Benedito Rodrigues da Silva Neto
(Organizador)

Pesquisa Científica e Tecnológica em Microbiologia 2

Atena Editora
Ano 2020

Benedito Rodrigues da Silva Neto
(Organizador)

Pesquisa Científica e Tecnológica em Microbiologia 2

Atena Editora
Ano 2020

2020 by Atena Editora

Editora Chefe: Profª Drª Antonella Carvalho de Oliveira
Diagramação: Karine de Lima
Edição de Arte: Lorena Prestes
Revisão: Os Autores

Atena Editora
Ano 2020

Atena Editora
Ano 2020

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

P474 Pesquisa científica e tecnológica em microbiologia 2 [recurso eletrônico] / Organizador Benedito Rodrigues da Silva Neto. – Ponta Grossa, PR: Atena Editora, 2020.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-85-7247-939-4
DOI 10.22533/at.ed.394202201

1. Microbiologia – Pesquisa – Brasil. I. Silva Neto, Benedito Rodrigues da.

CDD 579

Elaborado por Maurício Amormino Júnior – CRB6/2422

Atena Editora
Ponta Grossa – Paraná - Brasil
www.atenaeditora.com.br
contato@atenaeditora.com.br

APRESENTAÇÃO

Temos o prazer de apresentar o segundo volume da obra “Pesquisa científica e tecnológica em microbiologia”, contendo trabalhos e pesquisas desenvolvidas em diversos locais do país que apresentam análises de processos biológicos embasados em células microbianas ou estudos científicos na fundamentação de atividades microbianas com capacidade de interferir nos processos de saúde/doença.

Conforme destacamos no primeiro volume, a microbiologia é um vasto campo que inclui o estudo dos seres vivos microscópicos nos seus mais vaiados aspectos como morfologia, estrutura, fisiologia, reprodução, genética, taxonomia, interação com outros organismos e com o ambiente além de aplicações biotecnológicas. Como uma ciência básica a microbiologia utiliza células microbianas para analisar os processos fundamentais da vida, e como ciência aplicada ela é praticamente a linha de frente de avanços importantes na medicina, agricultura e na indústria. Os microrganismos são encontrados em praticamente todos os lugares, e hoje possuímos ferramentas cada vez mais eficientes e acuradas que nos permitem investigar e inferir as possíveis enfermidades relacionadas aos agentes como bactérias, vírus, fungos e protozoários.

O potencial desta obra é enorme para futuras novas discussões, haja vista que enfrentamos a questão da resistência dos microrganismos à drogas, identificação de viroses emergentes, ou reemergentes, desenvolvimento de vacinas e principalmente a potencialização do desenvolvimento tecnológico no estudo e aplicações de microrganismos de interesse.

Portanto apresentamos aqui temas ligados à pesquisa e tecnologia microbiana são com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela saúde em seus aspectos microbiológicos. Parabenizamos à todos os envolvidos que de alguma forma contribuíram em cada capítulo e cada discussão, com destaque principal à Atena Editora que tem valorizado a disseminação do conhecimento obtido nas pesquisas microbiológicas.

Assim desejo a todos uma ótima leitura!

Benedito Rodrigues da Silva Neto

SUMÁRIO

CAPÍTULO 11

MULTIPLEX PCR FOR THE DETECTION OF DIARRHEAGENIC *Escherichia coli* PATHOTYPES IN CHILDREN WITH ACUTE DIARRHEA

Data de submissão: 19/11/2019
Data de aceite: 10/12/2019

Daniela Cristiane da Cruz Rocha
Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil
http://lattes.cnpq.br/4294679098531496

Anderson Nonato do Rosario Marinho
Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil
http://lattes.cnpq.br/1184866913213283

Karina Lúcia Silva da Silva
Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil
http://lattes.cnpq.br/4308364152963260

Edvaldo Carlos Brito Loureiro
Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil
http://lattes.cnpq.br/2685418720563351

Eveline Bezerra Sousa
Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil
http://lattes.cnpq.br/3387101084207778

Corresponding address: Daniela Cristiane da Cruz Rocha. Instituto Evandro Chagas, Rodovia BR 316, km 07, s/n, Bairro Levilândia. CEP: 69.030-000. Ananindeua-PA Phone: 55 (091) 3214-2297. E-mail: danielarocha@iec.gov.br/danielaufpa@yahoo.com.br

ABSTRACT: Gastrointestinal tract diseases have a global distribution, affecting mainly children. In developing countries, they are important cause of morbidity and mortality, considering the bacterial etiology. Enterobacteria, as pathogenic Escherichia coli, are recognized as important etiology agents, being closely linked to endemic forms of childhood diarrhea. The objective of this work was to isolate and characterize E. coli pathotypes from fecal samples culture from diarrhea patients and healthy people (control group) attended at Public Health Units. 239 fecal samples were evaluated (152 diarrhea and 87 controls). For the isolation of enteropathogenic bacteria, we used selective indicator and enrichment media. Biochemical characterization was performed using API-20E Systems and serological through polyvalent and monovalent antisera. For the detection of pathogenic categories of E. coli (EPEC-enteropathogenic E. coli, ETEC-enterotoxigenic E. coli, STEC-Shiga toxin producer E. coli, EIEC-enteroinvader E.coli and EAEC-enteroaggregative E. coli) multiplex PCR assays (M-PCR) were performed using specific primers for each virulence factor. As for the pathogenic categories of E. coli, ETEC 7.2% (12/152) was the most common pathotype in cases of diarrhea followed by EAEC 5.9% (9/152), while in controls, the highest proportion 9.2% (8/87) was represented by EAEC followed

by EPEC 2.3% (2/87). Of the eight enteropathogenic E. coli (EPEC) identified, only one was typical EPEC and of the twelve enterotoxigenic E. coli (ETEC) isolates, five presented the ST-coding genes, four from LT and ST and three from LT. The multiplex PCR assays was therefore showed as important tools in the rapid identification of pathogenic E. coli categories, and can be routinely used in the molecular diagnosis of these categories.
KEYWORDS: Pathogenic Escherichia coli, diarrhea; Multiplex PCR.

PCR MULTIPLEX PARA DETECÇÃO DOS PATOTIPOS DE *Escherichia coli* DIARREIOGÊNICAS EM CRIANÇAS COM DIARREIA AGUDA

RESUMO: Doenças do trato gastrointestinal têm uma distribuição global, afetando principalmente crianças. Nos países em desenvolvimento, figuram como importante causa de morbimortalidade, considerando-se a etiologia bacteriana. Enterobacterias, como as Escherichia coli patogênicas, são reconhecidas como importantes agentes etiológicos estando estreitamente ligadas as formas endêmicas de diarreia infantil. O objetivo deste trabalho foi isolar e caracterizar patotipos de E. coli a partir de culturas de amostras fecais de pacientes com diarreia atendidos em Unidades de Saúde Pública e de pessoas saudáveis (grupo controle). Foram avaliadas 239 amostras de fezes (152 diarréicas e 87 controles). Para o isolamento de bactérias enteropatogênicas, utilizou-se meios seletivos indicadores e de enriquecimento. A caracterização bioquímica foi realizada utilizando os Sistemas API-20E e a sorológica, através de antissoros polivalentes e monovalentes. Para a detecção das categorias patogênicas de E. coli (EPEC- E. coli enteropatogênica, ETEC- E. coli enterotoxigênica, STEC- E. coli produtora de toxina de Shiga, EIEC- E. coli enteroinvasora e EAEC-E. coli enteroagregativa) foram executados ensaios de PCR Multiplex (M-PCR) utilizando-se primers específicos para cada fator de virulência. Quanto às categorias patogênicas de E. coli, a ETEC 7,2% (12/152) foi o patotipo mais frequente nos casos de diarreia aguda, seguido de EAEC 5,9% (9/152), enquanto nos controles, a maior proporção 9,2% (8/87) foi representada pela EAEC seguido de EPEC 2,3% (2/87). Das oito E. coli enteropatogênicas (EPEC) identificadas, apenas uma foi EPEC típica e das doze E. coli enterotoxigênica (ETEC) isoladas, cinco apresentaram os genes codificadores de ST, quatro de LT e ST e três de LT. Os ensaios da PCR multiplex mostraram-se, portanto uma ferramenta importante na identificação rápida das categorias de E. coli patogênicas, podendo ser utilizados rotineiramente no diagnóstico molecular dessas categorias.
PALAVRAS-CHAVE: Escherichia coli patogênica, diarreia; PCR Multiplex.

1 I INTRODUCTION

Diarrheal diseases are an important public health problem, being one of the most important causes of mortality and morbidity among children under five years old, mostly in developing countries, and with predisposing factors as early breastfeeding

abandonment, low educational level and unsatisfactory sanitary hygienic conditions (Fiedoruk et al, 2015). In Brazil, the regions with the highest occurrence of these diseases are the North and Northeast, as they concentrate the poorest populations in the country (Bühler et al., 2014).

A wide variety of pathogens (viruses, bacteria and protozoa) are implicated in the genesis of diarrheal disease, presenting higher prevalence and importance in childhood, especially rotavirus (Lanata et al., 2013). Regarding bacterial etiology, E. coli is considered to be the pathogen most often related to endemic forms of acute diarrhea in children (Bonkoungou et al., 2013). In addition to enteropathogenic E. coli (EPEC) related to intestinal infections, other categories of E. coli are involved such as enterotoxigenic E. coli (ETEC), Shiga toxin producer E. coli (STEC), enteroinvader E. coli (EIEC), enteroaggregative E. coli (EAEC) and diffusely adhering E. coli (DAEC) (Nejma et al., 2014).

Enteropathogenic E. coli is divided into two categories, typical EPEC: presents eaeA gene and plasmid EAF and atypical EPEC: presents only eaeA gene. Typical EPEC is well recognized as a major cause of childhood gastroenteritis; on the other hand, the role of atypical EPEC in childhood diarrhea remains controversial. However, many studies have shown some significant association between atypical EPEC and diarrhea. STEC induced diarrheal manifestations are attributed to two enterotoxins, called SLT-I and SLT-II (Shiga Like Toxin), also known as Verotoxins (VT-1 and VT-2) and Shiga toxin (Stx-1 and Stx-2) (Croxen et al., 2013).

The mechanism which ETEC promotes diarrhea is well known: adherence in the intestinal mucosa and elaboration of enterotoxins. Adherence to the intestinal mucosa is related to adhesins known as Pili type 1 and CFA I and II (colonization factors). From the colonization begins the elaboration of enterotoxins. ETEC strains produce two types of enterotoxins: thermolabile toxins LT - I and LT - II (heat-labile toxin) and thermostable toxins STa and STb (heat-stable toxin) (Dubreuil; Isaacson; Schifferli, 2016).

The typical disease caused by EAEC is characterized by secretory, mucoid and watery diarrhea and in some cases has little or no symptoms such as vomiting and low fever. The most studied virulence factor is aggR, the main regulator of EAEC virulence gene, which controls the expression of adhesion factors, dispersin protein, and a set of genes encoded by the EAEC chromosome. However, the frequency of these pathogens is underestimated because its detection requires more sensitive and specific diagnostic methods that are not used in clinical practice, which restricts the use of efficient ways for epidemiological control and prevention (Croxen et al., 2013).

The diagnosis of DEC is made difficult by the fact that these organisms cannot be identified based only on biochemical criteria since they are generally indistinguishable from non-pathogenic E. coli. Differentiation between E.coli pathotypes requires the use of immunoassays, cell culture or molecular techniques (Croxen et al., 2013).

In molecular assays using the PCR technique it is possible to detect genes involved with the pathogenicity of several bacterial isolates, allowing rapid identification

with accurate results since this technique has high sensitivity and specificity (Wang et al ,2014). In this way, the present study used the multiplex PCR methodology to evaluate the applicability of this assay to determine the diarrheagenic E. coli categories isolated from individuals with and without diarrhea.

2 I MATERIAL AND METHODS

2.1 SAMPLING

From February and June 2008 to July 2009, 850 isolated samples from 239 individuals (152 diarrheics-three or more evacuations/ day and 87 controls-absence of diarrhea in the last 15 days) were evaluated for virulence factors. of the E. coli diarrheagenic categories, from different age groups (54.4% were under 10 years old) and sex attended at three health units and a public hospital in the county of Juruti, State of Pará.

The study was approved by the Human Research Ethics Committee of the Evandro Chagas Institute, SVS / MS, under Protocol CEP/IEC-No. 0013/2009, CAAE No. 0016.0.072.000-09, of August 27, 2009.

2.2 DIARRHEAGENIC E. coli MOLECULAR RESEARCH

E. coli samples previously stored on Luria agar (Difco) were biochemically reidentified and then cultured on nutrient agar (Difco) at 35 - 37 ° C for 18 - 24 hours. The reference strains used as positive controls were Escherichia coli: EPEC E2348 / 69 (eae and bfpA gene), EAEC O42 (aggR gene), ETEC H10407 (elt and est gene), EIEC EDL1284 (ipaH gene) and EHEC EDL931 (stx gene) and Escherichia coli K12 DH5α as negative control. All of them were kindly provided by Dr. Tânia Vaz of the Adolfo Lutz Institute/SP.

2.2.1 Bacterial DNA Extraction and Primer Selection

The DNA from the phenotypically characterized isolates as E. coli and from the reference strains of the positive and negative controls was extracted by boiling and freezing method, following the recommendations of Starnbach et al. (1989) e Baloda et al (1995). Different oligonucleotide sequences were selected based on the work developed by Aranda et al. (2007) (Table 1).

Designation	Oligonucleotides sequence (5´- 3´)	Target gene	Amplification products	References
eae-1 eae-2	CTGAACGGCGATTACGCGAA CGAGACGATACGATCCAG	eae	917 pb	Aranda et al. (2004)
BFP-1 BFP-2	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	b*f*pA	326 pb	Aranda et al. (2004)
aggRks-1 aggRksa-2	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	aggR	254 pb	Toma et al. (2003)

LT-f LT-r	GGCGACAGATTATACCGTGC CGGTCTCTATATTCCCTGTT	elt	450 pb	Aranda et al. (2004)
ST-f ST-r	ATTTTTMTTTCTGATTTRTCTT CACCCGGTACARGCAGGATT	est	190 pb	Aranda et al. (2004)
IpaH-1 IpaH-2	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	ipaH	600 pb	Aranda et al. (2004)
VTcom-u VTcom-d	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	stx1/ stx2	518 pb	Toma et al. (2003)

Table 1 - Oligonucleotides used in multiplex PCR and their respective amplification products.

2.2.2 Multiplex PCR

The PCR reaction was performed from 2 µL of each extracted DNA and 23 µL of the mix solution, containing between 0.5 to 1.5 µL according to each primer (Invitrogen, Brazil), 10 mM dNTP mix dATP, dCTP, dGTP, dTTP (Invitrogen, Brazil Invitrogen, USA), 0.5 U Taq DNA Platinum Polymerase, Taq 1X Buffer, 50 mM MgCl2 (Invitrogen, Brazil) and ultra pure sterile water to a final volume of 25 μL. The multiplex PCR preparations were placed on the VeritiTM 96-Well Thermal Cycler (Applied Biosystems - USA) automatic gradient thermal cycler model and subjected to specific amplification cycles consisting of 1 2 min step at 50 °C (Hot-Start), 1 step 5 min. at 95 °C (Initial Denaturation) followed by 40 cycles of 1 s at 95 °C, 50 °C and 72 °C and 1 final extension step of 7 min at 72 °C.

Amplicons were analyzed by electrophoresis on 2% agarose gel, stained with ethidium bromide (10 mg / mL) in TBE buffer (0.89M Tris base, 0.45M Boric Acid, 1mM EDTA, pH 8.4) and visualized under UV light with the aid of a transluminator (Vilber Lourmat, France). As molecular size marker was used the 1 Kb plus-Invitrogen Ladder, Brazil. Subsequently, the gel was photographed by a photodocumentation system, Biomaging Systems (UPV, U.S.A).

3 I RESULTS

The results obtained by multiplex PCR were able to reproduce the results generated by the PCR protocols developed by Aranda et al. (2007) highlighting the cost savings due to the use of a more affordable Taq DNA polymerase. We identified 39 diarrheagenic E. coli isolates (17 EAEC, 12 ETEC, 8 EPEC, 1 EIEC and 1 STEC), distributed among patients with acute diarrhea with 17.8% (27/152) and 13, 9% in the control group (12/87) (Table 1). There was no statistically significant difference between the diarrheagenic E. coli distribution in the diarrheal and control groups ($p = 0.5340$); on the other hand, the frequency of pathogenic E. coli categories was statistically different between them ($p < 0.0001$). The largest number of positive samples was observed in the age group of 1-9 years old, followed by 20-29 years.

In the multiplex PCR assay all seven molecular targets were amplified (Figure 1). Enterotoxigenic E. coli was the most frequent (7.2%) in patients with acute diarrhea,

while in controls, the highest proportion (9.2%) was represented by enteroaggregative E. coli (Table 1). However, none of the diarrheagenic E. coli categories were associated with the diarrheal group (Table 2).

Of the 24 positive samples, diarrheagenic E. coli infections were unique: 10 ETEC, 8 EAEC, 5 EPEC and 1 STEC. Of the 8 enteropathogenic E. coli positive samples, 7 were atypical EPECs and 12 of the enterotoxigenic E. coli isolates, 5 had ST, 4 LT and ST and 3 LT coding genes.

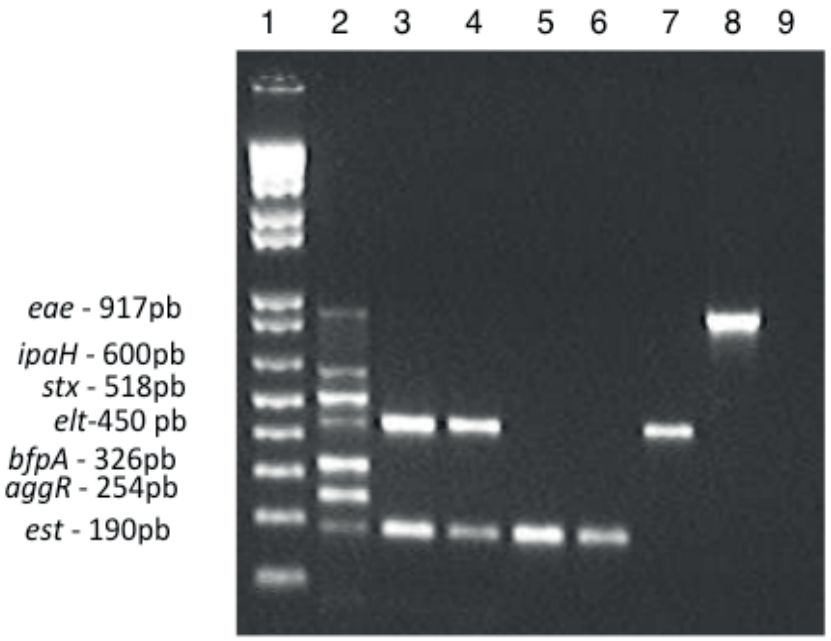

Figure 1 - Multiplex PCR assay, 1 - Ladder 1Kb, 2 - positive control (mix of the 5 standard diarrheagenic E. coli categories). Clinical samples: 3 and 4 - ETEC (elt, est), 5 and 6 - ETEC (est), 7 - ETEC (elt), 8 - EPEC (eae) and 9 - negative control (E.coli K12 DH5α).

Diarrheagenic E. coli	Group						(p)
	Diarrheal		Control		Total		
	%	(NP/NA)	%	(NP/NA)	%	(NP/NA)	
EAEC	5,9	(9/152)	9,2	(8/87)	7,1	(17/239)	0,4977
ETEC	7,2	(11/152)	1,2	(1/87)	5,0	(12/239)	0,0561
EPEC	4,0	(6/152)	2,3	(2/87)	3,3	(8/239)	0,7555
EIEC	0,7	(1/152)	-	(0/87)	0,4	(1/239)	0,7812
STEC	-	(0/152)	1,2	(1/87)	0,4	(1/239)	0,7812
Total	**17,8**	**(27/152)**	**13,9**	**(12/87)**	**16,2**	**(39/239)**	

Table 2 - Distribution of diarrheagenic Escherichia coli categories isolated from individuals in the diarrheal and control group, Juruti, Pará. February and June 2008 to July 2009.

* NP / NA: Number of positive samples/number of samples evaluated.

To demonstrate the effectiveness of the used multiplex PCR assays, we compared the results obtained from the 39 diarrheagenic E. coli samples identified with 39 non-diarrheagenic samples, both detected using the multiplex and monoplex PCR protocol, respectively. Figure 2 shows the amplification products and molecular sizes of the

pathogenic E. coli virulence factor gene fragments of the reference strains and one representative of the clinical sample for each target searched. Table 3 shows the full agreement on multiplex and monoplex PCR results, demonstrating positive results for diarrheagenic E. coli and negative for non-diarrheagenic E. coli.

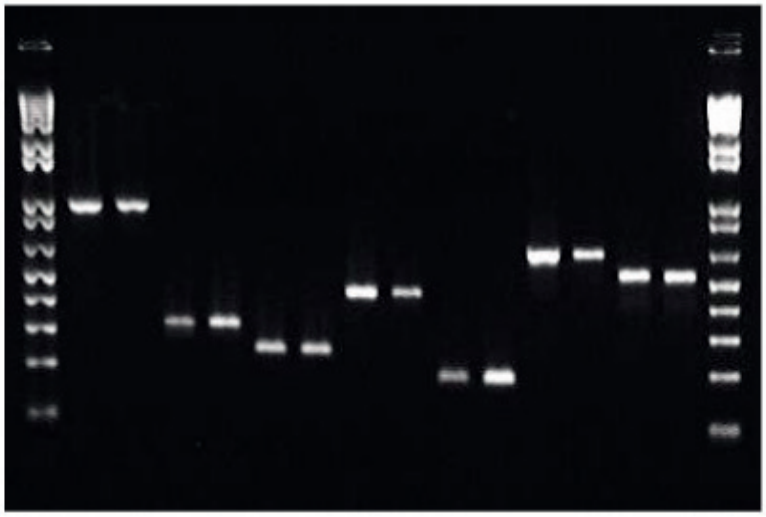

Figure 2 - Electrophoresis of conventional PCR products. 1kb ladder (1 and 16); EPEC E2348/69 - eae and bfpA (2 and 4); EPEC - eae and bfpA (3 and 5); EAEC O42 - aggR (6), EAEC (7); ETEC H10407 - elt and est (8 and 10), ETEC - elt and est (9 and 11); EIEC EDL1284 - ipaH (12), EIEC - (13); STEC EDL931 - stx1/stx2 (14) and STEC - stx1/stx2 (15).

Diarrheagenic Escherichia coli	**Numbe of samples**	**Targets**	**Multiplex**	**Monoplex**
Typical EPEC	1	eae, bfpA	1	1
Atypical EPEC	7	eae	7	7
ETEC	3	elt	3	3
	5	est	5	5
	4	elt, est	4	4
EAEC	17	aggR	17	17
EIEC	1	ipaH	1	1
STEC	1	stx-1/ srx-2	1	1
E. coli	39		39	39

Table 3 - Comparison of positive results obtained by multiplex and monoplex PCR.

4 I DISCUSSION

Diarrheagenic Escherichia coli are recognized as emerging etiology pathogen, as a cause of childhood diarrhea, especially in developing countries, and the epidemiological significance of each type of pathogenic E. coli in childhood diarrhea varies greatly with geographic area (Nguyen et al., 2006; Paniagua et al., 2007). The proportion of isolated diarrheagenic Escherichia coli in the diarrheal group was 12.8% (27-118) versus 5.7% (12-36) in the control group. Of the 39 pathogenic E. coli pathotypes isolated, 25 were present in children under 10 years of age. These data are similar to those observed by Nguyen et al. (2006) and Samal et al. (2008).

In patients with acute diarrhea, ETEC (7.2%) had the highest frequency, followed by EAEC (5.9%) and EPEC (4%). However, these E. coli pathotypes were not statistically associated with the diarrheal group, corroborating the findings of Bueris et al. (2007), where the prevalence of E. coli types identified in Bahia were not significantly different between individuals in the diarrheal and control group. However, these findings differed from most studies that the frequency of diarrheagenic E. coli in patients with acute diarrhea is higher compared to those without diarrhea (Nguyen et al., 2005; Rappelli et al., 2005; Orlandi et al., 2006; Paniagua et al., 2007; Hien et al., 2007).

In Mexico and other developing countries, ETEC is the most prevalent category of diarrheagenic E. coli. This bacteria is considered a major pathogen of childhood diarrhea, especially during the first 6 months of life, where isolation rates range from 10 to 30% and is also responsible for most episodes of gastroenteritis among travelers (Nguyen et al., 2006; Paniagua et al., 2007). In this study, the presence of ETEC in the diarrheal group was 7.2% and 1.2% in the control group. This finding was similar to that found by Nguyen et al. (2006). However in most studies, ETEC is strongly associated with patients in the diarrheal group (Haque et al., 2003; Rappelli et al., 2005; Hien et al., 2007; Paniagua et al., 2007; Al-Gallas et al. al., 2007).

Of the twelve enterotoxigenic E. coli (ETEC) isolated: five were positive for the ST toxin gene, four for both LT and ST toxin genes and three for LT. Diversity in ETEC strains can be observed in different studies in the literature that address molecular and epidemiological investigations. Among these studies, the question remains if the amount of gallbladder-linked LT is sufficient to induce ETEC diarrhea (Sánchez and Holmgren, 2005). A study done in Japan where PCR stool samples from people with symptoms such as diarrhea, fever and abdominal pain were found to have only the ST toxin genes (Tsuji et al., 2002).

In the present study, a low frequency of enteropathogenic E. coli was found in patients with acute diarrhea (4.0%) and a proportion of 2.3% in controls (Table 1). This result was similar to that found by Hien et al. (2007), that although EPEC was more frequent in cases (2.8%) than in controls (0.8%), it was not statistically significant associated with diarrhea. However, these findings differed from several studies, which point to EPEC as one of the major enteropathogens responsible for pediatric episodes of diarrhea, especially in poorly developed countries, where sanitary conditions are still very poor (Gunzburg et al., 1995; Dulguer et al., 2003; Alikhani et al., 2006; Orlandi et al., 2006; Nguyen et al., 2006; Paniagua et al., 2007; Ochoa et al., 2008).

Enteropathogenic E. coli is not common in developed countries, as it presents, for example, the best sanitation conditions. In the state of São Paulo, EPEC was one of the main pathogens observed in children, however, some evidence suggests that they are not very common in rural areas and/or smaller cities, which may explain the low frequency found at this study (Schnack et al., 2003).

In the control group, EAEC was the most frequent category (9.2%), followed by EPEC, with 2.3%. These results were similar to those of Nguyen et al. (2006), where

EAEC was the most frequent bacteria in controls (7.2%), followed by EPEC (4.4%). EAEC has been increasingly recognized as an important emerging enteric pathogen, widely distributed worldwide (Okeke et al., 2003; Huang et al., 2007);

When comparing the results between diarrheal and controls, it was observed that there was no statistically significant difference between the distribution of diarrheagenic E. coli in the diarrheal and control groups ($p = 0.5340$), on the other hand, the frequency of the E. coli pathogenic categories was statistically different between them ($p < 0.0001$). The results showed that the most frequent agent enteroaggregative E. coli (EAEC) was isolated in 5.9% of the population with diarrhea studied. In the present study, EAEC samples were also found in control group feces (9.2%). The results of the present study corroborate those found in a study conducted in Rio de Janeiro on the frequency of isolated diarrheagenic E. coli in children with and without diarrhea. EAEC was the most frequent category, with 14.6% of isolates. in patients with acute diarrhea and 11.1% in controls, suggesting that EAEC has a potential role as an enteric pathogen in the investigated community (Mangia et al., 2004).

In the present study enteroinvader E. coli was isolated in only 1 patient with acute diarrhea. This data was consistent with other studies in the literature, which estimate, for developing countries, including Brazil, isolation of this agent in proportions ranging from 1% to a maximum of 7% of cases (Tornieporth et al., 1995; Toporovski et al. al., 1999; Franzolin et al., 2005; Nguyen et al., 2006; Paniagua et al., 2007; Al-Gallas et al., 2007).

Shiga toxin producer Escherichia coli (STEC) is widely distributed worldwide, and is one of the most studied bacteria in the United States, causing more than 73,000 diarrheal episodes in the United States each year (Paniagua et al., 2007). It was not detected in any patient of the diarrheal group in the present study, but it was identified in one patient of the control group, similar results were observed by Toporovski et al. (1999) and Paniagua et al. (2007). This pathotype has recently been described as an emerging pathogen worldwide (Paniagua et al., 2007). However in Iran STEC is one of the most important causes of diarrhea, accounting for 44.7% of diarrheal isolates (Jafari et al., 2008).

In Brazil, STEC infections have been mainly associated with sporadic cases of non-bloody diarrhea, particularly in children (Guth et al., 2005). It is frequently detected in food and animal reservoirs in Brazil and other Latin American countries, and several animal serotypes have been identified associated with human diarrheal disease (Irino et al., 2004; Bastos et al., 2006; Bueris et al., 2007).

5 I CONCLUSION

Multiplex PCR assays have proved therefore to be an important tool for the rapid identification of pathogenic E. coli categories and can be routinely used in the molecular diagnosis of these categories in place of monoplex PCR. The present study also shows

the importance of diarrheagenic E. coli in cases of acute diarrhea in the municipality of Juruti, Pará, providing important support to diarrheal disease prevention and control measures in the region.

REFERENCES

AL-GALLAS, N., BAHRI, O., BOURATBEEN, A., HAASEN, A.B., AISSA, R.B. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on Diarrheagenic Escherichia coli: prevalence, phenotyping, and molecular epidemiology. **The American Society of Tropical Medicine and Hygiene, 77**: 571-582, 2007.

ALIKHANI, M.Y., MIRSALEHIAN, A., ASLANI, M.M. Detection of typical and atypical enteropathogenic Escherichia coli (EPEC) in Iranian children with and without diarrhoea. **Journal of Medical Microbiology, 55**: 1159-1163, 2006.

ARANDA, K.R.S., NETO, U.F., SCALETSKY, I.C.A. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. **Journal of Clinical Microbiology, 42**: 5849-5853, 2004.

ARANDA, K.R.S., FABBRICOTTI, S.H., NETO, U.F., SCALETSKY, I.C.A. Single multiplex assay to identify simultaneously enteropatogenic, entroaggregative, enterotoxigenic, enteroinvasive and Shiga toxin-producing Escherichia coli strains in Brazilian children. **Federation of European Microbiological Societies, 26**7: 145-150, 2007.

BALODA, S.B., KROVACEK, K., ERICSSON, L., LINNE, T., MANSSON, I. Detection of aerolysin gene in Aeromonas strains isolated from drinking water, fish, and foods by polymerase chain reaction. **Comparative Immunology and Microbiology Infectious Deseases, 18**: 17-26, 1995.

BASTOS, F.C., VAZ, T.M.I, IRINO, K., GUTH, B.E.C. Phenotypic characteristics, virulence profile and genetic relatedness of O157 Shiga toxin-producing Echerichia coli isolated in Brazil and other Latin American countries. **Federation of European Microbiological Societies, 265**: 89-97, 2006.

BONKOUNGOU, I.J.O.; HAUKKA, K.; ÖSTERBLAD, M.; HAKANEM , A; J.;TRAORÉ, A.; BARROS,N.; SIITONEN. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. **BMC Pediatrics**, 2013.

BUERIS, V., SIRCILI, M.P., TADDEI, C.R., SANTOS, M.F.D., FRANZOLIN, M.R., MARTINEZ, M.B., FERRER, S.R., BARRETO, M.L., TRABULSI, L.R. Detection of diarrheagenic Escherichia coli from children with and without diarrhea in Salvador, Bahia, Brazil. **Memórias do Instituto Oswaldo Cruz, 102**: 839-844, 2007.

CROXEN MA, LAW RJ, SCHOLZ R, KEENEY KM, WLODARSKA M, FINLAY BB. Recent advances in understanding enteric pathogenic Escherichia coli. **Clin Microbiol** Rev.26(4):822–880. 2013.

DUBREUIL J, ISAACSON R, SCHIFFERLI D. Animal Enterotoxigenic Escherichia coli, **EcoSal** Plus 2016.

DULGUER, M.V., FABBRICOTTI, S.H., BANDO, S.Y., FILHO, C.A.M., NETO, U.F., SCALETSKY, I.C.A. Atypical enteropathogenic Escherichia coli strains: phenotypic and genetic profiling reveals a strong association between enteroaggregative E. coli heat-stable enterotoxin and diarrhea. **The Journal of Infectious Diseases, 18**8: 1685-1694, 2003.

FIEDORUK, K.; DANILUK, T.; ROZKIEWICZ, D.; ZAREMBA, M.L.; OLDAK, E.; SCIEPUK, M.; LESZCZNSKA, K. Conventional and molecular methods in the diagnosis of community-acquired diarrhoea in children under 5 years of age from the north-eastern region of Poland. **International. J. of Infectious Diseases** 37: 145151, 2015.

FRANZOLIN, M.R. In: **Microbiologia**. Trabulsi, L.R. & Alterthum, F. (eds). Atheneu, 2005. p. 345-346.

GUNZBURG, S.T., TORNIEPORTH, N.G., RILLEY, L.W. Identification of Enteropathogenic Escherichia coli by PCR-Based Detection of the Bundle-Forming Pilus Gene. **Journal of Clinical Microbiology, 33**: 1375-1277, 1995.

GUTH, B.E.C., GOMES, T.A.T., VAZ, T.M.I., IRINO, K. Inability to decarboxylate lysine as a presumptive marker to identify Shiga toxin-producing Escherichia coli strains of serogroup O111. **Journal of Clinical Microbiology, 41**: 3450p. 2003.

HAQUE, R., MONDAL, D., KIRKPATRICK, B.D., AKTHER, S., FARR, B.M., SACK, R.B., PETRI, W.A. Epidemiologic and clinical characteristics of acute diarrhea with emphasis on Entamoeba histolytica infections in preschool children in an urban slum of Dhaka, Bangladesh. **The American Society of Tropical Medicine and Hygiene, 69(4):** 398-405, 2003.

HIEN, B.T.T., SCHEUTZ, F., CAM, P.D., SERICHANTALERGS, O., HUONG, T.T., THU, T.M., DALSGAARD, A. Diarrheagenic Escherichia coli and Shigella strains isolated from children in a Hospital Case-Control Study in Hanoi, Vietnam. **Journal of Clinical Microbiology, 46**: 996-1004, 2007.

HUANG, D.B., MOHAMED, J.A., NATARO, J.P., DUPONT, H.L., JIANG, Z.D., OKHUYSEN, P.C. Virulence characteristics and the molecular epidemiology of enteroaggregative Escherichia coli isolates from travelers to developing countries. **Journal of Medical Microbiology, 56**: 1386-1392, 2007.

IRINO, K., KATO, M.A.M.F., VAZ, T.M.I., RAMOS, I.I., SOUZA, M.A.C., CRUZ, A.S., GOMES, T.A.T., VIEIRA, M.A.M., GUTH, B.E.C. Serotypes and virulence markers of Shiga toxin-producing Escherichia coli (STEC) isolated from dairy cattle in São Paulo state, Brazil. **Veterinary Microbiology, 105**: 29-36, 2004.

JAFARI, F., SHOKRZADEH, L., HAMIDIAN, M., AHRABI, S.S., ZALI, M.R. Acute diarrhea due to enteropathogenic bactéria in patients at hospitals in Tehran. **Journal of Infectious Diseases, 61**: 269-273, 2008.

LANATA CF, FISCHER-WALKER CL, OLASCOAGA AC, TORRES CX, ARYEE MJ, BLACK RE. Global causes of diarrheal disease mortality in children, 5 years of age: a systematic review. **PLoS One** 2013.

MANGIA, A.H.R., GUTH, B.C., ANDRADE, J.R.C., IRINO, K., PACHECO, A.B.F., FERREIRA, L.C.S., ZAHNER, V., TEXEIRA, L.M. Genotypic and phenotypic characterization of enterotoxigenic Escherichia coli (ETEC) strains isolated in Rio de Janeiro city, Brazil. **Federation of European Microbiological Societies Immunology and Medical Microbiology, 40**: 155-162, 2004.

NEJMA, B.S.B.; ZAAFRANE, M. H.; HASSINE, F.; LOULIZI, K.S.; SAID, M.B.; AOUNI, M.; MZOOGHI, R. Etiology of Acute Diarrhea in Tunisian Children with Emphasis on Diarrheagenic Escherichia coli: Prevalence and Identification of E. coli Virulence Markers. **Iranian J Publ Health**, No. 7, Vol. 43, p. 947-960, Jul, 2014.

NGUYEN, T.V., VAN, P.L., HUY, C.L., GIA, K.N., WEINTRAUB, A. Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. **International Journal of Infectious Diseases, 10**: 298-308, 2006.

NGUYEN, T.V., VAN, P.L., HUY, C.L., GIA, K.N., WEINTRAUB, A. Detection and characterization of diarrheagenic Escherichia coli from young children in Hanoi, Vietnam. **Journal of Clinical Microbiology, 43**: 755-760, 2005.

OCHOA, T.J., BARLETTA, F., CONTRERAS, C., MERCADO, E. New insights into the epidemiology of enteropathogenic Escherichia coli infection. **National Institutes of Health, 102**: 852-856, 2008.

OKEKE, I.N., OJO, O., LAMIKANRA, A., KAPER, J.B. Etiology of acute diarrhea in adults in Southwestern Nigeria. **Journal of Clinical Microbiology, 41**: 4525-4530, 2003.

ORLANDI, P.P., MAGALHÃES, G.F., MATOS, N.B., SILVA, T., PENATTI, M., NOGUEIRA, P.A., SILVA, L.H.P.DA. Etiology of diarrheal infections in children of Porto Velho, Rondonia, Western Amazon region, Brazil. **Brazilian Journal of Medical and Biological Research, 39**: 507-517, 2006.

PANIAGUA, G.L., MONROY, E., GONZÁLEZ, O.G., ALONSO, J., NEGRETE, E., VACA, S. Two or more enteropathogens are associated with diarrhoea in Mexican children. **Annals of Clinical Microbiology and Antimicrobials,** 6: 17, 2007.

RAPPELLI, P., FOLGOSA, E., SOLINAS, M.L., DACOSTA, J.L., PISANU, C., SIDAT, M., MELO, J., CAPPUCCINELLI, P., COLOMBO, M.M. Pathogenic enteric Escherichia coli in children with and without diarrhea in Maputo, Mozambique. **Federation of European Microbiological Societies Imumunology and Medical Microbiology, 43**: 67-72, 2005.

SAMAL, S.K., KHUNTIA, S.H.K., NANDA, P.K., SATAPATHY, C.S., NAYAK, N.R., SARANGI, A.K., SAHOO, N., PATTNAIK, S.K., CHHOTRAY, G.P., PAL, B.B. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. **Journal Infectious Diseases, 61**: 350-355, 2008.

SÁNCHEZ, J.; HOLMGREN, J. Virulence factors, pathogenesis and vaccine protection in cholera and ETEC diarrhea. **Current Opinion in Immunology**, Volume 17, Issue 4, 2005.

SCHNACK, F.J., FONTANA, L.M., BARBOSA, P.R., SILVA, L.S.M., BAILLARGEON, C.M.M., BARICHELLO, T., PÓVOA, M.M., CAVASINI, C.E., MACHADO, R.L.D. Enteropatógenos associados com diarréia infantile (< 5 anos de idade) em amostra da população da área metropolitana de Criciúma, Santa Catarina, Brasil. **Caderno de Saúde Pública do Rio de Janeiro, 19:** 1205-1208, 2003.

STARNBACH, M.N., FALKOW, S., TOMPKINS, L.S. Species-specific detection of Legionella pneumophila in water by DNA amplification and hibridization. **Journal of Clinical Microbiology, 2**7: 1257-1261, 1989.

TOMA, C., LU, Y., HIGA, N., NAKASONE, N., CHINEN, I., BASCHKIER, A., RIVAS, M., IWANAGA, M. Multiplex PCR assay for identification of human diarrheagenic Escherichia coli. **Journal of Clinical Microbiology, 41**: 2669-2671, 2003.

TOPOROVSKI, M.S., MÍMICA, I.M., CHIEFF, P.P., PASCHOALOTTI, M.A., DIAS, A.M.G., SILVA, C.B. Diarréia aguda em crianças menores de 3 anos de idade: recuperação de enteropatógenos nas amostras fecais de pacientes comparada à grupo controle. **Journal of Pediatric, 75**: 97-104, 1999.

TORNIEPORTH, N.G., JOYLENE, J., SALGADO, K., JESUS, P., LATHAM, E., MELO, M.C.N.M., GUNZBURG, S.T., RILEY, L.W. Differentiation of pathogenic Escherichia coli strains in Brazilian children by PCR. **Journal of Clinical Microbiology, 33**: 1371-1374, 1995.

TSUJI, H.; OSHIMA, K.; ISHIKAWA, K.; HAYASHI, K. An outbreak of heat-stable enterotoxin-producing Escherichia coli O25:HNM resistant to nalidixic acid caused by ingestion of contaminated box-lunch in September-october 2002 in Hyogo and shiga Prefectures. **Japanese Journal Infection Disease**, v. 55, p. 2015-206, 2002.

Wang J. Z., Duan R., Liang J. R., Huang Y., Xiao Y. C., Qiu H. Y., et al. Real-time TaqMan PCR for Yersinia enterocolitica detection based on the ail and foxA genes. **J. Clin. Microbiol**. 52, 2014.

SOBRE O ORGANIZADOR:

Benedito Rodrigues da Silva Neto: Possui graduação em Ciências Biológicas pela Universidade do Estado de Mato Grosso (2005), com especialização na modalidade médica em Análises Clínicas e Microbiologia (Universidade Candido Mendes - RJ). Em 2006 se especializou em Educação no Instituto Araguaia de Pós graduação Pesquisa e Extensão. Obteve seu Mestrado em Biologia Celular e Molecular pelo Instituto de Ciências Biológicas (2009) e o Doutorado em Medicina Tropical e Saúde Pública pelo Instituto de Patologia Tropical e Saúde Pública (2013) da Universidade Federal de Goiás. Pós-Doutorado em Genética Molecular com concentração em Proteômica e Bioinformática (2014). O segundo Pós doutoramento foi realizado pelo Programa de Pós-Graduação Stricto Sensu em Ciências Aplicadas a Produtos para a Saúde da Universidade Estadual de Goiás (2015), trabalhando com o projeto Análise Global da Genômica Funcional do Fungo Trichoderma Harzianum e período de aperfeiçoamento no Institute of Transfusion Medicine at the Hospital Universitatsklinikum Essen, Germany. Seu terceiro Pós-Doutorado foi concluído em 2018 na linha de bioinformática aplicada à descoberta de novos agentes antifúngicos para fungos patogênicos de interesse médico.

Palestrante internacional com experiência nas áreas de Genética e Biologia Molecular aplicada à Microbiologia, atuando principalmente com os seguintes temas: Micologia Médica, Biotecnologia, Bioinformática Estrutural e Funcional, Proteômica, Bioquímica, interação Patógeno-Hospedeiro.

Sócio fundador da Sociedade Brasileira de Ciências aplicadas à Saúde (SBCSaúde) onde exerce o cargo de Diretor Executivo, e idealizador do projeto “Congresso Nacional Multidisciplinar da Saúde” (CoNMSaúde) realizado anualmente, desde 2016, no centro-oeste do país.

Atua como Pesquisador consultor da Fundação de Amparo e Pesquisa do Estado de Goiás - FAPEG. Atuou como Professor Doutor de Tutoria e Habilidades Profissionais da Faculdade de Medicina Alfredo Nasser (FAMED-UNIFAN); Microbiologia, Biotecnologia, Fisiologia Humana, Biologia Celular, Biologia Molecular, Micologia e Bacteriologia nos cursos de Biomedicina, Fisioterapia e Enfermagem na Sociedade Goiana de Educação e Cultura (Faculdade Padrão). Professor substituto de Microbiologia/Micologia junto ao Departamento de Microbiologia, Parasitologia, Imunologia e Patologia do Instituto de Patologia Tropical e Saúde Pública (IPTSP) da Universidade Federal de Goiás. Coordenador do curso de Especialização em Medicina Genômica e Coordenador do curso de Biotecnologia e Inovações em Saúde no Instituto Nacional de Cursos. Atualmente o autor tem se dedicado à medicina tropical desenvolvendo estudos na área da micologia médica com publicações relevantes em periódicos nacionais e internacionais. Contato: dr.neto@ufg.br ou neto@doctor.com

ÍNDICE REMISSIVO

V

X

2020