Phenotypic characteristics, virulence profile and genetic relatedness of O157 Shiga toxin-producing *Escherichia coli* isolated in Brazil and other Latin American countries

Flávia C. Bastos[1], Tânia Mara I. Vaz[1,2], Kinue Irino[2] & Beatriz Ernestina C. Guth[1]

[1]Universidade Federal de São Paulo–Escola Paulista de Medicina, São Paulo, Brazil; and [2]Instituto Adolfo Lutz, São Paulo, Brazil

Correspondence: Beatriz E.C. Guth, Disciplina de Microbiologia, Universidade Federal de São Paulo, Rua Botucatu 862/3 andar, CEP 04023-062, São Paulo, SP, Brazil. Tel.: +55 11 5576 4537; fax: 55 11 5572 4711; e-mail: becguth@ecb.epm.br

Received 5 July 2006; revised 30 August 2006; accepted 7 September 2006.
First published online 10 October 2006.

DOI:10.1111/j.1574-6968.2006.00472.x

Editor: Rob Delahay

Keywords
shiga toxin; *Escherichia coli*; O157:H7; virulence profile; genetic relatedness; Latin America.

Abstract

Thirty-eight Shiga toxin-producing *Escherichia coli* (STEC) O157:H7/H$^-$ strains isolated from human infections, cattle and foods in Brazil and in some other Latin American countries were compared with regard to several phenotypic and genotypic characteristics. The genetic relatedness of the strains was also determined by pulsed-field gel electrophoresis (PFGE). Similar biochemical behaviour was identified, regardless of the origin and country of the strains. Most (89.5%) strains were sensitive to the antimicrobial agents tested, but resistance to at least one drug was observed among bovine strains. Although a diversity of *stx* genotypes was identified, most (77.8%) of the human strains harboured *stx*$_2$ or *stx*$_2$*stx*$_{2c(2vha)}$, whereas *stx*$_{2c(2vha)}$ prevailed (64.2%) among strains isolated from cattle. *stx*$_1$ and *stx*$_1$*stx*$_{2c(2vha)}$ were the genotypes identified less frequently, and occurred exclusively among strains isolated from food and cattle, respectively. Despite differences in the *stx* genotypes, all strains carried *eae*-γ, *efa*1, *ehx*, *iha*, *lpf*$_{O157}$ and *tox*B sequences. Many closely related subgroups (more than 80% of similarity) were identified by PFGE, and the presence of a particular O157:H7 STEC clone more related to human infections in Brazil, as well as a common origin for some strains isolated from different sources and countries in Latin America can be suggested.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains are foodborne pathogens that have emerged worldwide, associated with a broad spectrum of human diseases, including diarrhoea, haemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS) (Griffin & Tauxe, 1991). STEC strains belong to a large number of O:H types, but O157:H7 was the first associated with bloody diarrhea, and is by far the most prevalent serotype associated with large outbreaks and sporadic cases of HC and HUS in several countries (Nataro & Kaper, 1998). Domestic ruminants, especially cattle, have been implicated as the principal reservoir. Therefore, STEC strains are mainly transmitted to humans through the consumption of undercooked meat, unpasteurized dairy products and by any other food contaminated with bovine feces (Meng & Doyle, 1998). However, transmission by water and from person-to-person, although less common, has also been documented (Nataro & Kaper, 1998).

Recognition of *E. coli* O157:H7 has initially been facilitated by its inability to ferment sorbitol after overnight incubation (March & Ratnam, 1986), but sorbitol-fermenting nonmotile O157 STEC strains (O157:H$^-$) have also emerged as important causes of human diseases in several European countries (Karch & Bielaszewska, 2001).

Although the most important STEC virulence characteristic is the production of one or more types of Stx toxins (Stx$_1$, Stx$_2$ or variants), several other virulence factors may contribute to the pathogenicity of these bacteria. A protein called intimin, encoded by the *eae* gene, which is located within the locus for the enterocyte effacement (LEE) pathogenicity island, is responsible for the intimate attachment to intestinal cells and causes attaching-and-effacing lesions in the intestinal mucosa (McDaniel & Kaper, 1997). Different types of intimin have already been described based on the heterogeneity of its aminoacid sequence in the C-terminal end, but a correlation between intimin types and STEC serotypes has been observed (Adu-Bobie *et al.*, 1998). Moreover, a plasmid-encoded enterohemolysin (Ehx), which acts as a pore-forming cytolysin on eukaryotic cells, may play a role in pathogenesis (Nataro & Kaper, 1998).

The search for additional virulence markers in these pathogens revealed several other proteins that were proposed to be novel adhesion factors, such as a protein called

ToxB that is required for full expression of adherence of O157:H7 strain Sakai, (Tatsuno *et al.*, 2001); Iha, a protein that confers adherence similar to *Vibrio cholerae* IrgA (Tarr *et al.*, 2000); enterohemorrhagic *E. coli* factor for adherence, called Efa1 (Nicholls *et al.*, 2000); and Saa, an autoagglutinating adhesin identified in LEE-negative strains (Paton *et al.*, 2001), and a long polar fimbriae (LPF) closely related to LPF of *Salmonella enterica* serovar Typhimurium (Doughty *et al.*, 2002; Torres *et al.*, 2002).

Currently, molecular methods are used for epidemiological investigation of outbreaks and for control and monitoring of the spread of potential pathogens. Pulsed-field gel electrophoresis (PFGE) is the most common molecular method used in the subtyping of STEC strains, and due to its high power of discrimination and reproducibility has proved to be very important for epidemiologic typing of O157 STEC strains all over the world (Izumiya *et al.*, 1997; Breuer *et al.*, 2001; Giammanco *et al.*, 2002).

Most outbreaks and sporadic cases of HC and HUS caused by O157 STEC have been reported from industrialized nations of the northern hemisphere, but its incidence in countries of the southern hemisphere, such as Argentina, Chile and Australia, has also been described (Nataro & Kaper, 1998).

In Brazil, O157:H7 STEC was first identified in cattle from Rio de Janeiro (Cerqueira *et al.*, 1999), and only in recent years has its occurrence in human diseases and in cattle from different Brazilian regions been reported (Irino *et al.*, 2002, 2005; Farah *et al.*, 2003; Gonzalez, 2003). Thus, there are no other reports analyzing the phenotypic and genotypic characteristics of O157 STEC strains isolated in Brazil from diverse sources and regions. Moreover, comparisons with several O157 STEC strains that had been isolated in other Latin American countries were also carried out.

Materials and methods

Bacterial strains

A total of 38 O157 STEC strains isolated from human infections ($n = 18$), cattle ($n = 14$), food ($n = 5$) and water ($n = 1$) were studied. The 18 Brazilian strains were isolated during different surveys conducted in our country (Cerqueira *et al.*, 1999; Irino *et al.*, 2002, 2005; Farah *et al.*, 2003; Guth *et al.*, 2003; unpublished data), and five of them, isolated from cattle in Rio de Janeiro, were kindly supplied by Dr J.R.C. Ramos, Universidade Estadual do Rio de Janeiro, Brazil (Gonzalez, 2003). Sixteen strains isolated in Argentina ($n = 8$), Chile ($n = 3$), Colombia ($n = 1$) and Uruguay ($n = 4$) were kindly provided by M. Rivas (Servicio Fisiopatogenia, Instituto Nacional de Enfermedades Infecciosas Dr Carlos G. Malbrán, Buenos Aires, Argentina), V. Prado (Instituto de Ciências Biomédicas, Universidad de Chile), D. Urbina (Laboratório de Posgrado de Microbiologia, Facultad de Medicina, Cartajena, Colômbia) and F. Schelotto (Departamento de Bacteriologia y Virologia, Facultad de Medicina, Montevideo, Uruguay), respectively. The isolation and identification of most of these strains had been previously described (Chinen *et al.*, 2001, 2003; Urbina *et al.*, 2003; Gadea *et al.*, 2004; Toma *et al.*, 2004). Two strains from the United States of America were kindly provided by Dr L.R. Trabulsi (Instituto Butantan, São Paulo, Brazil), and *E. coli* strains EDL932 and G5244 were also included as controls (Centers for Disease Control and Prevention reference strains).

Phenotypic characterization of the strains

The biochemical properties of the strains were determined by standard methods (Ewing, 1986). Fermentation of sorbitol within 24 h was tested as described previously (Guth *et al.*, 2003). The β-D-glucoronidase activity was investigated on fluorocult laurylsulfat–bouillon broth (Merck, Darmstadt, Germany) added with 2% of agar (Nagano *et al.*, 2002). Enterohemolysin production (Ehx) and expression of cytotoxicity activity on Vero cells were assayed as described by Beutin *et al.* (1989) and Gentry & Dalrymple (1980), respectively. The antimicrobial susceptibility to ampicillin, cefoxitin, ciprofloxacin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamide, tetracycline and trimethoprim was determined by the standard disk diffusion method (NCCLS, 2000).

Virulence profile

The primers and conditions used in the PCR assays for identification of gene sequences related to *stx*$_1$ and *stx*$_2$ (Pollard *et al.*, 1990), *eae* (Karch *et al.*, 1999), *eae*γ(Adu-Bobie *et al.*, 1998), *ehx*A (Schmidt *et al.*, 1994), *efa*1 (Nicholls *et al.*, 2000), *iha* (Tarr *et al.*, 2000), *saa* (Paton *et al.*, 2001), *lpf*$_{O113}$ (Doughty *et al.*, 2002), *lpf*$_{O157}$ (Torres *et al.*, 2002) and *tox*B (Tatsuno *et al.*, 2001) were as those reported. The differentiation of Stx$_1$ and Stx$_2$ variants was carried out as previously described (Cergole-Novella *et al.*, 2006). Nonmotile (H^-) strains were investigated for the flagellar antigen H7 (*fli*C) genes by RFLP-PCR (Machado *et al.*, 2000) using a motile O157:H7 strain as a standard. *Escherichia coli* strain EDL932 was used as a positive control for *stx*$_1$, *stx*$_2$, *fli*C, *eae*, intimin γ, *ehx*, *iha*, *efa*1, *lpf*$_{O157}$ and *tox*B; *E. coli* O113:H21 as a positive control for *saa* and *lpf*$_{O113}$, and *E. coli* DH5α as a negative control.

Genetic relatedness

PFGE was used to analyze the genetic relatedness of the strains studied. The method described by Gautom (1997)

was followed with some modifications. Cleavage of the agarose-embedded DNA was achieved with XbaI (Invitrogen) at 37 °C for 16 h, and pulse and run times were 5–50 s for 18 h and 50 min, performed in a CHEF-DR III System (Bio-Rad) apparatus. The PFGE patterns were analyzed using the GelCompar II program, and similarity between PFGE patterns was evaluated using the Dice coefficent similarity (tolerance, 1%).

Results

The phenotypic and genotypic characteristics identified among the O157 STEC strains are shown in Table 1. A similar biochemical behavior was observed in most of the strains, regardless of their source and country. β-D-Glucoronidase activity was not observed among the strains analyzed, but enterohemolysin production was detected in all of

Table 1. Phenotypic and genotypic characteristics among O157 STEC strains isolated in Brazil and other Latin American countries

Country*	Strain	Origin†	H type	Urease	LCD‡	Sorbitol	*stx* genotype§	Virulence profile *eae-γ, ehx,efa1,iha,lpf*$_{O157}$*,toxB*	Antimicrobial resistance¶
ARG	179/03	HUS	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	724/01	HUS	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	STR
	790/01	D	H7	+	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	145/98	C	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	146N/99	C	H7	–	+	–	*stx*$_1$*stx*$_{2c}$	+	STR
	438/99	C	H7	–	+	–	*stx*$_{2c}$	+	–
	109/96	F	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	148/97	F	H7	–	+	–	*stx*$_2$	+	–
BR	EC156/90	D	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	EC255/03	BD	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	EC622/03	BD	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	337/01	BD	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	385/01	BD	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	143/05	HUS	H7	–	+	–	*stx*$_2$	+	–
	B1/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
	B18/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
	EC393/01	C	H7	–	+	–	*stx*$_{2c}$	+	TRI
	GC148	C	H7	–	+	+	*stx*$_2$	+	–
	EC339/02	C	H7	–	+	–	*stx*$_2$*stx*$_{2c}$	+	–
	YB20	C	H$^-$	–	+	–	*stx*$_1$*stx*$_{2c}$	+	STR, SUL
	EC102/97	F‖	H7	–	+	–	*stx*$_2$	+	–
	581/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
	691/1	C	H7	–	–	–	*stx*$_{2c}$	+	–
	1728/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
	1770/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
	2004/1	C	H7	–	+	–	*stx*$_{2c}$	+	–
CH	GB2001	HC	H7	+	+	–	*stx*$_2$	+	–
	HUS 28	HUS	H7	–	+	–	*stx*$_2$	+	–
	HUS 32	HUS	H7	–	+	–	*stx*$_2$	+	–
COL	558	BD	H$^-$	–	+	–	*stx*$_1$*stx*$_2$	+	–
URU	C195	F	H7	–	+	–	*stx*$_1$	+	–
	C363	F	H7	–	+	–	*stx*$_1$*stx*$_2$	+	–
	M1.Col5	F	H7	–	+	–	*stx*$_1$*stx*$_2$	+	–
	HUS 56	HUS	H7	+	+	–	*stx*$_2$	+	–
USA	EDL932	HUS	H7	–	+	–	*stx*$_1$*stx*$_2$	+	–
	G5244	HUS	H7	–	+	–	*stx*$_2$	+	–
	O157/14	HUS	H$^-$	–	+	–	*stx*$_{2c}$	+	–
	O157/16	HUS	H7	–	+	–	*stx*$_{2c}$	+	–

*ARG, Argentina; BR, Brazil; CH, Chile; COL, Colombia; URU, Uruguay; USA, United States of America.
†D, human diarrhea; BD, bloody diarrhea; HC, hemorrhagic colitis; HUS, hemolytic uremic syndrome; C, cattle; F, food (ground meat).
‡LCD, lysine descarboxylase.
§*stx*$_{2c}$ corresponds to *stx*$_{2-vha}$ subtype.
¶ –, sensitive to all 10 antimicrobials tested; STR, streptomycin; TRI, trimethoprim; SUL, sulfonamide.
‖Strain isolated from water.

them. Most of the O157 STEC strains neither fermented sorbitol in 24 h (97.4%) nor presented urease activity (92%). Moreover, except for one strain isolated from cattle in Brazil, all the others were able to decarboxylate lysine. Three strains were nonmotile, but an *fli*C gene coding for flagellar type H7 was detected in all of them. Six O157 STEC strains did not express cytotoxic activity, and all of them were isolated from cattle in Brazil. Sensitivity to all the antimicrobial agents tested was observed in 34 (89.5%) of the O157 STEC strains. Considering the origin of the strains, susceptibility was found in 94% (17/18), 78.5% (11/14) and 100% of human, bovine and food strains, respectively. Two resistant strains were isolated from cattle in Brazil, and the other strains were isolated in Argentina from human and bovine. The antimicrobials, in which resistance was observed, were streptomycin, trimethoprim and sulfonamide.

Thirthy-seven of the 38 (97.4%) O157 strains harbored stx_2 toxin genes, either alone or combined with some stx_2 variant or with stx_1. The *eae* gene encoding intimin type γ was identified in all O157 STEC strains that were also positive for *efa*1, *ehx*, *iha*, lpf_{O157} and *tox*B genes, regardless of their source and country (Table 1). Restriction fragment analysis of *stx*-specific PCR products showed that none of the strains presented stx_{1c}, whereas all the strains, in which stx_{2c} was identified, presented only the 2vha subtype. The frequency and distribution of the *stx* genotypes identified in the O157 STEC strains isolated from different origins are presented in Table 2. Among the 18 human strains studied, 14 (77.8%) carried stx_2 or stx_2stx_{2c}, whereas 9 of 14 (64.2%) strains from cattle carried only the stx_{2c} genotype, and the stx_2 and stx_1stx_2 genotypes occurred at higher frequencies (33.3% each) among the strains isolated from ground meat.

Thirty-two different patterns were obtained by XbaI-PFGE, but most of the strains were grouped in the same cluster (A), which presented six subgroups (A1–A6) with 75–100% similarity (Fig. 1). Only three strains from human sources isolated in Brazil, Chile and Uruguay, and one cattle strain isolated in Brazil were more distantly related to the others (60% of similarity). Among the O157 STEC strains that showed 100% similarity, some were isolated from different sources and different countries. Strains C195, M1.col5 and C363, isolated from ground meat in Uruguay, showed the same PFGE pattern as strains 558, 790/01 and 724/01, isolated from human sources in Colombia and Argentina, respectively. Identical PFGE patterns were also identified among Brazilian strains isolated from cattle (strains 1728 and 1770) and from human infections (strains 385, 337 and 143/05). Moreover, it was interesting to observe that a Brazilian strain from human origin (156/90) was more closely related to O157 STEC strains from other Latin American countries, corresponding to a subgroup with more than 80% similarity (Fig. 1).

Discussion

Infections with O157:H7 STEC strains are a major public health concern, and studies on the characteristics of strains isolated from humans and the environment have helped to understand their epidemiology and ensure the establishment of efficient control measures.

In Brazil, such studies have not been carried out before because despite the first isolation of O157:H7 STEC from cattle in the late 1990s (Cerqueira *et al.*, 1999); only in recent years has it been identified as the cause of bloody diarrhea (Irino *et al.*, 2002) and HUS (Guth BEC, pers. commun.), as well as being isolated from bovines in different regions. Thus, in contrast to studies conducted so far the phenotypic and genotypic characteristics of O157:H7 STEC strains isolated in different Brazilian regions and from diverse sources were analyzed, and also compared for the first time with O157:H7 STEC strains isolated in other Latin American countries. Moreover, the genetic relatedness of the strains was also determined by PFGE.

Several similarities related to biochemical properties, susceptibility to antimicrobial agents and virulence profile were identified among the O157:H7 STEC strains studied. However, some differences especially related to *stx* genotypes were also identified in strains isolated in different countries and sources.

Nakano *et al.* (2001) suggested that the presence of urease gene (*ure*C) could be a useful genetic marker for the detection of O26, O111 and O157 STEC strains, which belong to the enterohemorrhagic *E. coli* (EHEC) group. Friedrich *et al.* (2005) also observed that except for one strain, all the O157:H7 EHEC strains they had analyzed have the *ure* gene. However, in both of these studies, urease

Table 2. Frequency and distribution of *stx* genotypes according to the origin of the O157 STEC strains

		No (%) of strains with					
Origin	Total No	stx_1	stx_2	stx_1stx_2	$stx_{2c(2vha)}$	$stx_1stx_{2c(2vha)}$	$stx_2stx_{2c(2vha)}$
Human	18	–	6 (33.3)	2 (11.2)	2 (11.2)	–	8 (44.5)
Cattle	14	–	1 (7.2)	–	9 (64.2)	2 (14.3)	2 (14.3)
Food	6	1 (16.7)	2 (33.3)	2 (33.3)	–	–	1 (16.7)
Total	38	1 (2.6)	9 (23.7)	4 (10.5)	11 (28.9)	2 (5.3)	11 (28.9)

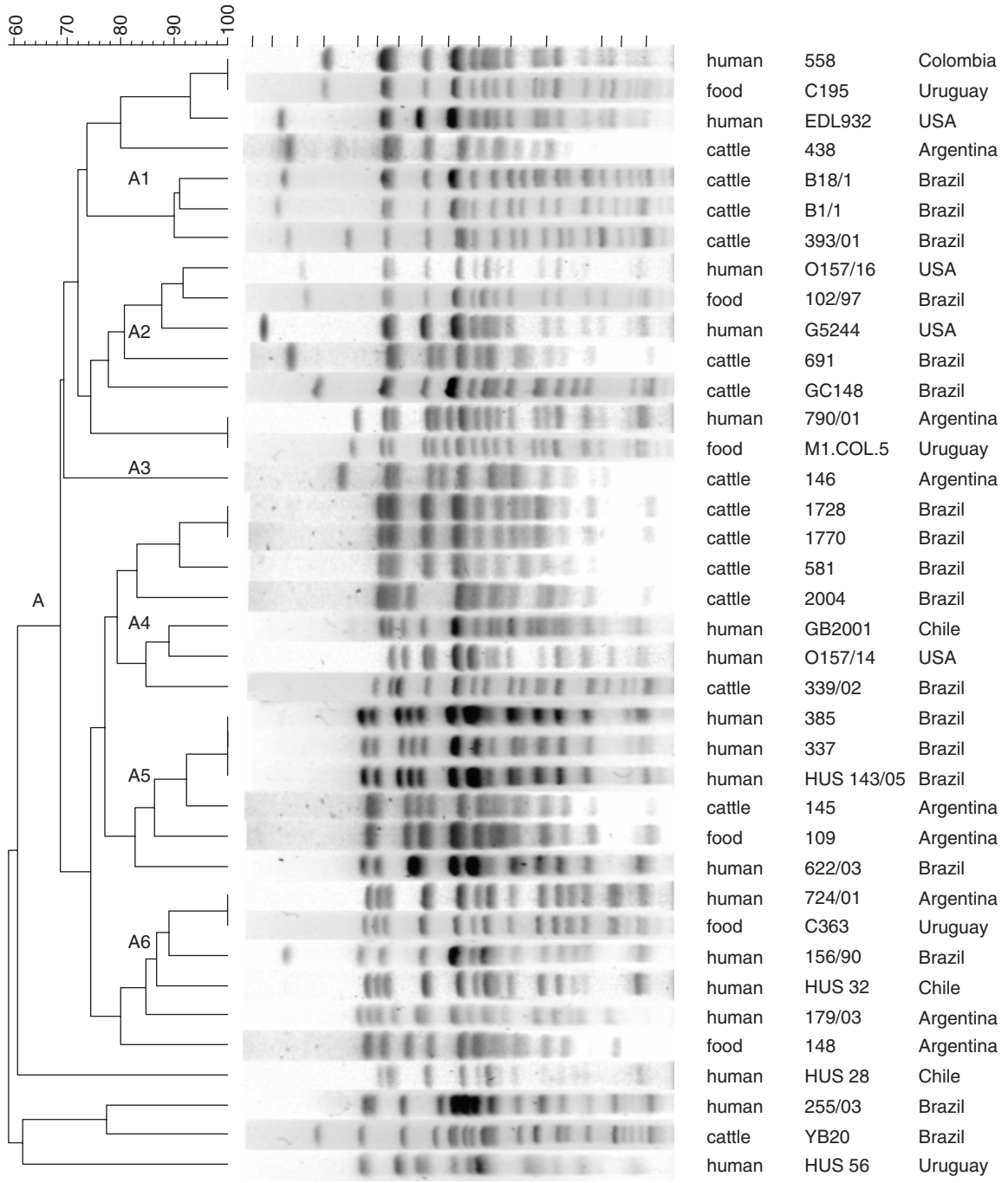

Fig. 1. Dendrogram outlining the relationship of O157:H7 STEC strains isolated in Brazil and other Latin American countries.

production could not be detected in most of the strains, despite their possession of the *ure*C gene. In the present study, the urease gene was not searched for, and although all strains isolated in Brazil and most of the O157 strains studied from other Latin American countries did not present urease activity, this property was identified in three human strains, each one isolated in Argentina, Chile and Uruguay. These results are similar to those previously reported (Nakano *et al.*, 2001; Friedrich *et al.*, 2005), where only a few O157 STEC strains showed urease activity.

Curiously, one O157:H7 STEC strain isolated from cattle in Brazil failed to decarboxylate lysine. Some other studies have documented the nondecarboxylation of lysine only in STEC strains belonging to the O111 serogroup (Vaz *et al.*, 2004; Torres *et al.*, 2005). On the other hand, all O157 STEC strains, regardless of the origin and country, were

β-glucoronidase negative, confirming the observations of Doyle & Schoeni (1984), but differing from the ones described by Hayes *et al.* (1995) and Nagano *et al.* (2002).

In the past years sorbitol-fermenting O157 strains have emerged as important causes of human diseases in several countries (Karch & Bielaszewska, 2001; Bettelheim *et al.*, 2002), and it is worth mentioning that all these strains were nonmotile (O157:H$^-$). In this study, fermentation of sorbitol was detected in only one O157 strain isolated from cattle in Brazil, but different from the previous observations this strain was O157:H7 as determined by standard seroagglutiantion assays (Ewing, 1986).

Recent studies have documented antimicrobial resistance among O157 STEC strains (Zhao *et al.*, 2001; Mora *et al.*, 2005). In contrast to these reports, a higher percentage of O157:H7 strains susceptible to antimicrobials was identified in the present study, and resistance to at least one drug occurred only among four of the 38 (10.5%) strains analyzed. Among these strains, three were from cattle source, isolated in Brazil (two strains) and in Argentina, and the other one was isolated in Argentina from humans. Other studies also showed that higher frequencies of resistance among O157:H7 STEC strains were recovered from bovine (Meng & Doyle, 1998; Zhao *et al.*, 2001).

In this study, besides the three bovine Brazilian O157:H7 STEC strains previously reported as unable to express Stx (Cerqueira *et al.*, 1999; Irino *et al.*, 2005), three other strains also isolated from cattle in Brazil showed no cytotoxic activity on Vero and HeLa cells' culture assays. On the other hand, this characteristic was neither observed among the human O157:H7 Brazilian STEC strains nor among the other O157 strains analyzed. No Stx expression in O157:H7 STEC strains isolated from cattle was also reported by Nielsen & Scheutz (2002).

Although a diversity of *stx* genotypes was presently identified, more than 80% of the O157:H7 STEC strains showed stx_2 and/or $stx_{2c(2vha)}$ genotypes, regardless of their source and country. Most (77.8%) of the human strains carried stx_2 or stx_2stx_{2c}, in agreement with several data described in literature that correlate stx_2 and/or stx_{2c} with more severe human diseases (Eklund *et al.*, 2002; Friedrich *et al.*, 2002). In addition, $stx_{2c(2vha)}$ prevailed (64.2%) among strains isolated from cattle, whereas stx_1 and stx_1stx_{2c} were the genotypes identified less frequently, and occurred exclusively among strains isolated from food and cattle, respectively. These data are in contrast to those obtained by Nielsen & Scheutz (2002), in which stx_1stx_{2c} predominated among O157:H7 STEC strains isolated from cattle, but similar to the results described by Zheng *et al.* (2005), who found stx_{2vha} as the dominant genotype among O157:H7 strains isolated from domestic animals in China.

All O157:H7 STEC strains analyzed in this study carried *eae*-γ, *efa*1, *ehx*, *iha*, lpf_{O157} and *toxB* sequences. This same virulence profile was identified in all O157:H7 STEC strains isolated from diverse origins in Argentina (Toma *et al.*, 2004) and Belgium (Tatarczak *et al.*, 2005). Therefore, despite differences in *stx* genotypes, a homogeneous distribution of other virulence factors could be observed in O157:H7 STEC strains isolated from diverse geographic regions and sources.

PFGE analysis grouped most of the O157:H7 strains studied into a same cluster, which was subdivided into several related groups (A1–A6, 75–100% similarity). Curiously, distinct strains isolated in Uruguay from ground meat samples presented identical PFGE patterns of human strains isolated in Argentina and Colombia, suggesting a common origin. Identical PFGE profiles were also observed among three human strains isolated in Brazil. Two of these three strains (strain 337 and 385) had recently been studied by Vaz *et al.* (2006), who proposed the first occurrence of an O157:H7 outbreak in Brazil. The other human strain (143/05), which presented the same PFGE pattern as those previously reported, was isolated from a child with HUS in São Paulo in 2005. Thus, all these data could probably indicate the maintenance of an O157 clone associated with human infections in our settings. A high degree of similarity (85–90%) between these three Brazilian human strains and two O157 STEC strains isolated from cattle and food in Argentina should also be highlighted. In addition, it was also interesting to observe that another Brazilian strain from human origin (156/90) was more closely related to O157 STEC strains from other Latin American countries, corresponding to a subgroup with more than 80% similarity. Guth *et al.* (2003) had previously analyzed by PFGE a few O157 STEC strains only isolated from animals and food samples in Argentina and Brazil, and observed that one Brazilian strain of animal origin was possibly related to some Argentinean bovine strains (80% similarity).

In summary, O157:H7 STEC isolates from Brazil and other Latin American countries share several similar phenotypic and genotypic characteristics. Moreover, analysis of the genetic relatedness of the O157 STEC strains suggested the presence of a particular clone more related to human infections in Brazil, as well as a common origin for some strains isolated from different sources and countries.

Acknowledgements

We thank Delfina Urbina, Felipe Schelotto, João R.C. Andrade, Marta Rivas and Valeria Prado for generously providing some of the STEC strains used in this study; we gratefully acknowledge the assistance of Sylvia P.C. Leão with the Gel Compar II programme and Lucilia S. Nishimura for helpful assistance. This work was partially supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP–N^o 01/07921-7), Conselho

Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília) and Programa de Apoio a Núcleos de Excelência PRONEX MCT/CNPq/FAPERJ. F.C.B. received a research fellowship from Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Adu-Bobie J, Frankel G, Bain C, Gonçalves AG, Trabulsi LR, Douce G, Knutton S & Dougan G (1998) Detection of intimins α, β, γ, and δ, four intimin derivatives expressed by attaching and effacing microbial pathogens. *J Clin Microbiol* **36**: 662–668.

Bettelheim KA, Whipp M, Djordievic SP & Ramachandran V (2002) First isolation outside Europe of sorbitol-fermenting verocytotoxigenic *Escherichia coli* (VTEC) belonging to O group O157. *J Med Microbiol* **51**: 713–714.

Beutin L, Montenegro MA, Ørskov I, Ørskov F, Prada J, Zimmerman S & Stephan R (1989) Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J Clin Microbiol* **27**: 2559–2564.

Breuer T, Benkel DH, Shapiro RL *et al.* (2001) A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emerg Infect Dis* **7**: 977–982.

Cergole-Novella MC, Nishimura LS, Irino K, Vaz TMI, Pestana de Castro AF, Leomil L & Guth BEC (2006) *Stx* genotypes and antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* strains isolated from human infections, cattle and foods in Brazil. *FEMS Microbiol Lett* **259**: 234–239.

Cerqueira AMF, Guth BEC, Joaquim RM & Andrade JRC (1999) High occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in healthy cattle in Rio de Janeiro State, Brazil. *Vet Microbiol* **70**: 111–121.

Chinen I, Tanaro JD, Miliwebsky E, Lound LH, Chillemi G, Ledri S, Baschkier A, Scarpin M, Manfredi E & Rivas M (2001) Isolation and characterization of *Escherichia coli* O157: H7 from retail meats in Argentina. *J Food Prot* **64**: 1346–1351.

Chinen I, Otero JL, Miliwebsky ES, Roldan ML, Baschkier A, Chillemi GM, Noboli C, Frizzo L & Rivas M (2003) Isolation and characterisation of Shiga toxin-producing *Escherichia coli* O157: H7 from calves in Argentina. *Res Vet Sci* **74**: 283–286.

Doughty S, Sloan J, Bennet-Wooa V, Robertson M, Robins-Browne RM & Hartland EL (2002) Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. *Infect Immun* **70**: 6761–6769.

Doyle MP & Schoeni JL (1984) Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl Environ Microbiol* **48**: 855–856.

Eklund M, Leino K & Siitonem A (2002) Clinical *E. coli* strains carrying *stx* genes: *stx* variants and *stx*-positive virulence profiles. *J Clin Microbiol* **40**: 4585–4593.

Ewing WH (1986) *The Genus Escherichia In: Edwards and Ewing's Identification of Enterobacteriacea*, 4th edn. pp. 93–134. Elsevier, Science Publishing, Inc., New York, NY.

Farah SMSS, Silva LR, Castilhos L, Kato MAMF, Ramos II & Irino K (2003) Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle in Paraná, Brazil. *5th Int. Symposium on Shiga toxin (verocytotoxin)-producing Escherichia coli infections*, abstract P212, p. 186, Edinburgh, UK.

Friedrich AW, Bielaszewka M, Zhang WL, Pulz M, Kuczius T, Ammon A & Karch H (2002) *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infect Dis* **185**: 74–84.

Friedrich AW, Köck R, Bielaszewska M, Zhang W, Karch H & Mathys W (2005) Distribution of the urease gene cluster among and urease activities of enterohemorrhagic *Escherichia coli* O157 isolates from humans. *J Clin Microbiol* **43**: 546–550.

Gadea MP, Varela G, Sirok A, Mota MI, Sabelli R, Grotiuz G, Schelotto F, Chinen I, Chillemi G & Rivas M (2004) Primer aislamiento en Uruguay de *Escherichia coli* productora de toxina Shiga del serotipo O157:H7 en una niña con síndrome urémico hemolítico. *Rev Med Uruguay* **20**: 79–81.

Gautom RK (1997) Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157: H7 and other gram-negative organisms in 1 day. *J Clin Microbiol* **35**: 2977–2980.

Gentry MK & Dalrymple JM (1980) Quantitative microtiter cytotoxicity assay for *Shigella* toxin. *J Clin Microbiol* **12**: 361–366.

Giammanco GM, Pignato F, Grimont F, Grimont PAD, Capriolli A, Morabito S & Giammanco G (2002) Characterization of Shiga toxin-producing *Escherichia coli* O157:H7 isolated in Italy and France. *J Clin Microbiol* **40**: 4619–4624.

Gonzalez AGM (2003). Phenotypic and genotypic characteristics of Shiga toxin-producing *Escherichia coli* (STEC*)* isolated from cattle in Rio de Janeiro State. Ph.D. thesis. 184 pp. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Griffin PM & Tauxe RV (1991) The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* **13**: 60–98.

Guth BEC, Chinen I, Milliwebsy E, Cerqueira AMF, Chillemi G, Andrade JRC, Baschkier A & Rivas M (2003) Serotypes and Shiga toxin genotypes among *Escherichia coli* isolated from animals and food in Argentina and Brazil. *Vet Microbiol* **92**: 335–349.

Hayes PS, Blom K, Feng P, Lewis J, Strockbine NA & Swaminathan BS (1995) Isolation and characterization of a β-D-glucuronidase-producing strain of *Escherichia coli* serotype O157:H7 in the United States. *J Clin Microbiol* **33**: 3347–3348.

Irino K, Vaz TMI, Kato MAMF, Naves ZVF, Lara RR, Marco MEC, Rocha MM, Moreira TP, Gomes TAT & Guth BEC (2002) O157: H7 Shiga toxin-producing *Escherichia coli* strains associated with sporadic cases of diarrhea in São Paulo, Brazil. *Emerg Infect Dis* **8**: 446–447.

Irino K, Kato MAMF, Vaz TMI, Ramos II, Souza MAC, Cruz AS, Gomes TAT, Vieira MAM & Guth BEC (2005) Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in São Paulo State, Brazil. *Vet Microbiol* **105**: 29–36.

Izumiya H, Terajima J, Wada A, Inagaki Y, Itoh K, Tamura K & Watanabe H (1997) Molecular typing of enterohemorrhagic *Escherichia coli* O157:H7 isolates in Japan by using pulsed-field gel eletrophoresis. *J Clin Microbiol* **35**: 1675–1680.

Karch H & Bielaszewska M (2001) Sorbitol-fermenting Shiga toxin-producing Escherichia coli O157:H$^-$ strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *J Clin Microbiol* **39**: 2043–2049.

Karch H, Schubert S, Zhang D, Zhang W, Schmidt H, Ölschläger T & Hacker J (1999) A genomic island, termed high-pathogenicity island, is present in certain non-O157 Shiga toxin-producing *Escherichia coli* clonal lineages. *Infect Immun* **67**: 5994–6001.

Machado J, Grimont F & Grimont PA (2000) Identification of *E. coli* flagellar types by restriction of the amplified fliC genes. *Res Microbiol* **151**: 535–546.

March SB & Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* **23**: 869–872.

McDaniel TK & Kaper JB (1997) A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K–12. *Mol Microbiol* **23**: 399–407.

Meng J & Doyle MP (1998) Microbiology of Shiga toxin-producing *Escherichia coli* in foods. *Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains* (Kaper JB & O'Brien AD, eds), pp. 92–108. ASM Press, Washington, DC.

Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, González EA, Bernárdez MI & Blanco J (2005) Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *Res Microbiol* **56**: 793–806.

Nagano H, Okui T, Fujiwara O, Uchiyama Y, Tamate N, Kumada H, Morimoto Y & Yano S (2002) Clonal structure of Shiga toxin (Stx)-producing and β-D-glucuronidase-positive *Escherichia coli* O157:H7 strains isolated from outbreaks and sporadic cases in Hokkaido, Japan. *J Med Microbiol* **51**: 405–416.

Nakano M, Iida T, Ohnishi M, Kurokawa K, Takahashi A, Tsukamoto T, Yasunaga T, Hayashi T & Honda T (2001) Association of the urease gene with enterohemorrhagic *Escherichia coli* strains irrespective of their serogroups. *J Clin Microbiol* **39**: 4541–4543.

Nataro JP & Kaper JB (1998) Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* **11**: 142–201.

National Committee for Clinical Laboratory Standards (2000). *Performance Standards for Antimicrobial Disk Susceptibility Tests*. Approved Standard. *M2-A7,* 7th edn. National Committee for Clinical Laboratory Standards, Wayne, PA.

Nicholls L, Grant TH & Robins-Browne RM (2000) Identification of a novel genetic locus that is required for *in vitro* adhesion of a clinical isolate of enterohemorrhagic *Escherichia coli* to epithelial cells. *Mol Microbiol* **35**: 275–288.

Nielsen EM & Scheutz F (2002) Characterization of *Escherichia coli* O157 isolates from Danish cattle and human patients by genotyping and presence and variants of virulence genes. *Vet Microbiol* **88**: 259–273.

Paton AW, Srimanote P, Woodrow MC & Paton JC (2001) Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-nagative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. *Infect Immun* **69**: 6999–7009.

Pollard DR, Johnson WM, Lior H, Tyler SD & Rozee KR (1990) Differentiation of Shiga toxin and Vero cytotoxin type 1 genes by polymerase chain reaction. *J Infect Dis* **162**: 1195–1198.

Schmidt H, Karch H & Beutin L (1994) The large sized plasmids of enterohaemorrhagic *Escherichia coli* O157:H7 strains encode hemolysins which are presumably members of the *E. coli* alpha-hemolysin family. *FEMS Microbiol Lett* **117**: 189–196.

Tarr PI, Bilge SS, James C, Varu JR, Jelacic S, Habeeb RL, Ward TR, Baylor MR & Besser TE (2000) Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. *Infect Immun* **68**: 1400–1407.

Tatarczak M, Wieczorek K, Posse B & Osek J (2005) Identification of putative adhesin genes in Shiga toxigenic *Escherichia coli* isolated from different sources. *Vet Microbiol* **110**: 77–82.

Tatsuno I, Horie M, Abe H, Miki T, Makino K, Shinagawa H, Taniguchi H, Kamiya S, Hayashi T & Sasakawa C (2001) *toxB* gene on pO157 of enterohemorrhagic *Escherichia coli* O157:H7 is required for full epithelial cell adherence phenotype. *Infect Immun* **69**: 6660–6669.

Toma C, Martinez Espinosa E, Song T, Miliwebsky E, Chinen I, Iyoda S, Iwanaga M & Rivas M (2004) Distribution of putative adhesins in different seropathotypes of Shiga toxin-producing *Escherichia coli*. *J Clin Microbiol* **42**: 4937–4946.

Torres AG, Giron JA, Perna NT, Burland V, Blattner FAF & Kaper JB (2002) Identification and characterization of lpfABCC′DE, a fimbrial operon of enterohemorrhagic *Escherichia co*li O157:H7. *Infect Immun* **70**: 5416–5427.

Torres AG, Vazquez-Juarez RC, Tutt CB & Garcia-Gallegos JG (2005) Pathoadaptive mutation that mediates adherence of Shiga toxin-producing *Escherichia coli* O111. *Infect Immun* **73**: 4766–4776.

Urbina D, Arzuza O, Young G, Parra E, Castro R & Puello M (2003) Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhea on the Colombian northern coast. *Int Microbiol* **6**: 27–32.

Vaz TMI, Irino K, Kato MAMF, Dias AMG, Gomes TAT, Medeiros MIC, Rocha MMM & Guth BEC (2004) Virulence properties and characteristics of Shiga toxin-producing *Escherichia coli* in São Paulo, Brazil, from 1976 through 1999. *J Clin Microbiol* **42**: 903–905.

Vaz TMI, Irino K, Nishimura LS, Novella MCC & Guth BEC (2006) Genetic diversity of Shiga toxin-producing *Escherichia coli* strains isolated in São Paulo, Brazil, from 1976 through 2003 as revealed by pulsed field gel electrophoresis. *J Clin Microbiol* **44**: 798–804.

Zhao S, White DG, Ge B, Ayers S, Friedman S, English L, Wagner D, Gaines S & Meng J (2001) Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. *Appl Environ Microbiol* **67**: 1558–1564.

Zheng H, Jing H, Wang H *et al.* (2005) $Stx_{2\text{–vha}}$ is the dominant genotype of Shiga toxin-producing *Escherichia coli* O157:H7 isolated from patients and domestic animals in three regions if China. *Microbiol Immunol* **49**: 1019–1026.