

Title: CHARACTERIZATION OF DIARRHEAGENIC *Escherichia coli* PATHOTYPES ISOLATED FROM CHILDREN WITH AND WITHOUT DIARRHEA IN BOTUCATU, SÃO PAULO STATE, BRAZIL

Authors Dias, R.C.B.¹, dos Santos, B.C.¹, dos Santos, L.F.², Vieira, M.A.¹, Oliveira, S.L.¹, Yamatogi R.S.³, Mondelli A.L.⁴, Sadatsune T.¹, Sforcin J.M.¹, Gomes T.A.T.⁵, Hernandez R.T.¹

Institution ¹Departamento de Microbiologia e Imunologia, Instituto de Biociências, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP, Brazil

²Centro de Bacteriologia, Instituto Adolfo Lutz (IAL), São Paulo, SP, Brazil

³Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia de Botucatu (FMVZ), Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP, Brazil

⁴Departamento de Patologia Clínica, Faculdade de Medicina, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP, Brazil

⁵Departamento de Microbiologia, Imunologia e Parasitologia da Universidade Federal de São Paulo (UNIFESP/EPM), São Paulo, SP, Brazil

Abstract:

Diarrheagenic *Escherichia coli* (DEC) comprise a major cause of childhood diarrhea worldwide. DEC is a leading cause of diarrheal disease in Brazil, especially among children. Therefore, this study aimed to investigate the prevalence of DEC pathotypes among diarrheal (patients) and healthy children (controls) up to five years of age, in the city of Botucatu, São Paulo, Brazil. Were analyzed stool samples from 200 children with diarrhea and 200 healthy children. *E. coli* isolates were classified in different pathotypes by detecting of virulence markers and then, characterized regarding their pattern of adherence to HeLa cells and antimicrobial resistance. Isolates carrying the locus of enterocyte effacement (*eae*⁺) were submitted to FAS (Fluorescence Actin Staining) test, to evaluate their ability to induce attaching and effacing lesion (AE). In addition, EPEC and STEC isolates had their somatic (O1-O181) and flagellar (H1-H56) antigens determined. DEC was isolated from 18.0% of the patients, and 19.0% of the controls, and none of the known DEC pathotypes could be individually associated with diarrheal disease. Enteroaggregative *E. coli* (EAEC) was the prevalent pathotype, being detected in equal proportion between patients and controls (10.0%), and the aggregative adherence pattern was observed in 70.0 % of these isolates. Among the enteropathogenic *E. coli* (EPEC) isolates, only one isolate was able to produce the localized adherence (LA) pattern to HeLa cells, being thus the only typical EPEC identified in this study. All the remaining EPEC were classified as atypical (aEPEC), and were detected in 8.0% and 8.5% of the patients and controls, respectively. Regarding the serotypes, 26.5% of the analyzed EPEC isolates belonged to the classical EPEC serogroups, and the only two STEC isolates found were serotyped as O26:H11 (patient) and O119:H7 (control). Antimicrobial susceptibility tests revealed that 44.6%, 29.7% and 2.7% of all the DEC isolates were resistant to ampicillin, cotrimoxazole and gentamicin, respectively. Our data indicate that DEC remains prevalent among children living in the city of Botucatu, being EAEC and aEPEC the most frequent pathotypes. The inability to associate the distinct DEC pathotypes with the diarrheal disease point out the need of additional studies to identify new potential virulence markers that could allow the identification of truly pathogenic isolates in this highly heterogeneous bacterial population.

Keywords: epidemiology, diarrheagenic *Escherichia coli*, pathogenicity, virulence markers

Development agency: Fapesp (Processo N° 2013/05170-1)