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Short Communication

Characterization of *Escherichia coli* isolated from carcasses of beef cattle during their processing at an abattoir in Brazil

E.C. Rigobelo [a], A.E. Stella [b], F.A. Ávila [b], C. Macedo [c], J.M. Marin [d,*]

[a] *Faculdade de Zootecnia de Dracena, Universidade Estadual Paulista, SP, Brazil*
[b] *Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista, SP, Brazil*
[c] *Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil*
[d] *Departamento de Morfologia, Estomatologia e Fisiologia, FORP, Universidade de São Paulo, Campus Ribeirão Preto, SP, Brazil*

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Abstract

Beef carcass sponge samples collected between March 2003 and August 2005 at an abattoir in Brazil were surveyed for the presence of Shiga toxin-producing *Escherichia coli* (STEC). Only one carcass among the 80 tested showed a STEC, *stx*2-encoding gene by PCR amplification. 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 30.0%, 70.0%, 27.5% in the rain season and of 22.5%, 55.0%, 17.5% during the dry season, respectively. The *E. coli* isolates exhibited a high level (45.0%) of multidrug resistance to two or more antimicrobial agents.

Keywords: *Escherichia coli*; Beef carcass; STEC; Abattoir; Multidrug resistance

1. Introduction

Escherichia coli forms 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 (Bell, 1997).

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 (α-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 (Butler and Clarke, 1994). LT I toxin does not occur in bovine samples (Blanco et al., 1993), but STa enterotoxin is quite common in bovine cattle (Blanco et al., 1993). CNF-producing *E. coli* has been isolated from animals with enteritis (De Rycke et al., 1987) and from humans with extra-intestinal infection (Caprioli et al., 1987); they have been rarely found in Brazil (Salvadori et al., 2003).

Shiga toxin-producing *E. coli* (STEC) has been implicated as the causative agent in several human diseases (Nataro and Kaper, 1998; Paton and Paton, 1998), ranging from mild diarrhea to very severe and life-threatening conditions like the hemolytic–uremic syndrome (HUS). The STEC strain most frequently associated with clinical disease in the United States and Europe is serotype O157:H7 (Nataro and Kaper, 1998). However several other serotypes (O26, O103, O111, O113 and O121) are commonly found in association with severe disease outbreaks; in some countries they are isolated from clinical cases more often than O157 (Nataro and Kaper, 1998; Acheson, 2000).

Cattle, considered primary reservoirs of both O157 and non-O157 STEC bacteria (Bettelheim, 2000), frequently carry

* Corresponding author. Tel.: +55 16 36024101; fax: +55 16 36330999.
E-mail address: jmmarin@forp.usp.br (J.M. Marin).

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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. However, Stx is a key factor in pathogenesis (Acheson, 2000). Two antigenically distinct classes of Shiga toxin have been identified, *Stx*1 and *Stx*2 (Nataro and Kaper, 1998). 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).

Although antimicrobial therapy is an important tool for infection treatment, resistance to antimicrobials is widespread and a cause of great concern in veterinary medicine (Monroe 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 (Schawarz and Chaslus-Dancia, 2001). From the human health perspective, the direct impact of antimicrobial resistance evolved from the use of antimicrobials in the treatment of animal infections, is not clear. Since the antimicrobials routinely used for the treatment of infections in humans are also used in animals for either 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).

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). 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. Materials and methods

2.1. Carcass samples

Two hundred and forty bovine carcass samples were collected between March 2003 and August 2005, at an abattoir in São Paulo State, in southwestern Brazil. Samples studied were from carcasses of 80 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 Dickinson) in a stomacher bag. Sponges were wrung out as much as possible within the bag, withdrawn 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; postevisceration 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 °C 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 (Koneman et al., 1997). 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 °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. Lysates were centrifuged as described above and 150 μl of the supernatants were used as DNA template for the polymerase chain reaction (PCR) (Wani et al., 2003). A total of 89 *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). The presence of *LT II* gene was assessed by PCR amplification using primer pairs and conditions described by Penteado et al. (2002). The *STa* gene was detected using the primer and conditions described by Jung (1997).

2.4. 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 α-hemolysin) and 24 h (for E-Hly), respectively. The reference strains used in this assay were *E. coli* U4–41 (positive control for α-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 recommend by the National Committee for Clinical Laboratory Standards (NCCLS, 1999). Eleven antimicrobial agents were selected for the tests: ampicillin, amoxicillin/clavulanic acid, cephalotin, ceftriaxone, tetracycline, gentamicin, streptomycin, amikacin, trimethoprim, 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 Table 1. *E. coli* distribution in the three stages of the sampling, show the same characteristics during the rain 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

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 2003 and August 2005

Carcass					
Collection	Season	Preevisceration	Postevisceration	Postprocessing	Total
1°	Raining	6/20[a]	13/20	6/20	25
2°	Raining	6/20	15/20	5/20	26
3°	Dryness	5/20	10/20	2/20	17
4°	Dryness	4/20	12/20	5/20	21
					89

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

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 one were negative for *stx*, *eae*, *LT II* and *STa* genes by PCR analysis, as well as for enterohemolysin expression. The only positive isolate was a *stx*2-encoding-strain. Toxin-profiling studies of O157: H7 clinical isolates by Ostroff et al. (1989) had shown that patients infected with isolates carrying only *stx*2 were 6.8 times more likely to develop severe disease than those infected with strains carrying *stx*1 or both *stx*1 and *stx*2. Therefore, isolates carrying *stx*2 could represent a potential increased threat to human health.

Rogerie et al. (2001) 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% (Leung et al., 2001); however, Artthur et al. (2002) 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 (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 work, the detected level of STEC strains (1.2%), matches those reported by others (Rogerie et al., 2001; Leung et al., 2001). 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 (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-O157 STEC strains detected. In Brazil 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: H7 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 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 (Zhao et al., 2001).

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 45.0% of the isolates and resistance to 2 or 3 antibiotics was most common among the isolates (Fig. 2). 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 therefore possible that resistant bacteria may be readily transferred to humans from animals used as food sources (Van den Bogaard and Stobberingh, 2000).

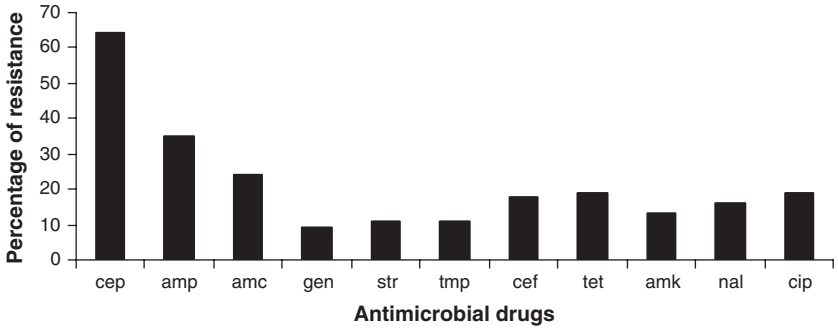

Fig. 1. Antimicrobial resistance patterns in 89 *Escherichia coli* strains taken from a cattle abattoir in Brazil. Amc — amoxicillin/clavulanic acid; amk — amikacin; amp — ampicilin; cef — ceftriaxone; cep — cephalothin; cip — ciprofloxacin; gen — gentamicin; nal — nalidixic acid; str — streptomycin; tet — tetracycline; tmp — trimethoprim.

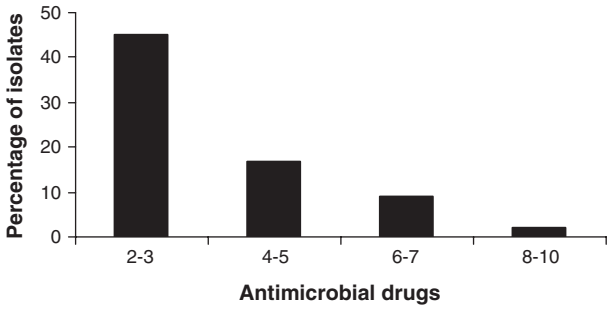

Fig. 2. Distribution of multidrug resistance to 11 antimicrobial drugs among 89 strains of *Escherichia coli* isolated from a cattle abattoir in Brazil.

To conclude, 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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