Virulence Markers of Vancomycin Resistant Enterococci Isolated from Infected and Colonized Patients

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ABSTRACT

Background: The aim of study was to find out the potential pathogenic role of virulence factors elaborated by strains of vancomycin resistant enterococci (VRE) isolated from clinical samples and VRE colonizing the gastrointestinal tract of hospitalized patients. **Materials and Methods:** Enterococci were isolated from various clinical samples and also from fecal specimens of colonized patients at the time of admission, after 48 h and after 5 days of admission. Various virulence determinants were detected by phenotypic tests. Vancomycin susceptibility in enterococci was detected by disc diffusion and agar screen method. Minimum inhibitory concentration was determined by agar dilution method. **Results:** Out of all the clinical and fecal samples processed, 12.0% isolates were either vancomycin resistant or vancomycin intermediate. Hemagglutinating activity against rabbit red blood cells was seen with 27.8% and 25.0% of clinical and fecal strains, respectively. Slime layer formation was seen with fecal VRE strains (37.5%) when compared to clinical VRE (27.8%). Among the clinical VRE strains the most prolific biofilm producers were *Enterococcus. fecalis* (92.9%) when compared to *Enterococcus. faecium* (52.9%). Biofilm formation/(presence of adhesions) was also seen in (29.2%) of the fecal VREs. In wound infection production of gelatinase, deoxyribonuclease (DNase), and caseinase (70.0% each) were the major virulence factors. The predominant virulence factors seen in the blood stream infection were adhesin, and hemolysin (44.4% each) and in catheter induced infection were DNase and adhesins (75.0% each). Adhesin (29.2%), slime layer (37.6%), DNAse (33.3%), gelatinase (25.0%), lipase (20.8%) and caseinase (16.6%) and hemolysin (8.3%) were produced the fecal isolates. **Conclusion:** An association between adhesin (as detected by biofilm formation) and urinary tract infection, adhesion and hemolysin with BSI, as also between DNase gelatinase & caseinase with wound infection was noted.

Key words: Blood stream infection, Catheter induced infection, Urinary tract infection, Vancomycin intermediate enterococci, Vancomycin resistant enterococci

INTRODUCTION

Enterococci are implicated in blood stream infections (BSI), endocarditis, urinary tract infections (UTI), pyogenic infections, intra-abdominal and pelvic infections.[1] Enterococci can infect humans because of its many virulence factors associated with biofilm formation including gelatinase, aggregation substance, capsule formation and enterococcal surface protein. Biofilms on medical devices favors disease sustenance because of restricted penetration of antimicrobials.[2-4]

Invasion is usually facilitated by damage to the host tissues and presence of bacterial virulence factors, which along with antibiotic resistance assist in advancement and further survival in newly infected places. Studies on adhesive properties of hemagglutinins produced by enterococci may contribute toward understanding the interaction of these organisms and the host cell surface and the mechanism of attachment.[5]

In recent years, an increase in the prevalence of *Enterococcus. faecium* has been seen which can be explained in part by the emergence of vancomycin resistant enterococci (VRE) and *Enterococcus. faecium* being the dominant detectable species among them.[6]

There is a paucity of information on the virulence factors distributed amongst enterococcal species.[7] The putative virulence markers in enterococcal strains isolated from various clinical sources and colonized patients and also the possible link between the presence of virulence factors and human infections was therefore investigated.

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MATERIALS AND METHODS

Study population

The study population included patients of both sexes and all age groups attending the outpatient and inpatient departments of a medical college hospital in eastern Bihar. The medical college caters to patients from the "Kosi region" in eastern Bihar and also to patients from adjoining areas. All clinical samples were processed for further study. Fecal samples were collected from the hospitalized patients at the time of admission, after 48 h and after 5 days of admission who were otherwise not suffering from any other infection, to look for colonization with VRE. Clearance from Institutional Ethics Committee was obtained to carry out this study.

Isolation and identification

Two hundred and fifty enterococci were isolated from various clinical samples (urine, pus, blood, catheter tip and tracheal aspirate) and another 250 were isolated from fecal specimens. The isolates were identified to species level using standard procedures.[8]

Hemolytic assay

Hemolytic activity of enterococci was assessed on two blood agar plates prepared with Muller-Hinton agar (HiMedia, Mumbai, India) containing 5% defibrinated sheep and human blood, by observation of zone of hemolysis around colonies after incubation for 24 h at 37ºC.[9]

Hemagglutination test

Enterococci were grown on brain heart infusion agar supplemented with 10% sheep blood. A loopful of bacteria was mixed on a glass slide with 25 µl of a 3% suspension of sheep, rabbit, human group A, human group O and human group B erythrocytes. *Vibrio cholerae* was used as positive control. After 5 min at room temperature, results were recorded as positive or negative.[10] — [Figure 1]

Physico-chemical properties of the culture filtrates

The effects of physic-chemical agents on hemagglutination test (HA) were investigated by performing HA test after treatment of the bacteria with trypsin, protease K, pepsin (HiMedia, Mumbai, India) . Bacterial suspensions of test strains were centrifuged and the deposit was added to separate test-tubes containing trypsin (1µg/ml), pepsin (1µg/ml) and proteaseK (1µg/ml) in phosphate buffered saline(PBS).The test-tubes were incubated at 37ºC for 60 min. For heat treatment bacterial suspensions were heated at 50ºC for 30 min. HA test was carried out with 20 µl of 3% erythrocyte suspension and 20 µl of enzyme treated and heated culture suspensions on glass slides. The suspensions were mixed, rotated gently for 30 seconds and results were recorded as either strong agglutination (+++ −), agglutination (− ++) or no agglutination.[11]

Caseinase production

Casein hydrolysis was detected on Muller-Hinton agar (HiMedia, Mumbai, India) containing 3% skimmed milk. Plates were streaked with test strains followed by incubation at 37ºC for 24 h. The presence of a transparent zone around the colonies indicated caesinase activity. Gelatinase production was detected by stab inoculating the test strain on nutrient agar supplemented with 3% gelatin (HiMedia, Mumbai, India) kept at 37ºC for 24 hours followed by refrigeration at 4ºC for half — an hour. Liquefaction of gelatin was considered as positive.[9,12]

Lipase production

Egg yolk agar (HiMedia, Mumbai, India) was used for lipase production. The test organism was spot inoculated on the medium and incubated at 37º C for 24 to 48 h. Positive test result was read as formation of thin iridescent pearly layer overlying the colonies and a confined opalescence in the medium, which was seen when the colonies were scraped off.[8]

Slime layer formation

Brain heart infusion agar (HiMedia,Mumbai,India) supplemented with 5% sucrose was used to determine the ability of enterococcus species to produce extracellular polysaccharide on the agar. Test strains grown in Todd Hewitt broth (HiMedia, Mumbai, India) was used as the

Figure 1: Hemagglutination test

inoculum. The colonies appeared mucoidal, runny or slimy due to production of polysaccharide.[12] — [Figure 2]

Deoxyribonuclease test

Test strains were inoculated on deoxyribonuclease (DNAse) agar (HiMedia, Mumbai, India). Clearing of the medium around the colonies indicated a positive test.[13]

Phosphatase test

Test strains were inoculated on phenolphlathelein phosphatase agar (HiMedia, Mumbai, India). The colonies turning pink on addition of ammonia solution were considered positive.[13]

Biofilm detection assa

The test strains were grown overnight at 37ºC in Brain Heart Infusion broth (HiMedia, Mumbai,India) plus 0.25% glucose. Culture was diluted 1:20 in the same media. 200 µL of this suspension was used to inoculate sterile 96 well polystyrene microtitre plates. After 24 h at 37ºC of static incubation, wells were washed with PBS, dried in inverted position and stained with 1% crystal violet for 15 min. The cells were rinsed once more and solubilized in 200 µl ethanol/acetone (80:20 v/v). The A_{630} was determined using microtitre plate reader. Biofilm formation was scored as non biofilm forming (−), weak- (+), moderate- (++), strong- (+++) corresponding to the A_{630} values ≤1, 1-≤2, 2-≤ 3 and > 3 respectively.[14]

Antimicrobial susceptibility & minimum inhibitory concentration tests

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method on Muller-Hinton agar. Minimum inhibitoru concentration of VRE was determined by agar dilution method using the following concentration of vancomycin 0.5 µg/mL to 64 µg/mL. The test was quality controlled using *Enterococcus. fecalis* ATCC 51299 and *Enterococcus. fecalis* ATCC 29212.[15,16]

Figure 2: Slime layer formation

Statistical analysis

Statistical analysis was performed using chi-square test. $P < 0.005$ was considered significant in the results.

RESULTS

A total of 500 samples (250 each clinical and fecal) were processed out of which 60 (36 from clinical and 24 from fecal samples) were either vancomycin resistant or vancomycin intermediate enterococci (VIE). 25 (69.4%) VRE and 11 (30.5%) VIE were isolated from clinical samples and 12 (50.0%) VRE and VIE each were isolated from fecal samples.

Amongst the 36 clinical VRE/ VIE, 13 (36.1%) strains were isolated from cases of UTI. 10 (27.7%) strains were isolated from wound infection, 9 (25.0%) strains were isolated from BSI and 4 (11.1%) cases from catheter induced infection (CII). Most of these clinical VRE were found to be multidrug resistant.

A total of 9 (25.0%) clinical VRE/VIE were hemolytic to sheep red blood cells (RBC) as compared to 2 (8.3%) fecal VRE/VIE. This finding was found to be statistically insignificant ($P = 0.102$)-[Table 1]. The

Table 1: Comparative evaluation of the virulence factors in clinical and faecal VRE/VIE

Phenotypic virulence markers	Clinical VRE/VIE *n* =36 (%)	Faecal VRE/VIE *n* = 24 (%)	*P* value
Hemolysis of sheep RBC	9 (25.0)	2 (8.3)	0.102
Hemolysis of human RBC	9 (25.0)	2 (8.3)	0.102
Hemagglutination of rabbit RBC	10 (27.8)	6 (25.0)	0.812
Hemagglutination of human "O" RBC	9 (25.0)	5 (20.8)	0.709
Hemagglutination of human "B' RBC	9 (25.0)	5 (20.8)	0.709
Production of deoxyribonuclease	14 (38.9)	8 (33.3)	0.662
Slime layer	10 (27.8)	9 (37.6)	0.428
Lipase	4 (11.1)	9 (37.6)	0.015
Gelatinase	14 (38.9)	6 (25.0)	0.264
Caseinase	11 (30.6)	4 (16.6)	0.224
Adhesins (responsible for biofilm formation)	22 (61.1)	7 (29.2)	0.015

Statistically significant (*P* value < 0.005); VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; RBC: Red blood cells

differences in the production of other virulence factors viz: hemagglutinins, DNase, slime layer, gelatinase and caseinase production in clinical and fecal isolates were not found to be statistically significant. Adhesin molecules responsible for biofilm formation on the other hand were seen in 22 (61.1%) clinical strains as compared to 7 (29.2%) fecal strains. This finding was statistically significant ($P = 0.015$)-[Table 1]. Of these 22 strains, 11 were isolated from cases of UTI, 4 each from wound and BSI and 3 from CII-[Table 5].

Production of lipase was significantly higher ($P = 0.015$) in fecal strains 9 (37.6%) when compared to the clinical strains 4 (11.1%)-[Table 1].

Of the clinical isolates that showed hemolysis to sheep RBC *Enterococcus. fecalis* and *Enterococcus. faecium* accounted for 4 (28.5%) and 4 (23.5%) and 1 (20.0%) was *Enterococcus. gallinarum* respectively. Hemolysis of human RBC showed the same findings as with that of sheep RBC from both clinical and fecal VRE/VIE-[Table 2].

In the HAs, 27.8% of clinical isolates agglutinated rabbit RBC and 25.0% each agglutinated human "O" group, human "B' group and sheep RBCs. None of the clinical strains agglutinated human "A" group RBCs. Of the fecal isolates 25.0 % and 20.8% strains each agglutinated rabbit RBC, human "O" group and "B" RBCs. None of the fecal strains agglutinated human "A" group RBC and sheep R-B-Cs-[Table 2].

Enzyme treatment of bacterial cells with pepsin, trypsin or protease had an inhibitory effect on the hemagglutinating activity of some strains of VRE. Heating of bacterial cells at 50º C for 30 mins also did not have any effect on HA activity in clinical and fecal VRE/VIE strains.

Production of other virulence factors like slime layer, lipase, gelatinase, serine caesinase were more common in *Enterococcus. faecalis* when compared to *Enterococcus faecium* and *Enterococcus. gallinarum* in clinical VRE/VIE-[Table 3]. None of the strains of *Enterococcus gallinarum*, however did not produce lipase and serine caesinase-[Table 3].

Thirteen (92.8%) out of the 14 isolates of *Enterococcus. fecalis* strains showed biofilm formation out of which 6 (46.2%) were urinary isolates, 3 (23.1%) were fromCII, 2 (15.4%) each from BSI and wound infections. 9 (52.9%) out of 17 *E. faecium* strains also produced biofilms, majority of the isolates being from the urinary tract. None of the strains of *E. gallinarum* were biofilm producers-[Table 4].

Table 2: Hemolysis pattern & heamagglutinating activity in VRE and VIE isolates

Species	Clinical VRE/VIE						Species	Fecal VRE/VIE					
	Hemolysis of sheep RBC (%)	Hemolysis of human RBC (%)	Hemagglutination tests					Hemolysis of sheep RBC (%)	Hemolysis of human RBC (%)	Hemagglutination tests			
			Rabbit RBC	Human-O RBC (%)	Human-B RBC (%)	Human-A RBC (%)	Sheep RBC (%)			Rabbit RBC (%)	Human-O RBC (%)	Human-B RBC (%)	Human-A RBC (%)	Sheep RBC (%)
E. faecalis n=14	4 (28.5)	4 (28.5)	5 (35.7)	4 (28.5)	4 (28.5)	0	4 (28.5)	*E. faecalis* n=12	1 (8.3)	1 (8.3)	3 (25.0)	3 (25.0)	3 (25.0)	0	0
E. fecium n=17	4 (23.5)	4 (23.5)	4 (23.5)	4 (23.5)	4 (23.5)	0	4 (23.5)	*E. fecium* n=8	1 (12.5)	1 (12.5)	2 (25.0)	2 (25.0)	2 (25.0)	0	0
E. gallinarum n=5	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	0	1 (20.0)	*E. gallinarum* n=4	0	0	1 (25.0)	0	0	0	0
Total=36	9 (25.0)	9 (25.0)	10 (27.8)	9 (25.0)	9 (25.0)	0	9 (25.0)	Total=24	2 (8.3)	2 (8.3)	6 (25.0)	5 (20.8)	5 (20.8)	0	0

VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; RBC: Red blood cells; *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*; *E. gallinarum: Enterococcus gallinarum*

Table 3: Other virulence factors detected in VRE and VIE isolates

Species	Clinical VRE/VIE					Species	Fecal VRE/VIE				
	DNase test (%)	Slime layer formation (%)	Lipase (%)	Gelatinase (%)	Serine caseinase (%)		DNase test (%)	Slime layer formation (%)	Lipase (%)	Gelatinase (%)	Serine caseinase (%)	Adhesin (%)
E. fecalis, n=14	6 (42.9)	6 (42.9)	3 (21.4)	6 (42.9)	5 (35.7)	*E. fecalis* n=12	4 (33.3)	4 (33.3)	3 (25.0)	3 (25.0)	3 (25.0)	4 (33.3)
E. fecium, n=17	7 (41.2)	3 (17.6)	1 (5.8)	6 (35.3)	6 (35. 3)	*E. fecium*, n=8	3 (37.5)	4 (50.0)	2 (25.0)	2 (25.0)	1 (12.5)	2 (25.0)
E. gallinarum, n=5	1 (20.0)	1 (20.0)	0	2 (40.0)	0	*E. gallinarum*, n=4	1 (25.0)	1 (25.0)	0	1 (25.0)	0	1 (25.0)
Total=36	14 (38.9)	10 (27.8)	4 (11.1)	14 (38.9)	11 (30.6)	Total=24	8 (33.3)	9 (37.6)	9 (37.6)	6 (25.0)	4 (16.6)	7 (29.2)

Percentages calculated horizontally; VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; DNase: Deoxyribonuclease; *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*; *E. gallinarum: Enterococcus gallinarum*

Adhesin molecules (84.6%) were the predominant virulence marker among UTI isolates. None of the UTI isolates were found to produce lipase or caesinase. Gelatinase, DNase and caesinase 70.0% each were produced by the isolates from wound infection. DNase and adhesions (75.0% each) were elaborated by VRE causing CII. In BSI adhesions and hemolysins were the predominant virulence factors elaborated (44.4%)-[Table 5].

DISCUSSION

Correlation between the presence of virulence markers in the clinical and fecal VRE and VIE were evaluated. The presence of these virulence determinants in *Enterococcus. fecalis, Enterococcus. faecium* followed by *Enterococcus. gallinarum* is necessary for adhesion, tissue invasion and causing disease.

In this study, only 25.0% of clinical VRE and VIE isolates and 8.3% of fecal VRE and VIE isolates produced hemolysis around colonies on BHI sheep and human blood agar plates. Some authors have reported that 75.0% of clinical *E. faecalis* were hemolytic to sheep RBCs.[9] Another study found higher number of hemolysin producers in clinical isolates (60.0%) when compared to fecal isolates (17.0%).[17] These findings suggest a role of hemolysin in the causation of human disease.

Among the clinical VRE/VIE strains production of hemolysin was higher in *Enterococcus. fecalis* (28.5%) than in *Enterococcus. faecium* (23.5%). In another study only 16% *Enterococcus. fecalis* isolates were hemolysin producers and none of the *Enterococcus. faecium* strains were found to produce hemolysin. Hemolysin producing strains of *Enterococcus. fecalis* are virulent in animal models and human infections with these strains are associated with increased severity of illness.

Elaboration of virulence markers like hemolysin was most common in the isolates from BSI (44.4%) followed by CII (25.0%), wound infection (20.0%), UTI (15.4%) and colonized patients (8.3%). In contrast, in yet another study of *Enterococcus. fecalis,* only 16% of endocarditis isolates, 32% of blood culture isolates and 20% of community acquired fecal isolates were found to be hemolytic leading to the conclusion that hemolysin is not an essential factor in the pathogenicity of enterococci causing these infections.[18] However, findings of this study shows that a much higher number of VRE/VIE strains causing BSI were hemolysin producers when compared to VRE strains causing other infections. Hemolysin production has been associated with the better ability of enterococci to reach bloodstream to induce septicemia and with fivefold increased risk of adverse terminal outcome in patients with enterococcal bacteremia.[19]

HA activity against rabbit RBC was more (26.7%) than against human RBC (23.3%) in both clinical and fecal strains. Contrasting results were shown in another study in which 12.7% of enterococci isolated from humans agglutinated human RBC as compared to 4.8% of rabbit RBCs.[10] Another study reported that 97.0% of *Enterococcus. fecalis* strains isolated from human blood were hemagglutination-positive, while all of 24 *Enterococcus.*

Table 4: Biofilm formation in the VRE/VIE isolated from infected patients

Biofilm formation in VRE/VIE isolated from clinical samples														
	E. faecalis, *n* = 14					*E. faecium*, *n* = 17					*E. gallinarum*, *n* = 5			
Biofilm forming capacity	UTI	Wound infection	Catheter induced infection	Blood infection	Total	UTI	Wound infection	Catheter induced infection	Blood infection	Total	UTI	Wound infection	Catheter induced infection	Blood infection
Weak	4	0	0	1	5	3	0	1	0	4	0	0	0	0
Moderate	2	2	2	1	7	2	0	0	2	4	0	0	0	0
Strong	0	0	1	0	1	0	0	1	0	1	0	0	0	0
Total	6	2	3	2	13	5	0	2	2	9	0	0	0	0

VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; UTI: Urinary tract infection; *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*; *E. gallinarum: Enterococcus gallinarum*

Table 5: Distribution of virulence markers in the VRE/VIE isolated from different types of infection

Type of infection/colonization	Adhesins (%)	Slime layer (%)	Hemolysin (%)	Gelatinase (%)	DNAse (%)	Lipase (%)	Caesinase (%)
UTI *n*=13	11 (84.6)	5 (38.5)	2 (15.4)	2 (15.4)	2 (15.4)	0	0
Wound infection *n*=10	4 (40.0)	3 (30.0)	2 (20.0)	7 (70.0)	7 (70.0)	3 (30.0)	7 (70.0)
BSI *n*=9	4 (44.4)	1 (11.1)	4 (44.4)	3 (33.3)	2 (22.2)	1 (11.1)	2 (22.2)
CII *n*=4	3 (75.0)	1 (25.0)	1 (25.0)	2 (50)	3 (75.0)	0	2 (50.0)
Total no of infections *n*=36	22 (61.1)*	10 (27.8)	9 (25.0)	14 (38.9)	14 (38.9)	4 (11.1)	11 (30.6)

Percentages calculated horizontally; *P = 0.015; VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; DNase: Deoxyribonuclease

faecium isolates were negative when tested with rabbit erythrocytes.[20] These differences might be based on the presence of different adhesion molecules of local strains. In our study, hemagglutinating *Enterococcus. fecalis, Enterococcus. faecium* and *Enterococcus. gallinarum* did not produce identical results with different erythrocytes tested, suggesting that binding was caused by the presence of different adhesins.[20]

Production of adhesins differed among Enterococcus species. Among the clinical VRE 92.8% (13/14) of *Enterococcus. fecalis*, 52.9% (9/17) of *Enterococcus. faecium* produced biofilm but none of the strains of *Enterococcus. gallinarum* were biofilm producers. Biofilm formation was also seen in 29.2% of faecal VRE/VIE. Similar findings were reported by other authors where the production of biofilm was more in *Enterococcus. fecalis* compared to *Enterococcus. faecium* and *Enterococcus. casseliflavus*.[21] Maximum number of biofilm production was seen with urinary VRE isolates suggestive of their role as potential virulence factors for colonization and persistence in the urinary tract.[21] Adhesin molecules were also detected in four cases each of BSI and wound infection and three cases of CII. Out of these two cases each of BSI and CII did not have favorable outcomes inspite of treatment with combination therapy of ceftriaxone, vancomycin and gentamicin.

Production of DNAse was seen in both clinical (38.9%) and fecal isolates (33.3%). Strains isolated from CII predominantly produced DNAse (75.0%) followed by strains causing wound infection (70.0%). DNAse production by enterococci may be responsible for virulence in these infections. Other reports have however, not considered DNAse production to be important as a virulence factor.[20]

Production of slime layer was more in the fecal isolates 37.6% as compared to clinical isolates 27.8%, which suggests that these isolates have the potential for colonizing the gastrointestinal tract by means of the slime produced which may then act as a nidus of infection in the body. Slime layer formation was also more frequent in isolates from UTI (38.5%) suggestive of their role in colonization of urinary tract.

The lipolytic activity in the fecal (20.8%) VRE was significant ($P = 0.015$) as compared to the clinical (11.1%) VRE. Lipase activity was seen only in strains predominantly causing wound infection The absence of lipase production in *Enterococcus. gallinarum* suggests that it is probably not associated with virulence in this species.

Gelatinase has been shown to contribute to the virulence of *Enterococcus. fecalis* in an animal model. The ability of gelatinase produced by enterococci, especially *Enterococcus. fecalis* to hydrolyze collagen and certain bioactive peptides suggests its participation in the initiation and propagation of inflammatory process. All the three species of enterococci viz: *Enterococcus. fecalis*, (42.8%), *Enterococcus. faecium*, (35.2%), and *Enterococcus. gallinarum,* (40.0%) isolated from clinical sources were gelatinase producers. In another study gelatinase activity was seen in (55%) *Enterococcus. fecalis* strains whereas none of the *Enterococcus. faecium* strains produced gelatinase.[20] Of the clinical VRE/VIE 38.9% clinical VRE were gelatinase producers as compared to 25.0% of fecal VRE which was statistically insignificant ($P = 0.264$). 70.0% of the isolates from wound and 50.0% from CII were found to produce gelatinase. In another study 54% of *Enterococcus. fecalis* from endocarditis, 68% of blood culture isolates and 27% of community-acquired fecal isolates were gelatinase producers.[21]

Serine caseinase activity was seen in fewer numbers of strains than gelatinase activity in both clinical and fecal VRE. In contrast to this other studies have reported serine caseinase activity in 75.0% *Enterococcus. fecalis* strains which showed no gelatinase activity.[9] Hence it has been suggested by many authors that gelatin hydrolyzing activity is different from caseinase activity.

CONCLUSION

Hospitalized patients may have a greater incidence of enterococcal infections not only because of virulence, but because the hospital itself is a hub. Numerous factors are associated with a greater risk of acquiring enterococcal infections. These factors including antimicrobial resistance and expression of virulence factors associated with infection-derived *Enterococcus. fecalis* strains, may account for the establishment and maintenance of this opportunistic pathogen in the nosocomial settings. On the other hand, the intestinal tract is an important reservoir for nosocomial pathogens such as enterococci and allows them to access to infectious sites through other means. The main virulent property of *Enterococcus. fecalis, Enterococcus. faecium* & *Enterococcus. gallinarum* is adherence to epithelial host cells, leading to biofilm formation and consequently the production of hemolysin, DNase, hemagglutinin, lipase which lead to human epithelial cell damage.

Majority of the isolates were recovered from urine, followed by wound infection, blood & catheter samples which is consistent with reports that Enterococci have become the leading cause of UTI, surgical wound infection, bacteremia &CII. HA activity indicates diversity in the surface structures involved in enterococcal

adhesion. Enzyme treatment of bacterial suspensions, which led to partial loss of HA activity, suggests that the hemagglutinins are protein in nature. On the other hand heating method did not have any effect on bacteria-erythrocyte interactions. These observations lead us to conclude that the hemagglutinins are thermostable. The abundance of slime layer among the fecal isolates is particularly important in the acquisition of resistance by promoting cell- cell contact and the conjugal transfer of plasmids harbouring resistance and virulence genes. Such strains appear to represent the entry gateway to new resistance genes into enterococcal species in the gastrointestinal tract and may contribute to spreading of such bacteria in the hospital settings. Adhesin molecules (responsible for biofilm formation) were the main virulence factor of the UTI causing isolates. This strongly reflects the affinity of enterococcal isolates towards the urinary tract epithelial cells, and explains the prevalence of enterococci as a causative agent of nosocomially acquired UTIs. Gelatinase, DNAse and caseinase were the main virulence factors isolated from wound infection. Thus our findings suggest that these virulence factors collectively, may promote the spread of VRE in wound infections.

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