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ORIGINAL ARTICLE

# Molecular and serotyping characterization of shiga toxigenic *Escherichia coli* associated with food collected from Saudi Arabia



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## KEYWORDS

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*E. coli*;  
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Shiga toxin genes;  
Intimin gene

**Abstract** Shiga toxin-producing *Escherichia coli* (STEC) strains are considered as one of the major food-borne disease agents in humans worldwide. STEC strains, also called verotoxin-producing *E. coli* strains. The objectives of the present study were serotyping and molecular characterization of shiga toxigenic *E. coli* associated with raw meat and milk samples collected from Riyadh, Saudi Arabia. A total of 540 milk samples were collected from 5 dairy farms and 150 raw meat samples were collected from different abattoirs located in Riyadh, Saudi Arabia. *E. coli* were recovered from 86 milk samples (15.93%), serotyping of *E. coli* isolates revealed, 26 (4.81%) strains O157: H7, 23 (4.26%) strains O111, 20 (3.70%) strains O113: H21, 10 (1.85%) strains O22: H8 and 7 (1.3%) strains O172: H21. Meanwhile, 17 (11.33%) strains of *E. coli* were recovered from raw meat samples, serotyping of *E. coli* isolates revealed, 6 (4%) strains O157: H7, 5 (3.33%) strains O111 and 4 (2.67%) strains O174: H2 and only two (1.33%) strains were identified as O22: H8. Shiga toxin2 was detected in 58 (67.44%) serotypes of *E. coli* recovered from milk samples and 16 (94.12%) serotypes of *E. coli* recovered from meat samples, while intimin gene was detected in 38 (44.186%) serotypes of *E. coli* recovered from milk samples and in 10 (58.82%) serotypes of *E. coli* recovered

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from meat samples. The results of this study revealed the efficiency of combination between serotyping and molecular typing of *E. coli* isolates recovered from food of animal origin for rapid detection and characterization of STEC.

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## 1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains have been identified as one of the major foodborne pathogens in humans, STEC strains, also called verotoxin-producing *E. coli* (Bandyopadhyay et al., 2012). Ruminant animals are considered as one of the most important reservoirs of STEC, which shed bacteria with their feces into the environment without showing any clinical manifestation of the disease and the organisms contaminate the raw milk and the meat causing foodborne disease for the human consuming such food (Alikhani et al., 2006; Mora et al., 2005). Therefore, products of animal origin, such as meat and milk, are at risk of contamination with STEC originating from animals (Jafari et al., 2009; Aitalhi and Hassan, 2009).

This raw, unpasteurized milk can carry dangerous bacteria such as *Salmonella*, *E. coli*, and *Listeria*, which are responsible for causing numerous foodborne illnesses (Alikhani et al., 2006; Jafari et al., 2009). In addition, CDC reported that unpasteurized milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalizations than illnesses involving pasteurized dairy products.

Enteropathogenic *E. coli* (EPEC) are a major cause of infantile diarrhea and it's considered to be one of the world's leading causes of morbidity and mortality (Jelacic et al., 2003; Alikhani et al., 2006). Transmission of EPEC is through the fecal-oral route, with contaminated hand or contaminated foods (Soomro et al., 2002; Kuczius et al., 2004; Vallance and Finlay, 2000).

STEC producing one or two potent toxins called Shiga toxin (Stx1, Stx2), may be able to produce intimin protein (De Buyser et al., 2001) For this reason, *E. coli* strains with the *eaeA* genotype which lack Shiga toxin gene (*stx1* and/or *stx2*) are classified as EPEC (Carneiro et al., 2006). Objectives of this study were serotyping and molecular characterization of shiga toxigenic *E. coli* associated with raw meat and milk samples collected from Riyadh, KSA.

## 2. Materials and methods

### 2.1. Samples

A total of 540 milk samples were collected from 5 dairy farms and 150 raw meat samples were collected from different abattoirs located in Riyadh, KSA, during the period of January, 2013 to March, 2014. The samples were placed on ice and transported immediately to the laboratory.

### 2.2. Standard microbiological techniques

Raw meat and milk samples were primary cultured on MacConkey agar medium, incubated aerobically at 37 °C as described by Quinn et al. (2002). Single typical well isolated

lactose fermenting colonies were tested for sorbitol fermentation by culturing on sorbitol MacConkey agar and sorbitol phenol red agar media, incubated at 37 °C overnight. Morphological, cultural and biochemical examinations were carried out according to methods described by Quinn et al. (2002). *E. coli* isolates were subjected to serological identification using diagnostic polyvalent and monovalent *E. coli* antisera (Welcome *E. coli* diagnostic antisera). Diagnostic *E. coli*-O157 antisera (Dif co code 2970-47-7) and H7 antisera (Difco code 2159-47-0) were used for serological identification of *E. coli* O157: H7.

### 2.3. Extraction of DNA

The DNA of the standard strains and of the other bacterial isolates yielded from bacteriological examination was extracted by hexadecyl trimethyl ammonium bromide (CTAB), according to Sambrook et al. (1989). Meanwhile, the extractions of DNA from milk and meat samples were carried out according to Riffon et al. (2001) and Meiri-Bendek et al. (2002).

### 2.4. Molecular characterization of the STEC

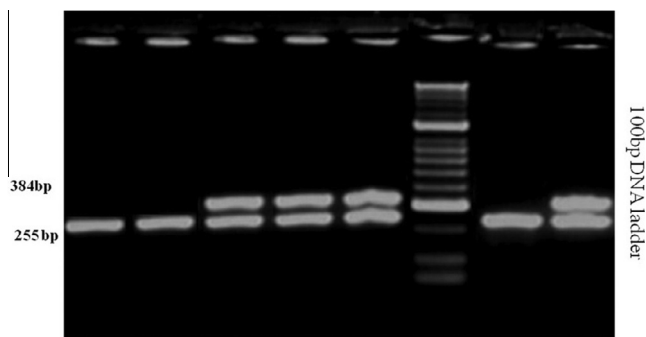
The recovered strains were identified by polymerase chain reaction according to Paton and Paton (1998), PCR primer pairs were designed with reference to published sequence data for shiga toxin type 2 (*stx2*) (Boerlin et al., 1999), intimin gene (*eaeA*) (Yu and Kaper, 1992). The extracted DNA of the standard strains and of the bacterial isolates yielded from bacteriological examination was tested with multiplex-PCR using the oligo nucleotide primers specific for (*stx2*) and (*eaeA*) genes. Concurrently the crude DNA extracted from milk samples were tested by the same primer pair. The PCR products were tested for positive amplification by agarose gel electrophoresis previously reported by Sambrook et al. (1989) using suitable molecular weight markers.

## 3. Results

Out of 540 collected milk samples, a total of 86 *E. coli* (15.93%), were isolated. Meanwhile, 17 (11.33%) strains of *E. coli* were recovered from raw meat samples. Serotyping of *E. coli* isolates from raw milk samples revealed, 26 (4.81%) strains O157: H7, 23 (4.26%) strains O111:K58, 20 (3.70%) strains O113: H21, 10 (1.85%) strains O22: H8 and 7 (1.3%) strains O172: H21.

Serotyping of *E. coli* isolates recovered from raw meat samples revealed, 6 (4%) strains O157: H7, 5 (3.33%) strains O111:K58 and 4 (2.67%) strains O174: H2 and only two (1.33%) strains were identified as O22: H8.

*E. coli* isolates recovered from raw milk samples were tested by multiplex PCR using *stx2* F & *stx2* R and *eaeA* F & *eaeA* R



**Figure 1** Agarose gel electrophoresis showing amplification of 384 bp fragments and 255 bp fragments of intimin and shiga toxin type two genes of shiga toxigenic *E. coli*, respectively.

primers. Positive amplification of 255 bp fragments of shiga toxin type 2 gene (*stx2*) was observed in 58 (67.44%) serotypes and 384 bp fragment of intimin gene from were in 38 (44.186%) strains only in all strains of O157: H7, while from serovar O111:K58 were 15 (65.22%) for *stx2*, 6 (26.09%) for *eaeA* and from serovar O113:H21 were 12 (60%) for *stx2*, 6 (30%) for *eaeA*. However, O22:H8 showed amplification of *stx2* only. No amplification to both genes could be observed with *E. coli* serovars O172:H21, as shown in Fig. 1 and Table 1.

*E. coli* isolates recovered from raw meat samples were tested by multiplex PCR using *stx2* F & *stx2* R and *eaeA* F & *eaeA* R primers. Positive amplification of 255 bp fragments of shiga toxin type 2 gene (*stx2*) was observed in 16 (94.12%) serotypes and 384 bp fragment of intimin gene from were in 10 (58.82%) strains only in all strains of O157: H7, while from serovar O111:K58 were 5 (100%) for *stx2*, 3 (60%) for *eaeA* and from serovar O174:H2 were 4 (100%) for *stx2*, 1 (25%) for *eaeA*. However, O22:H8 showed amplification of *stx2* only in one serotype, as shown in Fig. 1 and Table 2.

#### 4. Discussion

The isolation of *E. coli* strains out of 540 examined milk samples revealed 86 strains obtained from different farms with a prevalence of 15.93%. However, the presence of this pathogen in milk proved to be variable in different regions and these variations may be due to geographical location, season, farm

size, number of animals on the farm, hygiene status, farm management practices, variation in sampling, variation in types of samples evaluated, and differences in detection methods (Levine, 1987; Donnenberg and Kaper, 1992; Vallance and Finlay, 2000; De Buyser et al., 2001; Jelacic et al., 2003).

Serotyping of *E. coli* isolates yielded from bacteriological examination of milk samples revealed, 26 (4.81%) strains O157: H7, 23 (4.26%) strains O111, 20 (3.70%) strains O113: H21, 10 (1.85%) strains O22: H8 and 7 (1.3%) strains O172: H21. *E. coli* serovars yielded from bacteriological examination of milk samples were similar to *E. coli* serovars yielded from fecal samples, indicated that the serotypes causing bovine mastitis were similar to the serotype causing diarrhea or even associated with the fecal samples of apparently healthy calves. Our result confirms the conclusion of Padhye and Doyle (1991), Harmon et al. (1990) and Garber et al. (1999) who mentioned that *E. coli* serovars that causing bovine mastitis were similar to that of fecal isolates. Meanwhile, 17 (11.33%) strains of *E. coli* were recovered from raw meat samples, serotyping of the *E. coli* isolates revealed, 6 (4%) strains O157: H7, 5 (3.33%) strains O111 and 4 (2.67%) strains O174: H2 and only two (1.33%) strains were identified as O22: H8.

Most of these strains belonged to serotypes associated with severe illness in humans, such as O22:H8, O91:H21, O113:H21, O174:H2, and O174:H21 (Alikhani et al., 2006; Jafari et al., 2009).

Regarding Saudi Arabia, Salji et al. (1984) recorded coliforms as the main contaminants of raw milk. *E. coli* isolates and serovars *E. coli* with variable antigenic structure had been reported by Obied and Bagadi (1996) in Saudi camel's milk. Whereas Al Ghamdi et al. (1999), found 34.7% *E. coli* isolates resistant to ciprofloxacin in poultry meat.

*E. coli* serovars recovered by bacteriological examination were tested by multiplex PCR using *stx2* F & *stx2* R and *eaeA* F & *eaeA* R primers. Results observed in Fig. 1 revealed positive amplification of 255 bp fragment of shiga toxin type 2 gene in 58 (67.44%) serotypes of *E. coli* recovered from milk samples and 16 (94.12%) serotypes of *E. coli* recovered from meat samples, while intimin gene was detected in 38 (44.186%) serotypes of *E. coli* recovered from milk samples and in 10 (58.82%) serotypes of *E. coli* recovered from meat samples and 384 bp fragment of intimin gene from 38 (44.186%) serotypes of *E. coli* recovered from milk samples and in 10 (58.82%) serotypes of *E. coli* recovered from meat samples. Most of these strains belonged to serotypes associated with severe illness in humans (Gannon et al., 1993; Beutin et al., 2004). Enterohemorrhagic *E. coli* (EHEC) types

**Table 1** Multiplex PCR of *E. coli* serotypes recovered from milk samples.

Serovars	Samples from milk samples (540)		Multiplex PCR			
	N.	%	Positive for <i>stx2</i> gene		Positive for <i>eaeA</i> gene	
			N.	%	N.	%
O157: H7	26	4.81	26	100	26	100
O111: K58	23	4.26	15	65.22	6	26.09
O113: H21	20	3.70	12	60	6	30
O22: H8	10	1.85	5	50	0	0
O172: H21	7	1.3	0	0	0	0
Total	86	15.93	58	67.44	38	44.186

**Table 2** Multiplex PCR of *E. coli* serotypes recovered from raw meat samples.

Serovars	Samples from meat samples (150)		Multiplex PCR			
			Positive for <i>stx2</i> gene		Positive for <i>eaeA</i> gene	
	N.	%	N.	%	N.	%
O157: H7	6	4	6	100	6	100
O111: K58	5	3.33	5	100	3	60
O174: H2	4	2.67	4	100	1	25
O22: H8	2	1.33	1	50	0	0
Total	17	11.33	16	94.12	10	58.82

O157:H7, and STEC strains from food were positive for the *eae* gene, which is considered to be a marker gene for EHEC (Levine, 1987; Donnenberg and Kaper, 1992; Paton and Paton, 1998; Beutin et al., 2004; Caprioli et al., 2005). The *stx1* and *stx2* genes were detected in 3% and 6.1%, respectively of *E. coli* strains isolated from raw milk samples in Saudi Arabia (Altalhi and Hassan, 2009). In contrast, 58 (67.44%) of the food-borne STEC strains carried *stx2* genes, an indicator for potential high virulence of STEC for humans (Nataro and Kaper, 1998; Caprioli et al., 2005; Beutin et al., 2007). Most of these strains belonged to serotypes associated with severe illness in humans, such as O22:H8, O91:H21, O113:H21, O174:H2, and O174:H21 (Beutin et al., 2007).

## 5. Conclusions

The results of this study revealed the efficiency of combination between serotyping and molecular typing for detection and characterization of *E. coli* isolates recovered from food of animal origin.

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