Isolation and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil*

Isolamento e identificação sorológica de *Escherichia coli* enteropatogênica em leite pasteurizado

Zoraide N da Silva , Adriana S da Cunha, Márcia C Lins, Letícia de AM Carneiro, Angela C de F Almeida and Mara LP Queiroz

Departamento de Patologia e Laboratórios. Universidade do Estado do Rio de Janeiro. Rio de Janeiro, RJ, Brazil

Keywords
Escherichia coli, pathogenic.# Milk, microbiology.# Food microbiology.# – *Escherichia coli*, enteropathogenic. Pasteurized milk.

Abstract

Objective
To evaluate the microbiological quality of pasteurized milk commercialized in Rio de Janeiro, Brazil, and determine serologically enteropathogenic *Escherichia coli* (EPEC) strains in *E. coli* isolates obtained from milk samples.
Methods
Ninety samples of pasteurized milk – types B and C – of three different commercial brands, purchased in supermarkets and bakeries in Rio de Janeiro, were examined. The amount of total and fecal coliform bacteria was estimated using the Most Probable Number technique. Mesophilic, psychrotrophic, and thermoduric microorganism counts were determined by the Standard Plate Count technique. Isolation and identification of *E. coli* were carried out using conventional physiological tests. Commercial antisera were used for serological characterization of EPEC.
Results
The three milk brands analyzed revealed bacterial counts above the regulated values of the Brazilian government. It was found that among 208 strains of *E. coli* isolated, 46 (22.1%) were serologically classified as EPEC. The most common EPEC serogroup was O55 (15.2%).
Conclusions
Though recent studies on virulence factors indicate that not all strains serologically classified as EPEC are able to attaching/effacing lesion, it is believed that the isolation of EPEC serogroups from pasteurized milk represent a potential risk for children, as well as an indicative of the presence of other enteropathogens.

Descritores
Escherichia coli, *patogenicidade.# Leite, microbiologia.# Microbiologia de alimentos.#* – Escherichia coli, *enteropatogênica. Leite pasteurizado.*

Resumo

Objetivos
Avaliar a qualidade microbiológica de leite pasteurizado comercializado, isolar e identificar sorologicamente cepas de Escherichia coli enteropatogênica clássica (EPEC) nesse alimento.
Métodos
Foram estudadas 90 amostras de leite pasteurizado, de três marcas comerciais diferentes, dos tipos B e C, obtidas em padarias e supermercados do Rio de Janeiro,

Correspondence to:
Mara LP Queiroz.
Disciplina de Microbiologia e Imunologia
Departamento de Patologia e Laboratórios, UERJ
Av. 28 de Setembro, 87 fundos, 3º andar
20551-030 Rio de Janeiro, RJ, Brazil
E-mail: queiroz@uerj.br

*Presented on the XIX "Congresso Brasileiro de Microbiologia", Rio de Janeiro (Brasil), 1997, and in the 98th Meeting of the American Society for Microbiology, Atlanta, Georgia (USA), 1998.
Submitted on 29/6/2000. Reviewed on 10/1/2001. Approved on 2/3/2001.

Rev Saúde Pública 2001;35(4):375-9
www.fsp.usp.br/rsp

RJ. A contagem de coliformes totais e fecais foi estimada aplicando-se a técnica do número mais provável. A quantificação de microorganismos mesófilos, psicrotróficos e termodúricos foi determinada pela técnica da contagem padrão em placa. A identificação fisiológica de E. coli foi realizada por metodologia convencional e a identificação sorológica de EPEC, com a utilização de soros comerciais.

Resultados

As três marcas de leite pasteurizado apresentaram contagens bacterianas acima do padrão permitido pela legislação brasileira. Foram isoladas 208 cepas de E. coli, entre as quais 46 (22,1%) foram sorogrupadas como EPEC. O sorogrupo mais freqüente foi o O55 (15,2%).

Conclusões

Apesar de estudos recentes sobre fatores de virulência indicarem que nem todas as cepas sorogrupadas como EPEC são capazes de causar a lesão A/E (attaching/effacing), considerou-se que o isolamento de sorogrupos de EPEC, nesse alimento, representa risco potencial para crianças e, também, possível meio de veiculação de outros enteropatógenos.

INTRODUCTION

Pasteurized milk in Brazil is classified in the types A, B and C, according to their fat content and to the Brazilian standards of microbiological counts.[12] In this study, pasteurized milk samples of the types B and C of three commercial brands were analyzed. The maximum bacterial counts allowed by law are: type B pasteurized milk – mesophilic microorganisms: 8.0×10^4 CFU/ml; psychrotrophic and thermoduric microorganisms: 8.0×10^3 CFU/ml; total coliforms: 4 MPN/ml, fecal coliforms: 1 MPN/ml; type C pasteurized milk –mesophilic microorganisms: 3.0×10^5 CFU/ml; psychrotrophic and thermoduric microorganisms: 3.0×10^4 CFU/ml; total coliforms: 10 MPN/ml and fecal coliforms: 2 MPN/ml. Mesophilic, psychrotrophic, and thermoduric microorganisms count is an indicator of potential spoilage and a means of measuring food quality and safety. Coliform bacteria count in milk and dairy products is an indicator of the sanitary conditions or practices during production, processing and storage of the product.[2,12] Recovery and counting of *Escherichia coli* is used as an index of recent fecal contamination and suggests that other microorganisms of fecal origin, including pathogens, might be present.[15]

E. coli is responsible for several outbreaks of diarrhea in children and adults after ingestion of contaminated water and food, including milk and dairy products.[7]

Enteropathogenic *E. coli* (EPEC) have been implicated in food and waterborne human illnesses, especially as an important agent of infantile diarrhea in developing countries.[14,15] In Brazil, EPEC strains are recovered from 30% or more of the diarrhea cases in infants of low social economic status living in cities.[10]

In the present study the microbiological quality of pasteurized milk commercialized in Rio de Janeiro, Brazil, was investigated through mesophilic, psychrotrophic and thermoduric microorganism plate counting, counts of total and fecal coliform bacteria and serological identification of EPEC strains among *E. coli* isolates.

METHODS

Pasteurized milk samples of three different commercial brands were purchased in supermarkets and bakeries in Rio de Janeiro between June 1995 and February 1996. Food samples were acquired in regular consumer packages and immediately transported in ice bath to the laboratory, where they were processed within 2 hours. A total of 90 samples (1.000 ml each) of pasteurized milk of type B (15 samples of each brand) and type C (15 samples of each brand) were examined.

Standard plate and total and fecal coliform counts were performed according to the standard method for dairy products.[2]

Isolation and identification of *E. coli* were carried out using conventional physiological tests.[11,16] Serological characterization of EPEC was carried out using slide agglutination method with polyvalent and monovalent antisera. The isolates were first tested with OK polivalent antisera and then with O monovalent antisera for serogroups O26, O55, O111, O119, O114, O125, O142, O158, O86, O126, O127, and O128. The strains belonging to the same serogroups and isolated from the same samples were reported only once. Positive control strains were included in each experiment run.

RESULTS

Ninety milk samples of three commercial brands (I, II and III) that were in accordance with the Health

Table 1 - Standard plate counting of mesophilic, psychrotrophic and thermoduric microorganisms in pasteurized milk samples in Rio de Janeiro, Brazil.*

Brands							I					
	Type B						Type C					
	M		P		T		M		P		T	
Counts/ml	N	%	N	%	N	%	N	%	N	%	N	%
0 _/ 10			11	73.3					11	73.3		
10 _/ 10^2												
10^2 _/ 10^3			2	13.3					2	13.3	1	6.6
10^3 _/ 10^4	9	60.0	2	13.3	14	93.3	7	46.6	2	13.3	11	73.3
10^4 _/ 10^5	6	40.0			1	6.6	8	53.3			3	20.0
10^5 _/ 10^6												
10^6 _/6,5x10^6												
≥6,5x10^6												
Total	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0

Brands							II					
	Type B						Type C					
	M		P		T		M		P		T	
Counts/ml	N	%	N	%	N	%	N	%	N	%	N	%
0 _/ 10			6	40.0					5	33.3		
10 _/ 10^2												
10^2 _/ 10^3			2	13.3	2	13.3			3	20.0	1	6.6
10^3 _/ 10^4	5	33.3	1	6.6	8	53.3	5	33.3	2	13.3	10	66.6
10^4 _/ 10^5	9	60.0	4	26.6	4	26.6	7	46.6	1	6.6	4	26.6
10^5 _/ 10^6					1	6.6	1	6.6	1	6.6		
10^6 _/6,5x10^6	1	6.6					1	6.6				
≥6,5x10^6			2	13.3			1	6.6	3	20.0		
Total	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0

Brands							III					
	Type B						Type C					
	M		P		T		M		P		T	
Counts/ml	N	%	N	%	N	%	N	%	N	%	N	%
0 _/ 10			7	46.6								
10 _/ 10^2												
10^2 _/ 10^3			3	20.0	1	6.6			3	20.0		
10^3 _/ 10^4	6	40.0	2	13.3	12	80.0			8	53.3	2	13.3
10^4 _/ 10^5	8	53.3	3	20.0	2	13.3	9	60.0	3	20.0	11	73.3
0^5 _/ 10^6	1	6.6					5	33.3			2	13.3
10^6 _/6,5x10^6							1	6.6				
=6,5x10^6									1	6.6		
Total	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0

M - Mesophilic microorganisms
P - Psychrotrophic microorganisms
T - Thermoduric microorganisms

*Brazilian legislation - Maximum counting permitted

Type B	Type C
M - 8,0 x 10^4/ml	3,0 x 10^5/ml
P - 8,0 x 10^3/ml	3,0 x 10^4/ml
T - 8,0 x 10^3/ml	3,0 x 10^4/ml

Ministry's National Department of Sanitation Surveillance (Secretaria Nacional de Vigilância Sanitária do Ministério da Saúde – SNVS)[12] were analyzed.

Standard plate counts of mesophilic, psychrotrophic, and thermoduric microorganisms in pasteurized milk samples are summarized in Table 1. Type C milk samples of brand I showed microorganism counting within the SNVS maximum range, while type B milk samples of the same brand presented psychrotrophic and thermoduric counting above the SNVS maximum value, 13.3% and 5.6%, respectively. Types B and C milk samples of brands II and III had counting above the SNVS maximum value to all indicators microorganisms. Total and fecal coliform counts in milk samples are summarized in Figure. The total coliform MPN for types B and C milk samples of brand I were 33.3% and 20% of the samples respectively, above the SNVS maximum value, and fecal coliform MPN were 26% and 20%, respectively. Total coliform MPN were also above the SNVS maximum value for types B and C milk samples of brand II, i.e., 40% and 60%, respectively. For the same samples, fecal coliform MPN levels found were 40% and 53.3%, respectively. Types B and C milk samples of brand III presented a total (73% and 86.6%, respectively) and fecal (53.3% and 60%, respectively) coliform MPN above the regulated levels.

A total of 208 *E. coli* colonies were isolated from 41.1% of the 90 samples, and 46 (22.1%) strains were serologically identified as EPEC (Table 2). EPEC serogroups were isolated from all milk samples of the three brands. There were found 4 (8.6%) in brand I, 31 (67.3%) in brand II, and 11 (23.9%) in brand III. The most common EPEC serogroup found was O55 (15.2%). Type C milk samples showed

Rev Saúde Pública 2001;35(4):375-9
www.fsp.usp.br/rsp

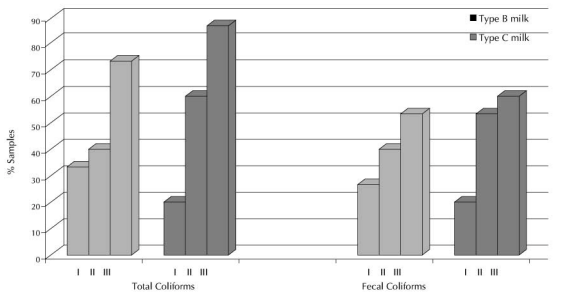

Figure - Enumeration of total and fecal coliforms above the levels permitted by SNVS[12] (1997) in pasteurized milk samples.

the highest rate of EPEC serogroups (58.5%). Thirty-five (76.08%) of these EPEC strains were isolated from 10 milk samples. EPEC strains were predominantly found in milk samples of brand II (Table 3).

DISCUSSION

The microbiological analysis of milk samples showed a significant deficiency in hygienic and sanitary quality conditions (Table 1 and Figure). Other studies on milk and/or dairy products showed similar results both in Brazil[3,6,9,17] and other countries.[1,5,8] It was observed that types B and C milk of brand III had a high coliform level (Figure) although EPEC strains prevailed in samples of brand II (Table 3). EPEC strains were also found in samples of brand I (Table 3), which showed to have the closest counts above the SNVS maximum value (Table 1 and Figure). It was also found that sample 2 of brand II and sample 3 of brand III presented at the same time microorganisms counting above the SNVS[12] (1997) maximum value, and EPEC serogroups, indicating a high contamination level of this products.

Forty-six (22,1%) out of 208 *E. coli* colonies isolated were positive for EPEC serogroups. Similar results were obtained by Tibana[17] (1981) when it was found 25.83% of positive EPEC serogroups among 78 of *E. coli* strains isolated from milk samples in Rio de Janeiro. However, in the city of Ouro Preto, Nascimento et al[13] (1988) isolated 605 strains of *E. coli* from one type of soft cheese and 9.8% were positive for EPEC serogroups. Frank & Marth[7] (1978), in the United States, did not detected EPEC serogroups in soft and semi-soft cheese samples. Simango[15] (1995) isolated 126 strains of *E. coli* from several foods and drinks consumed in a rural community, in Harare, Zimbabwe, and 7.5% of them were EPEC serogroups.

In the present investigation all EPEC serogroups were found in the milk samples tested and O55 was the most frequent one (15.2%). The O55 EPEC serogroup is one of most prevalent in children diarrhea in Brazil.[18]

Table 2 - Distribution of enteropatogenic *E. coli* serogroups isolated from pasteurized milk samples.

Serogroups		A*				B						C		Total
Brands / Types		O26**	O55	O111	O119	O114	O125	O142	O158	O86	O126	O127	O128	
I	B	1(2.1)***											1(2.1)	2(4.3)
	C					1(2.1)	1(2.1)							2(4.3)
II	B	1(2.1)	1(2.1)	1(2.1)	1(2.1)	2(4.3)			1(2.1)	1(2.1)		2(4.3)		10(21.7)
	C	2(4.3)	5(10.8)		1(2.1)		3(6.5)	1(2.1)	1(2.1)	2(4.3)	1(2.1)	3(6.5)	2(4.3)	21(45.6)
III	B		1(2.1)	1(2.1)			1(2.1)	1(2.1)	1(2.1)	2(4.3)				7(15.2)
	C	1(2.1)		1(2.1)		1(2.1)			1(2.1)					4(8.6)
Total		5(10.8)	7(15.2)	3(6.5)	2(4.3)	4(8.6)	5(10.8)	2(4.3)	4(8.6)	5(10.8)	1(2.1)	5(10.8)	3(6.5)	46(100.0)

*polivalent antisera **monovalent antisera ***per cent (percentage)

Table 3 - Distribution of different enteropatogenic *E. coli* serogroups isolated from pasteurized milk samples.

Brands	Types	Samples	Serogroups
I	B	1	0128
		2	026
	C	3	0114, 0125
II	B	4	026, 055, 0111, 0119, 0114, 0158, 086, 0127
		5	0114
		6	0127
	C	7	0128
		8	055
		9	055, 0125, 086
		10	026, 0119, 0142, 086
		11	055, 0125, 0127
		12	055, 0127
		13	026, 055, 0125, 0158, 0126, 0127, 0128
III	B	14	0158
		15	055, 0125
		16	086
		17	0111, 0142
		18	086
	C	19	0158
		20	0114
		21	026, 0111

Recent studies based on virulence properties rather than serological characteristics showed that EPEC serogroups are heterogeneous and although most of strains belonging to these serogroups are actually EPEC, but many of them are not.[4] Given that types B and C pasteurized milk are consumed mostly by the poor population in Rio de Janeiro, as well as in many Brazilian cities, due to its lower cost and nutritional value, the results showed that several EPEC serogroups can be easily isolated from pasteurized milk in the community. It is worth to emphasize the importance of surveillance in manufacturing pasteurized milk, since the presence of EPEC possibly indicates enteropathogens contamination.

REFERENCES

1. Abbar FM. Incidence of. fecal coliforms and serovars of enteropathogenic *Escherichia coli* in naturally contamined cheese. *J Food Prot* 1988;51:384-5.

2. American Public Health Association. *Standard methods for the examination of dairy products*. 16th ed. Washington (DC); 1992.

3. Baruffaldi R, Penna TCV, Machoshvilli IA, Abe LE. Condições higiênico-sanitárias do leite pasteurizado tipo "B" vendido na cidade de São Paulo, SP (Brasil), no período de fevereiro a agosto de 1982. *Rev Saúde Pública* 1984;18:367-74.

4. Campos LC. Conventional methods for the diagnosis of enteropathogenic *Escherichia coli* infections. *Rev Microbiol* 1996;27Suppl 1:50-3.

5. Crielly EM, Logan NA, Anderton A. Studies on the *Bacillus* of milk and milk products. *J Appl Bacteriol* 1994;77:256-63.

6. Falcão DP, Salgado Filho G, Nishida NK, Borges SR. Exame microbiológico de sorvetes não pasteurizados. *Rev Saúde Pública* 1983;17:2-8.

7. Feresu S, Nyati H. Fate of pathogenic and non - pathogenic *Escherichia coli* strains in two fermented milk products. *J Appl Bacteriol* 1990;69:814-21.

8. Frank JF, Marth EH. Survey of soft and semi soft cheese for presence of fecal coliforms and serotypes of enteropathogenic *Escherichia coli*. *J Food Prot* 1978;41:198-200.

9. Freitas AC, Nunes MP, Milhomem AM, Ricciardi ID. Occurrence and characterization of *Aeromonas* species in pasteurized milk and white cheese in Rio de Janeiro, Brazil. *J Food Prot* 1993;56:62-5.

10. Gomes TAT, Vieira MAM, Wachsmuth IK, Blake PA, Trabulsi LR. Serotype specific prevalence of *Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. *J Infect Dis* 1989;160:131-5.

11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Jr WC. *Color atlas and textbook of diagnostic microbiology*. 5th ed. Philadelphia: Lippincott - Raven Publishers; 1997.

12. Ministério da Saúde. Secretaria Nacional de Vigilância Sanitária (SNVS). Portaria nº 451 de 19 de setembro de 1997 - Regulamento Técnico. Princípios gerais para o estabelecimento de critérios e padrões microbiológicos para alimentos. *Diário Oficial da União*, Brasília (DF): 22 set. 1997. Seção 1. p. 21005.

13. Nascimento D, Sabioni JG, Pimenta N. Freqüência de *Escherichia coli* enteropatogênica clássica (EPEC) e enteroinvasora (EIEC) em queijo Minas Frescal da cidade de Ouro Preto, MG. *Rev Microbiol* 1988;19:258-61.

14. Ramos SRTS. Risk factors for EPEC infection. *Rev Microbiol* 1996;27Suppl 1:34-9.

15. Simango C. Isolation of *Escherichia coli* in foods. *Cent Afrc J Med* 1995;41:181-5.

16. Suassuna I, Suassuna IR. Duplo açúcar uréia (DAU). Um meio de triagem para enterobactérias. Nota técnica. *Rev Bras Patol Clin* 1978;14:201-3.

17. Tibana A. *Estudo bacteriológico do leite comercializado no Rio de Janeiro* [Tese]. Rio de Janeiro (RJ): Universidade Federal do Rio de Janeiro; 1981. p. 123.

18. Trabulsi LR, Campos LC, Whittam TS, Gomes TAT, Rodrigues J, Gonçalves AG. Traditional and non - traditional enteropathogenic *Escherichia coli* serogroups. *Rev Microbiol* 1996;27Suppl 1:1-6.