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DETECTION OF SHIGA TOXIN-PRODUCING *Escherichia coli* IN MEAT MARKETED IN CASABLANCA (MOROCCO)

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Abstract

The contamination of meat and meat products with Shiga toxin-producing O157:H7 and non-O157 *Escherichia coli* (STEC), obtained from markets in Casablanca, Morocco, was investigated. A total of 460 meat and meat products were sampled between March 2004 and July 2006 analysed and 176 strains of *E. coli* were isolated from these samples. The presence of the *stx1*, *stx2*, *eae* and *ehxA* genes, recognized as major virulence factors of STEC, was tested in *E. coli* isolates by polymerase chain reaction (PCR). STEC was detected in 4 (0.9%) samples. The result of serotyping by molecular method showed that two of these STEC isolates corresponded to the serotype O157:H7. The others Shiga toxin-producing *E. coli* non-O157 corresponded to O6:H21 and O76:H19. The presence of O157:H7 and non-O157 STEC in meat and meat products marketed in Casablanca, Morocco, emphasizes the importance of implementing the Hazard Analysis and Critical Control Point (HACCP) system, as well as the need for implementing, evaluating, and validating antimicrobial interventions to reduce the presence of potential pathogenic microorganisms.

Key words: Meat, STEC, Morocco.

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Abbreviations: **CDC**: Center for Disease Control and Prevention; **EHEC** : Entero-haemorragic *E.coli;* **HUS**: hemolytic uremic syndrome; **HACCP**: Hazard Analysis and Critical Control Point; **LEE**: locus for enterocyte effacement; **PCR**: Polymerase Chain Reaction; **PCR-RFLP**: polymerase chain reaction-restriction fragment length polymorphism; **STEC** : Shiga toxin-producing *Escherichia coli*; **STX1**: Shiga toxin 1; **STX2**: Shiga toxin 2.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), also called verotoxin-producing *E. coli* (VTEC), are a major cause of food borne illness capable of causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) in children (23). STEC include serotype O157:H7 and more than 100 non-O157 serotypes such as O111 and O26. Recently, several of these non-O157 STEC serovars have been linked to an increasing number of gastroenteritis infections and HUS in humans. According to the Center for Disease Control and Prevention (CDC), it is estimated that *E. coli* O157 causes 73,000 cases and 61 deaths, and non-O157 STEC lead to 36,000 infections and 30 deaths annually in the USA (18). It is considered that in North America and Europe around 90% of children with HUS show evidence of STEC infection, with the O157:H7 serotype responsible for 70% of the cases (4, 14).

Shiga toxins (Stx) are considered the major virulence factors of STEC, which are responsible for vascular endothelial damage. These toxins, encoded by lysogenic bacteriophages, are classified into two main types, *Stx1* and *Stx2*. STEC strains may produce Stx1 or Stx2, or both types. Another virulence factor of STEC is a 94-kDa outer membrane protein, called intimin, essential for cellular attachment. It is encoded by an *eae* gene, present on a 34-kb chromosomal pathogenecity island termed the locus for enterocyte effacement (LEE) (16). An additional virulence marker carried by some STEC strains is enterohemorraghic hemolysin (EHEC-Hly), encoded by a large plasmid-borne (90-kb) EHEC-*hlyA* gene (22) which seemed to be associated with severe clinical disease in humans.

STEC strains have been isolated from domestic animals as well as wild-living animals. However, these microorganisms rarely cause disease in animals, and ruminants are recognized as their main natural reservoir, especially cattle (5). The microorganism is carried as part of the native microbiota in the intestine of cattle and can contaminate meat and the slaughterhouse environment (2). Feces and hides are significant sources of bacterial carcass contamination (8, 10, 17).

In Morocco, meat is an important part of the human diet. Moroccan official data reported that between 2000 and 2004, an average of 152000 tons of beef, 338000 tons of white meat are produced yearly, with more than 12% of meat production in Casablanca.

During 2000 to 2004, 7118 cases of food borne diseases have been reported among which 86% were of bacterial origin (Morocco food borne disease outbreaks, searchable data 2000-2005. yearly reports 2000-2004). According to the same report, red meat and meat products caused 21.5% of the bacterial foodborne diseases and 14.7% occurred in the city of Casablanca (6). But only a few studies have reported the isolation and the characteristics of STEC in meats (3, 11), the aims of this study were: (i) to determine the occurrence of Shiga toxin-producing *E. coli* O157:H7 and non-O157 on retail raw meats obtained from markets in Casablanca, Morocco, (ii) to serotype the STEC isolates by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and (iii) to determine the verocytotoxicity of STEC isolated.

MATERIALS AND METHODS

Samples collection and isolation of E. coli

A total of 460 samples, composed by ground beef (n = 140), turkey (n = 200), sausage (n = 120), were collected randomly from markets between March 2004 and July 2006 in Casablanca, Morocco.

Samples were sent to the laboratory in sterile bags at 4 °C for ≤ 2 h. A portion of (25g) of each sample was placed into a separate sterile stomacher bag with 225 ml of buffered peptone water and then pummeled with a MIX I mixer (AES Laboratory, Combourg, France). One milliliter of each sample was streaked on Violet Red Bile Lactose Agar (Biorad) and incubated at 37 °C for 24 h. Colonies showing *E. coli* characteristics were submitted to Gram staining and identified by standard biochemical tests: oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative (13). The isolates were confirmed *E. coli* using the *Enterobacteriaceae* API 20E commercial kit (Biomerieux, Marcy l'Etoile, France).

PCR

For PCR, primers were selected based on previously published information for *stx1* and *stx2*, *hlyA* and *eaeA* genes (Table 1). DNA was extracted from bacterial cultures with Instagene matrix (Bio-Rad Laboratories, Richmond, CA) as described by the manufacturer.

Table 1. The primers used for detection of the various genes by PCR.

Gene	Primer	Oligonucleotid sequence (5'-3')	Fragment size (BP)	References
stx1	stx1F	ATA AAT CGC CAT TCG TTG ACT AC	180	(19)
	stx1R	AGA ACG CCC ACT GAG ATC ATC		
stx2	VT2 425 (VT2a)	TTA ACC ACA CCC CAC CGG GCA GT	524	(20)
	VT2 952 (3'II)	GGA TAT TCT CCC CAC TCT GAC ACC		
eaeA	SK1	CCC GAA TTC GGC ACA AGC ATA AGC	864	(12)
	SK2	CCC GGA TCC GTC TCG CCA GTA TTC G		
hlyA	hlyAF	GCA TCA TCA AGC GTA CGT TCC	534	(19)
	hlyAR	AAT GAG CCA AGC TGG TTA AGC T		

Molecular Serotyping of STEC

The identification of O- and H-antigens was carried respectively by restriction of the amplified O-antigen gene cluster (*rfb-RFLP*) and *fliC* gene (*fliC-RFLP*) by the methods described by (1, 7, 15). Molecular serotyping was accomplished by the agglutination test in slide; the antiserums were obtained from the Statens Serum Institute (Copenhagen, Denmark).

Verocytotoxicity essay of STEC

For production of Shiga toxins, one loopful of each strain was inoculated in 50-ml Erlenmeyer flasks containing 5 ml of tryptone soy broth (pH 7.5) with mitomycin C and incubated for 20 h at 37 °C (shaken at 200 rpm) and then centrifuged (6000g) for 30 min at 4 °C. The Vero cell culture assays were performed using nearly confluent cell monolayers grown in plates with 24 wells. At the time of assay, the growth medium (RPMI with polymyxin sulphate) was changed (0.5 ml per well) and 75 µl undiluted culture supernatant added. Cells were incubated at 37 °C in a 5% CO_2 atmosphere. The cells were examined microscopically for cytopathic effects compared to a positive control (purified *Stx* incubated with the Vero cells) and a negative control (PBS incubated with the Vero cells) (9).

RESULTS AND DISCUSSION

As shown in Table 2, three of the 140 ground beef samples and one of the 120 sausage samples were positive for *stx* gene by PCR. None of the 200 turkey samples was positive by PCR for *stx*. Among the 4 STEC isolated in this study, 2 (0.43%) strains were O157:H7, showing *stx1*, *stx2*, *eae*, and *ehxA* genes, and 2 non-O157 serotypes O6:H21, O76:H19.

These results demonstrate the presence of STEC in meat and meat products of serotypes O157 and non-O157. The cells, examined microscopically for cytopathic effects compared to a positive control (purified Stx incubated with the Vero cells) and a negative control (PBS incubated with the Vero cells), showed that all 4 STEC strains included in this study were cytotoxic to Vero cells.

STEC form part of the flora of the gastrointestinal tract of sheep and cattle produced for meat. As there is always the possibility of some transfer of faecal material to carcasses at slaughter by a variety of means, there is a potential for contamination of meat and meat products with these bacteria. The contamination rate of the samples analysed by STEC in our study is 0.9%. Several studies have reported the prevalence of *E. coli* O157H7; Elder et al. (2000) (8) detected O157 STEC in 2% of 330 beef carcasses, while non-O157 STEC was isolated from 8.3% of 326 beef carcasses by (24). In Canada, (21) isolated *E. coli* O157:H7 from 1.6% of 125 beef carcasses. Observed differences in prevalence among studies may be due to different sampling and isolation procedures and to variability in sampled populations. *E. coli* O157:H7 and other STEC strains are not naturally occurring in carcasses, but they can be present as a direct result of cross-contamination during the slaughter process. The presence of the pathogen in hides and feces has been found to correlate with carcass contamination (8, 10).

Results of this study suggest that there may be a lack of adequate control strategies during post-slaughter operations. Such inadequate controls may include: lack of good hygienic practices, inadequate sanitation during manufacturing, transport, storage, and post-production handling, as well as inadequate maintenance of adequate cold chain management during distribution. Consistent with other countries, Morocco is experiencing newer consumer trends in the culinary traditions with an obvious tendency to adopt international and further processed foods, some of which are uncooked or undercooked. Such a tendency combined with poor hygienic practices may contribute to increase the prevalence of pathogens in foods, thereby increasing the risk of food borne disease for consumers (6).

Table 2. Genotypic characteristics of STEC isolates

		Virulence genes detected by PCR			
Sample type	No. positive/tested	*stx1*	*stx2*	*eaeA*	*hlyA*
ground beef	3/140	+	+ (2/3)	+ (2/3)	+
sausage	1/120	+	-	-	-
turkey	0/200	-	-	-	-

Results obtained in this study provide evidence which may be used by the Moroccan government to adopt regulations enforcing the application of the hazard analysis critical control points (HACCP) system as a means to identify and control the hazards in foods and especially in meat products. Furthermore, these results may promote the acceptance of programs such as HACCP by the meat industry in an attempt to provide safer and more wholesome products.

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