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Short communication

Isolation of shiga toxigenic *Escherichia coli* from raw beef in Palestine

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Abstract

Shiga toxigenic *Escherichia coli* (STEC) isolated from raw beef samples in northern Palestine during a 1-year period were characterized for virulence genes by a polymerase chain reaction (PCR) assay and screened for their antibiotic resistance. STEC was identified in 44 (14.7%) of 300 raw beef samples. Twelve (27.3%) of the STEC isolates were serotype O157. Nine of those were isolated during summer. The majority of STEC isolates (70.5%) harbored both stx_1 and stx_2 genes, while the others harbored either stx_1 or stx_2. High levels of resistance against different antimicrobial agents were detected. Resistance to at least three drugs was found in 55% of the isolates.
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Keywords: STEC; *E. coli* O157; Antibiotic resistance; stx_1; stx_2; Raw beef

1. Introduction

Shiga toxigenic *Escherichia coli* (STEC) have been implicated as the causative agent in several human diseases including mild nonbloody or severe bloody diarrhea (hemorrhagic colitis), hemolytic uremic syndrome (HUS) and renal failure (Paton and Paton, 1998; Wong et al., 2000). Cattle are considered to be the principal natural reservoir of this pathogen (Gansheroff and O'Brien, 2000), but strains of this pathogen are also prevalent in the gastrointestinal tracts of other domestic animals, particularly ruminants (Beutin et al., 1993). Consumption of foods of bovine origin, particularly raw or undercooked ground beef products and raw milk contaminated with bovine feces, has been associated with large food poisoning outbreaks in which this organism was identified as the etiologic agent (WHO, 1997). In Palestine, an outbreak due to STEC infection has been reported (Adwan et al., 2002), but the sources of these cases have not been identified. The objective of this study was to determine the prevalence of STEC in raw beef samples in Northern Palestine, as this has not been investigated previously.

2. Materials and methods

Samples of meat from beef carcass surfaces were purchased from local butchers' shops in northern Palestine between 1st December 2001 and 30th November 2002, with 75 samples being obtained during

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each 3-month season. Fifteen grams of each sample were homogenized with 135 ml of trypticase soy broth (TSB, Sigma, St. Louis, MO, USA). The homogenate was incubated overnight at 37 °C. A portion of the TSB broth was spread on a plate of eosin methylene blue agar (EMB, Defco Laboratories, Detroit, MI, USA), which was incubated overnight at 37 °C. At least 10 *E. coli*-like colonies were picked from the plate and were suspended in 0.5 ml of sterile distilled water. The suspension was boiled for 10 min. Following centrifugation of the boiled suspension at 12,000 × *g* for 2 min, the supernatant was tested by a polymerase chain reaction (PCR) assay for the presence of stx_1 and stx_2 genes, as described previously (Paton and Paton, 1998). Isolates were confirmed as *E. coli* by the API 20E system (bioMérieux, Marcy L'Etoile, France) and tested for sorbitol fermentation on sorbitol MacConkey agar (SMAC, Oxoid, Hampshire, England). The O157 antigen of isolates was confirmed by agglutination with a specific latex reagent (Oxoid).

The STEC strains were tested for antibiotic resistance using the disk diffusion method (Bauer et al., 1966). Antibiotic disks (Oxoid) used were chloramphenicol (30 μg), tetracycline (30 μg), kanamycin (30 μg), amikacin (30 μg), ceftriaxone (30 μg), norfloxacin (10 μg), ampicillin (10 μg), streptomycin (10 μg), gentamicin (10 μg), ceftazidime (10 μg), and ciprofloxacin (5 μg). Zones of inhibition were determined in accordance with procedures of the National Committee for Clinical Laboratory Standard (NCCLS), 1999).

3. Results and discussion

STEC were identified in 44 (14.7%) of the 300 beef samples. Twelve (27.3%) of the STEC isolates were O157. All isolates that tested positive for the O157 antigen carried the stx_1 virulence gene and nine carried the stx_2 virulence gene. Twenty-two non-O157 isolates carried both stx_1 and stx_2 genes, whereas 10 isolates harbored only one or the other (Table 1). Nine of the *E. coli* O157 were isolated during summer.

Table 1
Shiga toxin gene profiles of 44 shiga toxigenic *E. coli* (STEC) isolates recovered from raw beef samples in Palestine

Shiga toxin genes	No. of STEC strains	
	STEC O157	STEC non-O157
stx_1	3 (25%)	6 (19%)
stx_2	0 (0%)	4 (12%)
stx_1 and stx_2	9 (75%)	22 (69%)

Table 2
Antibiotic resistance of 44 shiga toxigenic *E. coli* (STEC) isolates isolated recovered from raw beef samples in Palestine

Antibiotic	Resistant strains	
	No.	%
Streptomycin	29	66
Tetracycline	31	70
Ampicillin	22	50
Kanamycin	13	30
Amikacin	15	34
Chloramphenicol	18	41
Norfloxacin	13	30
Ciprofloxacin	18	41
Gentamicin	11	25
Ceftazidime	1	2
Ceftriaxone	2	5

The antibiotic resistance profiles of the STEC isolates are presented in Table 2. The most common resistance was to tetracycline and/or streptomycin. Resistance to ceftriaxone or ceftazidime was rare. Only three isolates were sensitive to all 11 antibiotics. Resistance to at least three drugs was found in 55% of the isolates.

In this study, the majority of the STEC isolates carried both stx_1 and stx_2 genes. These results were consistent with a previous report from India, where 44.5% of the STEC isolates harbored both stx_1 and stx_2 genes (Khan et al., 2002a). However, these results were in contrast to other studies from Germany, France and Japan where STEC isolates usually carried one or other of the *stx* genes (Akiba et al., 1999; Schmidt et al., 1999; Pradel et al., 2001).

The STEC prevalence in of raw beef samples was 14.7%. If the immunomagnetic separation method had been used, more accurate picture on STEC prevalence could be achieved because of this method increases the recovery of STEC from the selective enrichment broth (Zhou et al., 2002). The prevalence of STEC in beef samples reported for countries such as Belgium, New Zealand, India and USA has ranged from 1.8% to 50% (Pie'rard et al., 1997; Brooks et al., 2001; Khan et al., 2002b; Samadpour et al., 2002). The total STEC prevalence on samples from beef carcasses has

been reported as 71.9% before evisceration and 10.1% after processing (Arthur et al., 2002).

Results showed that 4% fresh meat beef samples were contaminated with serotype O157. This result is in agreement with data from China which showed that STEC O157 strains were isolated from 5% of beef (Zhou et al., 2002). Other studies reported prevalence of *E. coli* O157 in beef which ranged from 1.1% to 13.4% (Chapman et al., 1997, 2000, 2001). Most of these *E. coli* O157 strains were isolated during summer (Chapman et al., 1997; Van Donkersgoed et al., 1999; Arthur et al., 2002). Thus, carcass contamination with STEC may be much less frequent at other times of the year (Arthur et al., 2002).

The antimicrobial susceptibility results of the STEC isolates are a cause for concern as more than 50% of the isolates were resistant to three or more drugs. Similar incidences of resistance have been reported for isolates obtained elsewhere (Maidhof et al., 2002; Khan et al., 2002a). The high incidence in this study may be due in part to selective pressure resulting from incorporation of antibiotics into animal feeds. Two isolates of STEC O157 showed the same patterns of resistance (data not shown). This may have been due to cross contamination of meat by contact with workers hands or tools during evisceration of or hide removal from carcasses, or perhaps by direct contact between carcasses during transport.

To our knowledge this is the first survey of the prevalence of STEC in raw beef for human consumption in Palestine. As expected, beef in Palestine is contaminated by this pathogen as in other countries. Detection of either stx_1 or stx_2 genes does not necessary entail that the strains are pathogenic to man. Thus, expansion of this study to include genes encoding putative accessory virulence factors, such as intimin or the plasmid-encoded hemolysin (Arthur et al., 2002), is necessary to further evaluate significance of STEC strains in human disease in Palestine.

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