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PRESENCE OF EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI* IN BUTCHERIES IN TAQUARITINGA, SP, BRAZIL

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SHORT COMMUNICATION

ABSTRACT

The study was conducted in twenty-three butcheries in the city of Taquaritinga, State of São Paulo, Brazil, surveyed during a 10 months period. Among two hundred and eighty-seven *Escherichia coli* strains isolated from samples of ground beef, meat-grinding-machines and the hands of manipulators, five were recognized as extraintestinal pathogenic *E. coli* (ExPEC), showing virulence factors (P and S fimbriae, hemolysin and aerobactin) and presenting multidrug resistance. Retail-sold food may constitute an important vehicle for the dissemination of ExPEC in communities, giving rise to reasons for concern.

Key words: *Escherichia coli*, ExPEC, ground beef, butchery

In humans, strains of *Escherichia coli* may be commensal and/or cause of various infectious intestinal and extraintestinal diseases (2,8,14). The barrier between commensalism and virulence results from a complex balance involving host condition and the presence and expression of virulence factors. In general, pathogenicity has been correlated with the presence of genes encoding virulence factors (VFs) organized in large blocks called pathogenicity islands. Such factors may be horizontally disseminated among distinct *E. coli* strains (6), leading to infections of the urinary tract (UTI), meningitis and bacteremia; they are distinct from most intestinal commensal *E. coli* as well as from diarrheagenic *E. coli* types (4,16). These specialized extraintestinal pathogenic *E. coli* (ExPEC) strains acquire their unique pathogenicity from distinctive VFs that include adhesins, toxins, siderophores, polysaccharide coating and invasions (8,9), causing the host organism to overcome or subvert its defenses, colonize key anatomical sites, and disturbing host physiology by invading tissues causing disease. Several studies have suggested that food may give raise to human acquired antimicrobial-resistant *E. coli* and/or ExPEC (11,19). Meat products at slaughtering centers may be extensively contaminated with *E. coli* of animal origin, including strains that express ExPEC-associated O antigens (11). The objective of the present study was to survey retail meat markets by systematic sampling and examination for the presence of ExPEC in ground beef (GB), meat-grinding machines (MGM) and the hands of meat manipulators (HMM).

Meat samples and swabs from grinding-machines and the hands of meat manipulators were collected on various occasions, over a 10-month period ranging from March of 2004 to January 2005. Twenty-three butcheries located in Taquaritinga a city in the northwest of the State of São Paulo, Brazil, were surveyed. Immediately following collection, samples were kept on ice and processed on the day of arrival in the laboratory. A 25 g sample of ground meat and swabs from the grinding-machines and from the hands of the machine-operators were obtained. Ground beef was manually homogenized by shaking for 5-10 min in 225 ml of sterile 0.1% (wt/vol) peptone water in a stomacher bag

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(Oxoid Ltd, Basingstoke, Hampshire, UK). Swabs from the grinding machine and operator hands were mixed with 10 ml of 0.1% peptone water. One ml from each treatment was diluted in 9 ml of lauryl sodium sulfate broth (Difco Laboratories, Detroit, USA) and incubated for 24-48 h at 35ºC. One hundred µl of suspensions from tubes showing bacterial growth were mixed with 5 ml of Brilliant Green broth (Difco) or EC broth (Difco) and incubated for 24 h at 35ºC. Ten µl of the content of tubes positive for coliform growth were plated on Eosin Metylene Blue agar (EMB-Difco). At least 5 isolated colonies from each plate were submitted to further analysis by standard methods for *E. coli* identification (5). Bacterial strains (*E. coli* isolates) grown overnight in nutrient broth (Sigma Chemical Co, St Louis, USA) at 37ºC were tested for the presence of *pap, sfa* and *afa* using the polymerase chain reaction (PCR) protocol of Le Bouguenec *et a*l. (13). DNA templates were prepared by pelleting 1 ml of cultures, enriched by centrifugation at 12000xg. The cell pellet was resuspended in 250 µl of sterile distilled water and boiled for 10 min at 100ºC. The suspensions obtained were again centrifuged and their supernatants used for PCR, performed in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany); *pap, sfa* and *afa* genes were detected using primers and PCR conditions in the above-mentioned protocol. The amplified DNA products were separated by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and examined under ultraviolet light. Hemolysin production was assayed by growing the isolates overnight (16 h), at 37ºC in Luria-Bertani Broth (LB); 50 µl samples were then spot inoculated onto a sheep blood agar base, incubated at 37ºC overnight, and hemolysin production verified by the presence of a clear halo of hemolysis around the inoculated site. Production of aerobactin was assayed by growing isolated strains in LB medium containing 200 µM of α-α dipyridyl at 37ºC for 24 h, without shaking. The growths produced were spun for 3 min at 12000xg, supernatants were filtered through a nitrocellulose membrane (0.22 µm) and 50 µl aliquots of the filtrate were added to orifices made in LA medium previously seeded with strain LG 1522 (3). The plates were incubated at 37ºC for 48 h and the production of aerobactin was visualized by the growth of strain LG 1522 around the orifices. Antimicrobial susceptibility testing of ExPEC isolates was carried out by the disk diffusion method using commercial disks (Cefar, São Paulo, Brazil), according to the guidelines of the National Committee for Clinical Laboratory Standards (15). Antimicrobials tested and loads on the disks were 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.

During the 10-months survey, 69 samples from GB, MGM and HMM were cultured; 287 *E. coli* strains isolated from these samples were submitted to PCR to detect *pap, sfa* and *afa* genes as well as to traditional methods to detect hemolysin and aerobactin production. Five ExPEC strains were found: three of them carrying the *pap* gene (2 from GB and 1 from MGM) and two carrying *pap-sfa* genes (1 from GB and 1 from MGM). Most of the isolates possessed both hemolysin and aerobactin genes. The isolates presented genes with combinations of adhesins (P and S fimbriae), an iron-acquisition system (aerobactin) and toxins (hemolysin), which are at present collectively regarded as extraintestinal virulence factors (Table 1). During retail meat processing, bacteria present on the surface of food animal tissue may be transferred to meat surfaces via worker's hands and knives (7). There is also the possibility of cross-contamination of meat and equipment during meat processing (1). Recently, an unexplained dissemination of ExPEC clones has been reported (10,11).

The results of antimicrobial susceptibility testing, shown on Table 1, indicate that only one isolate (647) was susceptible to all antimicrobial agents tested. Among the other strains resistance to tetracycline, streptomycin, ampicillin and cephalothin was the most frequently observed. These findings agree with data from previous studies showing that such

Table 1. Virulence factors and antimicrobial resistance of ExPEC isolated from ground meat, meat-grinding-machines and hands of the machine-operators in Taquaritinga, São Paulo State, Brazil.

Isolates	Virulence Factors					ResistancePhenotype
	pap	sfa	Afa	hemolysin	aerobactin	
647	+	-	-	+	+	susceptible
685	+	-	-	+	+	Gen,Cot,Cipro, Ami, Amp, Nal, Str, Cep, Amo, Tet
738	+	+	-	-	+	Cot, Amp, Nal, Str, Tet
822	+	-	-	+	+	Amp, Str, Cep, Tet
919	+	+	-	+	+	Str, Cep, Tet

Susceptible- susceptible to all antimicrobials tested; Gen- gentamicin, Cot- cotrimoxazole; Cipro- ciprofloxacin; Ami- amikacin; Amp- ampicillin; Nal- nalidixic acid; Str- streptomycin; Cep- cephalothin; Amo- amoxicillin; Tet- tetracycline.

resistance is common among strains isolated from food animals and meat (18) including ExPEC strains (11). One isolate (685) was resistant to ten antimicrobial drugs, a quite uncommon finding.

ExPEC are responsible for millions of episodes of UTI, for a high number of deaths from sepsis and for a high annual cost for health-care systems throughout the world (17). Thus, even if only a small fraction of extraintestinal *E. coli* infections is due to foodborne ExPEC, these may rival diarrheagenic *E. coli* strains in importance as foodborne pathogens (11). To conclude, in this work it was found that GB and MGM could be contaminated with ExPEC strains presenting multidrug-resistance, indicating that food supplies could represent a new vehicle for the dissemination of these important pathogens. A better understanding of the impact on health of this new route of dissemination of ExPEC is required for the sake of taking adequate measures to prevent it.

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RESUMO

Escherichia coli patogênica extraintestinal em açougues em Taquaritinga, SP, Brasil

O trabalho foi desenvolvido em 23 açougues em Taquaritinga, Estado de São Paulo, Brasil, durante um período de 10 meses. De duzentas e oitenta e sete cepas de *E.coli* isoladas de carne moída, moedor de carne e mãos de manipuladores de carne, cinco foram caracterizadas como *E. coli* patogênica extraintestinal (ExPEC) apresentando fatores de virulência (fimbria P e S, hemolisina e aerobactina), assim como multiresistencia a drogas antimicrobianas. Retalhos de carne podem ser um veiculo importante para a disseminação de ExPEC, o que representa um motivo de preocupação.

Palavras-chave: *Escherichia coli*, ExPEC, carne moída, açougue

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