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Virulence factors of *Escherichia coli* strains belonging to serogroups O127 and O142

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SUMMARY

A total of 102 *Escherichia coli* strains belonging to serogroups O127 and O142 were examined for genotypic and phenotypic characteristics. The most frequent serotypes found were O127:H21, O127:H40 and O142:H34. The virulence properties were evaluated by adhesion to HeLa cells and hybridization with gene probes for diarrhoeagenic *E. coli*. Most strains in the two serogroups were categorized as enteropathogenic *E. coli*, but enteroaggregative *E. coli* was also detected in both serogroups. All strains that carried the *eae* sequence presented the LEE region inserted in *selC*. Five ribotypes were detected in serogroup O127 and four in serogroup O142 and a correlation between serotypes and ribotypes was observed mainly in serogroup O142.

INTRODUCTION

The role of *Escherichia coli* as a cause of diarrhoea was established when Bray [1] demonstrated that *E. coli* strains were the agent of epidemic and sporadic infantile diarrhoea. The term enteropathogenic *E. coli* (EPEC) was proposed by Neter et al. [2], in 1955. In 1987 the World Health Organization recognized EPEC to comprise strains of 12 serogroups: O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158 [3]. EPEC strains constitute one of the main causes of infantile diarrhoea in developing countries [4]. In Brazil, EPEC strains are recovered from 30% or more cases of diarrhoea in infants of low socio-economic status [5, 6]. Historically, EPEC strains have been identified by the distinct combination of somatic (O) and flagellar (H) antigens, which have been epidemiologically associated to infantile diarrhoea [7]. However, in recent years molecular basis of EPEC pathogenesis has been elucidated and specific virulence genes discovered. EPEC are capable of causing the attaching-and-effacing (A/E) lesion, which is characterized by localized destruction of intestinal microvilli and by the intimate adhesion of bacteria to the intestinal epithelium in a pedestal-like structure. Some EPEC strains carry a large plasmid known as *E. coli* adherence factor (EAF) plasmid, which encodes the bundle-forming pilus, responsible for interbacterial interactions that leads to microcolony formation, and the plasmid-encoded regulator, that activates virulence genes in a regulatory cascade. EPEC strains that hybridize with a cryptic fragment of the EAF plasmid are known as typical EPEC, while EAF non-reactive strains are known as atypical EPEC [4].

Consequently, a number of studies have shown that EPEC serogroups are heterogeneous with respect to virulence factors found among the strains of these serogroups and that not all strains are pathogenic [8–13]. Recently our laboratories have collaborated to investigate the virulence properties that define EPEC strains in the serogroups O55, O86, O111, O119, O125, O126 and O128 [8–13]. Although serogroups

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O127 and O142 are epidemiologically significant in Brazil [5, 6] and other countries [14–16], their virulence properties have not been investigated. Therefore, the purpose of this study was to characterize the virulence properties of *E. coli* strains of serogroups O127 and O142 and to investigate the relationship between serotypes and ribotypes in these serogroups.

MATERIALS AND METHODS

Strains and serotyping

This study involved children younger than 5 years old, with diarrhoea, visiting Health Centres or Public Hospitals that provide free medical care to urban children coming from different parts of São Paulo city, between 1977 and 1991. The stool specimens were collected at these centres and sent to Instituto Adolfo Lutz, São Paulo, Brazil for isolation and identification.

All strains of *E. coli* belonging to serogroups O127 (75 strains), and O142 (27 strains) were inoculated in a suitable solid medium and kept at room temperature. Prototype strains of serogroups O127 (E2348/69, O127:H6) [4] and O142 (C771, O142:H6) [17], were included. Strains were cultured in Triptic Soy Broth (TSB) and streaked out on Nutrient Agar for serogroup confirmation and determination of H antigens [18].

Adherence to HeLa cells

Adherence was tested by the method described by Cravioto et al. [19]. All non-adherent (NA) strains in the 3-h assay were tested in the 6-h assay.

Hybridization with DNA probes

All strains were submitted to colony hybridization assays by the method described by Maas [20], using specific radiolabelled DNA probes for *eae* (*E. coli* attaching and effacing gene encoding intimin), EAF (*E. coli* adherence factor), *bfpA* (bundle forming pilus structural gene), *daaC* (accessory gene for F1845 fimbriae biogenesis), EAEC (enteroaggregative *E. coli* adherence plasmid), INV (*E. coli* invasiveness plasmid), LT-I and II (heat-labile enterotoxin type I and II), ST-I h and p (heat-stable enterotoxin type I of human and porcine origin), Stx-I and II (Shiga toxin I and II genes) [12].

Locus of Enterocyte Effacement (LEE) insertion site

Insertion of the LEE region downstream of the gene encoding the tRNA for selenocysteine (*selC*) in the *E. coli* chromosome was assayed using primers for the right junction (K255/K260) and for the left junction (K295/K296) [21]. A total of 30 strains of serotypes O127:H6 (3 strains), O127:H21 (2 strains), O127:H40 (10 strains), O142:H2 (3 strains), O142:H6 (4 strains) and O142:H34 (8 strains), that carried the *eae* sequence were analysed. PCR reactions were performed as described by Sperandio et al. [22].

Ribotyping

Genomic DNA of 36 strains of serogroups O142 (15 strains) and O127 (21 strains), carrying different H antigens and virulence markers, as well as the prototype strains C771 (serotype O142:H6) and E2348/69 (serotype O127:H6), was extracted [10]. Approximately 2 μg of DNA were digested with *Bgl*I (Sigma) and electrophoresed with the genomic DNA of *Haemophilus influenzae* biogroup aegyptius (strain 320/86) digested with *Eco*RI, as size marker [10]. DNA was transferred to nylon membranes and hybridized with a cDNA probe prepared by reverse transcription of 16S and 23S rRNA (Boehringer, Germany), labelled with digoxigenin (Boehringer, Germany). Strains showing identical fingerprints were designed as ribotypes (RTs).

RESULTS

Serotypes and virulence markers

Serogroup O127

Five serotypes were detected among the 75 studied strains of serogroup O127:H40 (20 strains), H21 (36 strains), H16 (1 strain), H10 (9 strains), and H6 (4 strains). Five strains were non-motile (O127:H-). Table 1 presents the distribution of these serotypes throughout the years of isolation, and demonstrates that O127:H21 and O127:H40 were the most prevalent serotypes.

Table 2 shows the combination of virulence genes and the phenotypic characteristics of the strains of each serotype. None of the strains studied reacted with the Stx-1, Stx-2, LT-I, LT-II, ST-Ih, ST-Ip, INV and the *daaC* probes. Two distinct patterns of adherence to HeLa cells were found in the strains of this serogroup: the localized adherence (LA) pattern, i.e. production of distinct microcolonies over the cells [23], and the aggregative adherence (AA) pattern, i.e. presence of bacteria adhering to both cell and coverslip surfaces, assuming a characteristic stacked-brick pattern [4]. Non-adherent strains were also

Table 1. *Distribution of* E. coli *strains belonging to six serotypes of the O127 serogroup and five serotypes of the O142 serogroup between 1977 and 1991 in the city of São Paulo, Brazil*

	No. of strains per year of isolation														
Serotype	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
O127:H40	4	3	1	—	1	—	1	3	1	2	—	3	—	—	1
O127:H21	4	9	3	2	1	3	4	—	—	2	4	1	—	2	1
O127:H16	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
O127:H10	1	1	1	3	—	1	1	—	—	—	1	—	—	—	—
O127:H6	—	—	—	—	—	—	—	—	—	1	—	3	—	—	—
O127:H-	—	—	—	3	—	—	—	—	—	—	—	—	2	—	—
O142:H34	—	—	—	—	—	—	—	3	4	1	1	2	2	2	—
O142:H6	—	—	—	—	—	—	—	5	—	1	—	1	—	—	—
O142:H2	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—
O142:H-	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
O142:H?	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—

Table 2. *Adherence patterns and virulence DNA sequences presented by* E. coli *strains of serogroup O127*

Serotype	No. of strains	Virulence DNA sequences (no. of strains)	Adherence patterns* (no. of strains)
H40	20	*eae*, *bfpA*, EAF (10)	LA† (8) NA (2)
		eae, *bfpA* (2)	LA† (1) NA (1)
		eae (4)	AA‡ (1) NA (3)
		bfpA, EAF (1)	NA (1)
		None (3)	NA (3)
H21	36	None (33)	NA (33)
		eae; *bfpA*, EAF (1)	LA† (1)
		eae, *bfpA* (1)	NA (1)
		EAEC (1)	AA† (1)
H16	1	None (1)	NA (1)
H10	9	None (9)	NA (9)
H6	4	*eae*, *bfpA*, EAF (3)	LA† (3)
		EAEC (1)	AA† (1)
H-	5	*eae*, *bfpA*, EAF (1)	LA† (1)
		None (4)	NA (4)
H6§	1	*eae*, EAF, *bfpA*	LA†

* LA, localized adherence pattern; AA, aggregative adherence pattern; NA, non-adherent.
† 3-h adhesion assay.
‡ 6-h adhesion assay.
§ Prototype strain E2348/69 (serotype O127:H6).

found. A close relationship between the combination of virulence genes and adherence properties was observed. Typical EPEC strains, were found among strains of serotypes O127:H40, O127:H21, O127:H6 and O127:H-. Three of the four strains of serotype O127:H40 that carried only the *eae* gene were non-adherent, and one presented the AA pattern. Among the three strains carrying the *eae* and *bfpA* sequences, one presented LA (serotype O127:H40), and two were non-adherent (serotypes O127:H40 and O127:H21). Most strains of serotype O127:H21, all O127:H10 strains and one O127:H16 strain lacked all gene sequences searched for and were non-adherent.

Serogroup O142

Three distinct serotypes were identified among the 27 studied strains of serogroup O142:H34 (15 strains), H6 (7 strains), H2 (3 strains). The H antigen of one strain could not be determined (O142:H?) and one strain was non-motile (O142:H-). Table 1 presents the distribution of these serotypes throughout the years of isolation, demonstrating that the O142:H6 serotype was the most prevalent in 1984 and the O142:H34 serotype was the most frequent between 1985 and 1990. Table 3 shows the combination of virulence genes and the phenotypic characteristics of each serotype. None of the strains studied reacted with the Stx-1, Stx-2, LT-I, LT-II, ST-Ih, ST-Ip, INV, and the *daaC* probes. The strains of this serogroup presented the LA and AA pattern on HeLa cells. Moreover, strains presenting non-characteristic adhesion (NC), with a few dispersed bacteria adhering to the cells, were also found.

A close relationship between the combination of virulence genes and adherence properties was observed. Typical EPEC strains were found within serotypes O142:H34 and O142:H2. All the O142:H6 strains and one O142:H? strain were devoid of the EAF sequence, but carried *eae* and *bfpA*, and presented LA. Except for one strain (O142:H34) that

Table 3. *Adherence patterns and virulence DNA sequences presented by* E. coli *strains of serogroup O142*

Serotype	No. of strains	Virulence DNA sequence (no. of strains)	Adherence pattern* (no. of strains)
		eae, *bfpA*, EAF (10)	LA† (9), NA (1)
H34	15	*eae* (4)	AA‡ (1), NA (3)
		eae, EAEC (1)	AA‡ (1)
H6	7	*bfpA*, *eae* (7)	LA† (7)
H2	3	*eae*, *bfpA*, EAF (2)	LA† (2)
		eae (1)	NC‡ (1)
H-	1	None (1)	NA (1)
H?	1	*bfpA*, *eae* (1)	LA† (1)
H6§	1	*eae*	NC‡ (1)

* LA, localized adherence pattern; AA, aggregative adherence pattern; NC, non-characteristic adherence pattern; NA, non-adherent strain.
† 3-h adhesion assay.
‡ 6-h adhesion assay.
§ Prototype strain C771 (serotype O142:H6).

presented AA, the O142 strains that carried only the *eae* sequence, displayed a NC pattern (one O142:H2 strain and the prototype strain C771), or were non-adherent (three O142:H34 strains). Interestingly, a single O142:H34 strain hybridized with both the *eae* and EAEC probes and displayed the aggregative pattern of adhesion.

Insertion site of LEE region

Regarding the LEE insertion site, all 30 strains analysed, presented LEE inserted in *selC*, since the primers for the right junction amplified a fragment of 418 bp, as in the prototype strain E2348/69 [21]. The strains of serotypes O142:H2, O127:H21 and O127:H40 did not amplify any detectable fragment using the primers for the left junction, while all other strains amplified the same fragment as in the prototype strain E2348/69 (418 bp).

Ribotyping

The 36 strains submitted to ribotyping were grouped in 9 RTs, named from A–I (Fig. 1). The O142:H6 and O142:H? strains, as well as the prototype strain C771 (O142:H6), belonged to RT-A. The O142:H34 strains belonged to RT-B, and the O142:H- and the O142:H2 strains belonged to RT-C and RT-D, respectively. All the O127:H40, one O127:H- and two O127:H21 virulent strains belonged to RT-E.

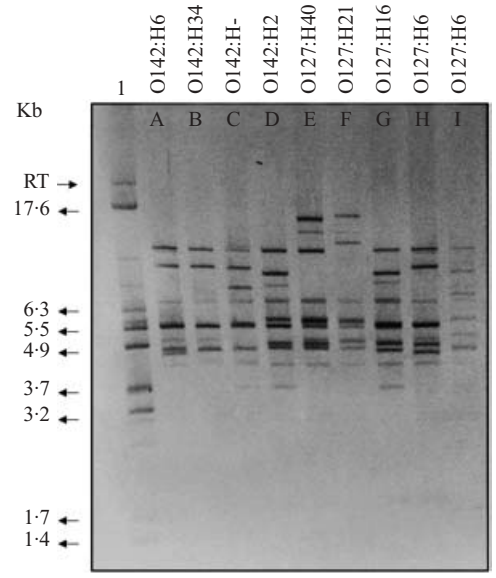

Fig. 1. Serotypes and ribotypes of *E. coli* strains, serogroups O127 and O142 isolated in São Paulo, Brazil. Slot 1 Marker; Slot 2 RT-A, O142:H6, O142:H?, C771; Slot 3 RT-B, O142:H34; Slot 4 RT-C, O142:H-; Slot 5 RT-D, O142:H2; Slot 6 RT-E, O127:H40, O127:H21, O127:H- (strain with virulence markers); Slot 7 RT-F, O127:H21, O127:H10 (strains with no virulence markers); Slot 8 RT-G, O127:H16; Slot 9 RT-H, O127:H6 (EPEC E2349/69); Slot 10 RT-I, O127:H6 (EAEC).

The O127:H21, O127:H10, O127:H- with no virulence markers and the O127:H21 EAEC strains belonged to RT-F. The only O127:H16 strain belonged to RT-G. The RT-H and RT-I comprised EPEC and the prototype strain E2348/69 (O127:H6) and EAEC strains of serotype O127:H6 respectively (Table 4).

DISCUSSION

In this study, we examined a collection of *E. coli* strains belonging to serogroups O127 and O142 isolated from faeces of children younger than 5 years old with diarrhoea, between 1977 and 1991 in São Paulo, Brazil. The strains were submitted to the flagellar antigens typing and the serotypes most commonly identified were O127:H21, O127:H40 and O142:H34, all of which were considered as non-classical EPEC serotypes [5]. These serotypes were also found in several studies conducted in Brazil and other countries [5, 14–16]. Classical EPEC serotypes O127:H6 and O142:H6 were also identified [5]. Among the strains of serogroup O127, the serotypes O127:H21 and O127:H40 were the most prevalent along the studied period. It was observed that the serotypes O142:H6

Table 4. *Relationship among serotypes, ribotypes and virulence markers*

Serotype	Ribotype (RT)	Virulence markers
O142:H6; O142:H?;C771	A	*eae*, *bfpA*; *eae*
O142:H34	B	*eae* only
		eae, EAEC
		eae, *bfpA*, EAF
O142:H-	C	*
O142:H2	D	*eae only*
		eae, *bfpA*, EAF
O127:H40, O127:H21, O127:H-	E	*eae*, *bfpA*, EAF
		eae, *bfpA*
		eae only
		*
O127:H21; O127:H10	F	EAEC
		*
O127:H16	G	*
O127:H6	H	*eae*, *bfpA*, EAF
O127:H6	I	EAEC

* Lack of virulence markers.

and the O142:H34 prevailed along the period of isolation. The serotype specific prevalence and distribution of EPEC and other categories of diarrhoeagenic *E. coli* found in São Paulo may differ from those found in other locations in Brazil and other countries, since these findings may probably be attributed to epidemiological circumstances such as age, socioeconomic status, clinical conditions and geographic origin of the population studied [6, 24].

The characteristics presented by the O127 strains were unusual. Fifty per cent of the O127:H40 strains reacted with the *eae*, *bfpA* and EAF probes, and 40% of them presented LA and were classified as typical EPEC [4]. The remaining 50% included strains presenting different combinations of virulence factors or those lacking any virulence property. All O127:H40 strains belonged to RT-E, therefore presenting the same clonal origin, an observation that suggests they originally harboured the *eae*, *bfpA* and EAF sequences, the virulence variants resulting from the loss of either the entire EAF plasmid (*eae* positive strains) or the EAF probe region (*eae* and *bfpA* positive strains) [11].

It is notable that 33 of the 36 O127:H21 studied strains were devoid of all virulence markers searched for, although they have been isolated from cases of diarrhoea. Among the three virulent strains, one reacted with the EAEC probe and two reacted with the *eae* and *bfpA* probes and the *eae*, *bfpA* and EAF probes, respectively. It has been reported that this serotype comprises EAEC strains or strains lacking any virulence markers [16], but the finding of EPEC strains has not been reported in this serotype. The results of this study could suggest that strains of serotype O127:H21 either lost the EAEC or the EPEC virulence genes or present a new virulence mechanism. The O127:H6, one of the most studied EPEC serotype, was not the most prevalent in the present study. Interestingly, the presence of EAEC markers in this serotype has not been reported before. The single O127:H16 and all the O127:H10 strains did not show any virulence marker, suggesting that they could be originally avirulent. We have not observed this kind of variation in the EPEC serogroups previously studied in our laboratories, since they belonged to a single ribotype [10] or to a group of related electrophoretic types [8, 11, 12]. The presence of 33% of virulent strains is a strong contrast with other EPEC O serogroups, in which 90% of the strains presents virulence markers [8–13].

The O142 serogroup were represented mainly by classical and non classical EPEC serotypes that possessed EPEC virulence characteristics. As observed among the O127:H40 strains, the O142:H34 strains that carried only *eae* presented the same ribotype and belonged to the same serotype of the EAF positive strains, suggesting they were derived of them and lost the EAF plasmid during storage, a fact that is relatively frequent in strains of other EPEC serotypes kept in laboratory for long periods [11, 12]. All the O142:H6 and the only O142:H? identified in this study were EAF negative, but presented LA pattern. These observations indicate that although the strains mentioned above were devoid of the EAF region they still carried the EAF plasmid, since they expressed Bfp (data not shown) and presented the LA pattern. Moreover, the O142:H2 is a typical EPEC serotype apparently not described before.

The 30 studied strains presented the LEE region inserted downstream of *selC*, as previously observed in strains of other serogroups by Sperandio et al. [22]. Our results show that these serotypes are evolutionary convergent with serotype O127:H6 where the LEE region was first described [21]. The finding of strains lacking fragment amplification for the left junction could be attributed to a deletion at the LEE left junction, as previously suggested by McDaniel et al. [21].

We have observed an association between O:H serotype and ribotype mainly in serogroup O142, as previously found in *E. coli* strains of the O86 serogroup [10]. In contrast, the serogroup O127 presented an interesting association between ribotype and virulence markers. Strains with different serotypes but harbouring the same virulence markers belonged to the same ribotype and the same serotype included two different diarrhoeagenic categories associated to distinct ribotypes. This indicates that ribotyping can not replace serotyping in this serogroup, but it is an useful complementary assay. Finally it should be emphasized that in our study, specific DNA probes associated with virulence properties contributed to the identification of possible pathogens among strains of the non-classical serotypes (carrying EAF, *eae* and *bfpA*) and strains of classical serotypes that carried *eae* alone, *eae* and *bfpA*, or with a sequence related with other diarrhoeagenic categories. It is important to mention that serotype and ribotype do not define pathogenesis and detection of virulence factors, and ideally their expression is what determines pathogenicity in EPEC.

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