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Full Length Research Paper

Contamination of cattle carcasses by *Escherichia coli* shiga like toxin with high antimicrobials resistence

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During processing of cattle carcasses, contamination may occurs with the transfer of microbiota of animals feaces to carcasses. This contamination many times may be by *Escherichia coli* carriers of virulence factor as *stx* and *eae* genes being classified as Shiga like toxin. Shiga toxin-producing *Escherichia coli* (STEC) is recognized wordwide as human pathogen. A survey was performed to determine the sensibility profile to several antimicrobial drugs of STEC in carcasses obtained from an abattoir in Brazil between March 2008 and August at 2009. A total of 120 STEC were isolated. All isolates were confirmed as being *E. coli* by their biochemical analysis and submitted to polymerase chain reaction (PCR) for detection of *stx*, *eae* and *ehly* genes. No strains was isolated being carriers of *ehly* gene. The number of isolates carriers of *eae* gene were 48/120. The most frequent resistance was seen against cephalothin (84.0%), streptomycin (45.0%), nalidixic acid (42.0%) and tetracycline (20.0%). Multidrug resistance (MDR) to three or more antimicrobial agents was observed in 46 (38.3%) *E. coli* isolates. The findings of STEC and MRD show that cattle carcasses may be a reservoir of pathogenic bacterial for the consumer public.

Key words: Multi-drug resistance, *Escherichia coli*, shiga toxin-producing *Escherichia coli* (STEC).

INTRODUCTION

Pathogenic *Escherichia coli* are classified at different groups of strains that cause a common disease using common and remarkable assortments of virulence factors (Kaper et al., 2004). One such pathotype, the STEC is the causative agent of severe clinical syndromes in humans such as haemolytic uremic syndrome (HUS) and haemorrhagic colitis. However the transmission of STEC occurs by waterborne, from person to person and also may be transmitted by food borne (Nataro and Kaper, 1998). *E. coli* is regarded as an indicator of fecal contamination when isolated from carcass processing. 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 (Bell, 1997).

Cattle, considered primary reservoirs of both O157 and non-O157 STEC bacteria (Bettelheim, 2000), frequently carry STEC without showing any pathological symptoms (Blanco et al., 1997). The full list of bacterial virulence determinants necessary for STEC's pathological effects is not known. Two types of Shiga like toxin, *stx1* and *stx2* (encoded by *stx1* and *stx2* genes), are associated with

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Abbreviations: STEC, Shiga toxin-producing *Escherichia coli*; **PCR,** polymerase chain reaction; **MDR,** multidrug resistance; **HUS,** haemolytic uremic syndrome; **PR,** Paraná.

human disease. These toxins vary in their amino-acid sequence (Kaper et al., 1998) antigenicity, and in their activation and receptor specificity (Schmitt et al., 1999). *E. coli* acquire *stx* genes, and the subsequent ability to produce toxins, following infection with temperate bacteriophages (James et al., 2001). The ability of *E. coli* to adhere to intestinal epithe-lium 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 (Donnenberg et al., 1992), causing characteristic attaching/effacing lesions (Paton et al., 1998). This attachment also enables Shiga toxins to be injected into the epithelial cytoplasm through a type III secretion system (Kaper, 2004). Other virulence factors such as intimin (eae*)* and hemolysin (*hly A*) are thought to enhance pathogenicity, but are not required for strains to produce severe disease, including HUS (Bonnet et al., 1998; Acheson, 2000). Antimicrobial therapy is an important tool for infection treatment, resistance to antimicrobials is a cause of great concern in veterinary medicine (Monro and Polk, 2000). Indeed, a close association between the use of antimicrobial agents for the treatment of infections in animals and the observed levels of resistance exists (Chaslus-Dancia, 2001). The use of antibiotics in animal agriculture has been a controversial issue due to the potential transfer of antibiotic resistance from animals to humans. This could have several public health implications that may cause treatment failure, including death and illness prolonga-tion, as well as increase in the associated costs (Kelly et al., 2004).

The direct impact of resistance evolved from the use of antimicrobials in treatment of animal infection, is not clear. Since the antimicrobials routinely used for the treat-ment of infections in humans are also used in animals for both therapy and prevention or as growth promotion factors, it is not easy to describe the relative contributions of animal derived resistant strains to human *E. coli* disease (Maynard et al., 2004). Outbreaks have been associated with consumption of STEC contaminated and undercooked hamburgers, subsequent to both animal and foods (Erickson and Doyle, 2007). This probably occurs because 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 (Bell, 1997; Barkocy-Gallagher et al., 2001). Some studies found a high prevalence of STEC in feces of healthy cattle, in Brazil, (Irino et al., 2005), Rio de Janeiro (Cerqueira et al., 1999), Rio Grande do Sul (Moreira et al., 2003; Timm et al., 2007) and also in Paraná (PR), (Farah et al., 2007; Pigatto et al., 2008) and a prevalence of 1 to 2% of STEC in cases of diarrhea in humans was reported by Vaz et al. (2004), De Toni et al. (2009). The objective of this study was to determine the virulence profiles and the antimicrobial drug resistance of *E. coli* isolates from beef carcasses at an abattoir in Brazil.

MATERIALS AND METHODS

Carcass samples

Six hundred carcass samples were collected an abattoir in São Paulo State, in southwestern Brazil, between March 2008 and August 2009. Samples studied were from carcasses cattle raised at pastures. Sampling of 150 feedlot cattle was done on 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 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. All samples were taken to the laboratory in an ice-cooled bag and kept for 12 h at room temperature.

Bacterial isolates

One hundred microliters of each sample was streaked on MacConkey agar plates (Oxoid Limited) 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 (Koneman et al., 1997). The isolates were serotyped for O157 using Latex Agglutination test kit (Oxoid, Basingstoke, UK). Negative strains were considered non-O157 strains.

PCR screening of samples

Bacterial strains, grown overnight in nutrient broth (Sigma Chemical Company) at 37°C, 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. Lysate was centrifuged as described above and 150 µl of the supernatants were used as DNA for the PCR (Wani et al., 2003). A total of 120 *E. coli* isolates were subjected to PCR. *stx* 1, *stx* 2 and *eae* genes were detected using the primers and PCR conditions described by China et al. (1998) .

Expression of E-Hly

Expression of enterohemolysin was determined based on the method described by Beutin et al. (1989). Plates were incubated at 37°C 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).

Susceptibility testing

In vitro susceptibility testing was performed by a standardized disk diffusion method (CLSI 2008). *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 served as quality control strains. Four antimicrobial agents were selected for the tests: cephalothin, streptomycin, nalidixic acid and tetracycline. The antimicrobials used in this study were the same used by farmers in animal produce.

Table 1. Distribution of the *Escherichia coli* isolates at two different seasons collected between March 2008 and August 2009.

Carcass			
Collection	**Season**	***Stx* genes**	***eae***
1º	Rainy	35/150	23/150
2º	Rainy	47/150	12/150
3º	Dry	17/150	5/150
4º	Dry	21/150	8/150
Total		120 *Stx*+	48 *eae*+

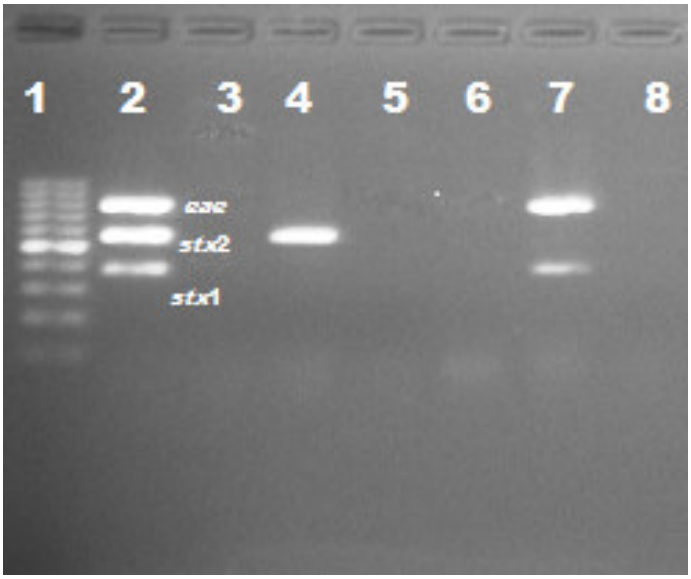

Figure 1. Photograph of a 1.5% agarose gel stained with ethidium bromide. Columns: 1 = 100 bp DNA ladder; 2 = positive control; 3 = negative control; 4 = strain positive for *eae;* 4, 6 and 8 = strain negative for all studied genes; 7 = strain positive for *stx*1 and *stx*2.

RESULTS

All isolates, confirmed as being *E. coli* by their biochemical analysis, 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. A total six hundred *E. coli* strains isolates the cattle carcasses were separate 120 isolates that carrying *stx*1, *stx*2 and *eae* genes. These isolates just 45 were carriers of *eae* gene (Table1 and Figure 1). All isolates were collected of preevisceration stage. There were not isolating of strains of pre-evisceration stages and neither of post-processing stage (data not show). The isolates number containing both *stx* and *eae* gene during rainy season were high than dry season (Table1) and also the isolates number that carried the *eae* genes were high than the number of isolates that carried *eae* genes. In no isolates was verified the expression of enterohemolisyn. All isolates were tested for this hemolytic toxin and also no isolated were isolates was serotyped as O157. *E. coli* strains were tested against ten antimicrobial agents. The resistance pattern observed was: cephalothin (84.0%), streptomycin (45.0%) and nalidixic acid (42.0%) and tetracycline (20.0%) (Figure 1), 24% of the isolates were resistant to all the antibiotics tested. Multidrug resistance was seen in 38.4% of the isolates and resistance to 2 or 3 antibiotics was common (Figure 2 and 3).

DISCUSSION

Among 600 strains analyzed only three were enterohemolysin positive. These results were similar to Rigobelo et al. (2008) that analyzed 216 samples from bovine carcasses and all of the isolates were negative for *ehly* gene. During raining season were found a high prevalence of STEC than dry season; probably the presence of water increased the spread of bacteria STEC. Some authors as Rogerie et al. (2001) reported lower post processing of nonO157 STEC prevalence (1.9%) on carcasses sampled during the summer in plants in France. Similarly, the non-O157 STEC prevalence on carcasses processed in Hong Kong was reported to be 1.7% (Leung et al., 2001), however, Arthur et al. (2002) reported higher level (54.0%) of contamination with nonO157 STEC in carcasses processed in the United States. Major sources of pathogens in processing of carcasses have been the hide and hair (Barkocy-Gallagher et al., 2001). 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. (1998) defend the idea that all STEC bacteria could be pathogenic under adequate circumstances. In the present study, the detected level of STEC strains (20%) did not match those reported by others (Rogerie et al., 2001; Leung et al., 2001; Mora et al., 2005). To the best of our knowledge, we could not find data from Brazil for comparison. Only Rigobelo et al. (2006) report of STEC (1.25%) and Rigobelo et al. (2008) report (1, 4%) of STEC. These differences were probably because of low hygienic conditions of abattoir where we collected the samples.

Some authors have reported the detection of STEC strains in fecal samples of dairy cattle (Irino et al., 2005), from diarrheic (Leomil et al., 2003) and from mastitic cattle (Lira et al., 2004) but none from abattoir samples. In all of them, the *stx 2* gene has been predominantly found, and the non-O 157 STEC strains detected. Only a small number of O157 strains have been detected among bovine fecal samples 0.6% as reported by Irino et al. (2005), they did not express the stx gene. Interestingly, the O157: H 7 strains isolated in São Paulo State from human infections, were all stx -producers (Vaz et al., 2004), predominantly presenting the *stx 1* gene. For more than four decades it has been a common practice on farms to use antimicrobial agents for disease

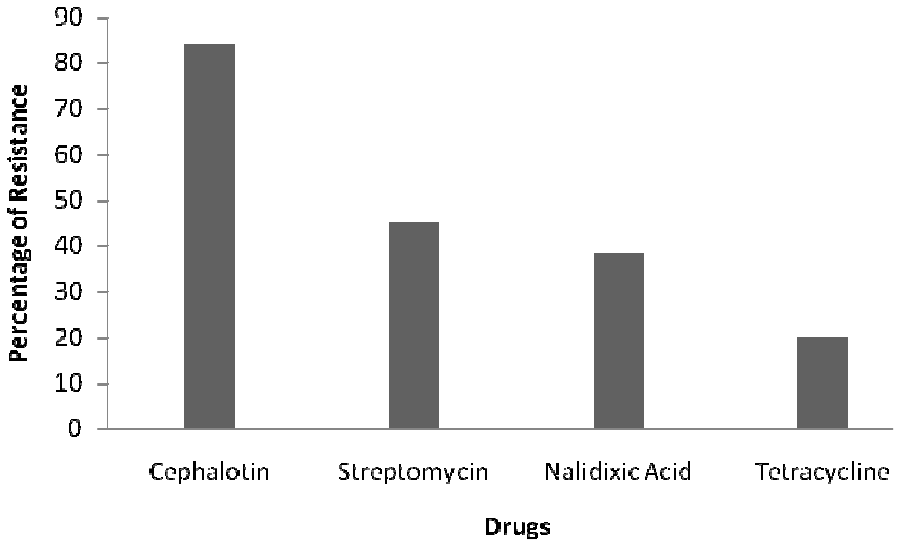

Figure 2. Antimicrobial resistance pattern of *Escherichia coli* isolate. CFL-cephalothin; STR - streptomycin – NAL-nalidixic acid; TET-tetracycline.

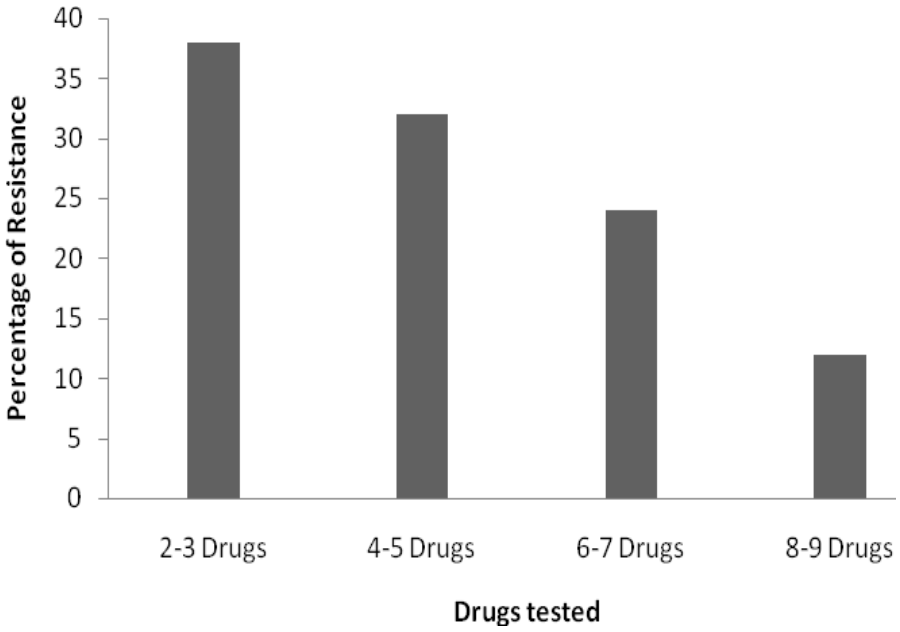

Figure 3. Distribution of multidrug resistance to four antimicrobial drugs among *Escherichia coli* strains (n=120).

prevention and growth promotion of animals. Widespread use of antimicrobial agents, 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 (Zhao et al., 2001).

Khan et al. (2002) 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 possible that resistant bacteria may be readily transferred to humans from animals used as food sources (Van den Bogaard and Stobberingh, 2000). During processing at an abattoir in Brazil we report a high level (20%) of occurrence of STEC strains on beef carcasses and also high

antimicrobial resistance suggesting poor hygienic conditions of slaughter of animals.

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