BRIEF COMMUNICATION

DETECTION OF NON-ENTEROTOXIGENIC AND ENTEROTOXIGENIC Bacteroides fragilis IN STOOL SAMPLES FROM CHILDREN IN SÃO PAULO, BRAZIL

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SUMMARY

Non-enterotoxigenic bacteria of the Bacteroides fragilis group and enterotoxigenic B. fragilis were identified from children with and without aqueous acute diarrhea. In this study, 170 stool samples from 96 children with and 74 without diarrhea were analyzed. Enterotoxin production and the toxin gene detection were detected by cytotoxicity assay on HT-29/C1 cells and by PCR, respectively. B. fragilis species was prevalent in both groups and enterotoxigenic B. fragilis strains were isolated from two children with diarrhea. More studies are important to evaluate the role of each bacteria of the B. fragilis group, including enterotoxigenic strains play in the diarrheal processes in children.

KEYWORDS: Bacteroides fragilis group; ETBF; Acute diarrhea; Children.
cellular alterations\textsuperscript{14}. Bacterial pellets mixed with 500 µl of Milli-Q water, washed twice (12,000 g, for 15 minutes), and resuspended in 500 µl of Milli-Q water were used for DNA extractions by boiling for 10 minutes. After centrifugation (14,000 g, 10 minutes) the supernatant was saved and used as template. Primers were synthesized according to PANTOSTI \textit{et al.}\textsuperscript{16} at the Biotechnology Branch, Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). For positive isolates the specific primer pair amplified a characteristic 294-bp fragment. DNA amplifications were performed in 25 µl containing 2.5 µl of 10 X PCR buffer (Gibco), 1.25 µl of MgCl\(_2\) (1.5 mM), 2.0 µl of dNTP mixture (0.2 mM) (Gibco), 0.25 µl of \textit{Taq} DNA polymerase (0.5 U) (Gibco), 1 µl of each primer (0.4 mM), 7 µl of ultrapure water (Milli-Q plus) and 10 µl of DNA template. Amplification was performed in a DNA thermal cycler (Perkin Elmer, Amp PCR System 2400) programmed for 94 °C (five minutes) followed by 35 cycles of 94 °C (one minute), 52 °C (one minute), 72 °C (one minute), and then 72 °C (five minutes). A negative control without template was included in each PCR run. Amplified products were visualized by electrophoresis in 1.6% agarose gel in 1X TBE buffer (1 M Tris, 0.9 M boric acid, 0.01 M EDTA, pH 8.4), at 80 v, for two hours. A 50 bp DNA Ladder (Gibco) was used as a molecular mass marker. Gels were stained with ethidium bromide (0.5 µg ml\(^{-1}\)) and photographed on a UV light transilluminator (Electrophoresis Documentation and Analysis System 120, Kodak Digital Science). Bacterial isolation from both patient and control groups was analyzed by using a \(\chi^2\) test.

The incidence of bacteria of the \textit{B. fragilis} group, non-enterotoxigenic \textit{B. fragilis} (non-ETBF), and ETBF isolated from hospitalized children with aqueous acute diarrhea and from healthy children without diarrhea can be observed in Table 1. Only children with diarrhea harbor ETBF species (2.08%). Non-ETBF \textit{B. fragilis} were isolated in 11 (11.45%) children with diarrhea and in 18 (24.3%) without diarrhea (Table 1). Also, the bacterial species that belonged to \textit{B. fragilis} group isolated from children with and without diarrhea are shown in Table 2. All the recovered bacteria from children with and without diarrhea were examined for enterotoxigenicity on HT-29/C1 cell and by PCR, and only ETBF were toxigenic and produced a characteristic 294 bp fragment. ETBF were isolated and detected in two children with diarrhea (8 months and 4 years old). No healthy children without diarrhea harbor ETBF.

\textit{B. fragilis} species are emerging as etiologic agents of diarrhea in farm animals and humans\textsuperscript{11}, ETBF detection from stools is amount-dependent of produced toxin, assay sensitivity, and toxin stability, but the toxin is susceptible for protease action\textsuperscript{11}.

Currently, the identification of enterotoxin production is achieved by culturing in selective medium (BBE) and by testing the isolates for the presence of enterotoxin by the cytotoxic assay with HT-29/C1 cells\textsuperscript{15} or lambs ileal loop test\textsuperscript{\textsuperscript{11}}. In this study, the bacterial isolation from both patient and control groups was not significant (\(P > 0.05\), Table 1). Hospitalized children were not using antimicrobial agents at the time of sampling. Although, it is suggested that some factors such as immunological alterations, age, nutritional conditions, genetic factors or pathologies could interfere in the \textit{B. fragilis} isolation\textsuperscript{14,20}.

Bacteria of the \textit{B. fragilis} group were isolated from 50% control group, in accordance with similar studies in Italian children (46%) and in Apache American children (50%)\textsuperscript{15,17}. Non-ETBF was observed in 24.3% of this control group. The presence of ETBF has been associated with acute diarrhea in children older than one year-old in USA, Italy, Sweden and Japan\textsuperscript{15,17,18,20}, and recently in Nicaraguan children younger than one-year old\textsuperscript{2}. Also, these studies showed that patients with or without diarrhea could harbor ETBF in their intestinal tract, but these organisms might be in a small number in the human intestinal indigenous microbiota\textsuperscript{15}. In our study, ETBF were isolated and detected from two children with diarrhea and its presence was not significant, however, other enteropathogens such as rotavirus, EPEC, ETEC, or \textit{Vibrio cholerae} were not found, but they could be implicated in diarrhoeal processes in Brazil\textsuperscript{1}. Moreover, these results indicate the need of more studies to evaluate the role that each bacteria of the \textit{B. fragilis} group, including non-ETBF and ETBF, play in the childhood diarrhea.

\textbf{RESUMO}

\textbf{Detecção de Bacteroides fragilis enterotoxigênicos e não enterotoxigênicos de amostras fecais de crianças em São Paulo, Brasil}

Bactérias não enterotoxigênicas do grupo \textit{Bacteroides fragilis} e \textit{B. fragilis} enterotoxigênicas foram isoladas e identificadas de crianças com e sem diarreia aguda aquosa. Neste estudo, 170 amostras fecais de 96 e 74 crianças, com e sem diarreia, respectivamente, foram analisadas. A produção de enterotoxina e a detecção do gene que media a produção da toxina foram determinadas por ensaios citotóxicos em células HT-29/C1, e por PCR, respectivamente. A espécie \textit{B. fragilis} foi prevalente em ambos
os grupos, e cepas de *B. fragilis* enterotoxigênicas foram isoladas de duas crianças com diarreia aquosa aguda. Maiores estudos são necessários para avaliar o papel de cada bactéria desse importante grupo bacteriano, incluindo-se o papel que as cepas enterotoxigênicas, desempenham no processo diarréico em crianças.

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