OCCURRENCE OF ENTEROCOCCAL VIRULENCE FACTORS GELATINASE, HEMOLYSIN AMONG CLINICAL ISOLATES

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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 hemolysin. Gelatinase- and hemolysin-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 hemolysin), phenotypically and co- relation between virulence factors with respect to different clinical specimens.

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 like 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 prevalence of virulence factors (gelatinase and hemolysin), phenotypically and co- relation between virulence factors with respect to different clinical specimens.

Material and Methods:-

Sample Collection: Clinical samples like urine, blood, pus, CSF, fluids and aspirates were collected from clinically suspected patients of Jhalawar medical college and attached hospital.

Laboratory procedures: All specimens were inoculated on MacConkey's agar and blood agar plates within 5 hrs of sample collection and were kept under incubation at 37°C for 48 hrs. Colony isolates were further confirmed by colony morphology on MacConkey's agar, Blood agar, Gram staining and Catalase test as per standard guidelines.

Identification of Enterococcal isolates :- Fifty isolates were identified as different species of Enterococcus by grown 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 like Arabinose, Mannitol, Raffinose, Sorbitol.)

Hemolysin production: - Hemolysin 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 37^0 c and evaluated at 24 and 48 hrs [12]. A clear zone of β-haemolysis around the streak on horse blood agar was considered to be positive for hemolysin 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 37^0 c and then cooled to ambient temperature for 2 hours. 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=38) were positive for gelatinase production and (n=28) were positive for haemolysin production and (n=11) were positive for production of both (gelatinase and haemolysin).

{Table-1} shows the age, sex and clinical cases (no. and %) from which Enterococcus spp. was isolated. Among them the age ranges of patients were from 2wks -70yrs. 45 were males and 55 were females. Among

clinical cases highest isolates were from UTI patients (42%), followed by wound infection patients (35%), septicaemia patients (11%), URTI patients (7%), Genital infection (5%).

{Table-2} shows production of gelatinase, haemolysin and both of the virulence factors. Results shows that among UTI patients (n=42), 20(47.6%) were produced gelatinase, 15(35.71%) 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%) were produced gelatinase, 2(28.5%) were produced haemolysin and 1(14.3%) were produced both of the virulence factor. Among wound infection patients (n=35), 11(31.5%) were produced gelatinase, 7(20%) were produced haemolysin and 3(8.5%) were produced both of the virulence factors. Among septicaemia patients (n=11), 3(27.2%) were produced gelatinase, 2(18.2%) were produced haemolysin, 1(9.1%) were produced both of the virulence factors. Among genital infection patients (n=5), 2(40%) were produced gelatinase, 2(40%) were produced haemolysin and 1(20%) were produced both of the virulence factors.

{Table-3} shows distribution of Enterococcus Spp. from various samples and their percentage in sample. A total 100 isolates of Enterococci from both outdoor and indoor patients of Jhalawar medical college, isolated during one year (January 2015 to December 2015) from various clinical samples were taken for the current study. All the isolates were identified up to species level by commercial biochemical identification panel, KB005A HiStrep^{TM} Identification Kit (Hi-Media) (Fig-1). In current study seven various species were identified which are *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* (1each) (table-1). Most isolates were obtained in urine (42) followed by pus (35), blood (11), throat swab (7) and vaginal swab (5)

Fig.1: commercial biochemical identification panel, KB005A HiStrep^{TM} 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.

DISCUSSION

We studied distribution of *Enterococcal* species among various clinical samples and prevalence of virulence factors like 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 2008. Prevalence of virulence factors among clinical isolates like gelatinase production (38%) and haemolysin production (28%) and both of the virulence factor production (11%), which is concordant to study done by Upadhyaya et al 2010. Mulik et al 2016 showed 15.58% gelatinase production and 35.71% haemolysin production. Banerjee et al 2015 showed that prevalence of gelatinase production was 9.03% and haemolysin was 31.61%.Urinary isolates were predominant in producing both the virulence factors this is consistent with other studies [14; 17; 18]. Unusual enterococcal species also produced haemolysin and gelatinase; this is similar to study conducted previously. [16; 20].

CONCLUSION

The present study has shown that enterococcal infections are quite prevalent, among which *Enterococcus faecalis* species are identified in most of the cases.

Virulence factors production is highest in urinary tract isolates followed by genital and wound isolates.

Virulence factor production determined high pathogenicity followed by more severe symptoms of the infection.

So by determining virulence factor production, severity of infection and suitable course of antimicrobial therapy can be monitored.

Development of mechanism to overcome production of virulence factors may provide alternate method of therapy to the patients.

Further study on the other virulence factor will throw some more light on the mechanism of pathogenesis in *Enterococcus spp*.

Table 1

Demographic and clinical characteristics of 100 patients

Characteristics	Value no.(%)
Age ,Median years (range)	2wks-70 yrs
Sex ,no. of males/no. of females	45/55
UTI(urinary tract infection)	42(42%)
URTI(upper respiratory tract infection)	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 (%)	No virulence production
UTI(urinary tract infection)(n=42)	20(47.6)	15(35.71)	5(11.9)	2(4.76)
URTI(upper respiratory tract infection) (n=7)	2(28.6)	2(28.6)	1(14.3)	2(28.57)
Wound infection(n=35)	11(31.5)	7(20)	3(8.5)	14(40)
Septicaemia(n=11)	3(27.2)	2(18.2)	1(9.1)	5(45.45)
Genital infection(n=5)	2(40)	2(40)	1(20)	0(0)

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)	20 (35.08)	23(40.03)	7 (12.2)	4 (7.01)	3 (5.26)	57
E. faecium (N=33)	10(30.30)	16(48.48)	3 (9.09)	2 (06.06)	2 (06.06)	33
E .raffinosus (N=4)	2 (50.0)	1 (25.0)	0	1(25.0)	0 (0)	4
E. durans (N=2)	0	1 (50.0)	1 (50.0)	0	0	2
E. mundtii (N=2)	0	2 (100.0)	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

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