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Study of vancomycin and high-level aminoglycoside-resistant *Enterococcus* species and evaluation of a rapid spot test for enterococci from Central Referral Hospital, Sikkim, India

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Abstract:

BACKGROUND: Enterococcus is an important pathogen, and with its emergence of resistance to multiple antimicrobials, the management of infection is becoming increasingly difficult.

AIM: The aim of the study is to determine the prevalence, antibiotic resistance, and risk factors associated with enterococcal infection or colonization.

MATERIALS AND METHODS: In this prospective study, samples from inpatients were screened for resistant enterococci. Antibiotic susceptibility testing was performed using the disc diffusion method and minimum inhibitory concentration by the agar dilution method. A modification of a test tube method of sodium chloride-esculin hydrolysis to a spot test was evaluated for its rapidity and reliability in the presumptive diagnosis of enterococci.

STATISTICAL ANALYSIS USED: Fisher's exact test was used for continuous (Student's *t*-test) and categorical variables. Multivariate analysis was performed with logistic regression using IBM SPSS 20.0 software (Armonk, NY, USA).

RESULTS: *Enterococcus* species were isolated from 182 samples: *Enterococcus faecalis* (68.7%), *Enterococcus faecium* (20.9%), *Enterococcus gallinarum* (6%), and *Enterococcus durans* (4.4%). Maximum resistance was to ciprofloxacin (59.3%) and least to linezolid (0.5%). The isolation rate of vancomycin-resistant enterococci (VRE) was 13.7%; 30.2% and 20.9% were of high-level gentamicin and streptomycin, respectively. All 182 *Enterococcus* species gave positive results within 30–60 min by the rapid spot test.

CONCLUSIONS: Overall, high-level aminoglycoside resistance (HLAR) was observed more than glycopeptide resistance. Surveillance strategies need to be upgraded and implemented in order to prevent the emergence and further spread of not only VRE but also HLAR enterococci in the hospital. The spot test gave reliable and rapid results in presumptive identification of enterococci.

Key words:

Enterococcus, high-level aminoglycoside resistance, rapid spot test, vancomycin-resistant enterococci

Introduction

Enterococcus species which forms part of the commensal flora of gastrointestinal tracts of humans, avian and veterinary origins

are also known to be pathogenic organisms of medical importance.^[1-3] They have the ability to acquire resistance (plasmid mediated) and are intrinsically resistant to commonly used antibiotics (such as clindamycin, cephalosporins, low-level aminoglycoside, and co-trimoxazole).

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Hence, enterococci are often considered as a “pathogen in training.”^[2,4,5]

Enterococcus is a ubiquitous organism; often, the ecology depends on how actively enterococci are distributed and controlled.^[2,6] In Europe, the reservoirs of resistant enterococci, particularly vancomycin-resistant enterococci (VRE), shifted from a veterinary to a community-acquired ecology whereas, in the USA, the source is often the nursing home/hospital environment.^[2] However, in India, the source of resilient enterococci is not well-defined. Similarly, in this region, there is a paucity of information on the prevalence of enterococci in hospitals as well as community-acquired settings. The aim of this study was to actively screen and identify the isolated *Enterococcus* species and determine its antimicrobial resistance pattern at a tertiary hospital in East Sikkim. The objective of this study was to detect glycopeptide-resistant and high-level aminoglycoside-resistant (HLAR) enterococci among patients and analyze the risk factors associated with infection or colonization. In addition, we sought to assess an in-house rapid spot test for the presumptive identification of enterococci (modification of a test first described by Qadri *et al.*) and its ability to distinguish enterococci from similar streptococci (catalase-negative Gram-positive cocci).^[7]

Materials and Methods

Study design

This is a cross-sectional study (prevalence study) conducted in the Department of Microbiology, Central Referral Hospital (CRH), East Sikkim, India, from November 2016 to April 2018. The study was reviewed and approved by the Institution Ethics Committee.

Study population

All patients admitted to CRH during the study period whose samples were sent to the Microbiology Department for other investigations were analyzed.

Methodology

The patients enrolled in the study were those (a) with fever $>38^{\circ}\text{C}$, (b) without fever, and (c) who developed a fever during their hospital stay. The diagnosis of infection was based on the guidelines formulated by the Centers for Disease Control and Prevention.^[8] The diagnosis of enterococcal infection was established when at least three criteria were met: (a) positive culture, (b) clinical signs and symptoms of fever $>38^{\circ}\text{C}$, (c) >10 leukocytes per high-power field in a preliminary Gram staining report, and/or (d) white blood cell >12000 or <4000 cells/ mm^3 .

Nosocomial infection was defined as an infection occurring in patients with >48 h of hospital stay or infection in those

with a history of recent hospitalization (2 weeks). Patients with a positive culture without signs and symptoms of infection were deemed to be colonizers.

Detailed history pertaining to demography, immune status (comorbidities and immunosuppressive therapy), antibiotic therapy, location of patient, duration of stay, invasive procedures (such as Foley’s catheter and central venous catheters), or surgery and history of recent hospitalization or intensive care unit (ICU) stay (≤ 30 days ago) were recorded for samples positive for enterococci. Length of hospital stay and assessment of clinical outcomes were recorded from the day of admission till discharge or death. Primary and secondary bloodstream infections (BSI) were defined accordingly.^[8] To overcome repetitive sampling, >1 *Enterococcus* isolates of the same patient but from different sites and on multiple occasions were considered as a single sample, i.e., only the first isolate was considered.

Microbiological sample processing

All samples from inpatient wards, sent for culture to the Department of Microbiology, were selected according to the criteria proposed in the study methodology. For blood culture, paired samples were inoculated in blood culture bottles (BacT/Alert FA Plus for adults and PF Plus for pediatric patients) and cultured in BacT/Alert systems (bioMérieux, France) for a period of at least 7 days. For urine culture, colony counts of $\geq 10^5$ colony-forming units per ml (CFU/ml) were evaluated. Selected samples were cultured in conventional media and screened for presumptive vancomycin resistance on a VRE screen agar and prepared using bile esculin azide agar supplemented with 6% (6 $\mu\text{g}/\text{ml}$) vancomycin (HiMedia Laboratories, Mumbai, India).^[4,9,10] Identification of *Enterococcus* species by conventional biochemical reactions (growth on potassium tellurite agar, 6.5% sodium chloride (NaCl) broth, 1% sugar fermentation tests, esculin hydrolysis, and arginine hydrolysis) and results interpreted by the identification scheme proposed by Facklam and Collins.^[2,3,11]

Antibiotic susceptibility testing

Antimicrobials against enterococci were tested by the Kirby–Bauer disc diffusion method with antibiotic discs (HiMedia Laboratories, Mumbai, India) for ampicillin (10 μg), ciprofloxacin (5 μg), vancomycin (30 μg), teicoplanin (30 μg), high-level gentamicin (HLG, 120 μg), high-level streptomycin (HLS, 300 μg), and linezolid (30 μg). Nitrofurantoin (300 μg) was tested for urine isolates only. Minimum inhibitory concentration (MIC) for glycopeptides was determined by the agar dilution method on brain–heart infusion agar (MIC: 0.125–256 $\mu\text{g}/\text{ml}$). Isolated organisms on 6% VRE screen agar were tested for their breakpoint MIC values by the vancomycin agar dilution method.

Test for detection of HLAR by the agar dilution method was done using gentamicin (500 µg/ml) and streptomycin (2000 µg/ml). In all the agar dilution methods, the presence of >1 colony indicated resistance. All the MIC determinations were performed as per the guidelines set by the Clinical and Laboratory Standards Institute (CLSI-M100S, 26th edition).^[10] The reference strains of *Enterococcus faecalis* ATCC[®] 29212[™] (sensitive) and ATCC[®] 51299[™] (resistant) were used as control while testing the antimicrobial susceptibility testing (AST) and MIC values.^[10] VRE and high-level gentamicin resistance (HLGR) findings by agar dilutions were also consistent with VITEK 2 systems reporting, using VITEK 2 GP and VITEK 2 AST-P628 cards (bioMérieux, France). VITEK 2 AST-P628 cards do not report HLS findings. Therefore, a comparison with this card could not be ascertained.

Sodium chloride-esculin hydrolysis rapid spot test

The medium was prepared as per the formulation of Qadri *et al.* who described it as a tube test method, but an impregnated filter paper method is evaluated in this study.^[7] The test solution is a 0.2% esculin and 5% NaCl medium, composed of 2 g esculin (HiMedia Laboratories, Mumbai, India), 0.5 g ammonium ferric citrate, 50 g NaCl, 0.4 g K₂HPO₄, and 0.1 g KH₂PO₄ in 1000 ml distilled water (pH 5.6 ± 0.2). About 100 ml was prepared at a time. The precipitate that formed when stored at 4°C –6°C went into solution when lightly heated. A 1.8 cm × 1.8 cm of Whatman filter paper (no. 2) was cut to fit a standard microscope. About 0.25 ml of solution was pipetted over the paper, and a colony of Gram-positive catalase-negative cocci, from a 24-h culture in blood agar, was rubbed in the center of the square. The slides were placed in an 11 cm petri dish, atop supporting glass rods, and incubated aerobically at 37°C for at least 30 min. To avoid drying of the paper during incubation, 2–5 ml water was added to the dish. The appearance of black color over the spot-inoculated area indicated a positive response. In this test, *Enterococcus* species and *Streptococcus* species were evaluated with the positive control (*E. faecalis* ATCC[®] 29212[™] or 51299[™]) and negative control (*Staphylococcus aureus*). Validation

of the spot test was done by comparing it with the growth of *Enterococcus* species in bile esculin azide (BEA) agar and 6.5% NaCl broth.

Statistical analysis

Continuous values were expressed as mean ± standard deviation (SD) and compared using the Student's *t*-test. Categorical values were assessed for key variables with the GraphPad software (San Diego, CA, USA) (risk ratio with 95% confidence interval [CI]). Multiple independent variable analysis was performed using binary logistic regression using IBM Corp. SPSS statistics for Windows, version 20.0 (Armonk, NY, USA). Logistic regression was done to ascertain the effects of antibiotic exposure, hospital-related, host-related, and outcome-related factors on the likelihood of acquiring resistant enterococcal infection or colonization. All the tests were two-tailed, and *P* ≤ 0.05 was considered statistically significant.

Results

During the study period, a total of 3208 selected samples were screened and 182 *Enterococcus* species (5.7%) were isolated. The overall mean age of the study population was 33.29 ± 18.25 years (SD); 96 (52.7%) were male and 86 (47.3%) female. Four *Enterococcus* species were isolated and identified as *E. faecalis* (125, 68.7%), *Enterococcus faecium* (38, 20.9%), *Enterococcus gallinarum* (11, 6.04%), and *Enterococcus durans* (8, 4.4%) [Table 1]. A total of 27.5% (50/182) enterococcal infections were from new admissions (40 – urinary tract infection [UTI] and 10 – pus), 20.9% (38/182) developed nosocomial infection (12 – BSI and 26 UTI), and 51.6% (94/182) were potential colonizers (44 – urine, 3 – pus, and 47 – respiratory secretions).

Overall, antibiogram of the isolates showed a high resistance to ciprofloxacin at 59.3% (108/182), followed by ampicillin at 53.8% (98/182), HLG at 34.1% (62/182), HLS at 26.9% (49/182), vancomycin at 14.3% (26/182), teicoplanin at 9.9% (18/182), and linezolid at 0.5% (1/182); urine isolates showing resistance to nitrofurantoin were 28.2% (31/110) [Figure 1]. Bar diagram represents

Table 1: Isolation of *Enterococcus* species from clinical samples

Sample (%)	Bacterial isolates			
	<i>Enterococcus faecalis</i> (%)	<i>Enterococcus faecium</i> (%)	<i>Enterococcus gallinarum</i> (%)	<i>Enterococcus durans</i> (%)
Urine: 110 (60.4)	81 (73.6)	19 (17.3)	7 (6.4)	3 (2.7)
Blood: 12 (6.6)	8 (66.7)	4 (33.3)	0 (0)	0 (0)
Respiratory fluids: 47 (25.8)	29 (61.7)	12 (25.5)	3 (6.4)	3 (6.4)
Sputum	20	5	3	2
Throat swab	6	5	0	0
Endotracheal tube	3	2	0	1
Pus: 13 (7.1)	7 (53.8)	3 (23.1)	1 (7.7)	2 (15.4)
Total: 182	125 (68.7)	38 (20.9)	11 (6.04)	8 (4.4)

Figures in parentheses are in percentages

the percentage of antimicrobial resistance of the four *Enterococcus* species.

By the agar dilution methods, enterococcal resistance to vancomycin was 13.7% (25/182) and for teicoplanin 10.9% (20/182). Resistance to glycopeptides was seen highest in *E. faecium*, followed by *E. faecalis* and *E. gallinarum*. Two isolates of *E. faecium* had MIC values at 64 and 128 µg/ml, indicating high resistance. Overall, HLGR and high-level streptomycin resistance (HLSR) were 30.2% (55/182) and 20.9% (38/182), respectively, seen highest among strains of *E. faecalis*, followed by *E. faecium*, *E. gallinarum*, and *E. durans* [Table 2]. HLAR was recorded in all VRE isolates except *E. durans*. HLGR and HLSR among vancomycin-sensitive (VS)

isolates were 22.7% (27/119) and 12.6% (15/119) in VS *E. faecalis*, 23.1% (6/26) and 11.5% (3/26) in VS *E. faecium*, respectively, and only 25% (2/80) HLGR in VS *E. durans*. No HLAR was observed in VS *E. gallinarum*.

Rapid sodium chloride-esculin hydrolysis spot test

A total of 230 isolates of enterococci and streptococci were tested [Table 3]. All the 182 enterococcal isolates were positive by the rapid spot test within 30–60 min [Figure 2]. All spot test-positive enterococcal results were positive for growth in BEA agar. Streptococci that did not grow in BEA agar were negative by this rapid spot test. However, two streptococci (with growth on BEA agar) gave positive spot reactions 24 h later. Therefore, to avoid false-positive results, the test was discarded after 90 min.

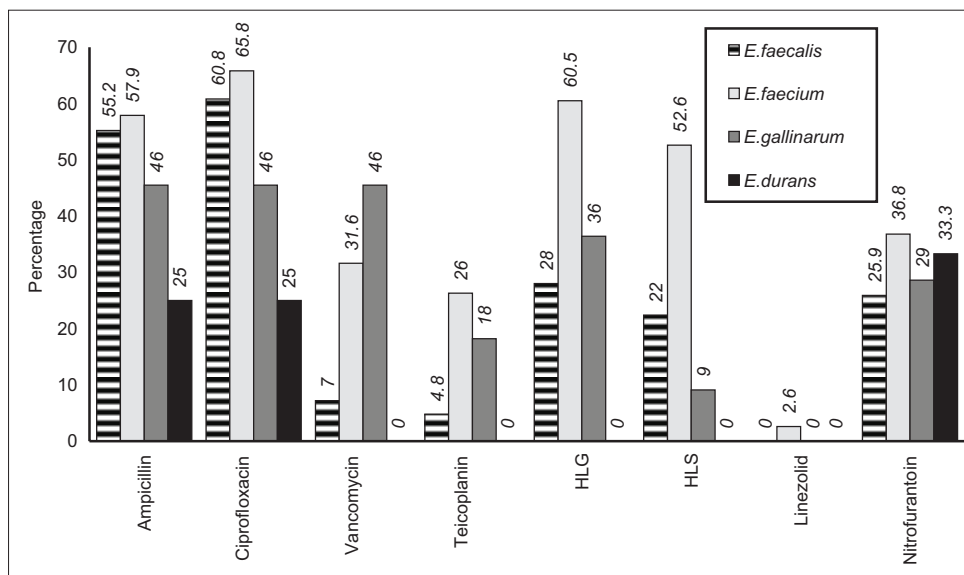


Figure 1: Percentage of resistant *Enterococcus* species by disc diffusion method

Table 2: Minimum inhibitory concentration values of resistant *Enterococcus* species (n=182)

Isolates	Vancomycin	Teicoplanin	HLG	HLS
<i>Enterococcus faecalis</i> (n=125)	6 (24)	8 (40)	33 (60)	21 (55.3)
<i>Enterococcus faecium</i> (n=38)	12 (48)	9 (45)	18 (32.7)	15 (39.5)
<i>Enterococcus gallinarum</i> (n=11)	7 (28)	3 (15)	2 (3.6)	2 (5.3)
<i>Enterococcus durans</i> (n=8)	0 (0)	0 (0)	2 (3.6)	0 (0)
Total	25 (13.7%)	20 (10.9%)	55 (30.2%)	38 (20.9%)

Results in parentheses indicate percentage. MIC determined by agar dilution method. HLG=High-level gentamycin (500 µg/ml), HLS=High-level streptomycin (2000 µg/ml). Range of glycopeptide MIC: 0.125 to 256 µg/ml. Breakpoints for vancomycin: [S]: ≥ 4 µg/ml; [I]: 8-16 µg/ml; [R]: 8-16µg/ml. Breakpoints for teicoplanin: [S]: ≥8 µg/ml; [I]: 16 µg/ml; [R]: 16 µg/ml. MIC=Minimum inhibitory concentration

Table 3: Rapid NaCl-esculin hydrolysis spot test

Organism	Number of isolates	Number positive				
		30 minutes	1 h	2 h	4 h	24 h
<i>Enterococcus</i> species ^a	182	74	108	-	-	-
Bile esculin-positive streptococci ^b	15	0	0	0	0	2
<i>Streptococcus pyogenes</i>	8	0	0	0	0	0
<i>Streptococcus pneumoniae</i>	5	0	0	0	0	0
Viridans streptococci	20	0	0	0	0	0

^aAll *Enterococcus* spp. were positive by both conventional bile-esculin azide agar culture and rapid spot test, ^bBile esculin-positive streptococci were positive by conventional bile-esculin azide agar culture. Two isolates were positive by the rapid spot test at 4 h and three-24 h later

Results of Statistical analysis

In univariate analysis, risk factors for colonization or infection by enterococci were in patients with bacteremia, skin and soft-tissue infections, indwelling intravenous



Figure 2: Sodium chloride-esculin spot test. Filter paper impregnated with sodium chloride-esculin solution and placed on a standard microscope slide. Top slide: Negative control used is *Staphylococcus aureus*. Positive control used are *Enterococcus faecalis* ATCC® 29212™ and a known *Enterococcus faecalis* (VITEK 2 systems identified). 2nd and 3rd slide: Positive reactions are indicated by the inoculation spot turning black 30–60 minutes later

catheters, hospitalization (≤ 30 days ago), exposure to vancomycin, and multiple antibiotics [Table 4]. Patients were at greater risk of infections or colonization by VRE, HLGR enterococci, and HLSR enterococci in the presence of indwelling intravenous catheters (relative risk [RR]: 2.96, 95% CI: 1.95–4.49; RR: 2.59, 95% CI: 1.66–4.07; and RR: 2.13, 95% CI: 1.35–3.35, respectively) and in those using multiple antibiotics (RR: 2.39, 95% CI: 1.82–3.15; RR: 1.97, 95% CI: 1.43–2.72; and RR: 1.64, 95% CI: 1.18–2.30, respectively). Acquiring VRE and HLGR enterococci were associated with patients who had vancomycin exposure (VRE – RR: 3.14, 95% CI: 1.41–7.01 and HLGR enterococci – RR: 4.95, 95% CI: 2.14–11.46), and the use of aminoglycoside posed a greater risk of acquiring HLGR enterococci (RR: 1.9, 95% CI: 1.23–2.96).

Outcome related

Prolongation of hospitalization (due to complications during hospital stay) was seen in 16% of patients with VRE (RR: 4.19, 95% CI: 1.27–13.79), 12.7% with HLGR enterococci (RR: 5.39, 95% CI: 1.45–20.07), and 13.2% with HLSR enterococci (RR: 3.16, 95% CI: 1.02–9.79). Among them, one patient who had an isolate resistant to all the antibiotics tested died due to functional status deterioration [Table 4].

Table 4: Univariate analysis of risk factors association with various resistant *Enterococcus*

Variables	VRE isolates (n=25)	P	HLGRE isolates (n=55)	P	HLSRE isolates (n=38)	P
Age, mean±SD	36.7±21.9	0.29	35.4±18.9	0.30	36.3±19.5	0.25
Source of sample related						
Blood samples	8 (4.3)	<0.0001*	11 (6.04)	<0.002*	7 (3.8)	0.003*
Urine samples	10 (5.5)	0.07	21 (11.5)	0.0008*	17 (9.3)	0.05*
Respiratory samples	3 (1.6)	0.14	14 (7.7)	0.94	8 (4.3)	0.46
Pus samples	4 (2.2)	0.045*	9 (4.9)	0.004*	6 (3.3)	0.03*
Host related						
Diabetes mellitus	9 (4.9)	0.027*	9 (4.9)	0.39	10 (5.5)	0.20
Surgical procedure	8 (4.3)	0.27	23 (12.6)	0.0002*	13 (7.1)	0.07
Gastrointestinal disease	3 (1.6)	0.49	5 (2.7)	0.939	3 (1.6)	0.73
Skin and soft tissue infection	4 (2.2)	0.019*	6 (3.3)	0.046*	5 (2.7)	0.03*
Antibiotics related						
Multiple antibiotics ^a	21 (11.5)	<0.0001*	35 (19.2)	<0.0001*	23 (12.6)	0.004*
Vancomycin	7 (3.8)	0.005*	15 (8.2)	0.0002*	10 (5.5)	0.13
Aminoglycosides	5 (2.7)	0.39	24 (13.2)	0.0038*	8 (4.3)	0.24
Cephalosporin	9 (4.9)	0.14	16 (8.8)	0.36	16 (8.8)	0.003*
Anaerobic drugs	5 (2.7)	0.24	23 (12.6)	0.053	13 (7.1)	0.66
Hospital related						
Recent hospitalization (≤ 30 days)	13 (7.1)	<0.0001*	14 (7.7)	0.12	12 (6.6)	0.02*
Recent ICU stay (≤ 30 days)	4 (2.2)	0.27	6 (3.3)	0.76	3 (1.6)	0.65
Mechanical ventilator use	3 (1.6)	0.38	4 (2.2)	0.89	2 (1.1)	0.54
Indwelling intravenous catheter	3 (1.6)	<0.0001*	27 (14.8)	<0.0001*	18 (9.9)	0.001*
Indwelling urinary catheter	13 (7.1)	0.12	22 (12.1)	0.86	19 (10.4)	0.10
Outcome related						
Length of hospital stay (days)	7.4±5.9	0.018*	9.2±4.1	0.0009*	9.6±4.6	0.0004*
Prolongation of hospital stay ^b	4 (2.2)	0.02*	7 (3.8)	0.01*	5 (2.7)	0.046*
Death	1 (0.5)	0.07	1 (0.5)	0.24	1 (0.5)	0.67

Results in parentheses indicate overall percentage of infection. ^aMultiple antibiotic included >2 antibiotics, ^bProlongation of hospital stay of ≥ 14 days due to complications; *Significant value ($P \leq 0.05$). VRE=Vancomycin-resistant enterococci, HLGRE=High-level gentamicin-resistant enterococci, HLSRE=High-level streptomycin-resistant enterococci, SD=Standard deviation, ICU=Intensive care unit

In Table 5, the logistic regression analysis revealed that the presence of an indwelling intravenous catheter was the common independent risk factor associated with resistant enterococcal infection or colonization. Antibiotic usage such as vancomycin was more likely to predispose the patients at risk to acquire HLAR enterococci than VRE, whereas multiple antibiotic uses were the risk factors associated with VRE and HLSR enterococci. History of recent hospitalization was a risk factor associated with VRE, and the presence of indwelling urinary catheter was associated with VRE and HLSR enterococci.

Discussion

In this study, the prevalence of enterococcal infection was 2.7% (88/3208). Isolation of vancomycin resistance, high-level gentamycin and high-level streptomycin resistance among the *Enterococcus* isolated was 13.7% (25/182), 30.2% (55/182), and 20.9% (38/182), respectively. All the enterococci causing bacteremia were hospital acquired, and the presence of indwelling intravenous catheter was a common independent risk factor predisposing the patients to resistant enterococci.

In this study, a significant risk of acquiring resistant enterococci, among hospitalized patients, is attributed to exposure to multiple antibiotics, and the majority of isolates showed >30% resistance to high-level aminoglycosides *in vitro*. HLGR was observed more than HLSR in both groups of VRE and VSE, especially among *E. faecalis* which is similar to a study by Hayakawa *et al.*^[12] Similarly, high percentages of HLAR were also reported in other studies.^[13-15] In this study, *E. faecium* was the predominant VRE isolate (48%), which is in agreement with similar studies.^[16-18] On the contrary, *E. faecalis* as the predominant VRE have been reported in other studies.^[14,19,20] Overall, isolation of HLAR was more than VRE in our study, similar to findings in other studies.^[14,15]

Studies on the prevalence of drug-resistant enterococci are mostly hospital based, and the implication of disease burden in the community extrapolated.^[2-4,6] The clinical significance of VRE is often difficult to

ascertain (e.g., isolation from healthy individuals or when recovered in mixed cultures with other pathogens) as false-negative results confound a diagnosis.^[4,6,21,22] Moreover, selective identification of enterococci from only sterile body sites often overlooks the possibility of drug-resistant enterococcal colonization from other sources, thereby underestimating the burden and potential transmissibility of resistant enterococci in the facility.^[6,23]

Although the rate of asymptomatic gastrointestinal colonization by enterococci far exceeds the rate of infection, the role of colonizers in nosocomial infections is well documented.^[2,4-6] Gut colonization, by resilient enterococci, persists for months to years, and patients often have the same organism colonizing their skin.^[4] Transmission in a hospital environment can occur readily from such expanded reservoir, particularly of resistant enterococci, which explains the significant findings from our study in patients with the presence of catheters *in situ*, history of recent hospitalization, and increase in the duration of hospital stay by the univariate analysis.^[5,6] Moreover, in the multivariable analysis of independent risk factors, indwelling catheters and recent hospitalization history were found to be associated with acquiring resistant enterococci. However, in this study, increase in the duration of hospital stay (>7 days) and history of recent ICU stay did not reveal any significant results (by the multivariable analysis) which are in contrast to other studies.^[12,19,23,24] Studies have implicated longer duration of hospital stay to increased risk of acquiring resistant enterococci, as there are higher chances of prolongation or receiving multiple antibiotics and longer exposure time for transmission.^[19,24]

Various methods of enterococcal identification range from 4 to 48 hours to interpret; these are either expensive, labour-intensive or time-consuming. The NaCl-esculin hydrolysis test is a method of rapid identification with a turnaround time of 1–2 hours by the test tube method, but by the filter paper method, a positive reaction was seen within 30–60 minutes.^[7] The principle behind the test is that only those organisms that hydrolyze esculin and survive the 5% NaCl environment will give a positive

Table 5: Independent risk factors associated with infection or colonization by resistant enterococci (binary logistic regression)^a

Variables	VRE		HLGRE		HLSRE	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Multiple antibiotic exposure	34.56 (9.3-128.7)	<0.0001*	2.11 (0.8-5.5)	NS	4.77 (1.7-13.3)	0.003*
Vancomycin	1.73 (0.5-6.5)	NS	3.82 (1.3-11.1)	0.013*	5.07 (1.4-18.9)	0.016*
Recent hospitalization	10.69 (2.8-40.4)	<0.0001*	1.42 (0.6-3.5)	NS	2.29 (0.9-6.02)	NS
Indwelling intravenous catheter	61.35 (7.9-474.1)	<0.0001*	5.32 (2.2-13.01)	<0.0001*	5.37 (1.9-15.2)	0.001*
Indwelling urinary catheter	12.49 (2.1-73.6)	0.005*	1.84 (0.8-4.1)	NS	3.50 (1.3-9.2)	0.011*

^aFactors associated with risk for acquiring resistant enterococci compared with sensitive enterococci, *Significant value ≤0.05. NS=Not significant, CI=Confidence interval, VRE=Vancomycin-resistant enterococci, HLGRE=High-level gentamicin-resistant enterococci, HLSRE=High-level streptomycin-resistant enterococci. OR (95% CI)=Odds ratio (95% CI)

reaction, for example, salt tolerant, bile esculin-positive *Enterococcus* species.^[7] Validation by comparison of the spot test with the growth of enterococci on BEA agar was because both the tests have a similar composition. However, false positive with other organisms which hydrolyze esculin should be excluded.^[25] Therefore, identification of *Enterococcus* species must still be confirmed by other methods. Nonetheless, laboratories seeking a presumptive identification of enterococci, to distinguish it from other streptococci, could utilize this method of NaCl-esculin hydrolysis spot test. The test is relatively inexpensive and rapid and gave reliable results (among Gram-positive catalase-negative cocci).

The major limitation of this study is that only the samples of hospitalized patients were taken into consideration and hence may not be representative of organisms in the community. However, the risk of enterococcal infections/colonization is increased in a hospital due to factors, namely prevalent resilient organisms, instrumentation, indwelling catheters, and decreased host immune response, and accordingly, our study results are still meaningful and relevant.^[12,17,19]

Conclusions

To prevent nosocomial transmission of resistant enterococci, the judicious use of antibiotics, handwashing of health-care providers, isolation wards for VRE confirmed patients, and surveillance strategies have to be implemented in hospital infection control methodology.

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Conflicts of interest

There are no conflicts of interest.

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