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Shiga Toxin–Producing *Escherichia coli* from Beef Carcass

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Abstract A survey was performed to estimate the frequency of *Escherichia coli* and Shiga toxin–producing *E. coli* (STEC) in carcasses obtained from at an abattoir in Brazil. The aim of present study was to verify the presence of virulence gene in *E. coli* strains isolated from cattle carcasses and also verify the antimicrobial resistance level these isolates. Thus, three carcass among the 80 tested showed a STEC, *stx2*-encoding gene. The frequency of carcass contamination by *E. coli* during processing was tested at three situations, respectively: preevisceration, postevisceration and postprocessing, during the rain and dry seasons. The prevalence of E. coli at the three points was of 14.67%, 37.61%, 14.67% in the rain season and of 7.33%, 11.0%, 14.67% during the dry season, respectively. The highest antimicrobials resistances were to cephalotin, followed by ampicilin and amikacin. The lowest were to trimethoprim, tetracycline and streptomycin. These findings suggest that the cattle carcasses can be vehicles from STEC and also from strains with high antimicrobials resistance for consumers.

Keywords *Escherichia Coli,* Cattle, Carcasses

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) can cause a range of serious disease syndromes in humans, including diarrhoea, haemorrhagic colitis and life threatening, haemol ytic–uraemic syndrome (HUS) (1). Transmission of STEC can be foodborne, waterborne or from person to person (2).

Escherichia coli form part of the bacterial population of the cattle's gastrointestinal tract. In beef carcass processing, *E. coli* is regarded as an indicator of fecal contamination. Levels of *E. coli* associated with cattle carcasses can increase or decrease during processing according to factors such as the levels of fecal contamination of live cattle, efficiency of evisceration and hygienic practices in the abattoir. *E. coli* is regarded as a pathogen of major worldwide importance in commercially produced beef, its presence can lead to significant economic loss (3).

Bovine E. coli strains can produce heat labile (LT) or heat stable (st) enterotoxins, Shiga-like toxins (stx), cytotoxic necrotizing factors (CNF1 and CNF2) and hemolysins (a-Hly and E- Hly). Enterotoxin-producing E. coli (ETEC) has been identified as the causative agent of several important diarrheal diseases in animals and humans and are capable of producing thermolabile (LT-I and LT-II) and thermostable (sta and stb) enterotoxins (4). LT I toxin does not occur in bovine samples (4), but sta enterotoxin is quite common in bovine cattle (5). CNF-producing *E. coli* has been isolated from animals with enteritis (6) and from humans with extraintestinal infection (7).

Cattle, considered primary reservoirs of both O-157 and non-O157 STEC bacteria (8), frequently carry STEC without showing any pathological symptoms (9). The full list of bacterial virulence determinants necessary for STEC's pathological effects is unknown. However, *stx* is a key factor in pathogenesis (10). Two types of Shiga toxin, *stx1* and *stx2* (encoded by *stx1* and *stx2* genes), are associated with human disease. These toxins vary in their amino-acid sequence (11) antigenicity, and in their activation and receptor specificity (12).

The ability of *E. coli* to adhere to intestinal epithelium is crucial in the colonization of the intestine, and therefore the progression of disease in humans. The protein intimin, encoded by the *eae* gene, enables intimate attachment of *E. coli* to intestinal cells (13), causing characteristic attaching/effacing lesions (14)

During the processing of the carcass, fecal contamination or transfer of bacteria from the animal's hide to the carcass can facilitate transmission of pathogenic *E. coli* to food supplies (3). The objective of this study therefore was to determine the virulence profiles and the antimicrobial drug resistance of *E. coli* isolates from beef carcasses at an abattoir in Brazil.

2. Material and Methods

2.1. Carcass samples

Three hundred and forty bovine carcass samples were

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collected between March 2008 and August 2009, at an abattoir in São Paulo State, in southwestern Brazil. Samples studied were from carcasses of 90 feedlot cattle raised at pastures. Sampling of 20 feedlot cattle was done in four different occasions, two in the rain season and two in the dry season. Each sample was obtained using a Specie- Sponge (3M- Brazil) moistened with 25 ml of Brilliant Green (BBL/Becton Dickins on) in a stomacher bag. Sponges were wrung out as much as possible within the bag, with drawn and used to swab each area. Each carcass was followed along the processing and sampled at three different stages always at the same site of the rump, near the anus over an area of 10 × 30 cm, delineated by a sterile metal template, from the same half of each carcass. Preevisceration samples were taken immediately after complete hide removal; postvisceration samples were collected after splitting and trimming; postprocessing samples were taken after washing of the carcasses hanging in the cooler. All samples were taken to the laboratory in an ice-cooled bag and kept for 12 h at room temperature.

2.2. Bacterial isolates

One hundred microliters of each sample was streaked on MacConkey agar plates (Oxoid Ltd) and incubated at 37℃ for 24 h. Colonies showing *E. coli* characteristics were submitted to Gram staining and identified by standard biochemical tests as oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative (15). The isolates were serotyped for the O serotype O157 using the O157 Latex Agglutination test kit (Oxoid, Basingstoke, UK). Negative strains were considered non-O157 strains.

2.3. PCR Screening of Samples

Bacterial strains were grown overnight in nutrient broth (Sigma Chemical Co.) at 37 ℃, were pelleted by centrifugation at 12, 000 g for 1 min, resuspended in 200 µl of sterile distilled water and lyzed by boiling for 10 min. Lysates were centrifuged as described above and 150 µl of the supernatants were used as DNA template for the polymerase chain reaction (PCR) (16). A total of 89 *E. coli* isolates were subjected to PCR; *stx1*, *stx2* and *eae* genes were detected using the primers and PCR conditions described by (17). The presence of LT-II gene was assessed by PCR amplification using primer pairs and conditions described by (18). The *sta* gene was detected using the primer and conditions described by (19).

2.4. Expression of E-Hly

Expression of enterohemolysin was determined based on the method described by (20). Plates were incubated at 37 ℃ for 24 h and observed for hemolysis after 3 h (for expression of a-hemolysin) and 24 h (for E- Hly), respectively. The reference strains used in this assay were *E. coli* U4– 41 (positive control for a -hemolysin), *E. coli* 32511 (STEC O157: H7) (positive control for E-Hly), and *E. coli* K12 (negative control).

2.5. Susceptibility Testing

Antimicrobial disk susceptibility tests were performed using the disk diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (21). Eleven antimicrobial agents were selected for the tests: ampicillin, amoxicillin/clavulanic acid, cephalotin, ceftriaxone, tetracycline, gentamicin, streptomycin, amikacin,tr imethoprim, nalidixic acid and ciprofloxacin.

3. Results and Discussion

The distribution of positive carcass responses for *E. coli* corresponding to each sampling season is shown in Table1. *E. coli* distribution in the three stages of the sampling, show the same characteristics during the rainy season and the dry season; however, the number of positive carcasses obtained in the rain season was higher than in the dry season. All isolates were confirmed as being *E. coli* by their biochemical analysis and were submitted to PCR for the detection of sequences of virulence genes. From each MacConkey agar plate a loopful from a confluent bacterial growth was collected and analyzed. All isolates except four were negative for *stx*, eae, LT-II and *sta* genes by PCR analysis, as well as for enterohemolysin expression. The four positive isolate was a three *stx2*- and one *stx1* encoding strain. Toxin- profiling studies of O-157: H7 clinical isolates by (22) had shown that patients infected with isolates carrying only *stx2* were 6.8 times more likely to develop severe disease than those infected with strains carrying *stx1* or both *stx1* and *stx2*. The before, isolates carrying *stx2* could represent a potential increased threat to human health. The number of isolates was high 109 strains during rainy season and dry. (Table1).

Table 1. Distribution of the Escherichia coli isolates at three different stages of processing of 80 beef carcasses at an abattoir in two different climatic seasons in Brazil between March 2008 and August 2009

Carcass					
Collection	Season	Pree-viscera tion	Poste-viscera-tion	Post-process-ing	Total
1º	Raining	7/20a	18/30	9/20	34
2º	Raining	9/20	23/20	7/20	39
3º	Dryness	4/20	5/20	8/20	17
4º	Dryness	4/20	7/20	8/20	19
					109

[a] Values are the number of samples positive for *E. coli*/among the total number of samples taken.

Rogerie et al. (23) reported a lower postprocessing of non-O157 STEC prevalence (1.9%) on carcasses sampled during the summer in processing plants in France. Similarly, the non-O157 STEC prevalence on carcasses processed in Hong Kong was reported to be 1.7% (24) however, (25) reported a high level (54.0%) of contamination with non-O157 STEC in carcasses processed in the United States.

The hides and feces of animals presented for slaughter have been shown to be major sources of pathogens in processing plants (26). It is not clear what proportion of non-O157 STEC bacteria detected in cattle feces or on beef carcasses is able to cause disease in humans. Gyles et al. (27) defend the idea that all STEC bacteria could be pathogenic under adequate circumstances.

In the present work, the detected level of STEC strains (1.2%), matches those reported by others (23; 24). To the best of our knowledge, we could not find data from Brazil for comparison. Some authors have reported the detection of STEC strains in fecal samples of dairy cattle (28), from diarrheic (29) and from mastitic cattle (30) but none from abattoir samples. In all of them, the *stx2* gene has been predominantly found, and the non-O 157 STEC strains detected. In Brazil only a small number of O157 strains have been detected among bovine fecal samples, 0.6% as reported by (28) they did not express the *stx* gene. Interestingly, the O157: H7 strains isolated in São Paulo State from human infections, were all *stx* -producers (31), predominantly presenting the *stx1* gene. For more than four decades it has been a common practice on farms to use antimicrobial agents for disease prevention and growth promotion of animals. The widespread use of antimicrobial agents would select for resistance enhancement and may have promoted the increasing frequency of STEC strain's multidrug resistance in bovines. This could result in STEC population increases and perhaps greater shedding which could lead to higher contamination of animal food products with STEC (32).

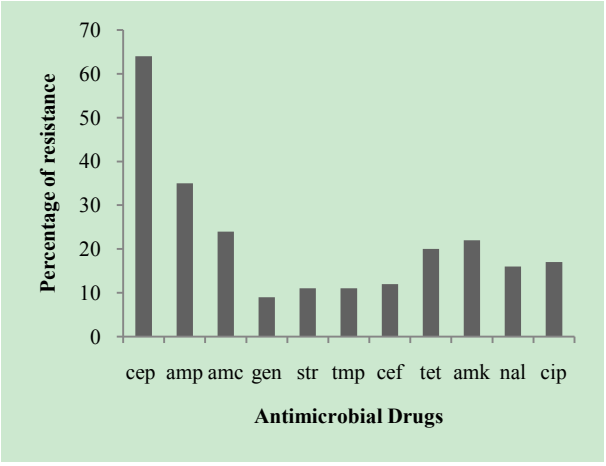

Figure 1. Antimicrobial resistance patternsin 109 *Escherichia coli* strains taken from a cattle abattoir in Brazil. Amc — amoxicillin/clavulanic acid; amk — amikacin; amp — ampicilin; cef — ceftriaxone; cep — cephalothin; cip — ciprofloxacin; gen — gentamicin; nal — nalidixic acid; str — streptomycin; tet — tetracycline; tmp — trimethoprim

An *E. coli* colony from each positive plate was tested against eleven antimicrobial agents. Most commonly, resistance was observed to cephalothin (64.0%), ampicillin (35.0%) and amoxicillin/clavulanic acid (24.0%) and less frequently to gentamicin (9.0%), streptomycin (11.0%) and trimethroprin (11.0%) (Fig.1). Twenty-four percent of the isolates were sensible to all the antibiotics tested. Multidrug resistance was seen in 55.0% of the isolates and resistance to 2 or 3 antibiotics was most common among the isolates (Fig. 2). Khan et al. (33) reported resistance to one or more antibiotics in 49.2% of STEC strains in India, with some strains exhibiting multidrug resistance.

Antimicrobial resistant bacteria from animals may colonize human population via the food chain; it is therefore possible that resistant bacteria may be readily transferred to humans from animals used as food sources (34). These findings suggest the cattle carcasses may be a good vehicle to transfer pathogenic bacteria specifically STEC presenting high antimicrobial resistance to the consumers.

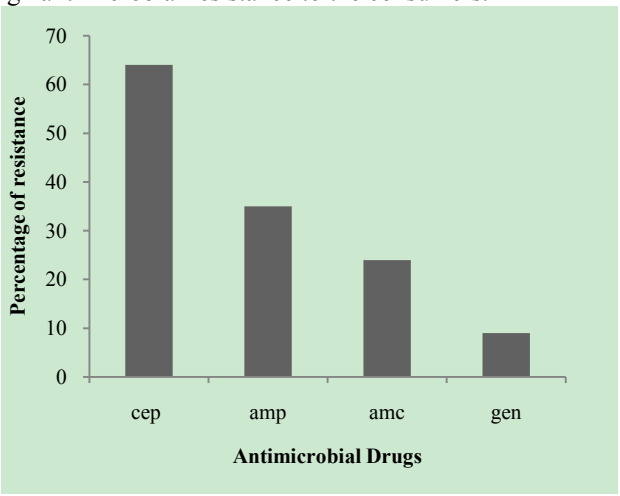

Figure 2. Distribution of multidrug resistance to 11 antimicrobial drugs among 109 strains of *Escherichia coli* isolated from a cattle abattoir in Brazil

4. Conclusions

We report here a small level (1. 2%) of occurrence of STEC strains on beef carcasses during processing at an abattoir in Brazil. However the *E. coli* isolates analyzed showed a high level of multidrug resistance capable of causing concern.

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