

Characterization of enteroaggregative *Escherichia coli* among diarrheal children in Western Brazilian Amazon

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ABSTRACT – Background – Enteroaggregative *Escherichia coli* (EAEC) is one of the main acute and chronic diarrhea causes both in children and adults, mainly in developing countries. **Objective** – The aim of the present study is to characterize EAEC strains isolated from faecal samples and to identify genes potentially contributing to virulence, biofilm production and antimicrobial resistance in children admitted to a pediatric hospital in Porto Velho, Rondônia State. **Methods** – The total of 1,625 *E. coli* specimens were isolated from 591 children in the age group 6 years or younger who were hospitalized in Cosme and Damião Children Hospital in Porto Velho, between February 2010 and February 2012, with acute gastroenteritis. Colonies suggestive of *E. coli* were subjected to polymerase chain reaction testing in order to identify the virulence factors. The in vitro adherence assays using HEP-2 adherence were tests. Biofilm detection through spectrophotometry and antimicrobial susceptibility tests were conducted in the disk diffusion method. **Results** – The mentioned study examined 591 stool samples from children with diarrhea. Diarrheogenic *E. coli* was found in 27.4% (162/591) of the children. EAEC was the diarrheagenic *E. coli* most frequently associated with diarrhea 52.4% (85/162), which was followed by enteropathogenic *E. coli* 43.8% (71/162), enterotoxigenic *E. coli* 2.4% (4/162), and enterohemorrhagic *E. coli* 1.2% (2/162). The *aggR* gene was detected in 63.5% (54/85) of EAEC isolates; moreover, statistically significant correlation was observed among typical EAEC (*aggR*) and *aatA* ($P < 0.0001$), *irp2* ($P = 0.0357$) and *shf* ($P = 0.0328$). It was recorded that 69% (59/85) of the 85 analyzed EAEC strains were biofilm producers; 73% (43/59) of the biofilm producers carried the *aggR* gene versus 42.3% (11/26) of non-producers ($P = 0.0135$). In addition, there was association between the *aatA* gene and biofilm production; 61% (36/59) of the samples presented producer strains, versus 19.2% (5/26) of non-producers ($P < 0.0004$). Antibiotic sensitivity test evidenced that most EAEC were ampicillin 70.6% (60/85), sulfamethoxazole 60% (51/85), tetracycline 44.7% (38/85) and cefotaxime 22.4% (19/85) resistant. **Conclusion** – As far as it is known, the present study is pioneer in Northern Brazil to investigate EAEC virulence factors and to show the antimicrobial susceptibility of EAEC strains isolated from children with diarrhea.

HEADINGS – *Escherichia coli*, classification. Infantile diarrhea. Child. Virulence, genetics.

INTRODUCTION

According to the World Health Organization (WHO), diarrhea is the second leading morbidity and mortality cause among children in the age group 5 years or younger. Diarrhea resulted in 530,000 deaths in 2015, or in approximately 1,400 deaths per day, worldwide⁽¹⁾. The main diarrheal-syndrome causes in children include inappropriate hygiene, insufficient water and food sanitation, besides lack of adequate health infrastructure⁽²⁾. Several viral, bacterial and parasitic agents are associated with diarrhea, among them bacteria belonging to species *Escherichia coli* (which is a natural species composing the intestinal flora). This species is strongly associated with diarrhea cases in children due to the acquisition of specific virulence factors contributing to its pathogenicity, the so-called diarrheagenic *E. coli* (DEC)⁽³⁾.

Diarrheagenic *E. coli* is divided in six pathotypes depending on the pathogenicity mechanism, namely: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli*, enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E. coli*⁽⁴⁾. The EAEC is associated with chronic and persistent diarrhea cases in developing countries, besides being one of the most relevant opportunistic pathogens affecting HIV patients. However, despite the several advances concerning the understanding about this bacterium species, its infection mechanism is not entirely understood because the heterogeneity of the pathogen impairs a proper diagnosis, as well as the understanding about its pathogenicity⁽⁵⁾.

Previous studies have described the three-stage model applied to EAEC pathogenesis: 1- initial adherence to mucosal surface, 2- biofilm formation, and 3- inflammatory response induction and

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toxin release. Several virulence factors affect the infection process; however, they can be used as pathogenesis identification markers⁽⁶⁾.

The EAEC strains are defined in HEp-2 cells through their aggregative adherence (AA) or “stacked brick” phenotype, which is the gold standard method to identify such pathogen. However, the adherence test is not able to distinguish pathogenic from nonpathogenic EAEC strains. In addition, molecular biology assays based on virulence factor detection have been widely used to identify and characterize DEC isolates⁽⁷⁾. The use of molecular techniques, and of epidemiological studies on this isolates, to investigate virulence markers in EAEC is important. Studies have been demonstrating that EAEC strains are heterogeneous and complex groups associated with many virulence factors. Different gene targets have been used to detect EAEC by using polymerase chain reaction (PCR). The knowledge about this genes and their function has been making the development of diagnostic methods and the understanding about the pathogenicity of EAEC possible⁽⁸⁾.

Some studies adopted the PCR technique to conduct EAEC molecular identification of DEC categories or to assess the presence of specific virulence factors associated with genes such as aggregative adherence fimbriae (AAFs), transcriptional activator (*aggR*)⁽⁹⁾, plasmid-encoded toxin (*pet*)⁽¹⁰⁾, Shigella enterotoxin 1 (ShET1)⁽¹¹⁾, EAEC heat-stable enterotoxin (EAST1)⁽¹²⁾, mucinase activity (Pic)⁽¹¹⁾, secreted proteins (*aap*)⁽¹³⁾, dispersin transporter (*aatA*)⁽¹⁴⁾, and yersiniabactin system (*irp2*)⁽¹⁵⁾ associated with genes in EAEC. The *aggR* gene is important for the pathogenesis and adherence properties of EAEC; moreover, the presence or absence of *aggR* is used to classify EAEC as typical or atypical, respectively.

The biofilm formation has been linked to several human diseases. Biofilms are highly organized communities of microorganisms structured within an array of exopolysaccharides (EPS)⁽¹⁶⁾. The bacterial arranged in biofilms tend to be more resistant to antimicrobial therapy, and the ability to biofilm formation in combination with the heterogeneity of virulence genes have been evidenced in EAEC strains⁽¹⁷⁾.

In addition to mechanisms directly related to virulence, the antimicrobial resistance has been identified through clinical and non-clinical EAEC sources. The antimicrobial resistance has been seen as one of the most important factors to help assessing the impact this pathogen has on public health, and EAEC associated with multidrug resistant (MDR) was reported from different parts of the world⁽¹⁹⁾. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories⁽¹⁹⁾.

Since EAEC is one of the main agents causing diarrhea, mainly in developing countries, it is worth conducting characterization and epidemiological surveillance studies, mainly in Northern Brazil, where public and environmental health is directly associated with poverty. Porto Velho is the capital of Rondônia State (Western Brazilian Amazon); the city is among the worst capitals when it comes to basic sanitation. Such poor sanitation condition has strong impact on local health indicators, which depict the epidemiological scenario in the region, as well as the high incidence of diarrheal diseases and infant mortality rates. Given the need of setting the profile of aetiological agents causing acute gastroenteritis, the aim of the present study was to characterize EAEC strains isolated from faecal samples and to identify genes potentially contributing to virulence, biofilm production and antimicrobial resistance in children admitted to a pediatric hospital in Porto Velho, Rondônia.

METHODS

Study site and patients

The total of 1,625 *E. coli* specimens were isolated from 591 children in the age group 6 years or younger who were hospitalized in Cosme and Damião Children Hospital in Porto Velho, between February 2010 and February 2012, with acute gastroenteritis. Cases were defined as acute gastroenteritis when patients presented liquid or semi-liquid stools, and three or more evacuations within 24-h periods. Sample collection was carried out three times a week, for two consecutive years. One fecal sample was collected from each child participating in the experiment. A sterile universal collector was used during the collection procedure. The samples were registered, labeled and stored at -80°C . The experiment was approved by the Ethical Committee of Rondônia Tropical Medicine Research Centre (protocol N. 0113/2010).

Bacteriology

E. coli strains were selected from MacConkey, Salmonella-Shigella and xylose lysine deoxycholate selective agar provided by HiMedia U.S.A selective agar. All colonies were processed through routine microbiological and biochemical tests purchased at bioMérieux France (API20E system). Five colonies suggestive of *E. coli* were subjected to PCR testing in order to identify the virulence factors.

HEp-2 adherence test

All *E. coli* isolates were subjected to HEp-2 adherence tests⁽²⁰⁾. The EAEC 042 was used as aggregative adherence positive control.

Analysis of *E. coli* virulence factors through multiplex polymerase chain reaction (PCR)

The EAEC virulence factors *aggR*, *astA*, and *pic* were identified through PCR by using specific primers as previously described by Müller et al.⁽¹⁸⁾. The *aatA*, *shet1A*, *shf*, *irp2*, and *pet* virulence factors were identified according to the method described by Mohamed et al.⁽¹⁷⁾. The EAEC 042 was used as positive control. Non-pathogenic *E. coli* strain HB101 was used as negative control, as well as to monitor PCR contamination.

Biofilm detection through spectrophotometry

The 96-well polystyrene microtiter plates were used to detect biofilms on polystyrene, according to previously described procedures⁽²¹⁾. The following strains were used to assure the quality of the biofilm assay: *Pseudomonas aeruginosa* PAOI, EAEC 042 – which is a strong biofilm producer, and the non-pathogenic *E. coli* strain HB101 (negative control).

Antimicrobial sensitivity test

Antimicrobial susceptibility tests were conducted in Mueller-Hinton agar (HiMedia U.S.A) through the disk diffusion method, according to Clinical and Laboratory Standards Institute guidelines⁽²²⁾. Gentamicin (GEN 10 μg), imipenem (IMP 10 μg), piperacillin/tazobactam (TZP 10 μg), tetracycline (TET 30 μg), trimethoprim/sulfamethoxazole (SXT 25 μg), amoxicillin/clavulanic acid (AMC 30 μg), amikacin (AMI 30 μg), ampicillin (AMP 10 μg), cefotaxime (CTX 30 μg), and ceftazidime (CFZ 30 μg) antibiotic disks (Sensifar-cefar[®], Brazil) were used. The *E. coli* strain ATCC 25922 was used for quality control in all tests.

Statistical analysis

Data were analyzed through Fisher's exact test conducted in GraphPad Prism 5.0. Results were significant at *P*-values < 0.05.

RESULTS

Pathogens associated with diarrhea

Pathogenic agent isolates analyzed in the study were taken from a study conducted from February 2010 to February 2012 in Porto Velho, Rondonia, Brazil. The mentioned study examined 591 stool samples from children with diarrhea. Diarrheogenic *E. coli* was found in 27.4% (162/591) of the children. EAEC was the DEC most frequently associated with diarrhea – 52.4% (85/162), which was followed by EPEC 43.8% (71/162), ETEC 2.4% (4/162), and EHEC 1.2% (2/162). Among other enteropathogenic bacteria, *Salmonella* sp was found in 7.1% (42/591) cases and *Shigella* species were recorded in 2.1% (13/591) of the cases.

The mean age of the patients was 17.2 months; nonetheless, this pathotype incidence was higher in children under 2 years old, although there was not statistically significant difference.

The total of 85 EAEC isolates (all of them presenting aggregative adherence pattern) from 51 patients were identified through cell assays in order to characterize cellular adhesion. All EAEC strains were subjected to the PCR technique in order to find the virulence factors. Infection caused by EAEC was detected in both male 60.8% (31/51) and female 39.2% (20/51) patients. Typical EAEC (*aggR*-positive) was found in 63.5% (54/85) and atypical EAEC (*aggR*-negative) in 36.5% (31/85) of the strains (TABLE 1).

TABLE 1. Distribution of epidemiological factors among children infected by EAEC.

	Age (in months)				<i>P</i>
	0-6 (n = 134)	7-12 (n = 162)	13-24 (n = 194)	>25 (n = 101)	
Gender					
Male	75 (56)	92 (56.8)	102 (52.6)	56 (55.4)	0.6475
Female	59 (44)	70 (43.2)	92 (47.4)	45 (44.6)	
EAEC	17 (12.7)	23 (14.2)	36 (18.6)	9 (8.9)	
Typical	12 (70.6)	17 (73.9)	20 (55.6)	5 (55.6)	0.4452
Atypical	5 (29.4)	6 (26.1)	16 (44.4)	4 (44.4)	
Other enteropathogens					
EPEC	14 (10.4)	18 (11.1)	26 (13.4)	13 (12.9)	0.8351
EHEC	1 (0.7)	0 (0)	1 (0.5)	0 (0)	-
ETEC	1 (0.7)	0 (0)	2 (1)	1 (1)	-
<i>Salmonella</i> spp	2 (1.5)	4 (2.5)	6 (3.1)	1 (1)	0.621
<i>Shigella</i> spp	7 (5.2)	12 (7.4)	16 (8.2)	7 (6.9)	0.7701

EAEC: enteroaggregative *E. coli*; EPEC: enteropathogenic *E. coli*; EHEC: enterohemorrhagic *E. coli*; ETEC: enterotoxigenic *E. coli*.

All children participating in the study presented typical enteropathogen infection symptoms including diarrhea, vomiting and fever. Bloody diarrhea was recorded in 23.5% (12/51) of the cases and EAEC was the only pathogen detected in 58.3% (7/12) of these cases.

Prevalence of virulence genes

One or more virulence marker genes were detected in all EAEC isolates. The *irp2* gene was the most commonly identified one, since it was recorded in 75.3% (64/85) of the isolates; it was followed by *astA*, *aggR*, and *aatA*, which were found in 64.7%, (55/85), 63.5% (54/85), and 48.2% (41/85) of the isolates, respectively. In addition, many other genes were involved in EAEC pathogenesis, including *set1A*, *afa1*, *shf*, *pic*, and *pet*, these genes were more often found in typical EAEC strains than in atypical EAEC strains. There was statistically significant correlation between typical EAEC and the presence of *aatA* (*P*<0.0001), *irp2* (*P*=0.0357) or *shf* (*P*=0.0328) genes (TABLE 2).

TABLE 2. Incidence of virulence genes in typical and atypical enteroaggregative *E. coli* strains.

Virulence factors	Typical	Atypical	<i>P</i> -value	OR (IC 95%)
	n (%)	n (%)		
<i>set1A</i>	19 (35.2)	14 (45.2)	0.4883	0.66 (0.24–1.8)
<i>astA</i>	37 (68.5)	18 (58.1)	0.3548	1.56 (0.57–4.31)
<i>afa1</i>	19 (35.2)	15 (48.4)	0.2574	0.58 (0.21–1.57)
<i>aatA</i>	36 (66.7)	5 (16.1)	<0.0001	10.08 (3.13–39.43)
<i>Pet</i>	7 (13)	1 (3.2)	0.2483	4.41 (0.52–207.77)
<i>irp2</i>	45 (83.3)	19 (61.3)	0.0357	3.11 (1.01–9.94)
<i>Shf</i>	24 (44.4)	6 (19.4)	0.0328	3.29 (1.08–11.42)
<i>Pic</i>	15 (27.8)	7 (22.6)	0.7975	1.31 (0.43–4.39)
biofilm	43 (79.6)	16 (51.6)	0.0134	3,603 (1.253–10.789)

Association with biofilm production

Of the 85 analyzed EAEC strains, 69.4% (59/85) were biofilm producers; therefore, there was statistically significant relation between biofilm production and the presence of *aggR* gene (TABLE 2). It is worth highlighting that 73% (43/59) of the biofilm producers had the *aggR*, whereas this gene frequency in non-producer strains was 42.3% (11/26; *P*=0.0135 [OR=3.665; IC95%=1.393 to 9.639]). Similarly, there was association between the presence of *aatA* gene and biofilm producers (61% [36/59]), versus 19.2% (5/26) association in non-producers; *P*=0.0004 [OR=6.574; IC95% 2.173 to 19.89]. No correlation was observed between biofilm production and the presence of other virulence genes such as *set1A*, *afa1*, *irp2*, *pic*, *astA*, *pet*, and *shf* (FIGURE 1).

DISCUSSION

Studies about the interaction and importance of different pathogenic *E. coli* strains associated with acute diarrhea in Brazil have highlighted EPEC as the major etiologic agent of infant diarrhea^(23,24). However, studies have been reporting EAEC as an emerging enteric pathogen that causes persistent diarrhea and malnutrition in children living in developed countries⁽²⁵⁾.

With regard to the current study, 8.6% (51/591) of childhood gastrointestinal singular infection or co-infection by other enteropathogens could be attributed to EAEC. Studies conducted abroad reported EAEC infection prevalence from 2% to 24%^(26,27). Previous studies carried out in Brazil showed that 0.5% to 41% of acute gastroenteritis cases requiring hospitalization were caused by this pathotype⁽²⁸⁻³⁰⁾. The high incidence of diarrhea cases in developing countries can be attributed to standard fecal-oral contamination routes resulting from deficient sanitary infrastructure, low education level, nutritional deficiency and inappropriate personal and food-related hygiene practices. Such incidence results in high economic burden for national public health systems^(31,32). It is worth emphasizing that patients in the current study presented critical social patterns related to low per capita income and untreated water intake.

Diarrheagenic *E. coli* infections in the current study most affected children in the age group 2 years or younger. Besides diarrhea, the major clinical symptoms associated with DEC among the assessed children were vomiting and fever. This outcome is consistent with reports from India⁽³³⁾, Tanzania⁽³⁴⁾, Libya⁽³⁵⁾, Cambodia⁽³⁶⁾, Panama⁽³⁷⁾, and Israel⁽³⁸⁾.

There are just few reports about bloody diarrhea cases associated with EAEC and they rarely concern children^(5,33). With regard to the current study, bloody diarrhea was observed in 23.5% (12/51) of the assessed cases and EAEC was identified as the only pathogen in 58.3% (7/12) of them; however, other studies have reported conflicting results. A study conducted in Nigeria did not identify bloody stool as symptom of childhood EAEC infection⁽²⁶⁾. The prevalent virulence genes in the herein analyzed isolates were *irp2*, in 75.3% (64/85) of the cases; *astA*, in 64.7% (55/85); and *aggR*, in 63.5% (54/85). Previous studies have reported *irp2* genes in EAEC samples but it was rarely found in EPEC, enteroinvasive *E. coli* and ETEC, besides being absent in EHEC, *Shigella* and *Salmonella enterica*^(17,39). The *irp2* gene is part of the High-pathogenicity island found in the chromosome of Yersinia species (*Y. pestis*, *Y. pseudotuberculosis* serotype O1 and *Y. enterocolitica* biotype 1B) involved in iron uptake, which is mediated by the siderophore yersiniabactin and found in EAEC 042.

Elias et al., 2002, used the EAEC probe and found that *irp2* was the second most prevalent marker (91.4%) among the 70 EAEC probe + strains⁽⁴⁰⁾. There was statistically significant association between the typical EAEC *aatA* ($P < 0.0001$) and *irp2* ($P = 0.0357$) genes. Patzi-Vargas conducted a study about DEC carrying supplementary virulence genes; he showed that the *aatA* genes are significantly more common in EAEC isolates than in non-DEC strains⁽²⁵⁾. Tokuda et al⁽⁴¹⁾ showed that *aatA* and *irp2* genes are more prevalent in typical EAEC isolates than in atypical ones. Results in the present study evidenced a whole variety of virulence genes combinations in typical EAECs, fact that was not so common in atypical EAECs.

Some researchers have assumed that several virulence gene combinations can be directly associated with diarrhea or with strain virulence^(35,42).

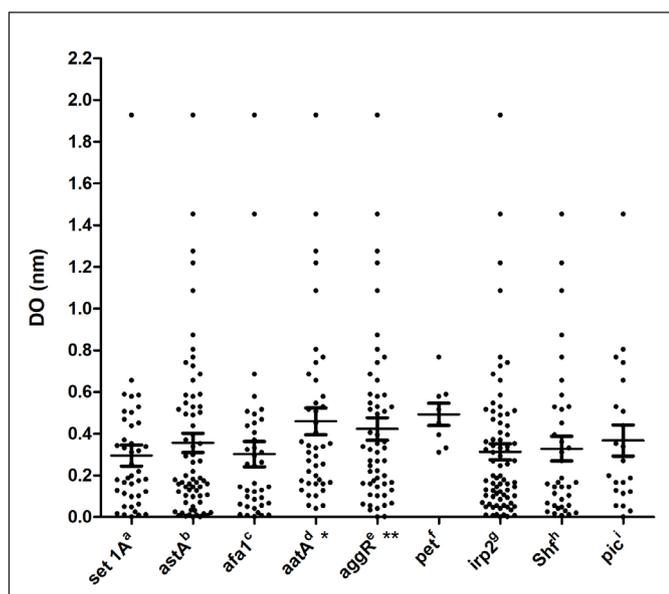


FIGURE 1. Biofilm formation and presence of virulence genes in EAEC isolates among diarrheal children. ^a shigella enterotoxin 1; ^b enteroaggregative heat-stable enterotoxin 1; ^c aggregative adherence; factor; ^d dispersin transporter; ^e transcriptional activator; ^f plasmid encoded toxins; ^g yersiniabactin biosynthesis; ^h cryptic open reading frame; ⁱ mucinase activity genes. *P* values represent correlation between biofilm production and virulence genes in EAEC: * $P = 0.0004$; ** $P = 0.0135$ (Fisher's exact test).

Antibiotic sensitivity in EAEC isolates

All 85 EAEC isolates were tested against 10 different antibiotics; the susceptibility profiles are shown in TABLE 3. At least one strain resistant to each of the tested antibiotics was identified. Results evidenced that most EAEC isolates were resistant to AMP 70.6% (60/85), SXT 60% (51/85), TET 44.7% (38/85), and CTX 22.3% (19/85). Multidrug resistance (MDR) was detected in 36.5% (31/85) of the tested strains. The MDR strains were resistant to antibiotics belonging to the most common chemotherapy classes, including AMP 96.8% (30/31), SXT 96.8% (30/31), TET 61.3% (19/31), CTX 54.8% (17/31).

TABLE 3. Antimicrobial profiles of EAEC: enteroaggregative *E. coli* isolates.

Antibiotic	Sensitive n (%)	Resistant n (%)
Amoxicilin/clavulanic acid (AMC)	61 (71.8)	24 (28.2)
Amikacin (AMI)	83 (97.6)	2 (2.4)
Ampicillin (AMP)	25 (29.4)	60 (70.6)
Cefotaxime (CTX)	66 (77.6)	19 (22.3)
Ceftazime (CRX)	70 (82.4)	15 (17.6)
Gentamicin (GEN)	72 (84.7)	13 (15.3)
Imipenem (IPM)	80 (94.1)	5 (5.9)
Piperacillin/tazobactam (PPT)	75 (88.2)	10 (11.8)
Tetracycline (TET)	47 (55.3)	38 (44.7)
Sulfamethoxazole (STX)	34 (40)	51 (60)

Pathogenic implications of *aggR* gene have been assessed in a number of studies worldwide^(17,41). Interestingly, current results showed that children over one month old presented greater risk of being infected by typical than by atypical EAEC ($P=0.0155$). These findings suggest that children have greater probability of being exposed to the most virulent strains as they age.

Andrade and collaborators showed that EAEC strains found in small intestine and colon mucosa produce large amounts of biofilm and cause changes in the epithelia, fact that possibly explains the long duration of diarrheal episodes⁽⁴³⁾. Biofilm production, adhesiveness and other EAEC virulence factors are directly associated with certain genes, including the regulatory gene *aggR*. The *aggR* is one of the most studied genes since it controls the expression of other genes related to EAEC pathogenesis. The presence of *aggR* in the present study was indeed statistically correlated with biofilm production ($P=0.0135$); this finding corroborates findings reported in studies conducted in Mexico and Mongolia, which have also indicated that the presence of *aggR* gene is associated with biofilm formation^(44,45). Although it was possible recording significant association with the presence of *aatA* gene and biofilm production ($P=0.0004$), the *shf* gene and biofilm production were not correlated to each other. This results were different from those evidenced in a previous study that correlated *shf* with biofilm formation, even when it was associated with *aggR*⁽⁴⁶⁾.

Antimicrobial resistance, in EAEC populations, mainly MDR, has been reported in several studies^(47,48). Results in the present study evidenced greater EAEC resistance to AMP, SXT, TET and CTX than to other antibiotics. The resistance to these antibiotics was expected since they are the low-cost chemotherapeutic drugs of choice in many studies⁽⁴⁹⁾. Antibiotics, mainly β -lactams, are often used to treat DEC infections, mainly in persistent diarrhea cases when the infectious agent is not identified, although these infections are self-limiting and only require oral-rehydration therapy. Most of

the herein tested EAECs showed MDR phenotype, and this result is consistent with other studies. The most common MDR phenotypes recorded in the current study included resistance to β -lactams, TET, aminoglycosides, SXT, and GEN. There was no association between antimicrobial resistance and biofilm production, although this correlation was previously described by other researchers⁽⁵⁰⁾.

As far as it is known, the present study is pioneer in showing the pathogenic potential and heterogeneity of virulence EAEC genes and co-infections caused by other enteric pathogens in children with gastroenteritis living in Rondônia. Data presented in the current study may contribute to the better understanding about the role played by EAEC in children with diarrheal illnesses living in Porto Velho. The results may help developing strategic plans to control antimicrobial resistance in these poor regions.

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Authors' contribution

Taborda RLM, Silva LA, and Rodrigues RS: collected data for the study, and carrying out the cell adhesion assays and biofilm formation. Batista FS, and Orlandi PP: reviewers of the current article. Matos NB: design and execution of the project.

Taborda RLM, Silva LA, Orlandi PP, Batista FS, Rodrigues RS, Matos NB. Caracterização de *Escherichia coli* enteroagregativa entre crianças com diarreia na Amazônia ocidental brasileira. Arq Gastroenterol. 2018;55(4):390-6.

RESUMO – Contexto – A *Escherichia coli* enteroagregativa (EAEC) é um dos principais agentes causadores de diarreia aguda e crônica em crianças e adultos, principalmente em países em desenvolvimento. **Objetivo** – Caracterizar cepas de EAEC isoladas de amostras fecais e identificar genes que potencialmente contribuem para a virulência, produção de biofilme e resistência antimicrobiana em crianças internadas em um hospital pediátrico em Porto Velho, Rondônia. **Métodos** – Um total de 1.625 cepas de *E. coli* foram isolados de 591 crianças com gastroenterite aguda na faixa etária de 6 anos que foram internadas no Hospital Infantil Cosme e Damião na cidade de Porto Velho, entre fevereiro de 2010 e fevereiro de 2012. Colônias sugestivas de *E. coli* foram submetidas a reação em cadeia da polimerase para identificação de fatores de virulência. O ensaio de adesão *in vitro* foi desenvolvido com célula HEP-2. A detecção de biofilme foi realizada através do teste de espectrofotometria e os testes de susceptibilidade aos antimicrobianos foram realizados através do método de difusão em disco. **Resultados** – A *E. coli* diarreioagregativa foi encontrada em 27,4% (162/591) das crianças e a EAEC foi a *E. coli* diarreioagregativa mais frequentemente associada à diarreia com 52,4% (85/162), seguida pela *E. coli* enteropatogênica 43,8% (71/162), *E. coli* enterotoxigênica 2,4% (4/162) e *E. coli* enterohemorrágica 1,2% (2/162). O gene *aggR* foi detectado em 63,5% (54/85) dos isolados de EAEC com correlação estatisticamente significativa entre esse gene com os genes *aatA* ($P<0,0001$), *irp2* ($P=0,0357$) e *shf* ($P=0,0328$). Neste estudo 69% (59/85) das cepas de EAEC eram produtoras de biofilme, destas 73% (43/59) possuíam o gene *aggR*, ao passo que entre as não produtoras 42,3% (11/26) possuíam o gene ($P=0,0135$). Essa associação também foi observada com o gene *aatA*, presente em 61% (36/59) das cepas produtoras e em 19,2% (5/26) das não produtoras ($P<0,0004$). O teste de sensibilidade aos antibiogramas evidenciou que a maioria das EAEC eram resistentes a ampicilina 70,6% (60/85), ao sulfametoxazol 60% (51/85), a tetraciclina 44,7% (38/85) e a cefotaxima 22,4% (19/85). **Conclusão** – Este é o primeiro estudo no Norte do Brasil sobre a investigação dos fatores de virulência de EAEC mostrando a susceptibilidade antimicrobiana de cepas de EAEC isoladas de crianças com diarreia.

DESCRITORES – *Escherichia coli*, classificação. Diarreia infantil. Criança. Virulência, genética.

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