Molecular Detection of cylA and Investigation of Heamolysin Type in *Enterococcus faecalis*

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Abstract

In this study, 39 isolates of *Enterococcus faecalis* were collected from different source (stool, urine and vagina). It was seen that 79.4% of the isolates are non –hemolyticon human blood agar, where20.6% were hemolytic. Since,5.1% revealedα-hemolysis and 15.5% β-hemolysis. All β-hemolytic isolates were from urine and vagina samples but not from stool samples .

Moreover, investigation for cytolysinA(*cylA*)by using specific primer and PCR technique was showed that all isolates from different sources gave positive amplicons with *cylA* primer.

cytolysinA may be part from Hly activity in *Enterococcus faecalis.*

الخلاصة

تم فيهذه الدراسة تم عزل٣٩عزلة من المكورات المعوية البرازية من مصادر مختلفة(الخروج والادرار والمهبل).وقد اظهرت الدراسة بأن79.4% من العزلات غير محللة للدم على اكار الدم البشري،بينما 20.6%من العزلات كانت محللة. حيث اظهر5.1% تحلل من النوع الفا و 15.5% تحلل من نوع بيتا.

وتبين من الدراسة ان التحلل من نوع بيتا شمل عينات الادرار والمهبل فقط ولم يظهر بين عينات الخروج.

وعند التحري عن السايتولايسن(*cylA*) بأستخدام بادئ متخصص وتقنية تفاعل البلمرة المتسلسل(PCR). ظهر أن جميع العزلات من المصادر المختلفة اعطت نتيجة موجبة لبادئ (*cylA*.) .حيث يعتبر السايتولايسن جزء من نشاط الهيمولايسن فيكتريا *Enterococcus faecalis*.

Introduction

Enterococci are common inhabitants of the human intestinal microflora and the genitourinary tract of men and women[1].They are now recognized as important causes of health care-associated infections including urinary tract infections, postsurgical wound infections, bacteremia, endocarditis, meningitis, neonatal sepsis [2].

The general interest for Enterococci and treatment of Enterococcal infections has increased due to the appearance of antibiotic multi-resistant strains and especially to the occurrence of Vancomycin resistant strains [3].

The production of hemolysin, adhesions, aggregation substances and bacteriocins are the most studied [4].

The *cylA* is a bacterial toxin expressed by many strains of *E.faecalis* and *E.faecium* that cause lysis of horse, rabbit, human blood cell , the genes for the production of which are located on pheromone-responsive plasmids[5].

CylA has β-hemolytic properties in humans and is bactericidal against other Gram-positive bacteria and associated with bacteriocin activity and its believed that the Hly and bacteriocin are mediated by the same genetic determinant[6].

The cytolysin operon is encoded on mobile elements thatoccur within a subset of *E. faecalis* strains [7,8]. It is unique among bacterial haemolytic toxins as well as bacteriocins in having incorporated both these activities into a single system. The relationship of the cytolysin to streptolysin S, produced by *Streptococcus pyogenes* , and also to the lantibiotic class of bacteriocins is evident both structurally and functionally [9].

Aim of the study:

To investigate of hemolysis type and cytolysinA gene among local isolates of *Enterococcus faecalis*

Materials and Methods

Collection of specimens:

The study was conducted at Teaching General Hilla Hospital in Babylon Governorate. 96 samples collected from different sources during the period from October 2012 to January 2013. Only 39isolates of *Enterococcus faecalis* were obtained from patients with gastro- enteritis, urinary tract infection and vaginal discharge by standard bacteriological methods. Since,(18isolates were collected from stool samples ,14 from urine, and 7 from vaginasamples).

Bacterial identification:

The samples were processed on blood agar and selective media (Bile esculin sodium azide agar) were incubated at 37 ºC performed by standard biochemical methods (catalase test, oxidase test, tolerance to bile-esculin, growth in 6.5% NaCl, hemolysin, and growth at 10-45c_o) according to Bergy's Manual for Determinative Bacteriology [10].

Phenotypic Detection of Hly in *E.faecalis*:

For detection of hemolysin activity, the brain-heart infusion agar (Difco,USA) supplemented with 5% human blood. Agar plates were incubated for 24hrs at 37C, and cytolytic activity was observed as beta-hemolysis surrounding bacterial colonies[11].The result shown in Table(1).

DNA extraction for Gram positive bacteria:

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company(Geneaid, UK).

Detection of cylA markers by PCR:

The primers and PCR conditions used to amplify cylA genes encoding virulence factors with PCR are listed in table (2). Each 25µl of PCR reaction contained 2.5µl of each upstream and downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 70V. The size of the amplicons were determined by comparison to the 100 bp allelic ladder (promega, USA).

Table 1 Primers sequences and PCR condition

Genes	Primer sequence (5′-3′)	Size of product bp	PCR condition	Reference
cylA F *cylA* R	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	688	94ºC 2min 1x 94ºC 1min 54ºC 1min 30x 72ºC 1min 72ºC 10min 1x	*Creti *et al.*, 2004

Results and Discussion

All isolates of *E. faecalis* tested phenotypically for revealed the hemolysin activity on human blood agar and the results showed that *E.faecalis* was mostly non-hemolytic at a rate (79.4%). However, some isolates gave α- hemolysis (5.1%)or β-hemolysis (15.5%) as shown in Table (2).All stool isolates gave no hemolysis on human blood agar , whereas urine and vagina isolates gave different mode of hemolysis where three gave β-hemolysis , one α- hemolysis and other with no hemolysis.It is known that *E.faecalis* is mostly non hemolytic,where hemolysin is not produced extracellularly but some studies indicated that this bacteria may produce different type of Hly.

Absent of hemolytic activity in Enterococcus not necessarily mean that these bacteria are not virulent beacause Hly is not turn virulence , it is classified withinvirulence associated factors [12].

Table 2 Distribution of hemolysin production according to Enterococci isolates

Type of haemolysis	**Source of *E.faecalis* isolates**			
	Urine (14)	**Stool (18)**	**Vagina (7)**	**Total %**
Gamma	**10**	**18**	**3**	**31(79.4%)**
Alpha	**1**	**0**	**1**	**2(5.12%)**
Beta	**3**	**0**	**3**	**6(15.3%)**
Total	**14**	**18**	**7**	**100%**

However, the frequency of hemolysis production among *Enterococcus* from feces of healthy individual was low compared with that of clinical isolates.

None of the stool isolates gave α or β - hemolysin only γ- hemolysis was seen. This may reflect that this bacteria don't need the production of Hly when present as normal flora in the human intestine.

However, some isolates which were isolated from urine or vagina also gave γ- hemolysis . this means

that the source of these bacteria which were isolated from urine or vagina was from the human intestine mostly in women.

Moreover, it was seen that some urine or vagina isolates gave β- hemolysis, this may be due to the presence of large plasmid which confer β- hemolytic on blood agar which in turn will enhance the virulence of these isolates. Also it was mentioned by Tomita*et al* .,[13] who pointed out that hemolysin produced by *E.faecalis* has bacteriocin activity ,this will increase the bacterial virulence and pathogenic

These results are in agreement by Archimbaud *et al* .,[14] and Dupont *et al*.,[15] Who found that hemolysin was more common in clinical isolates than among community fecal isolates, indicating that intestinal Enterococci may differ in this respect from clinical strains.

However, specific PCR primers were used for detection of *cylA* gene. It was found that *cylA* gene was present in all *E.faecalis* isolates . This means that this gene present among all isolates from different sources. as shown in Figure(1).

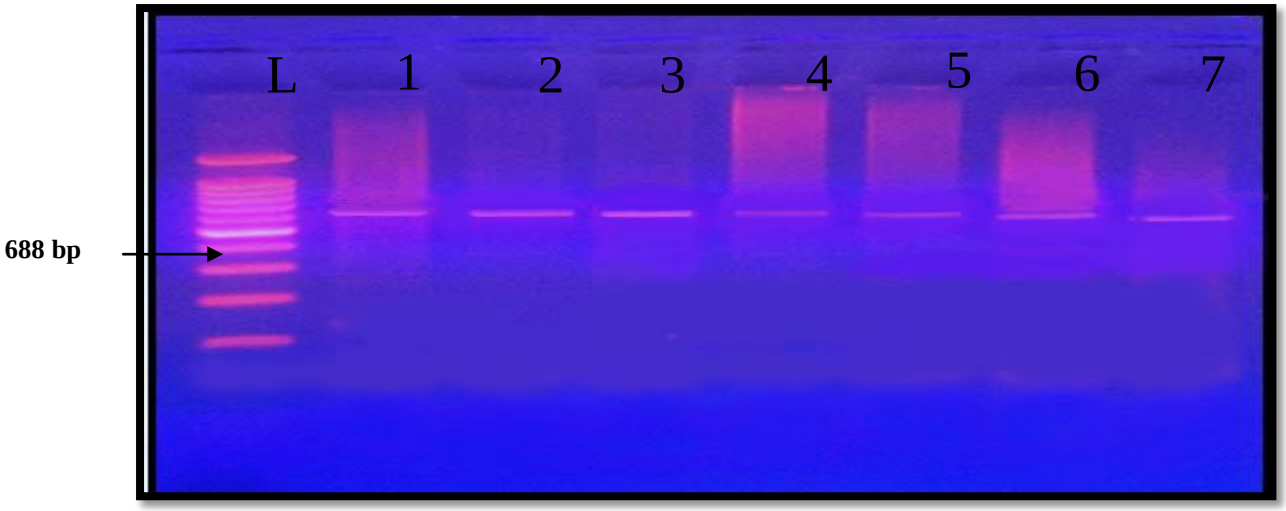

Figure 1 Gel electrophoresis of PCR of *cylA* amplicon product.
L: ladder; 1, 2 3, 4,5,6,7 : no. of isolates obtained from urine ,stool, and vagina with positive result for *cylA*.

This result is in agreement with the result obtained byCreti *et al.*, [16] who found all isolated of *E.faecalis*expressed of *cylA* gene.

The Enterococcal *cylA* including a large family of toxins and bacteriocins, It enhance Enterococcal virulence in infection and associated with patient mortality because it is lethal for a broad range of prokaryotic and eukaryotic cell by lyses a wide range of gram-positive bacteria as well as human, rabbit, and horse erythrocytes [17].

Moreover, the *cylA*has been associated with lethality to humans following analysis of an outbreak of multiple antibiotic resistant *E. faecalis* [18]. High resistance to antibiotics in hemolytic isolates may be due to the possession of the isolates of Hly plasmid which confers resistance to antibiotics possibility that conjugative Hly plasmid highly play a significant role in the dissemination (mobilization) of resistance determinants.

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