Available online at www.sciencedirect.com

SCIENCE DIRECT®

International Journal of Food Microbiology 97 (2004) 81–84

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

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

Ghaleb M. Adwan*, Kamel M. Adwan

Department of Biology and Biotechnology, An-Najah N. University, P.O. Box (7)-Nablus, Palestine, Palestine Territory, Israel

Received 31 May 2003; received in revised form 23 March 2004; accepted 23 March 2004

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.

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

* Corresponding author. Fax: +972-9-2675891.
E-mail address: ghal1@yahoo.com (G.M. Adwan).

0168-1605/$ - see front matter
doi:10.1016/j.ijfoodmicro.2004.03.032

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.

References

Adwan, K., Abu-Hasan, N., Essawi, T., Bdir, M., 2002. Isolation and characterization of Shiga toxigenic *Escherichia coli* from northern Palestine. Journal of Medical Microbiology 51, 332–335.

Akiba, M., Masuda, T., Sameshima, T., Katsuda, K., Nakazawa, M., 1999. Molecular typing of *Escherichia coli* O157:H7 (H-) isolates from cattle in Japan. Epidemiology and Infection 122, 337–341.

Arthur, T.M., Barkocy-Gallagher, G.A., Rivera-Betancourt, M., Koohmaraie, M., 2002. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. Applied and Environmental Microbiology 68, 4847–4852.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Truck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 45, 493–496.

Beutin, L., Geier, D., Steinruck, H., Zimmermann, S., Scheutz, F., 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. Journal of Clinical Microbiology 31, 2483–2488.

Brooks, H.J., Mollison, B.D., Bettelheim, K.A., Matejka, K., Paterson, K.A., Ward, V.K., 2001. Occurrence and virulence factors of non-O157 Shiga toxin-producing *Escherichia coli* in retail meat in Dunedin, New Zealand. Letters in Applied Microbiology 32, 118–122.

Chapman, P.A., Siddons, C.A., Gerdan Malo, A.T., Harkin, M.A., 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiology and Infection 119, 245–250.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T., Harkin, M.A., 2000. A one year study of *Escherichia coli* O157 in raw beef and lamb products. Epidemiology and Infection 124, 207–213.

Chapman, P.A., Cerdan Malo, A.T., Ellin, M., Ashton, R., Harkin, M.A., 2001. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. International Journal of Food Microbiology 64, 139–150.

Gansheroff, L.J., O'Brien, A.D., 2000. *Escherichia coli* O157:H7 in beef cattle presented for slaughter in the U.S.: higher prevalence rates than previously estimated. Proceedings of the National Academy of Sciences of the United States of America 97, 2959–2961.

Khan, A., Das, S.C., Ramamurthy, T., Sikdar, A., Khanam, J., Yamasaki, S., Takeda, Y., Nair, G.B., 2002a. Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. Journal Clinical of Microbiology 40, 2009–2015.

Khan, A., Yamasaki, S., Sato, T., Ramamurthy, T., Pal, A., Datta, S., Chowdhury, N.R., Das, S.C., Sikdar, A., Tsukamoto, T., Bhattacharya, S.K., Takeda, Y., Nair, G.G., 2002b. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India. Emerging Infectious Diseases 8, 54–62.

Maidhof, H., Guerra, B., Abbas, S., Elsheikha, H.M., Whittam, T.S., Beutin, L., 2002. A multiresistant clone of shiga toxin-producing *Escherichia coli* O118:[H16] is spread in cattle and humans over different European countries. Applied and Environmental Microbiology 68, 5834–5842.

National Committee for Clinical Laboratory Standards (NCCLS), 1999. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals (M31-A) NCCLS, Pennsylvania, USA.

Paton, A.W., Paton, J.C., 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. Journal of Clinical Microbiology 36, 598–602.

Pie'rard, D., Van Damme, L., Moriau, L., Stevens, D., Lauwers, S., 1997. Virulence factors of verocytotoxin-producing *Escherichia coli* isolated from raw meats. Applied and Environmental Microbiology 63, 4585–4587.

Pradel, N., Boukhors, K., Bertin, Y., Forestier, C., Martin, C., Livrelli, V., 2001. Heterogeneity of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. Applied and Environmental Microbiology 67, 2460–2468.

Samadpour, M., Kubler, M., Buck, F.C., Depavia, G.A., Mazengia, E., Stewart, J., Yang, P., Alfi, D., 2002. Prevalence of Shiga toxin-producing *Escherichia coli* in ground beef and cattle feces from King County, Washington. Journal of Food Protection 65, 1322–1325.

Schmidt, H., Geitz, C., Phillips, I.T., Matthias, F., Karch, H., 1999. Non-O157 pathogenic shiga toxin producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. Journal of Infectious Diseases 179, 115–123.

Van Donkersgoed, J., Graham, T., Gannon, V., 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7 and salmonella in the feces and rumen of cattle at processing. Canadian Veterinary Journal 40, 332–338.

Wong, C.S., Jelacic, S., Habeeb, R.L., Watkins, S.L., Tarr, P.I., 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. New England Journal of Medicine 342, 1930–1936.

World Health Organization (WHO), 1997. Consultation on the Prevention and Control of Enterohaemorrhagic *Escherichia coli*. World Health Organization, Geneva, Switzerland, p. 39.

Zhou, Z., Nishikawa, Y., Zhu, P., Hong, S., Hase, A., Cheasty, T., Smith, H.R., Zheng, M., Haruki, K., 2002. Isolation and characterization of shiga toxin-producing *Escherichia coli* O157:H7 from beef, pork and cattle fecal samples in Changchun, China. Journal of Veterinary Medical Science 64, 1041–1044.