Kafrelsheikh Vet. Med. J. Vol. 10 No. 1 (2012) (127-135)

OCCURANCE OF ENTEROCOCCI IN MILK WITH SPECIAL REFERENCE TO ENTEROCOCCUS FAECALIS

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ABSTRACT

A total of 115 unheated milk samples (61 street peddlers samples and54 farmers milk samples), were collected from various localities in Kafr El-Sheikh Governorate (Egypt). The samples were examined bacteriologically for enterococci with percentages of (100% of street peddlers samples and 62.96% of farmers milk samples) while The percentage of Enterococcus strains in positive samples were [(100%) for Ent. faecalis, (21.31%) for Ent. faecium and (16.39%) for Ent. durans] in street peddlers samples and [(100%) for Ent. faecalis, (23.53%) for Ent. faecium and (5.88%) for Ent. durans] in farmers milk samples.

Amplification of 185 bp fragment specific for the groES gene of Enterococcus faecalis used for confirmation of its identification.

INTRODUCTION

Enterococci are generally accepted as an index of fecal contamination ***(El-Sayed and Ayoub, 1993).***

It is generally believed that the primary habitat of enterococci is the intestinal contents of warm-blooded animals yet, the gastrointestinal contents of cold blooded animals, including insects and birds, constitute other important habitats as well ***(Mannu et al., 2003).***

In accordance with their widespread occurrence in the intestinal tract of animals, enterococci and other group D-streptococci are present in many foods, especially in those of animal origin. Therefore, the isolation of *E. faecalis* and *E. faecium* in foods has often been used to indicate a 'primary' contamination with faeces ***(Klein, 2003).***

Enterococci are the leading cause of nasocomial infection (or secondary infection acquired in a hospital). They are responsible for approximately 110,000 cases of urinary tract infection, 25,000 cases of bacteremia, 40,000 wound infections, and 1, 100 cases of endocarditis yearly in the United States ***(Doe Joint Genome Institute, 2005).***

Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis and meningitis ***(Fisher and Phillips, 2009).***

Polymerase chain reaction (PCR) was used for *Enterococcus faecalis* identification ***(Teng et al., 2001).***

Therefore the goal of the present study was to detect enterococci in milk and confirmation of *E. feacalis* isolates by PCR.

MATERIAL AND METHODS

Collection of samples:

A total of 115 unheated milk samples (61 samples from street peddlers and 54 samples from farmers milk) were collected from different localities in Kafr El-Sheikh Governorate (Egypt) in clean, dry and sterilize containers during the period from April 2010 to September 2010.

Bacterological examination:

Isolation and identification of enterococci was carried out according to ***Quinn et al. , (1994).***

Uses of polymerase chain reaction (PCR) for confirmation of *E. Faecalis* was carried out according to ***Teng et al., (2001).***

RESULTS

Table (1): Prevalence of enterococci in the examined milk samples.

Type of samples	No. of samples	Positive samples	
		Enterococci	
		No.	%
Street peddlers	61	61	100
Farmers milk	54	34	62.96

Table (2): Prevalence of *Enterococcus* species in positive samples.

Enterococcus species	Street peddlers positive samples (61)		Farmers milk positive samples (34)	
	No.	%	No.	%
Ent. faecalis	**61**	**100**	**34**	**100**
Ent. faecium	**13**	**21.31**	**8**	**23.53**
Ent. durans	**10**	**16.39**	**2**	**5.88**

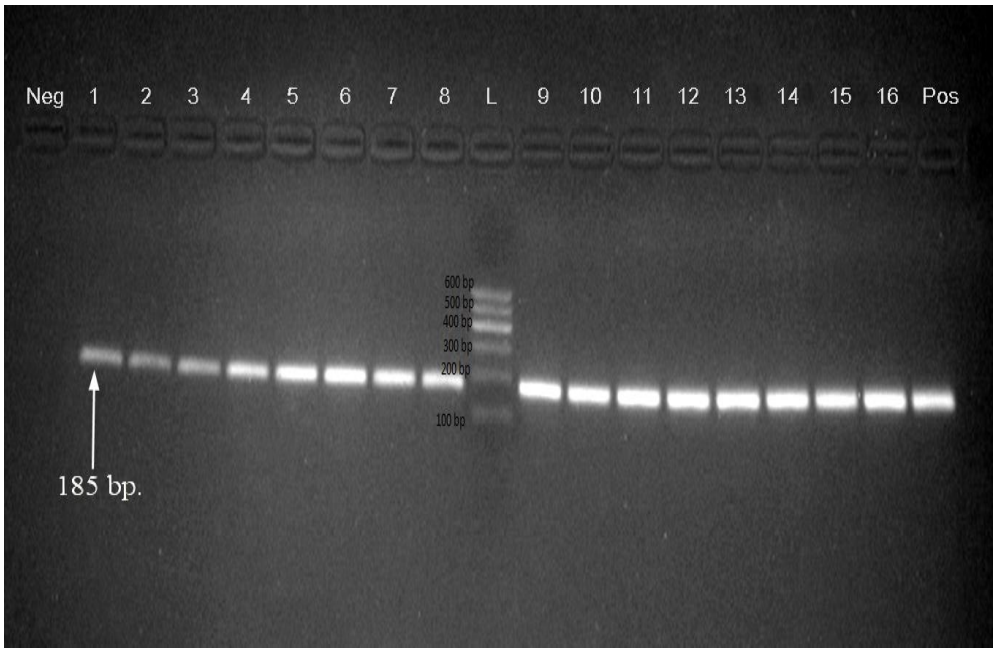

Photo (1): Agarose gel electrophoresis result of Ent. faecalis isolates (1:16) by conventional PCR which revealed the presence of groES gene of Ent. faecalis in all 16 isolates recovered from 16 positive milk samples (8 from street peddlers and 8 from farmers milk samples).

DISCUSSION

The data recorded in Table (1) showed that the enterotocci could be isolated by (100%) out of 61 street peddlers positive samples while it was isolated by (62.96%) out of 34 farmers milk positive samples. ***El-Sayed and Ayoub, (1993)*** in Zagazig isolated enterococci by 58.33% from street peddlers and dairy shops also ***Bogdanowicz and Nockiewicz, (1973)*** isolated 97.51% of enterococci from raw milk.The difference may be related to locality and hygenic measures.

Results recorded in Table (2) indicate that *Ent. faecalis* could be isolated by (100%) out of 61 street peddlers positive samples and also it was isolated by (100%) out of 34 farmers milk positive samples. Nearly similar results were obtained by ***El-Sayed (1985)*** who showed that *Strept. faecalis* was present in all samples of raw milk. On other side, the obtained results disagree with those reported by ***Moustafa et al., (1975).*** They recorded that *Strept. faecalis* isolated from street vendors and shops by 8%. This disagreement may be due to poor sanitation and bad handling. While *Ent. faecium* isolated by (21.31%) from street peddlers positive samples and by (23.53%) from farmers milk positive samples. It is interested that ***Jacobsen (1963)*** isolated *Strept. faecium* by (27.4%) from fresh milk.

Finally *Ent. durans* was isolated by (16.39%) from street peddlers positive samples and by (5.88%) from farmers milk positive samples. ***Thomas and Laxminarayana, (1972)***. isolated *Strept. durans* from cow's and buffalo's milk by (2.6%).

The results of investigation revealed that the prevalent *Enterococcus strains* was *Ent. faecalis* followed by *Ent. faecium* then *Ent. durans.* These results are in agreement with those reported by ***Hashimoto (1961)*** who reported that *Strept. faecalis* was commonly found in raw milk but *Strept. faecium* predominated in heat treated food.

The higher result of *Enterococcus faecium* in farmers milk positive samples than street peddlers positive samples may be due to that the *Enterococcus faecium* is a prevalent species in production animals ***(Klein ,2003).***

A PCR-based detection assay was used in this study for confirmation of *Ent. faecalis* which was carried out on 16 isolates of *Ent. faecalis* (8 from street peddlers and 8 from farmers milk samples) identified by conventional culture method and represented by 16 out of 16 isolates at percentage of 100% Photo (1). These results agree with ***Lee et al., (2001)***. They reported that PCR was positive for all the *Ent. faecalis* strains tested. On other side, the obtained results disagree with those reported by ***Farid et al., (2006)***. They reported that *Ent. faecalis* was obtained in only 2 strains from 8 tested at percentage of 25%.

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دراسات على تواجد المكورات السبحية المعوية في اللبن مع التركيز علي ميكروب الانتيروكوكس فيكالز

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أجريت هذه الدراسة على 115 عينة من اللبن الغير معامل حراريا (61 عينة من الباعة الجائلين بالأسواق و54 عينة من ألبان الفلاحين) جمعت عشوائيا من محافظة كفرالشيخ (مصر) ونقلت سريعا للمعمل حيث تم فحصها بكتريولوجيا لتحديد مدي تواجد المكورات السبحية المعوية وتبين من الفحص البكتريولوجي أن المكورات السبحية المعوية تواجدات بنسبة 100% فى العينات المأخوذة من ألبان الباعة الجائلين بالأسواق وبنسبة 62.96% من ألبان الفلاحين بينما تواجدت الميكروبات السبحية المعوية فى العينات الإيجابية بالنسب الآتية : وجد أن المكور السبحى فيكالز تواجد بنسبة 100% فى كل من العينات المأخوذة من الباعة الجائلين بالأسواق وعينات الألبان المأخوذة من الفلاحين بينما تواجد المكور السبحى فيكم بنسبة 21.31% و23.53% فى كل من العينات المأخوذة من الباعة الجائلين بالأسواق وألبان الفلاحين على التوالى أخيرا تواجد المكور السبحى ديورانس بنسبة 16.39% و5.88% في كل من ألبان الباعة الجائلين بالأسواق وألبان الفلاحين على التوالى.

كما تم أيضاً استخدام تفاعل البلمرة المتسلسل لتأكيد تواجد المكور السبحى فيكالز *Enterococcus faecalis* والذى أجرى على الحامض النووى باستخدام البادئ المتخصص لجين groES.