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PREVALENCE OF ENTEROPATHOGENIC AND ENTEROTOXIGENIC *Escherichia coli* IN FOODS OF ANIMAL ORIGIN IN SOUTHERN BRAZIL[1]

PREVALÊNCIA DE *Escherichia coli* ENTEROPATOGÊNICA E ENTEROTOXIGÊNICA EM ALIMENTOS DE ORIGEM ANIMAL NO SUL DO BRASIL

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SUMMARY

Prevalence of ***Escherichia coli*** *in foods of animal origin from the city of Pelotas, Brazil, was determined. The ocurrence of enterotoxigenic (ETEC) and classic enteropathogenic (EPEC) serogroups among* ***E. coli*** *isolates was determined.* ***E. coli*** *was isolated from 68% of the food samples surveyed. Of 36 food samples tested, 11(30%) and 24(66%) were positive for EPEC and ETEC strains respectively. However, of 187* ***E. coli*** *isolates tested, 30(16%) were EPEC compared to 75(40%) which were ETEC. The antibiotic resistance pattern revealed that the isolates were highly sensitive to all antibiotics tested.*

Key words: Escherichia coli, *EPEC, ETEC, foods, Brazil.*

RESUMO

Foi determinada a prevalência de ***Escherichia coli*** *em alimentos de origem animal na cidade de Pelotas, RS. Determinou-se a ocorrência de cepas enterotoxigênicas (ETEC) e enteropatogênicas clássicas (EPEC) entre os isolamentos de* ***E. coli****. Em 86% das amostras de alimentos analisadas foi detectada a presença de* ***E. coli****. De 36 amostras de alimentos testadas, 11(30%) e 24(66%) foram positivas para EPEC e ETEC, respectivamente. Entre os 187 isolamentos de* ***E. coli*** *testados, 30(16%) pertenciam a sorogrupos de EPEC e 75(40%) foram positivos para ETEC. O perfil de resistência à antibióticos revelou que os isolados foram altamente sensíveis a todos os antibióticos testados.*

Palavras-chave: Escherichia coli*, EPEC,ETEC, alimentos, Brasil*

INTRODUCTION

Foods contaminated with enteropathogenic bacteria are an important factor contributing to the high incidence of diarrhoea in developing countries with poor sanitary standards. In this respect, classic enteropathogenic and enterotoxigenic strains of ***Escherichia coli*** are of particular importance since they were involved in several outbreaks of diarrhoea associated with the consumption of contaminated foods and water (MARRIER ***et al.***, 1973; TAYLOR ***et al.***, 1983; WOOD ***et al.***, 1983a).

The mechanism of EPEC pathogenesis is not well understood but it is known that the bacterium requires a plasmid-encoded adherence factor and chromosome encoded factors to cause alterations in the intestinal epithelial cells (DONNENBERG & KAPPER, 1992). Enterotoxigenic ***E. coli*** (ETEC) strains adhere to and colonize the epithelial cells of the small intestine and secrete a heat stable (ST) and/or a heat labile (LT) enterotoxin that causes diarrhoea in humans and animals (LEVINE, 1987).

Several papers have reported the ocurrence of EPEC and ETEC in raw and processed foods and in water in Brazil (REIS ***et al.***, 1982; SATO ***et al.***, 1983;

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FRANCO *et al.*, 1985; FRANCO *et al.*, 1987), however most of these studies were done on foods from the Southeast region of the country. There are no data available on the contamination of foods by enteropathogenic bacteria in Southern Brazil, which is one of the regions with high standards of living in the country. In this paper we report on the ocurrence of EPEC and ETEC in foods of animal origin currently consumed in Pelotas, a medium size city in the extreme South of Brazil.

MATERIALS AND METHODS

A total of 16 samples of pasteurized milk, 10 samples of ground beef and 10 samples of white cheese (Minas type) were examined for the presence of EPEC and ETEC. Milk and meat samples were purchased directly from food stores. Cheese samples were bought from street vendors at "feiras livres" (open markets), a common way to commercialize foods in most Brazilian cities. Food products were transported to the laboratory in an ice box and cultured within 2h of purchase.

Food samples were initially enriched by mixing 25g or ml of food with 225ml of lauril-sulfate-tryptose broth (LST, Difco) and incubating at 37°C for 24h. Isolation of ***E. coli*** from enriched cultures was carried out on eosin-methylene blue agar (EMB-Levine, Difco) incubated at 35°C for 24h. Ten colonies with morphologic characteristics of ***E. coli*** were transferred into separate tubes containing triple-sugar-iron agar slants (TSI, Difco) and LST broth with a fermentation tube, and incubated at 35°C for 24h. Colonies that yelded yellow butt and slant, did not produce H_2S in TSI agar, and produced gas in LST broth were further characterized according to EDWARDS & EWING (1972). The ***E. coli*** isolates thus obtained were stored at - 20°C in trypitic soy broth containing 15% of glycerol. The isolates were propagated in sheep blood agar for serological, toxin and antibiotic resistance tests.

The production of ST enterotoxin was detected using the suckling mouse assay (DEAN *et al.*, 1972), and the production of LT enterotoxin was detected by indirect hemagglutination of sheep red blood cells (RICCI & PESTANA DE CASTRO, 1986). For EPEC detection the isolates were screened by slide agglutination with three polyvalent O antisera (poly A: O26, O55, O111, O119; poly B: O114, O125, O142, O158; poly C: O86, O126, O127, O128; Probac do Brasil, São Paulo). Isolates that were positive in the screening were then agglutinated with monovalent O antisera. The method of BAUER *et al.*,(1966) was used to determine the resistance of the isolates to the following antimicrobials: amikacin (30ug), nalidixic acid (30ug), ampicilin (10ug), cephalothin (30ug), chloramphenicol (30ug), trimethoprim-sulfamethoxazole (1,25 + 23,5ug), streptomycin (10ug), gentamycin (10ug), nitrofurantoin (30ug), polymixin B (300U), tetracycline (30ug), and tobramycin (10ug).

RESULTS AND DISCUSSION

A total of 36 food samples were examined. The frequency of food samples contaminated with ***E. coli***, EPEC and ETEC is shown in Table 1. The high number of samples positive for ***E. coli*** found in this survey was not unexpected since the Brazilian microbiological standards for foods allows the existence of fecal coliforms even in pasteurized milk and cheese (MINISTÉRIO DA SAÚDE, 1987). However, the number of food samples positive for EPEC (30.5%) and ETEC (66.6%) was not anticipated. These pathogens occur in foods throughout the world usually at low frequencies (DANIELSSON *et al.*, 1979; WOOD *et al.*, 1983; ABBAR, 1988; AHMED *et al.*, 1988). In Brazil, both EPEC and ETEC have been detected in foods of animal origin before, though not at the levels detected in this study (REIS *et al.*, 1980; FRANCO *et al.*, 1987). The methodology used for isolation of ***E. coli***

Table 1. Prevalence of ***Escherichia coli***, EPEC, and ETEC in samples of foods consumed in Pelotas, Brazil.

	No. of samples surveyed	No. positive for *E. coli*(%)	No. positive for EPEC(%)	No. positive for ETEC(%)
Milk	16	13(81)	4(25)	10(62)
Cheese	10	8(80)	3(30)	6(60)
Ground beef	10	10(100)	4(40)	8(80)
Total	36	31(86)	11(30)	24(66)

EPEC= Classic enteropathogenic ***E. coli***
ETEC= Enterotoxigenic ***E. coli***

in this study did not include enrichment at 44.5°C to prevent loss of plasmids encoding enterotoxin production (HILL & CARLISLE, 1981). However, this fact alone cannot explain the high isolation rates obtained. More than likely our data reflect poor hygienic practices during production of these foods. Milking of cows in the Southern region is usually done manually, under unsanitary conditions, and the milk remains several hours at room temperature before being transported to a processing plant. The type of cheese sampled in this study was made with non-pasteurized milk. The meat came from animals slaughtered at small facilities lacking proper sanitation.

The number of ***E. coli*** and of EPEC and ETEC isolates obtained, EPEC serogroups, ETEC phenotypes is shown in Table 2. The high proportion of EPEC (16%) and ETEC (40%) among the ***E. coli*** strains recovered is in disagreement with the relatively low frequencies found by others in different countries. In Brazil, REIS ***et al.*** (1980) found only 1.5 % of ETEC among *E. coli* isolates while FRANCO ***et al.*** (1985) reported 0.4% of EPEC and 1.2% of ETEC. WOOD ***et al.*** (1983b), in Mexico, found 10% of ETEC in isolates from commercial foods. However, AHMED ***et al.*** (1988) reported that 33% of the ***E. coli*** strains isolated from Egyptian white cheese were enteropathogenic.

Production of enterotoxins was very common among the ***E. coli*** strains isolated from all types of foods. The majority of ETEC produced only the ST enterotoxin, although producers of the LT toxin only and of LT and ST simultaneously were also found. This result is in contrast with previous findings in Brazil where LT producer strains were prevalent (REIS ***et al.***, 1980; FRANCO ***et al.***, 1987).

The EPEC serogroups 026, 055 and 0119 isolated from cheese are relatively frequent in infants with diarrhea in some Brazilian cities (QUEIROZ ***et al.***, 1985; GOMES ***et al.***, 1991). The other serogroups detected, although frequently isolated from foods (FRANCO ***et al.***, 1985; PETRI ***et al.***, 1989), are not commonly involved in cases of infant diarrhea. EPEC isolates from serogroups 0114, 055 and 0158 gave positive results in the suckling mouse assay. It seems that positive suckling mouse assay is common among EPEC isolates from developing countries (KORNACKI & MARTH, 1982).

The ***E. coli*** strains isolated from foods were sensitive to the antimicrobial agents tested. Our results confirm previous findings in Brazilian foods and water (REIS ***et al.***, 1980; SATO ***et al.***, 1983) and suggest that, in Brazil, the use of these drugs in medical and veterinary practices did not affect the resistance pattern of ***E. coli*** found in foods so far.

The epidemiological significance of these results is not clear at present since foodborne gastroenteritis caused by EPEC or ETEC have not yet been reported in Brazil. However, studies on the prevalence and epidemiology of diarrheal diseases in adults that include identification of etiological agents are rare in Brazil. Studies with infants up to the age of 5 years show that EPEC and ETEC are among the most frequently detected enteropathogens (TRABULSI ***et al.***, 1985; GOMES ***et al.***, 1991). Moreover, foodborne gastroenteritis caused by enteropathogenic strains of ***E. coli*** is relatively common all over the world, even in industrialized countries (MARRIER ***et al.***, 1973; DANIELSSON

Table 2. Properties of ***Escherichia coli*** isolated from foods in Pelotas, Brazil.

Food source	No. of ***E. coli*** isolates[a]	No. of EPEC isolates(%)[b]	EPEC sero groups(No.)	No. of ETEC isolates(%)[b]	ETEC phenotypes(%)		
					ST	LT	STLT
Milk	59/130	9(15)	0128(1) 0114(1) 0158(7)	36(61)	64	25	11
Cheese	58/80	9(15)	0114(5) 026(1) 055(2) 0119(1)	13(22)	85	15	0
Ground beef	70/100	12(17)	0126(1) 0158(11)	26(37)	46	42	12
Total	187/310	30(16)		75(40)	61	29	9

[a] ***E. coli*** confirmed colonies/total number of colonies tested.
[b] Number of EPEC or ETEC among the ***E. coli*** isolates.

et al., 1979; TAYLOR *et al.*, 1983). Thus, it is probable that foods of animal origin play an important role in the dissemination of EPEC and ETEC in our community.

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