acquired enterococci infections in two primary-care hospitals in osogbo, south-western Nigeria. Afr J Infect Dis. 2011;5(2):40-46.
[20] Sreeja S, Babu PRS, Prathab AG. The prevalence and the characterisation of the enterococcus species from various clinical samples in a tertiary care hospital. J Clin Diagn Res. 2012;6(9):1486-88.
[21] Jia W, Li G, Wang W. Prevalence and antimicrobial resistance of Enterococcus species: a hospital-based study in China. Int J Environ Res Public Health. 2014;11(3):3424-42.
[22] Goel V, Kumar D, Kumar R, Mathur P, Singh S. Community acquired enterococcal urinary tract infections and antibiotic resistance profile in north India. J Lab Physicians. 2016;8(1):50-54.
[23] Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and vancomycin resistant enterococci: a therapeutic problem. J Clin Diagn Res. 2017;11(8):GC07-GC11.
[24] Haghi F, Lohrasbi V, Zeighami H. High incidence of virulence determinants, aminoglycoside and vancomycin resistance in enterococci isolated from hospitalised patients in Northwest Iran. BMC Infect Dis. 2019;19(1):744.
[25] Saengsuwan P, Singkhamanan K, Madla S, Ingviya N, Romyasamit C. Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* clinical isolates in a tertiary care hospital in southern Thailand: a retrospective study. Peer J. 2021;9:e11478.
[26] Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterised patients. Indian J Med Microbiol. 2001;19(3):132-37.
[27] Brinkwirth S, Ayobami O, Eckmanns T, Markwart R. Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. Euro Surveill. 2021;26(45):2001628.
[28] Gardiner BJ, Stewardson AJ, Abbott IJ, Peleg AY. Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. Aust Prescr. 2019;42(1):14-19.
[29] Phukan C, Lahkar M, Ranotkar S, Saikia KK. Emergence of vanA gene among vancomycin-resistant enterococci in a tertiary care hospital of North-East India. Indian J Med Res. 2016;143(3):357-61.
[30] Udo EE, Al-Sweih N, Phillips OA, Chugh TD. Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. J Med Microbiol. 2003;52(Pt 2):163-68.

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