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ORIGINAL ARTICLE

Molecular characterization of *Escherichia coli* O157:H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia

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Abstract Raw meat can harbor pathogenic bacteria, potentially harmful to humans such as *Escherichia coli* O157:H7 causing diarrhea and hemolytic-uremic syndrome (HS). Therefore, the current study was carried out to evaluate the prevalence and the molecular detection characterization of *E. coli* serotype O157:H7 recovered from raw meat and meat products collected from Saudi Arabia. During the period of 25th January 2013 to 25th March 2014, 370 meat samples were collected from abattoirs and markets located in Riyadh, Saudi Arabia "200 raw meat samples and 170 meat products". Bacteriological analysis of the meat samples and serotyping of the isolated *E. coli* revealed the isolation of 11 (2.97%) strains of *E. coli* O157:H7. Isolation of *E. coli* O157:H7 in raw beef, chicken and mutton were 2%, 2.5%, and 2.5%, respectively, however, there was no occurrence in raw turkey. The incidences of *E. coli* O157:H7 in ground beef, beef burgers, beef sausage, ground chicken and chicken burgers were 5%, 10%, 0.0%, 5% and 0.0%, respectively. The multiplex PCR assay revealed that 3 (27.27%) out of 11 *E. coli* O157:H7 isolates from raw beef,

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chicken and mutton had *stx*1, *stx*2, and *eae* while 5 (45.45%) *E. coli* O157:H7 isolates from ground beef, ground chicken, and raw beef had both *stx*1 and *stx*2. However, from beef burgers, only one *E. coli* O157:H7 isolate had *stx*1 while two were positive for *hly*A gene. These results call for urgent attention toward appropriate controls and good hygienic practices in dealing with raw meat.

1. Introduction

Foodborne diseases are a major public health problem with growing concern. The epidemiological data point to an escalating incidence of infectious diarrhea (Osservasalute, 2009). *Escherichia coli* O157:H7 is one of the most important foodborne pathogens that causes significant losses among the human population in the past two decades. More than 75,000 cases of foodborne illness attributed to *E. coli* O157:H7 occur annually (Perna et al., 2001). The infection with this pathogen, frequently associated with hemorrhagic colitis (HC) is associated with hemolytic uremic syndrome and renal failure (Paton and Paton, 1998a,b).

Transmission of *E. coli* serotype O157:H7 is via fecal-oral route, due to improperly washed hands or following ingestion of contaminated foods from animal origin harboring the organism specially meat and the meat products as well as milk and dairy products which are not treated well by heat (Dilielo, 1982; Soomro et al., 2002).

The pathogenicity of *E. coli* O157:H7 mostly attributed to the ability of the microorganism to produce the shiga toxins (*stx*1 and *stx*2), and the presence of the intimin (*eae*) gene, which is essential for adherence of the organism to the intestinal epithelium(attaching and effacing mechanism) (Vallance and Finlay, 2000).

Hemolysins (*hly*) are an important virulence factor as they can induce extraintestinal lesions (Bhakdi et al., 1990) and have the ability to affect several cells, such as lymphocytes, granulocytes, erythrocytes, and renal cells causing severe effect. Several studies had been carried out in KSA to determine the ability of shiga toxigenic *E. coli* to cause diarrhea, but, there are no sufficient reports about the food contamination with *E. coli* O157:H7. Therefore, this study: was conducted to detect the prevalence and molecular characterization of *E. coli* O157:H7, molecular detection of virulence genes in raw meat samples and meat products (*stx*1, *stx*2, *eae* and *hly*A) collected from different localities and markets located in Riyadh, Kingdom of Saudi Arabia.

2. Materials and methods

2.1. Samples

Samples were collected during the period of 25th January 2013 to 25th March 2014 at the Microbiology laboratory of College of Science. 370 meat samples of either raw meat ($n = 200$) or meat products ($n = 170$) were collected; from abattoirs and markets located in Riyadh, Saudi Arabia. The raw meat included beef, mutton, chicken, and turkey meat samples while the meat products comprised ground beef, beef burgers, beef sausage, ground chicken and chicken burgers. The samples were kept refrigerated and transported to be examined in the laboratory.

2.2. Isolation and identification of E. coli

Primary isolation occurs on modified soy broth by blending 25 grams of the examined samples in 225 ml of modified tryptic soy broth mTSB modified using stomacher at medium speed for one minute and incubated aerobically at 37 °C for 24 h according to Cowan (1985), Ethelberg et al. (2009). 100 µl were cultured on Eosin Methylene Blue agar media (EMB) and incubated at 37 °C for 24 h; *E. coli* had been grown producing green metallic shine colonies, at the same time 100 µl were cultured on Sorbitol MacConkey agar (SMAC) to evaluate the ability of the organism to ferment sorbitol. Sorbitol non-fermenting bacteria produce colorless colonies). Morphological characters after staining, cultural and biochemical characters were carried out according to Quinn et al. (2002).

2.3. Serotyping of E. coli O157:H7

The isolates identified as *E. coli* by culture and biochemical characters were examined by serotyping using diagnostic antisera for *E. coli* and the antisera to identify the serotype O157 using antisera from Difco.

2.4. Extraction of DNA

Hexadecyltrimethyl ammonium bromide (CTAB) was used for extraction of DNA from the standard strains and from the recovered strains of *E. coli* O157:H7. Briefly, one ml of each culture was harvested (5000 rpm, 5 min, 4 °C), The sedimented colonies were washed five times using phosphate buffered saline and suspended in 1.0 ml of sterilized water and the whole genomic DNA was extracted by the CTAB method according to the method explained by Sambrook and Fritscgh (1989). The extracted DNA was suspended in DDW to be used for PCR.

2.5. PCR design and amplification conditions according to Fagan et al. (1999)

All the extracted DNA of the standard strains and of the recovered *E. coli* O157:H7 isolates by bacteriological examination were examined using multiplex-PCR for molecular typing of the toxic and virulence genes (*stx*1, *stx*2, *eae*, and *hyl*A) using specific oligo nucleotide primers. The sequence of the primers and the size of the amplified fragments are listed in (Table 1). The reaction mixture consisted of 1 µl (200 µg) of the extracted DNA from the bacterial isolates or from the standard strains, 5 µl of 10× PCR buffer (BIO TOOLS) (75 mM Tris base-HCI, pH 9.0, 2 mM $MgCI_2$, 50 mM KCI, 20 mM $(NH_4)_2$ SO_4, 1 µl dNTPs (40 µM) (BIOTOOLS), 1 µl (l U Amplitaq DNA polymerase) (Qiagen), 1 µl (50 pmol) of

Table 1 PCR primers used for multiplex PCR.

Oligonucleotides sequence (5′–3′)	Specificity	Amplicon size
F-GTGGCGAATACTGGCGAGACT R-CCCCATTCTTTTTCACCGTCG	Intimin gene	890
F-CAACACTGGATGATCTCAG R-CCCCCTCAACTGCTAATA	Shiga toxin type 1	349
F-ATCAGTCGTCACTCACTGGT R-CTGCTGCTGTCACAGTGACAAA	Shiga toxin type 2	110
F-ACGATGTGGTTTATTCTGGA R-CTTCACGTGACCATACATAT	*hylA*	165

the forward and reverse primers and the final volume made up to 50 µl using deionized distilled water (DDW). 40 µl paraffin oil was added and after denaturation firstly occurs for 5 min at 96 °C, followed by 35 PCR cycles that consist of denaturation at 95 °C/for 3 min, annealing at 55 °C/45 s, extension at 72 °C/45 s, and final extension at 72 °C/7 min. Agarose gel electrophoresis was carried out according to Sambrook and Fritscgh (1989) to evaluate the amplified fragments using standard PCR markers and 100 bp ladder.

3. Results

Standard microbiological examination of the meat samples either raw or processed samples collected from different markets and serotyping of the isolated *E. coli* revealed the isolation of 11 (2.97%) strains of *E. coli* serotype O157:H7 organisms out of a total of 370 examined samples. The incidence of *E. coli* serotype O157:H7 in raw meat samples was 2%. It was found that, the incidence of *E. coli* serotype O157:H7 in 100 raw beef, 40 raw chicken and 40 raw mutton samples were 2%, 2.5% and 2.5%, respectively, however, there was no incidence in raw turkey (20 samples) as shown in (Table 2). While 7 (4.12%) strains of *E. coli* serotype O157:H7 were recovered from the 170 tested meat product samples. The incidences of *E. coli* serotype O157:H7 in ground beef, beef burgers, beef sausage, ground chicken and chicken burgers were 5%, 10%, 0.0%, 5% and 0.0%, respectively as depicted in (Table 2).

The present study was carried out to detect the presence of *stx*1, *stx*2, *eae* and *hly*A genes in the recovered strains by multiplex Polymerase Chain Reaction. The amplified fragments by PCR revealed that 3 out of 11 (27.27%) *E. coli* serotype O157:H7 isolates from raw beef, raw chicken, and raw mutton had *stx*1, *stx*2 and *eae* while 5 out of 11 (45.45%) *E. coli* O157:H7 isolates from ground beef, ground chicken, and raw beef had both *stx*1 and *stx*2. However, only one *E. coli* serotype O157:H7 isolate had *stx*1 recovered from beef burgers, as well as two, were positive for *hly*A gene isolated from beef burgers and ground beef.

4. Discussion

E. coli O157 is one of the most important enterohemorrhagic strains of *E. coli* (EHEC) affecting human health. It is a leading cause of numerous foodborne illnesses and infantile diarrhea (Levine, 1987; Donnenberg and Kaper, 1992), and is considered to be the principal cause of high morbidity and high mortality of rats around the world (World Health Organization, 1995). Therefore, our study was carried out to detect the prevalence and molecular characterization of *E. coli* O157 and virulence maker genes (*stx*1, *stx*2, *eae*, *hly*A) in meat and meat products collected from the various markets of Riyadh, Saudi Arabia.

In the present study, 370 random samples of raw meat and processed meat products were investigated for the presence of *E. coli* O157. It is evident from the results displayed in (Table 2), that the prevalence of *E. coli* O157 in raw meat samples was 2%, and in meat products was 4.12%, accounting for a combined occurrence of 2.97% in meat and processed meat products.

Foods recovered from animal origin have been ascribed to be prime sources of EHEC infection moreover, raw meat and processed meat have been considered as the principal source of transmission of such organisms to human beings (Roberts et al., 1995). The incidence of *E. coli* O157 in milk and dairy products proved to be variable in different localities as a result of variation in seasons, number of animals in the farm, type of ration, hygienic measures in such farms, farm management practices, incongruity in sampling, inconsistency in the type of samples evaluated, and divergence in detection methods (Donnenberg and Kaper, 1992; Vallance and Finlay, 2000). The occurrence of *E. coli* O157 in raw beef, raw chicken, and raw mutton were 2%, 2.5%, and 2.5%, respectively, however, there was no incidence in raw turkey. While the prevalence in ground beef, beef burgers, beef sausage, ground chicken and chicken burgers were 5%, 10%, 0.0%, 5% and 0.0%, respectively. *E. coli* O157 was recovered from dairy

Table 2 Characterization of the recovered *E. coli* O157:H7 by multiplex PCR from raw meat and meat product samples.

Types and number of collected meat samples	*E. coli* O157:H7	Multiplex PCR of *E. coli* O157:H7					
		*stx*1	*stx*2	*eae*	*hlyA*	*stx*1 and 2	*stx*1/2 and *eae*
Raw beef (100)	2 (2%)	0	0	0	0	1	1
Raw mutton (40)	1 (2.5%)	0	0	0	0	0	1
Raw chicken (40)	1 (2.5%)	0	0	0	0	0	1
Raw turkey (20)	0 (0.0%)	0	0	0	0	0	0
Ground beef (80)	4 (5%)	0	0	0	1	3	0
Beef burger (20)	2 (10%)	1	0	0	1	0	0
Beef sausage (30)	0 (0.0%)	0	0	0	0	0	0
Ground chicken (20)	1 (5%)	0	0	0	0	1	0
Chicken burger (20)	0 (0.0%)	0	0	0	0	0	0
Total (370)	11 (2.97%)	1 (36.36%)	0	0	2 (18.18%)	5 (45.45%)	3 (27.27%)

farms, young calves, chicken farms and from sheep (Doyle and Schoeni, 1987; Mermelstein, 1993; An-Hung et al., 1995). Our results are in agreement with those of De Giusti et al. (2010) who reported that *E. coli* O157 incidence in raw meat was 2.61% with the direct culture method that was also serologically confirmed by the National Institute of Health (ISS) of Rome-Italy.

When mutton samples were assayed for the presence of pathogenic *E. coli*, Malik and Memona (2010) found that 73 samples, including 33 cooked and 40 uncooked were positive for the pathogenic *E. coli* in total samples. These data substantiate that sheep are the main reservoir hosts of pathogenic *E. coli* as the bacteria predominantly comprise the alimentary flora of bovine species. Regarding the meat products, several authors have described the survival of *E. coli* O157 in ground beef (Doyle and Schoeni, 1987). More than half of the disease epidemic in the United States has been linked to under cooked ground beef contaminated with *E. coli* O157 (Gansheroff and O'Brien, 2000; Baran and Gülmez, 2003) where 7 strains of *E. coli* O157:H7 were detected. Also, higher prevalence rates of *E. coli* O157 than those observed in this study have been reported elsewhere. In South Africa and Malaysia, 74.5% and 36% respectively of the beef samples harbored this isolate (Vorster et al., 1994; Radu et al., 1998). On the contrary, in some studies, beef and beef product samples have been found to be entirely devoid of *E. coli* O157 (Uhitil et al., 2001), while still others found it in lower incidence rates in contaminated samples (Itoh et al., 1999; Tarr et al., 1999). Zhao et al. (2001), (Naylor et al., 2003) also isolated *E. coli* O157 strains from samples of hamburgers with vegetables.

Although, in this study, we have not isolated the microorganism from chicken burgers, but it was found in ground chicken. Doyle and Schoeni (1987) had isolated *E. coli* O157 from 4 (1.5%) of 263 poultry samples and affirmed that the organism is not a rare contaminant of poultry meat.

The pathogenicity of *E. coli* O157 is attributed to the production of shiga toxins (Stx1 and Stx2), previously known as verocytotoxins because of their toxicity on Vero cells (Griffin and Tauxe, 1991). In the current study, the isolated *E. coli* O157 isolates were characterized by the detection of shiga toxin type 1 and 2 (*stx*1 and *stx*2), *eae* and *hly*A genes by multiplex PCR. It is evident from the results obtained that specific shiga-like toxin genes (*stx*1 and *stx*2) were present in 5 (45.45%) out of 11 *E. coli* O157 isolates from ground beef, ground chicken, and raw beef while one *E. coli* O157 isolate recovered from beef burgers had *stx*1. Further, 3 out of 11 (27.27%) *E. coli* O157 isolates from raw beef, raw chicken, and raw mutton had *stx*1, *stx*2 and *eae* while two isolates from beef burgers and ground beef possessed *hly*A gene. The sensitivity of the PCR procedure was evaluated by Matise et al. (1995) for *E. coli* O157 and shiga toxin detection in ground beef and ground pork at contamination levels of 0.14, 1.4 and 14 colonies.

5. Conclusion

Multiplex Polymerase Chain Reaction is highly recommended for the rapid detection of virulence factors of special molecular detection of stx1, *stx*2, *eaeA* and *hly*A genes. In our study, most of the recovered *E. coli* O157 strains possess a combination of two or more of the virulence genes. The presence of more than one virulence gene in the recovered strains increases the ability of the organism to cause infection and severe illness in the infected individuals.

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