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High prevalence of antimicrobial drug-resistant diarrheagenic *Escherichia coli* in asymptomatic children living in an urban slum

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Summary *Purpose:* The aim of this study was to investigate the presence of diarrheagenic *Escherichia coli* and antibiotic resistance in asymptomatic school-age children living in an area with defective environmental sanitation, comparing with children registered at a private school, both in the city of Osasco, Brazil.
Methods: Seventy-nine school-age children between 5 and 10 years living in a slum and 35 children who attended a private school of the same city were included in the study.
Results: DEC was found in 58% of the children living in the slum and in 17% of the control group ($P = 0.001$). Resistance to at least one antimicrobial drug was found in 65% of DEC strains; resistant to two or more antimicrobial drugs was found in 46% of strains.
Conclusion: The high carriage status among the slum children point towards the widespread environment contamination in low socio-economic housing conditions, in conformance with the pediatric population at higher risk for developing DEC diarrhea.

Introduction

Diarrhea remains an important public health problem for children of low socio-economic level families in Brazil.[1–4] Low socio-economic levels often lead to living in conditions that lack basic sanitary facilities which are directly associated with environment contamination.

Escherichia coli strains are among the most important bacterial causes of childhood diarrhea.[1–4] Diarrheagenic *E. coli* (DEC) can be divided into six main categories on the basis of distinct epidemiological and clinical features,

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specific virulence determinants and an association with certain serotypes.[5]

The most commonly reported DEC strains in Brazilian children are enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).[1–4] EPEC harbor the locus of enterocyte effacement (LEE) pathogenicity island, which encodes factors responsible for the attaching and effacing (A/E) phenotype on host enterocytes. The EPEC strains can also harbor the EPEC adherence factor plasmid (EAF) comprising the cluster of genes encoding the bundle-forming pilus (BFP). EPEC strains with the EAF plasmid are classified as typical (tEPEC); whereas EPEC strains that do not possess the EAF plasmid are classified as atypical (aEPEC). EAEC are characterized by an aggregative adherence (AA) pattern on cultured epithelial cells, and production of fimbrial colonization factors called aggregative adherence factors (AAFs). DAEC show diffuse adherence (DA) pattern, and possess the F1845 fimbrial adhesin. The other three categories seem to be less prevalent in Brazilian children: enterotoxigenic *E. coli* (ETEC), which produces the heat labile (LT) and/or heat-stable (ST) enterotoxins; enteroinvasive *E. coli* (EIEC), which invades the colonic epithelium; and enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), which produces Shiga toxins 1 and 2 (Stx1 and Stx2) and in some strains the presence of the LEE region.[5]

In a previous study carried out in the 90s, Fagundes-Neto et al.[6] found a high prevalence of DEC, especially EPEC, among children living in a slum in São Paulo, Brazil, in both children with (42%) or without (37%) diarrhea, although at that time, not all six DEC categories were known.

Poor hygiene and poor sanitation facilities for sewage disposal are common in poor communities. These factors combine to facilitate the spread of these bacteria in such settings. There is little information on the extent socioeconomic conditions interfere with enteropathogen carriage among children in less developed countries. The aim of this study was to investigate the presence of the different *E. coli* groups in asymptomatic school-age children living in an area with defective environmental sanitation, comparing with children registered at a private school, both in the city of Osasco, Brazil.

Subjects and methods

Subjects

The study was carried out from August 2007 to October 2007 during scholar period. The investigation included 79 children randomly selected aged between 5 and 10 years living in the Colinas D'Oeste slum, who had no gastrointestinal symptoms in the previous 30 days or antibiotic use in the previous 15 days. These children were compared with a control group constituted by 35 children randomly selected aged between 5 and 10 years who attended a private school of the same city. The same prerequisites, absence of clinical gastrointestinal symptoms and no use of antibiotics, were also mandatory for this group.

The stool collection was performed with the consent of the children's parent, and the study was approved by the Ethical Committee of Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo.

Area of study

Colinas D'Oeste slum is located at the periphery of the city of Osasco, in the west region of the State of São Paulo, Brazil. The estimated population is 8700 inhabitants. The houses vary in size and construction material, but the great majority is made of wood or clay, and consists of one single room or two rooms at most. There is no sewage system, although sometimes a collective water supply, as well as electricity, can be found. Garbage collection is infrequent. The average family income is $180 per month (U.S. dollars).

In contrast, control group children lived in upper–middle class areas of the city of Osasco, in very comfortable houses with potable water, sewage facilities, and regular garbage collection. The average family income is >$1500 per month (U.S. dollars).

Microbiological methods

Stool samples were collected and placed in Cary-Blair transport medium, transported to a microbiology laboratory within 3 h of collection and inoculated on MacConkey agar plates for the isolation of *E. coli*. Three to five colonies from each sample, identified by biochemical assays as *E. coli*, were submitted to slide agglutination with polyvalent and monovalent antisera (PROBAC, São Paulo, Brazil) against O antigens of EPEC serogroups (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128ab, O142, O158), and EHEC O157. All *E. coli* strains were kept in nutrient agar slants at room temperature.

Detection of DEC by multiplex PCRs

All *E. coli* isolates were examined for the presence of the following virulence markers: *eae* (structural gene for intimin of EPEC and EHEC), *bfpA* (structural gene for the BFP of typical EPEC), *aggR* (transcriptional activator for the AAF/I and AAF/II of EAEC), *afaBC* (F1845 of DAEC), *elt* and *est* (LT and ST toxins of ETEC), *ipaH* (invasion plasmid antigen H found in EIEC), and *stx* (Stx1, Stx2, and variants of EHEC) using two multiplex PCR assays, as described previously with some modifications.[7,8] Assay 1 identified EAEC, DAEC, typical EPEC by the presence of *eae* and *bfpA*, and atypical EPEC by the presence of only *eae*. Assay 2 identified ETEC, EIEC, and EHEC.

Three to six bacterial colonies form each isolate were pooled for template DNA preparation immediately by prior to PCR testing, suspended in 300 μL of sterile water, and boiled for 10 min. A 5-μL aliquot of this suspension was added to 50 μL of PCR mixture (50 mM KCl, 10 mM Tris–HCl [pH 8.3], 1.5 mM $MgCl_2$, 2 mM of each deoxynucleoside triphosphate), 1.5 U of AccuPrime *Taq* DNA polymerase, and 5 μM of each set of primers except for ipaH primers, which were used at a concentration of 10 μM. The reactions were run in a thermal cycler (model system 2400; Perkin-Elmer Corporation, Norwalk, Conn.) with the following cycling conditions: 94 °C for 5 min, 40 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C (assay 1) or 50 °C (assay 2) for 1 min and primer extension at 72 °C for 2 min followed by a final extension at 72 °C for 7 min. PCR products (10 μL) were visualized after electrophoresis in 2%