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Detection of shiga toxin strains of Escherichia coli non O157 in different soft cheese by polymerase chain reaction

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A B S T R A C T

The objectives of the current study were detection and characterization of the isolated Escherichia coli non O157 using PCR assay. A total of 90 cheese samples high salt soft cheese, Kareish cheese and Tallaga cheese were examined for E. coli non O157 using modified vancomycin- trypticase soy broth and Sorbitol MacConkey agar plates. Serodiagnosis of E. coli non-O157 has been done using slide agglutination test. Toxigenic E. coli non-O157 isolates were detected using PCR assay. Results postulated that the detection rate of non O157 using biochemical technique was 63.33% in Kariesh cheese, 20% in high salt soft cheese cheese, and 33.33% in Tallaga cheese. Ten different serotypes of E. coli non O157 have been distributed as following O1 (4.44%), O18 (1.11%), O20 (1.11%), O25 (1.11%), O26 (3.33%), O125 (1.11%), O126 (1.11%), O127 (1.11%) and untyped E. coli (18.89%). Regarding PCR results, serotypes group (O1) was positive for Stx2 gene, (O1 and O20) were positive for both Stx2 and hly genes, one serotype group O25 was positive for eaeA gene, one serotype group O26 was positive for hly gene while no detection for Stx1and STa genes. From the aforementioned data, attention must be paid to the problems of E. coli non O157 in foods. Consequently, more restriction and preventive measures should be taken in milk herds, milk production and dairy factories in respect to quality control sanitation and health care.

Keywords: *E. coli non-O157, Stx1, Stx2, eaeA and hly genes .*

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1. INTRODUCTION

Diarrheaogenic E. coli (DEC) are some of the most frequently detected pathogens worldwide. There are six pathotypes of DEC: Enterotoxigenic E. coli (ETEC), Entero-aggregative E. coli (EAEC), Entero-pathogenic E.coli (EPEC), Enteroinvasive E. coli (EIEC), Entero-haemorrhagic E. coli (EHEC) or Verocytotoxin-producing E. coli and diffusely adherent E. coli (Bischoff et al., 2005 ; Vernacchio et al., 2006). Although many strains of non-O157 STEC appear to be less virulent than E. coli O157:H7. In 2008 an outbreak of STEC strain O111 in Oklahoma caused illness in at least 314 people, HUS in 17 cases, and one death (Mathusa et al., 2010).

Shiga toxins, the main virulence factors contributing to pathogenicity consist of two major types, Stx1 and Stx2, each including several variants (Scheutz and Strockbine, 2005). Non O157 Shiga toxin–producing E. coli (STEC) strains have been linked to outbreaks and

sporadic cases of illness worldwide. Illnesses linked to STEC serotypes other than O157:H7 appear to be on the rise in the United States and worldwide, indicating that some of these organisms may be emerging pathogens (Mathusa et al., 2010).

Cheeses were contaminated with different STEC serotypes (O26:H11,O103:H2 and O145:H28) at the milk preparation stage. STEC growth and survival were monitored on selective media during the entire manufacturing process. (Miszczycha et al., 2013).

2. MATERIALS AND METHODS

2.1. Samples collection:

Ninety soft cheese samples divided as Kareish cheese, high salt soft cheese and Tallaga cheese(30 of each) were collected from milk vendors and retail shops from Assuit Governorate, Egypt for detection of shiga toxin strains of Escherichia coli non O157 in different soft cheese by polymerase chain reaction.

2.2. Isolation and identification of E. coli non O157: Samples were prepared to isolate the E. coli according to standard Bacteriological Analytical Manual (BAM), U.S. Food and Drug Administration (USFDA) method (Kumar et al., 2008). The samples were enriched in modified vancomycin- trypticase soy broth (MVTSB) and incubated at 37°C for 24 h. (Samadpouret al., 1990). Loopful of culture inoculated into Sorbitol MacConkey (SMAC) agar plates and incubated at 37°C for 24 h. Suspected E. coli O157 colonies were sorbitol negative and appeared pale in colour as compared with bright pink sorbitol positive, these colonies produced by E. coli non-O157 and other enteric pathogens (De Boer and Heuvelink, 2000).Various biochemical tests were done for the confirmation of E. coli non-O157 as proposed by APHA (1992).

2.3. Serodiagnosis of E. coli non-O157:

It had been done in Clinical Microbiology unit in Central Health Laboratories of Ministry of Health and Serology unit in Animal Health Research Institute, Cairo, Egypt. The test sera/test reagents anti-coli intended for use in the serological detection was purchased from SIFIN (Institut für Immunpräparate und Nährmedien GmbH Berlin, Germay). The determination of the serovar E. coli strains isolated from soft cheese samples were done using slide agglutination test. The test sera are absorbed sera from immunized rabbits. The test reagents consist of a mixture of absorbed sera from immunized rabbits and monoclonal antibodies or contain only monoclonal antibodies.

2.4. Detection of toxigenic E. coli non-O157 isolates using PCR assay:

It had been done in Biotechnology unit in Animal Health Research Institute, Cairo, Egypt. Cycling conditions of the primers during PCR are shown in Table (1).Primers were supplied from metabion (Germany).They have specific sequence and amplify specific products as illustrated in Table (2).

Table (1): Cycling conditions of the primers during PCR.

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Stx1	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
Stx2	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
hly	94˚C 5 min.	94˚C 30 sec.	60˚C 50 sec.	72˚C 1 min.	35	72˚C 10 min.
STa	94˚C 5 min.	94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
eaeA	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

Table (2): Oligonucleotide primers encoding for 16SrRNA and clfA genes.

Target gene	Primer sequence (5'-3')	Length of amplified product	Reference
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	Dipineto *et al.*, 2006
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	
Hly	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177 bp	Piva *et al.*, 2003
STa	GAAACAACATGACGGGAGGT GCACAGGCAGGATTACAACA	229 bp	Lee *et al.*, 2008
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248 bp	Bisi-Johnson *et al.*, 2011

3. RESULTS

Results postulated in Table (3) indicated that the detection rate of E. coli non O157 using biochemical reactions was 63.33% in Kareish cheese, 20% in high salt soft cheese and 33.33% in Tallaga cheese.

Serological identification of isolated E. coli declared that the highly contaminated product was Kareish cheese (53.33%) followed by Tallaga cheese (30%) and high salt soft cheese (16.67%) (Table 4). Ten different serotypes of E. coli non O157 have been distributed as following O1 (4.44%), O18 (1.11%), O20 (1.11%), O25 (1.11%), O26 (3.33%), O125 (1.11%), O126 (1.11%), O127 (1.11%) and untyped E. coli (18.89%) (Table 5). Regarding PCR results, E. coli non O157 was detected in 5 out of 90 samples (5.56%) distributed as 10% in Kareish cheese and 6.67% in Tallaga cheese (Table 6). Serotypes group (O1) was positive for Stx2 gene, (O1 and O20) were positive for both Stx2 and hly genes, one serotype group O25 was positive for eaeA gene, one serotype group O26 was positive for hly gene while no detection for Stx1and STa genes(Table 7).

Table (3): Incidence of *E.coli* non O157 in the examined cheese samples based on biochemical reactions.

Samples	No. of examined samples	No. of positive samples	%
Kareish cheese	30	19	63.33
High salt soft cheese	30	6	20
Tallaga cheese	30	10	33.33
Total	90	35	38.89

Table (4): Incidence of *E.coli* non O157 in the examined cheese samples based on serology.

Samples	No. of examined samples	No. of positive samples	%
Kareish cheese	30	16	53.33
High salt soft cheese	30	5	16.67
Tallaga cheese	30	9	30
Total	90	30	33.33

Table (5): Serological differentiation of *E. coli* non O157 in the examined cheese samples.(n=30)

Samples	*E. coli O1*		*E. coli O18*		*E. coli O20*		*E. coli O25*		*E. coli O26*		*E. coli O125*		*E. coli O126*		*E. coli O127*		Untyped *E. coli*	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Kareish cheese	2	6.67	0	0	1	3.33	0	0	2	6.67	1	3.33	0	0	1	3.33	9	30
High salt soft cheese	2	6.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	10
Tallaga cheese	0	0	1	3.33	0	0	1	3.33	1	3.33	0	0	1	3.33	0	0	5	16.67
Total	4	4.44	1	1.11	1	1.11	1	1.11	3	3.33	1	1.11	1	1.11	1	1.11	17	18.89

Table (6): Incidence of *E. coli* non O157 containing toxigenic genes in the examined cheese samples according to PCR results. (n=30)

Samples	No. of examined samples	No. of positive samples	%
Kareish cheese	30	3	10
High salt soft cheese	30	0	0
Tallaga cheese	30	2	6.67
Total	90	5	5.56

Table (7): Differentiation of toxigenic genes among the isolated *E.coli* non O157 strains.

Samples	*E. coli O1*		*E. coli O20*	*E. coli O25*	*E. coli O26*
	Stx2	*Stx2 and hly*	*Stx2 and hly*	*eaeA*	*hly*
Karish cheese	1	1	1	0	0
High salt soft cheese	0	0	0	0	0
Tallaga cheese	0	0	0	1	1

4. DISCUSSION

Kareish cheese is one of the ancient Egyptian fresh white soft cheeses. It is consumed largely in Egypt due to its low price and high nutritive value. The incidence of E. coli non O157 was 63.33% in Kareish cheese, 20% in high salt soft cheese, 33.33% in Tallaga cheese (Table 3). The variation between such types of cheeses in results may be due to the difference in salt concentrations, acidity, and the method of manufacture. Additionally, ripening in brine solution, quality and heat treatment of milk used in the manufacture, handling method, hygienic practices, transportation condition, storage condition and distribution play an important role in its microbial quality.

Nearly similar result was detected by El- Bessery (2006).Lower result in kariesh cheese was reported by Hassan and Gomaa (2016). Higher incidence in kariesh cheese was reported by Ombarak et al.(2016) and Amin et al.(2017).

The higher rate of E. coli contamination rate of Kareish cheese observed is may be due to the differences in cheese making process and the characteristics of final product between cheeses (Ombarak et al., 2016).

Contamination of cheese with micro-organisms may originate from many sources. Such sources during cheese production might be: starter culture, brine, floor and packaging material, cheese vat, cheese cloth and curd cutting knife, cold room and production room air (Sharaf et al., 2014).

Serological identification of isolated E. coli declared that the highly contaminated product was Kareish cheese (53.33%) followed by Tallaga cheese (30%) and high salt soft cheese (16.67%) (Table 4). These results were not agree with those recorded by Elsherif (2014) who found that the contamination with E. coli in Tallaga cheese samples was 12%. In the current study, the strains of pathogenic E. coli isolated from examined soft cheese were illustrated as O1 (4.44%), O18 (1.11%), O20 (1.11%), O25 (1.11%), O26 (3.33%), O125 (1.11%), O126 (1.11%), O127 (1.11%) and untyped E. coli (18.89%) as shown in Table (5).

The highest number of E. coli strains isolated from Kareish cheese, 2 strains for each of O1and O26 (6.67%), one strain for each of O20, O125 and O127 (3.33%) and 9 (30%) untyped strains. one strain for each of O18, O25, O26 and O126 (3.33%) and 5(16.67%) untyped strains isolated from Tallaga cheese. Two strains belonged to O1 (6.67%) and 3(10%) untyped strains isolated from high salt soft cheese (Table 5). A large number of STEC strains (e.g., members of the serogroups O26, O91, O103, O111, O118, O145, and O166) have caused major outbreaks and sporadic cases of human illnesses that have ranged from mild diarrhea to the life-threatening hemolytic uremic syndrome (Hussein and Sakuma, 2005).

Different serotypes groups of STEC isolates were detected by PCR from the examined soft cheese. Serotypes group (O1) was positive for Stx2 gene, (O1 and O20) were positive for both Stx2 and hly genes, one serotype group O25 was positive for eaeA gene, one serotype group O26 was positive for hly gene while no detection for Stx1and STa genes (Table 7).

Nearly similar results have been reported by Hassan and Elmalt (2008) in Kareish cheese who showed that one strain encoded for Stx2gene and none for Stx1and eaeA. On another hand, out of 50 cheese samples, 4 (16.00%) isolates were positive for Stx1 gene only, while 5 (20.00%) isolates were positive for Stx2 gene and 2 isolates (8.00%) were positive for eaeA gene (Virpari et al., 2013).Other studies detected STEC grew in the two uncooked processed cheeses during the first 24 h of cheese making Then, STEC levels progressively decreased in cheeses that were ripened for more than 6 months. In lactic cheese with along acidic coagulation step, STEC did not grew (Miszczycha et al., 2013).

Detection of Stx genes or prevalence of E. coli harboring Stx genes in dairy products were reported at various degree from different countries such as 0.45% in Spain (Quinto and Cepeda, 1997), 0.87% in Canada (Steele et al., 1997), 3.9% in Germany (Klie et al., 1997), 6% in Brazil (Paneto et al., 2007) and13% in France (Vernozy-Rozand et al., 2005).

5. CONCLUSION

High detection rate of non O157 using biochemical technique was recorded in Kariesh cheese followed by Tallaga cheese and high salt soft cheese. On the other hand PCR results indicated that E. coli non O157 was detected in 5 out of 90 samples (5.56%) soft cheese samples. So, PCR has advantages in terms of sensitivity, specificity, cost and ease of implementation. Multiplex PCR assay presented here is a practical and rapid diagnostic tool for identification of enterotoxigenic E. coli in a single reaction tube.

From the aforementioned data, attention must be paid to the problems of E. coli non O157 in foods. Consequently, more restriction and preventive measures should be taken in milk herds, milk production and dairy factories in respect to quality control sanitation and health care. Moreover, a preventive strategy based on thorough analysis of conditions, which ensure that objectives of the quality assurance program are met, is recommended.

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