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ISOLATION OF SHIGA TOXIGENIC *ESCHERICHIA COLI* FROM BUTCHERIES IN TAQUARITINGA CITY, STATE OF SÃO PAULO, BRAZIL

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ABSTRACT

Shiga toxigenic *Escherichia coli* (STEC) has been implicated as the cause of several human diseases. Samples (ground beef, grinding-machines and human hands) from 23 butcheries were assayed for *E. coli* using standard microbiological methods, and 287 isolates were submitted to polymerase chain reaction for the detection of *stx* 1, *stx* 2 and *eae* genes. Four STEC isolates were recovered, two from ground beef and two from grinding-machines; all harbored the *stx* 2 gene and were negative for the *eae* gene. All *E. coli* isolates including the four STEC were screened for antibiotic resistance. High levels of resistance against several antimicrobial agents were detected; those most commonly observed were to tetracycline (76.6%), amoxicillin (64.1%) and cephalothin (58.8%). Such high levels of antimicrobial resistance highlight the need for a more rational use of these agents in cattle.

Key words: *Escherichia coli*, STEC, *stx* 2, antibiotic resistance, ground beef, cattle

INTRODUCTION

Shiga toxigenic *Escherichia coli* (STEC) has been implicated as the cause of several human diseases including mild or severe bloody diarrhea (hemorrhagic colitis), hemolytic syndrome (HUS) and renal failure (10,22). The STEC strain most frequently associated with clinical disease in the United States and Europe is the serotype O157:H7 (4,16). However, several other serotypes have been associated with severe disease outbreaks, and in some countries they have been isolated from clinical cases more often than O157 (13,16). Cattle is considered the primary reservoir of both O157 and non-O157 STEC bacteria (2). Several human infections have been linked to STEC in undercooked ground beef (10). During processing, fecal contamination of the carcass or transfer of bacteria found on the animal hide to the carcass could facilitate transmission of STEC to food (4,6).

Pathogenic STEC strains known to produce one or two toxins resembling those from *Shigella dysenteriae* (19), are Shiga Toxin 1 (Stx 1) and Shiga Toxin 2 (Stx 2). Although different proteins, encoded by different genes (*stx* 1 and *stx* 2, respectively), they have similar biological activities (18). Strains possessing *stx* 2 are potentially more virulent than those carrying *stx* 1 or even strains carrying both *stx* 1 and *stx* 2 (3,16). Another virulence factor, intimin (*eae*) is encoded in a pathogenicity island called LEE (locus of enterocyte effacement). LEE-encoded genes appear to enhance STEC virulence and have been associated with severe human disease (13).

Antimicrobial agents for therapy or prophylaxis aimed at animal growth promotion, have favored propagation of resistant bacteria (27). Intestinal resistant bacteria due to fecal contamination during slaughtering may be transferred to meat products (6). If subsequently transmitted to human food (27), they become reason for concern.

This study is aimed at the determination of STEC occurrence in ground beef and the characterization of antimicrobial resistance of *E. coli* isolated from ground beef, meat-grinding machines and hands of meat manipulators.

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MATERIALS AND METHODS

Sampling

Meat samples and swabs from grinding-machines and hands of meat manipulators were collected on separate occasions, over a 10-month period (March 2004 to January 2005), from twenty-three butcheries in Taquaritinga, a city in the northwest of the State of São Paulo. Samples were kept on ice between collection and testing. On the same day of collection, 25g samples of ground meat were homogenized by hand in 225 ml of sterile 0.1% (wt/vol) peptone water (Oxoid Ltd, Basingstoke, Hampshire, UK), in a stomacher bag, for 5-10 min. Swabs from machines or hands of operators were manually mixed with 10 ml of 0.1% peptone water. One ml samples from each suspension were diluted in 9 ml of lauryl sodium sulfate broth (Difco Laboratories, Detroit, USA) and incubated for 24-48h at 35ºC. One hundred µl samples from tubes showing bacterial growth were added to 5 ml of Brilliant Green broth (Difco) or EC broth (Difco) and incubated as above. Ten µl of the broths positive for coliform growth were plated on Eosin Metylene Blue (EMB-Difco) agar and incubated for 24h at 35°C. At least five colonies were taken from the EMB plates for further identification (7).

Determination of *stx* genes

E. coli isolates were grown overnight in nutrient broth (Sigma Chemical Co, St Louis, USA) at 37ºC, and tested for *stx* genes (*stx* 1 and *stx* 2) using the polymerase chain reaction (PCR) protocol of Orden *et al.* (20). DNA templates were prepared by pelleting 1 ml of each culture by centrifugation (12000xg), ressuspension in 250µl of sterile distilled water and boiling for 10 min. After centrifugation, supernatants were used for PCR in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). The amplified DNA products were separated by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and detected under ultraviolet light. Reference *E. coli* strains used as controls were EDL 933 (O157:H7, *stx*1, *stx* 2, *eae*) and DH5α (negative control).

Characterisation of isolates

Isolates were confirmed as *stx*+ and tested for accessory virulence marker (*eae*) using the PCR protocol of China *et al.* (5).

O157 latex agglutination

STEC isolates were serotyped for the O serotype O157 using the O157 Latex Agglutination test kit (Oxoid, Basingstoke, Hampshire, UK). The EDL 933 strain was used as a positive control. Strains negative for agglutination were considered non-O157.

Antibiotic susceptibility tests

STEC and non-STEC isolates were submitted to the disk diffusion method according to National Committee for Clinical Laboratory Standards (17), using commercial disks (Cefar, São Paulo, Brazil), loaded as follows: nalidixic acid (30 µg), amikacin (30 µg), amoxicillin (10 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), streptomycin (10 µg), gentamicin (10 µg) and tetracycline (30 µg). *E. coli* reference strains ATCC 25922 and ATCC 35218 were used for strain quality control.

RESULTS

Among 287 *E. coli* isolates submitted to PCR to detect *stx* 1, *stx* 2 and *eae* genes, four STEC strains were isolated. Two of them were from the same butchery (ground beef and grinding-machine) and the remaining two (one from ground beef and one from a grinding-machine) were from two different stores (Table 1). All isolates carried the *stx* 2 gene and were negative for the presence of *eae* gene.

Antibiotic resistance patterns of the isolates (n=287) are presented in Table 2. Isolates presenting intermediary resistance were also classified as resistant. The most frequent resistances were to tetracycline, amoxicillin, cephalothin and streptomycin. Resistances to amoxicillin + clavulanic acid and ceftriaxone were rare. Thirty (10.4%) isolates were sensitive to all antibiotics tested.

Resistance to at least three drugs was found in 67.0% of the isolates and 22.0% were resistant to more than 6 antibiotics. The resistance patterns of the STEC isolates are presented in Table 3. One of the isolates (strain 530) was resistant to 7 antibiotics; another (strain 426) was susceptible to all drugs.

Table 1. Isolation of STEC from butcheries in Taquaritinga city, State of São Paulo, Brazil.

Origin	Examined samples	Positive samples (%)	stx genotype
Ground beef	91	2 (2.1)	stx 2
Manipulator hands	42	0	0
Grinding-machines	154	2 (1.2)	stx 2

DISCUSSION

STEC strains are part of the microbiota of the gastrointestinal tract of cattle raised for meat consumption. Transfer of fecal material to carcasses at slaughter leads to potential contamination of raw meat (6), as also does cross-contamination of meat and processing equipment (1).

All STEC isolated in this study carried the *stx* 2 gene, in agreement with Brazilian (11,14) and Argentinean reports (21) showing that *stx* 2 is more frequent than *stx* 1 in cattle. Absence

Table 2. Antibiotic resistance of 287 *E. coli* isolates recovered from ground beef, grinding-machines and hands of meat manipulators in 23 butcheries in a Taquaritinga city, State of SãoPaulo, Brazil.

Antibiotic	Resistant strains *	
	n	%
Tetracycline	220	76.6
Amoxicillin	184	64.1
Cephalothin	169	58.8
Streptomycin	147	51.2
Nalidixic acid	90	31.3
Ampicillin	68	23.6
Amikacin	47	16.3
Ciprofloxacin	44	15.3
Cotrimoxazole	35	12.2
Gentamicin	28	9.7
Ceftriaxone	21	7.3
Amoxicillin + clavulanic acid	10	3.4

*Isolates presenting full and intermediary resistance were combined and considered resistant.

Table 3. Resistance patterns of the four STEC isolates recovered from butcheries.

Isolate	Resistance pattern*
426	none **
393	amox *
883	nalid; ceph; amox; tet
530	cipro; amik; amp; nalid; ceph; amox; tet

*-amox- amoxicillin; nalid- nalidixic acid; ceph- cephalothin; tet- tetracycline; cipro- ciprofloxacin; amik- amikacin; amp- ampicillin.
**- Susceptible to all tested antibiotics.

or rarity of the *eae* gene in STEC isolates coincides with earlier reports (11,14). Absence of serotype O157: H7 in STEC isolates is not unexpected; it is extremely rare (0.6%) in Brazilian cattle (11). Interestingly, two recovered isolates did not express *stx* genes (11).

Hussein and Bollinger (10), in a review of the prevalence of STEC in beef, reported prevalence of non-O157 STEC ranging between 1.7% and 58.0% in samples from packing plants and between 3.0% and 62.5% in supermarket samples; the USA, England, Canada and India showed the highest frequencies on isolation. In the present study, we found 1.3%, a value lower than those reported earlier (11).

Two of the STEC isolates, strains 393 and 426, were recovered from the same butchery, one from ground beef and the other from a meat-grinding machine. This is a matter of concern because unsatisfactory cleaning of meat grinders have been proven to be a source of contamination during processing (1,8). Warriner *et al.* (26) demonstrated that the same clone of *E. coli* was isolated from a pork carcass and from the equipment used during the carcass processing.

The emergence and dissemination of antimicrobial resistance among *E. coli*, especially STEC strains from cattle, may have potentially negative clinical implications for humans. Thus continued surveillance of emerging antimicrobial resistance among zoonotic food-borne pathogens, including STEC is required to ensure public health protection. Although STEC O157: H7 had been considered sensitive to many antibiotics (24), recent results demonstrated resistance of STEC O157:H7, and in special the non-O157 STEC strains (9,12). Results of antimicrobial susceptibility testing (Table 2) showed high resistance to tetracycline, cephalothin, amoxicillin and streptomycin. These findings agree with data from previous studies showing that resistance is common among strains isolated from food, animals and meat (23,25).

Despite of low number of non-O157 STEC strains isolated in the present study (n = 4) some of these strains (Table 3) were resistant to four or more antibiotics, in agreement with the recent report of Mora *et al.* (15), who described that among 581 non-O157 STEC strains, 239 (41.0%) were resistant to at least one of the antibiotics tested. These authors detected multiple resistance in 71 strains, which were resistant to five or more antimicrobial agents.

Antimicrobial-resistant bacteria in food may be a source of resistance genes transferable to human intestinal microbiota (28). Control strategies should therefore be introduced by veterinary authorities to safeguard public health.

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RESUMO

Isolamento de *Escherichia coli* Shiga toxigênica em açougues na cidade de Taquaritinga, Estado de São Paulo, Brasil

Escherichia coli Shiga toxigênica (STEC) tem sido responsabilizada como o agente etiológico de diversas doenças nos seres humanos. Neste estudo foram analisadas amostras provenientes de 23 açougues (carne moída, moedor de carne e mãos de manipuladores) visando o isolamento de cepas de *E. coli* utilizando os métodos microbiológicos tradicionais. Um total de 287 cepas de *E. coli*, isoladas destas amostras, foram submetidas a reação em cadeia da polimerase para a detecção

dos genes *stx* 1, *stx* 2 e *eae*. Foram identificadas 4 cepas STEC, 2 provenientes de carne moída e 2 provenientes do moedor de carne, todas as cepas apresentando o gene *stx* 2 e negativas para a presença do gene *eae*. Todas as cepas de *E. coli*, incluindo as 4 cepas STEC, foram examinadas para verificar a resistência antimicrobiana. Foram detectados altos níveis de resistência a diferentes agentes antimicrobianos, sendo as resistências mais elevadas para a tetraciclina (76,6%), amoxicilina (64,1%) e cefalotina (58,8%). Estes altos índices de resistência ressaltam a necessidade de uma utilização mais racional destes agentes no gado bovino.

Palavras-chave: *Escherichia coli*, STEC, *stx* 2, resistência a antibióticos, carne moída, gado

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