

Random amplification of polymorphic DNA reveals clonal relationships among enteropathogenic *Escherichia coli* isolated from non-human primates and humans

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) strains are important agents of infantile diarrhea all over the world, gaining even greater importance in developing countries. EPEC have also been isolated from various animal species, but most isolates belong to serotypes that differ from those recovered from humans. However, it has been demonstrated that several isolates from non-human primates belong to the serogroups and/or serotypes related to those implicated in human disease. The objective of this study was to evaluate the genetic differences between thirteen strains isolated from non-human primates and the same number of strains isolated from human infections. Human isolates belonged to the same serogroup/serotype as the monkey strains and the evaluation was done by analysis of random amplified polymorphic DNA. Dendrogram analysis showed that there was no clustering between human and monkey strains. Human and non-human isolates of the EPEC serotypes O127:H40 and O128:H2 shared 90 and 87% of their bands, respectively, indicating strong genomic similarity between the strains, leading to the speculation that they may have arisen from the same pathogenic clone. To our knowledge, this study is the first one comparing genomic similarity between human and non-human primate strains and the results provide further evidence that monkey EPEC strains correlate with human EPEC, as suggested in a previous investigation.

Key words

- *Escherichia coli*
- Enteropathogenic *Escherichia coli*
- Attaching and effacing *Escherichia coli*
- Attaching and effacing
- Monkey
- Random amplified polymorphic DNA

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Enteropathogenic *Escherichia coli* (EPEC) strains are defined by the ability to induce the lesion called “attaching and effacing” combined with the inability to produce Shiga toxins, in such a way that diarrhea is the result of pathogen-host membrane interactions (1,2).

EPEC strains are agents of infantile diarrhea all over the world, with even greater importance in developing countries, where the rate of infection and consequent infantile mortality reach higher levels (1,3).

Attaching and effacing *E. coli* (AEEC)

have been isolated from healthy and diarrheic animals of various species including pigs (4,5), cows (5,6), sheep and goats (5,7), dogs and cats (5,8), monkeys (9,10), and rabbits (11,12).

It has been recently demonstrated that several isolates from non-human primates belong to serogroups and/or serotypes related to those implicated in human disease, including the traditional EPEC serogroups O26, O127, O128, and O142. Carvalho et al. (9) speculated that the isolates from non-human primates may have the same origin as the human strains. Thus, the objective of the present study was to obtain complementary genetic evidence testing this assumption by evaluating genomic differences between strains isolated from humans and from non-human primates using the analysis of polymorphisms obtained by the random amplified polymorphic DNA (RAPD) technique.

Thirteen EPEC strains isolated from healthy and sick non-human primates, mostly marmosets, were compared with human strains belonging to the same serogroup/serotype as the monkey isolates. Thirteen human strains were obtained from children with diarrhea and selected from the culture collection of Instituto Adolfo Lutz, São Paulo,

SP, Brazil. The complete phenotypic and genotypic characterization of the monkey strains was described elsewhere (9,13) and is summarized here in Table 1.

E. coli strains were grown in 2 mL brain heart infusion broth and incubated aerobically at 37°C overnight with shaking. DNA extraction was performed according to the recommendations of the Invitrogen-Easy-DNA™ kit, version E (Carlsbad, CA, USA). DNA concentration and purity were evaluated by electrophoresis on a 0.8% submerged agarose gel stained with ethidium bromide. The high DNA mass ladder (Invitrogen) was used as a molecular size marker.

RAPD-PCR was performed according to a protocol described elsewhere (14) using the primers OPR-04 (5'-CCCGTAGCAC-3'), OPR-06 (5'-GTCTACGGCA-3') and OPR-08 (5'-CCCGTTGCCT3'). Each bacterial strain was analyzed in at least three independent reactions. PCR was carried out in an MJ Research model PTC-100™ thermal cycler (Watertown, MA, USA). The RAPD products were visualized by electrophoresis on 1.4% agarose gels in 1X TBE (0.1 M Tris, 90 mM boric acid, and 1 mM EDTA, pH 8.3) stained with ethidium bromide. A 1-kb DNA ladder (Invitrogen) was

Table 1. Non-human primate EPEC strains isolated in the present study in Brazil.

Strain No.	Species	Clinical status	Serotype	<i>bfpA</i> gene/BFP expression
1	<i>Callithrix penicillata</i>	HP	O128:H2	-/-
2	<i>Callithrix jacchus</i>	HP	O127:H40	-/-
3*	<i>Saguinus fuscicollis</i>	SP	O127:H40	-/-
4	<i>Callithrix penicillata</i>	HP	O33:H34	-/-
5	<i>Callithrix penicillata</i>	HP	O167:H9	-/-
6	<i>Callithrix penicillata</i>	SP	O167:H6	+/-
7	<i>Callithrix penicillata</i>	SP	O142:H6	+/+
8	<i>Callithrix jacchus</i>	HP	O132:H31	+/+
9	<i>Callithrix penicillata</i>	HP	O132:H31	+/+
10*	<i>Saguinus fuscicollis</i>	SP	O26:H7	-/-
11**	<i>Callithrix penicillata</i>	SP	O142:H6	+/+
12**	<i>Callithrix penicillata</i>	SP	O142:H6	-/-
13	<i>Callithrix jacchus</i>	SP	O142:H6	-/-

*/**Strains recovered from the same animal. HP = healthy non-human primate strain; SP = sick non-human primate strain. The *bfpA* gene was detected by PCR and BFP expression was tested by Western blotting with BFP antiserum (9).

used as a molecular size marker.

The RAPD-PCR profiles were recorded and analyzed with the NTSYS-pc program (NTSYS-ps, 1992-Numerical Taxonomical and Multivariate Analysis System, Exeter Software, Setauked, NY), version 1.7.

The RAPD technique has been used in the study of the clonal relationship between bacterial populations and is considered to be a useful tool for epidemiological studies (15,16). The aim of the present study was to compare human and non-human primate strains isolated in São Paulo State. The use of the three primers generated 111 polymorphisms which were used in the construction of a binary matrix based on the presence or absence of shared bands. The dendrogram generated from the use of Dice coefficients is shown in Figure 1.

The results obtained showed that the distribution of human and monkey EPEC occurred uniformly among the two main clusters, A and B, in contrast to the data reported by Venieri et al. (16), who demonstrated a difference between *E. coli* saprophyte strains isolated from animal and human sources.

Cluster B comprises all strains of sero-

type O26 studied, independent of their origin, as well as serotypes O142:H6 and O132:H8 isolated from monkeys and humans, respectively. This group included strains recovered from sick individuals only.

Cluster A consisted of two other subgroups, A1 and A2. Subgroup A1 mainly consisted of isolates originating from healthy monkeys and subgroup A2 consisted predominantly of isolates originating from sick primates.

The monkey serotypes O128:H2, O127:H40, O167:H9, and O132:H31 were detected in cluster A1. The first two serogroups are widely referred to in the literature as the cause of diarrhea in children and the serotypes are prevalent in Brazil (3,17). Due to their characterization regarding the presence and expression of *bfpA*/BFP and the intimin subtype, in a previous study by Blanco et al. (13) the monkey isolates of the present study were considered to be human serotypes.

The genomic profile determined by the RAPD technique in strains of serotypes O128:H2 and O127:H40 demonstrated a close correlation between the isolates from monkeys and humans, corroborating previ-

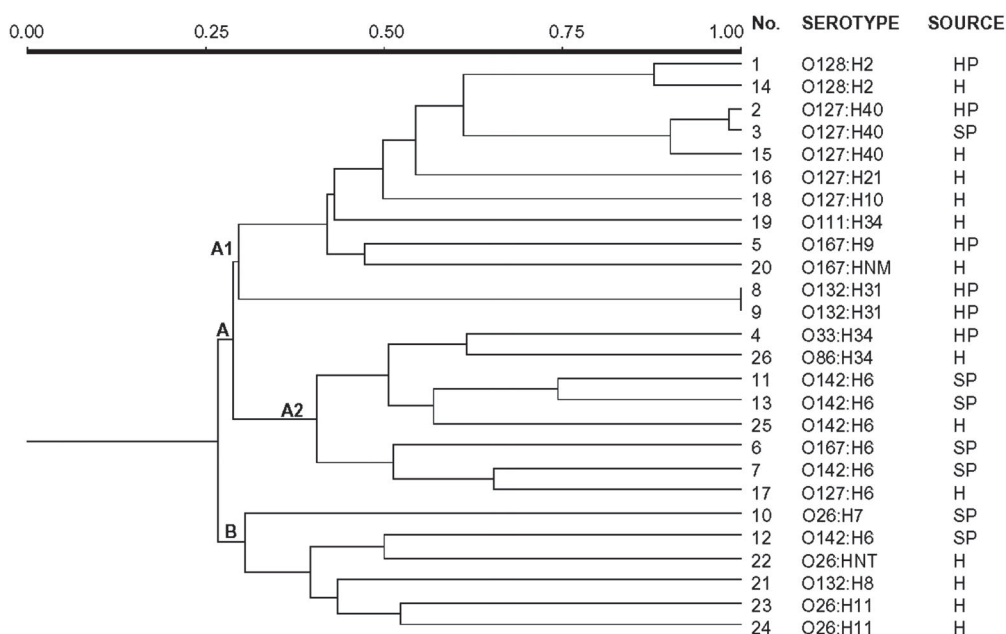


Figure 1. Dendrogram based on UPGMA cluster analysis of Dice coefficients. Clusters are indicated by the letters A, A1, A2 and B. HP = healthy non-human primate strain; SP = sick non-human primate strain; H = human strain.

ous results (9). Strains belonging to serotype O128:H2 shared 87% of the band profiles while strains belonging to serotype O127:H40 shared 90% of the bands, a fact leading to the speculation that these strains could have a common ancestor pathogen strain. On the other hand, strains of serotype O167:H9 presented approximately 50% similarity to the human strain of the same serogroup. Although not included among the AEEC serotypes, O167:H9 was characterized as such in an outbreak involving a large number of children in Japan (18).

The two isolates of serotype O132:H31 were shown to be identical even though they were recovered from different individuals belonging to the same institution (data not shown), supporting the idea that potentially pathogenic clones may disseminate among individuals sharing the same environment. These O132:H31 strains had approximately 30% band identity with the remaining cluster strains. Both presented and expressed the *bfpA* gene, showing a subtype of intimin β , LEE, inserted in *phe U* and an FAS-positive reaction (9,13). Despite showing human EPEC characteristics, the serotype is not included among human EPEC; however, serogroup O132 is related to infections in rabbits by REPEC (rabbit EPEC) in Brazil (12).

All isolates with the flagellar antigens H6 and H34 were included in cluster A2, with the majority of strains being recovered from sick monkeys. These results are similar

to those obtained by other investigators who studied the genetic relationship between human EPEC serotypes (17,19). The phenotypic and genotypic characteristics of the strains with these flagellar antigens allowed the authors to classify them as typical EPEC. In the present study, however, some of the isolates did not present the *bfpA* gene (O33:H34 and O142:H6) nor did they express it in *in vitro* studies (O167:H6), despite their clonal relationship.

This is the first study comparing genomic similarity between human and non-human primate strains. The genomic similarity between serotypes O128:H2 and O127:H40, isolated from humans and monkeys, which are prevalent in Brazil in children with diarrhea (3,17), supports the previously raised hypothesis that monkeys carry human EPEC strains (9,13). These serotypes are classified as atypical EPEC and have been considered to be emergent human pathogens in developing countries (3).

Non-human primates are important models for the study of human disease, due to their phylogenetic proximity and similar susceptibility to human pathogens (20). The verification that these animals carry human EPEC strains opens the possibility of using these primates as models to study these infections, since Vallance and Finlay (2) emphasized the need to study the pathogenesis of EPEC in models genetically and immunologically more closely related to humans.

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