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A Study for the Presence of Enterococcal Virulence Factors Gelatinase, Haemolysin among Clinical Isolates in a Tertiary Care Hospital

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Abstract

Enterococcus, considered a normal commensal of intestinal tract, is fast emerging pathogen causing serious infection. Despite the increasing importance of Enterococcus as opportunistic pathogens, their virulence factors are still poorly understood. The potential virulence factors of Enterococci include production of enterococcal surface protein (Esp), gelatinase, and haemolysin. Gelatinase- and haemolysin-producing strains of Enterococcus faecalis have been shown to be virulent in animal models of enterococcal infections. This study was undertaken to determine the prevalence of virulence factors (gelatinase and haemolysin), phenotypically and correlation between virulence factors with respect to different clinical specimens.

Keywords: *Enterococcus, gelatinase, haemolysin*

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INTRODUCTION

Enterococci exert dual functions both as commensals and as pathogens. When inside the body, they are well adapted to an ecological complex niche in the gut, genitourinary tract, and oral cavity which is enriched with low redox potential [1]. Enterococci are increasingly important cause of nosocomial infection. They are intrinsically resistant to or tolerant of many antibiotics and are readily able to acquire resistance to antibiotics, either by mutation or by acquisition of plasmids or transposons containing genetic sequences that confer resistance in other bacteria [2].

Virulence factors such as gelatinase, haemolysin, and aggregation substance protein production are associated with pathogenic isolate of Enterococci than environmental isolate [3–5]. These factors have been associated with the virulence of *Enterococcus faecalis* in animal models [3, 6–8].

Hemolysin is a cytolytic protein capable of lysing human, horse, and rabbit erythrocytes. Hemolysin producing strains of *E. faecalis* have been shown to be virulent in animal models and human infections [7–9] and to be associated with increased severity of infection [4].

Gelatinase is a protease produced by *E. faecalis* that is capable of hydrolyzing gelatine, collagen, casein, haemoglobin, and other peptides [10]. Gelatinase-producing strains of *E. faecalis* have been shown to contribute to the virulence of endocarditis in an animal model [11].

Considering the above facts the present study evaluates the prevalence of virulence factors (gelatinase and hemolysin), phenotypically and correlation between virulence factors with respect to different clinical specimens.

MATERIALS AND METHODS

Collection of Specimen

Clinical specimen viz. urine, blood, pus, cerebrospinal fluid (CSF), fluids and aspirates were collected aseptically, from patients of Jhalawar Medical College and attached hospital, Jhalawar, Rajasthan, India.

Processing in Laboratory

All specimens were streaked on preincubated MacConkey's agar and blood agar plates

within 5 h of sample collection and were kept under incubation at 37 °C for 48 h. Colony isolates were further confirmed by colony morphology on MacConkey's agar, Blood agar, Gram staining and Catalase test as per the standard guidelines.

Confirmation, Identification of Enterococcal Isolates

Fifty isolates were confirmed as different species of Enterococcus by growing them on M-enterococcus agar base and bile esculin agar and identified them by using semi-automated identification system containing (VP, Esculin hydrolysis, PYR test, ONPG, Arginine utilization, fermentation of carbohydrates such as Arabinose, Mannitol, Raffinose, Sorbitol).

Haemolysin Production

Haemolysin production was detected by inoculating Enterococci on freshly prepared blood agar base (HiMedia) which had beef heart infusion agar which was supplemented by 5% horse blood. Plates were incubated overnight at 37 °C and evaluated at 24 h and 48 h [12]. A clear zone of β-haemolysis around the streak on horse blood agar was considered to be positive for haemolysin production.

Gelatinase Production

Gelatinase production was detected by inoculating the organism onto freshly prepared nutrient agar (peptone yeast and beef extract agar) containing 30 g/l of gelatine [12]. Plates were incubated overnight at 37 °C and then cooled to ambient temperature for 2 h. The appearance of a turbid halo or zone around the colonies was considered to be a positive indication of gelatinase production.

RESULTS

Among the hundred (n=100) clinical isolates, (n=44) were positive for gelatinase production and (n=31) were positive for haemolysin production and (n=10) were positive for production of both (gelatinase and haemolysin).

Table-1 shows the age, sex and clinical cases (number and %) from which Enterococcus spp. was isolated. Among them the age ranges of patients were from 2 weeks to 70 years. A total of 45 isolates were from males and 55 were from females. Among clinical cases highest isolates were from urinary tract infection (UTI) patients (42%), followed by wound infection patients (35%), septicaemia patients (11%), upper respiratory tract infection (URTI) patients (7%), and genital infection (5%).

Table-2 shows the production of gelatinase, haemolysin and both of the virulence factors. Results shows that among UTI patients (n=42), 26 (61.9%) produced gelatinase, 18 (42.9%) produced haemolysin and 5 (11.9%) produced both of the virulence factors, that is highest among all the clinical isolates. Among URTI infection patients (n=7), 2 (28.6%) produced gelatinase, 2 (28.5%) produced haemolysin and 1 (14.3%) produced both of the virulence factor. Among wound infection patients (n=35), 11 (31.5%) produced gelatinase, 7 (20%) produced haemolysin and 3 (8.5%) produced both of the virulence factors. Among septicaemia patients (n=11), 3 (27.2%) produced gelatinase, 2 (18.2%) produced haemolysin, and 1 (9.1%) produced both of the virulence factors. Among genital infection patients (n=5), 2 (40%) produced gelatinase, 2 (40%) produced haemolysin and 1 (20%) produced both of the virulence factors.

Table-3 shows distribution of Enterococcus spp. from various samples and their percentage in sample. A total of 100 isolates of Enterococci from both outdoor and indoor patients of Jhalawar Medical College, Jhalawar, Rajasthan, India were isolated during one year (January 2015 to December 2015). These 100 isolates of Enterococcus spp. were isolated from various clinical samples taken for the current study. All the isolates were identified up to species level by commercial biochemical identification panel, KB005A HiStrep™ Identification Kit (Hi-Media) (Figure 1). In the current study, seven various species were identified which were *E.faecalis, E.faecium, E.raffinosus, E.durans, E.mundtii, E.gallinarum,* and *E.solitarus.* Among all the species *E.faecalis* (57) was the predominant isolates from all the clinical samples followed by *E.faecium* (33) then *E.raffinosus* (4), *E.durans* and *E.mundtii* (2 each), *E.gallinarum,* and *E.solitarus* (1 each) (Table 1). Most isolates were obtained in pus (44) followed by urine (32), blood (12), throat swab (6) and vaginal swab (6).

Fig. 1: *Commercial Biochemical Identification Panel, KB005A HiStrep™ Identification Kit (Hi-Media) Wells; from left to right Voges Proskauer, Esculin Hydrolysis, PYR Test, ONPG, Arginine Dihydrolase Test, Glucose, Lactose, Arabinose, Sucrose, Sorbitol, Mannose and Raffinose Fermentation Depicting Reaction of E. faecalis.*

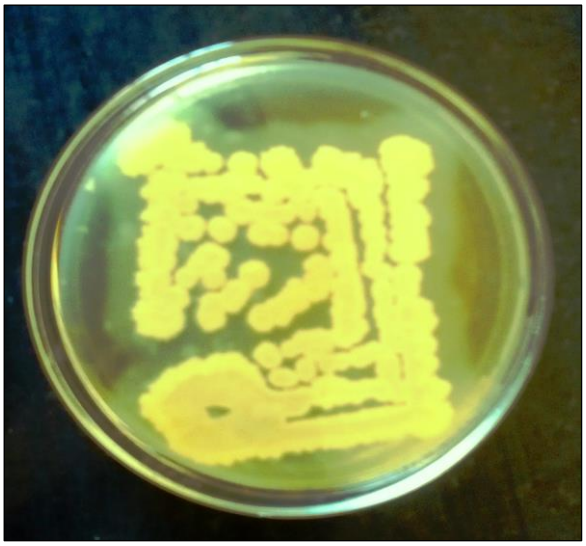

Fig. 2: *A Nutrient Agar Plate Containing Gelatine Showing Turbid Halo Around the Colonies of E. faecalis Indicating Gelatinase Production.*

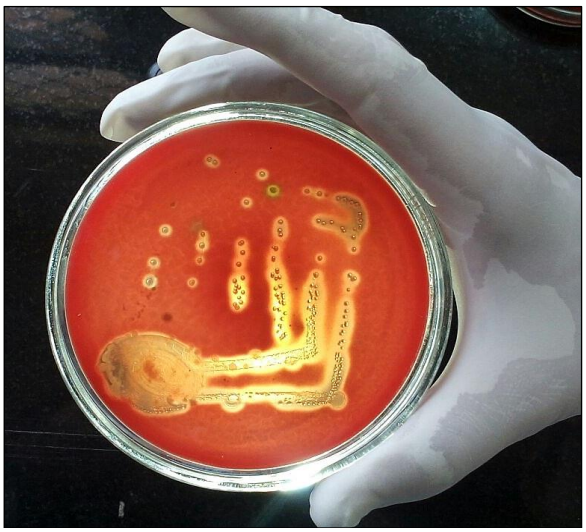

Fig. 3: *A Blood Agar Plate Containing 5% Horse Blood Showing β-Haemolysis Around the E. faecalis Colonies Indicating Haemolysin Production by Isolates.*

Table 1: *Demographic and Clinical Characteristics of 100 Patients.*

Characteristics	Value no. (%)
Age, Median years (range)	2 weeks–70 years
Sex, number of males/number of females	45/55
Urinary tract infection (UTI)	42 (42%)
Upper respiratory tract infection (URTI)	7 (7%)
Wound infection	35 (35%)
Septicaemia	11 (11%)
Genital infection	5 (5%)

Table 2: *Production of Gelatinase, Haemolysin and Both Virulence Factors in Isolates from Different Clinical Conditions.*

Clinical samples	Gelatinase (%)	Haemolysin (%)	Gelatinase+Haemolysin (%)
Urinary tract infection (UTI) (n=42)	26 (61.9)	18 (42.9)	5 (11.9)
Upper respiratory tract infection (URTI) (n=7)	2 (28.6)	2 (28.6)	1 (14.3)
Wound infection (n=35)	11 (31.5)	7 (20)	3 (8.5)
Septicaemia (n=11)	3 (27.2)	2 (18.2)	1 (9.1)
Genital infection (n=5)	2 (40)	2 (40)	1 (20)

Table 3: *Distribution of Enterococcus spp. from Various Samples and their Percentage in Sample.*

***Enterococcus* spp.**	**Pus (%)**	**Urine (%)**	**Blood (%)**	**Throat swab (%)**	**Vaginal swab (%)**	**Total**
E. faecalis (N=57)	25 (43.8)	18 (31.5)	7 (12.2)	4 (07.0)	3 (05.2)	57
E. faecium (N=33)	16 (48.4)	10 (30.3)	3 (9.09)	2 (06.1)	2 (06.06)	33
E .raffinosus (N=4)	2 (50.0)	1 (25.0)	0	0	1 (25.0)	4
E. durans (N=2)	0	1 (50.0)	1 (50.0)	0	0	2
E. mundtii (N=2)	0	1 (50.0)	1 (50.0)	0	0	2
E. gallinarum (N= 1)	1 (100)	0	0	0	0	1
E.solitarus (N=1)	0	1 (100)	0	0	0	1

DISCUSSION

Enterococcal infections are one of the most important global health problems causing considerable morbidity in the general population. We studied distribution of Enterococcal species among various clinical samples and prevalence of virulence factors such as gelatinase and haemolysin in those clinical isolates. Among all Enterococcal isolates, *E.faecalis* is most prevalent followed by *E.faecium* which is concordant to study done by Sood *et al* [13]. Prevalence of virulence factors among clinical isolates such as gelatinase production (44%) and haemolysin production (31%) and both of the virulence factor production (10%), was concordant to study done by Upadhyaya *et al* [14].

CONCLUSION

To conclude Enterococci are Gram-positive cocci presenting as harmless commensal now become a virulent pathogen. We determined the prevalence of haemolysin and gelatinase among clinical isolates.

The present study showed that isolates from UTI patients has highest prevalence of both virulence factor production. Isolates from genital infection and wound infection were second and third highest, respectively.

Study also evidenced that isolation *of E.faecalis* was highest among all clinical samples which were taken in count.

Given the importance of Enterococcus as an emerging pathogen, nosocomial pathogen and increasing multidrug-resistant (MDR) Enterococcus as shown by other studies [15], the identification of virulence factors associated with invasiveness and disease severity has become an important subject for

research. Development of mechanism to overcome production of virulence factors may provide an alternate method of therapy to the patients. Further study on other virulence factors will throw some more light on the mechanism of pathogenesis in Enterococcus spp.

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