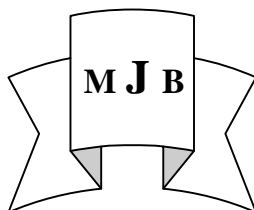


Virulence Factors of *Enterococcus faecalis*

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Abstract

Two hundred seventy six samples collected from different sources. The samples were divided into three groups; first included 40 stool samples collected from healthy individuals; second group included 125 clinical samples from patients who admitted to teaching general Hilla hospital. The third group included 111 samples collected from environment of same hospital .

The morphological characterization and biochemical reactions showed 33 isolates diagnosed as *Enterococcus faecalis* , of which , 15 isolates from normal flora of intestine, 11 isolates from clinical cases ,and 7 isolates from hospital environment. The results of detection of virulence factors showed that *E.faecalis* possessed the followed factors; adhesion factors (45.4%), haemagglutination (87.8%), hemolysin (15.1%), gelatinase (9.0%) , lipase (6.0%) and bacteriocin (90.9%). The significance of these factors in the pathogenesis of enterococcal infection needs to be elucidated in further studies.

Introduction

The term enterococcus derives from the name enterocoque was first used by Thiercelin in a paper from France published in 1899[1]. The name was proposed to described a new gram-positive coccus of intestinal origin[2].

Enterococcus species were formerly classified in the genus *Streptococcus*, but in 1984 the *Enterococcus* was complete separated from the genus *Streptococcus* by studies of Schleifer and Kilpper[3] .

Streptococcus faecalis was first used by Andrews and Horder in 1906 to identify an organism of faecal origin, who isolated this organism from a patient with endocarditis ,the organism can be fermented mannitol and lactose but not raffinose[4].

The enterococci are primarily commensal residents of the intestine of human and animals. Enterococci , especially *E.faecalis* in human clinical infections has been reviewed very

thoroughly by Murray[1]. The most common infections are UTI, in which enterococci are implicated in about 10% of cases, and up to 16% of nosocomial UTI. The species is responsible for significant proportion of cases of bacterial endocarditis , accounting for 5-20% of reported series. Bacteraemias due to this organism are more common than infective endocarditis , often occurring in elderly patients with serious underlying medical conditions or in immunocompromised individuals who have undergone antimicrobial therapy[5]. Several properties of enterococci have been suggested as potential virulence factors reviewed by Jett *et al.*[6]. The production of hemolysin, adhesions, aggregation substances and bacteriocins are the most studied. The virulence ; hemagglutination , lipase and gelatinase were studied by Elsner *et al.*[7].

Very little is known about virulence factors and pathogenicity of *E.faecalis*. This research focuses on virulence factors. The further information about virulence factors of *E.faecalis* is given in this paper for the first time in Iraq.

Materials and Methods

Collection of specimens

The study was conducted at teaching general Hilla hospital, Babylon governorate. 276 samples collected from different sources during the period from 20 March to 1 August, 2003. Of which, 125 patients were admitted to the hospital who suspected with enterococcal infections, and 111 samples were collected from environment of hospital, and 40 stool samples are collected from healthy persons.

Different specimens (blood, stool, and urine) were taken from the patients by standard procedures as described in [7], whereas other specimens (Acne, bed sore, vaginal discharge, ear discharge, wounds, hospital floor, and medical devices) were collected by swabbing, while the sample of tap and sewage water were collected by standard methods that described in [8].

Bacteriological examinations:

The diagnosis was based on bacteriological examinations such as: Gram stain and bacterial cultures, and identified by biochemical tests for each isolates.

The culture for isolation of *E.faecalis* was carried out by standard technique [9], including use of selective media, Brain-heart infusion agar (Oxoid /UK) supplemented with sodium azide and crystal violet. *E.faecalis* isolates were phenotypically identified with colonial morphology. Gram stain was performed as described in [10], and final identification of each isolate with a series of conventional

biochemical tests which described in MacFaddin manual [11].

Detection of virulence factors:

For detection of hemolysin activity, the brain-heart infusion agar (Difco, USA) supplemented with 5% blood was used. Agar plates were incubated for 24hr at 37C, and cytolitic activity was observed as beta-hemolysis surrounding bacterial colonies [9].

Proteinase (gelatinase) activity detected using the gelatin agar (Difco). *E.faecalis* were incubated at 37C for 48hr., and gelatinase activity was observed as transparent halo around the colonies after flooding the plate with Frazier solution [12].

Haemagglutination test was performing using modification of the method described by Korhonen and Finne [13]. *E.faecalis* isolates were incubated for 24hr. at 37C on Columbia agar (Difco) supplemented with 10% blood. A loop of bacteria was picked up with a plastic rod and mixed gently on a glass slide with 25 microns of 3% erythrocytic suspension in phosphate buffered saline (pH 7.4). After 5 min at room temperature, the haemagglutination was recorded as positive or negative.

For detection of lipase activity, enterococci were incubated for 72hr at 37C on Spirit blue agar (Difco) supplemented with lipase reagent [9].

Detection of adhesion factor according to standard procedure that described by Guzman *et al.* [14], urine samples from women were centrifuged to sedimentation of epithelial cells. These cells incubated with bacteria, and examined under microscope.

Bacteriocin production was detected by cup assay procedure that described in [15]. The result of bacteriocin activity were recorded after incubation at 37C for 24hr.

Results and Discussion

The primary isolation of *E.faecalis* was performed by streaking all collected samples on crystal violet-azide BHIA medium. Thirty three isolates were identified as *E.faecalis*, in which 15 isolates from stool of healthy persons, 11 isolates from clinical cases , and 7 isolates from hospital environments (see table-1).

Thirty three isolates of the *E. faecalis* tested possessed at least one of the six virulence factors hemolysin, gelatinase, haemagglutination, adherence, lipase and bacteriocin (see table-2), whereas other isolates belong to other species and none of these classic factors were present. This correspond to the results of previous study [16] in which potential virulence factors of *E.faecalis* include hemolysin , aggregation substances, gelatinase, lipase.

In Germany[7] , results of the study reveal approximately 16% of *E.faecalis* isolates were found to be hemolysin producers. Similar results were found in study of Coque *et al.*[17]. We concluded that hemolysin is not an essential virulence factor or has limited role in pathogenicity of enterococcal infections.

The production of lipase and gelatinase were observed in 3% and 6% respectively in all isolates of *E.faecalis* . The activity of these factors have been described previously in *E.faecalis* isolated from soil [18], and isolates from clinical cases. The low prevalence of these virulence factors suggest that they are probably not associated with virulence in this species.

Agglutination of erythrocytes by these bacteria is a convenient measure of adherence [14]. In our study we found approximately 88% positive cases for haemagglutination . Haemagglutination has been previously described in 97% of

E.faecalis isolates [7]. We conclude this factor may contribute to attachment to host cells and has role in pathogenicity of *E.faecalis*.

Adherence ability of *E.faecalis* to epithelial cells of host body occur in most clinical cases .we conclude the ability of *E.faecalis* to colonize in epithelial cell means that these microorganisms have adhesion factors. Bacterial adherence to host tissues is a crucial first step in the infection process[1,14]. During the process of tissues invasion , *E.faecalis* encounter an environment vastly different than those at sites of colonization. The surface proteins ,surface carbohydrates and lipoteichoic acid of *E.faecalis* cell wall are interfering with binding to target site[6,19] .

Thirty isolates reveal that 90.9% were able to produce a bacteriocin . These results are in agreement with the findings of Al-Barazangi in 2001[20], who found a bacteriocin-producing strains of *E faecalis*. in most clinical isolates. The result is indicate these substances have important role in bacterial interferences as strategy for control and prevention of certain microorganisms that cause infectious disease.

Conclusions

In this study; Adherence factor , hemagglutination and bacteriocin were seen in feces of healthy persons because the *E. faecalis* bacteria are commonly found in intestine of human as commensally organisms, also these factors are essential for colonization of enterococci in human gut. The colonization of host tissues may a play role in pathogenesis of enteroocci.

Several properties of *E.faecalis* which indicate as potential virulence factors such as hemolysin, bacteriocin, lipase, gelatinase, adhesions in most clinical cases. Since nosocomial isolates of *E.faecalis* have displayed virulence

factors , it with likely become excellent opportunistic invaders.

In present study, *E.faecalis* has little virulence factors among types isolated from hospital environments. It is well adapted for survival and persistence in a variety of adverse environments, including sites of infection and inanimate hospital surface .

Future Directions

More research is needed to clarify the pathogenesis of *E.feacalis* in experimental model, and additional studies on the pathogenesis to characterize the molecular and cellular between virulence factors and infection.

Table-1 Types of specimens and number of isolates of *Enterococcus faecalis*

Source of isolation	Number of isolates	
Normal flora:		
Stool(40 samples)	15	45.4%
Clinical specimens(125):	-	-
Urine (30)	7	21.1%
Blood(20)	0	0.0%
Acne(12)	1	3.0%
Bed sores(16)	1	3.0%
Wounds(18)	0	0.0%
Vaginal discharge(20)	1	3.0%
Ear discharge(9)	1	3.0%
Environmental samples(111):	-	-
Tap water(20)	0	0.0%
Sewage water(21)	4	12.1%
Patients beds(15)	2	6.0%
Hospital floor(30)	0	0.0%
Medical devices(25)	1	3.0%
Total number: 276	33	100%

Table 2 Some virulence factors in 33 isolates of *Enterococcus faecalis* .

Virulence factors	Source of isolation			Total number
	stool	clinical	environmental	
Adherence factors	5(15.1%)	10(30.3%)	0(0.0%)	15(45.4%)
Heamagglutination	12(36.3%)	11(33.3%)	6(18.2%)	29(87.8%)
Hemolysin	0(0.0%)	4(12.1%)	1(3.0%)	5(15.1%)
Gelatinase	0(0.0%)	2(6.0%)	1(3.0%)	3(9.0%)
Lipase	0(0.0%)	2(6.0%)	0(0.0%)	2(6.0%)
Bacteriocin	13(39.3%)	11(33.3%)	6(18.2%)	30(90.9%)

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