

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2017.601.097>

## Prevalence of Enteropathogenic *Escherichia coli* in Maize (*Zea mays*) or Millet (*Pennisetum glaucum*) Flours and Porridges

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### ABSTRACT

#### Keywords

Cereal,  
Enteropathogenic  
*Escherichia coli*,  
flour, porridge,  
prevalence.

#### Article Info

Accepted:  
22 December 2016  
Available Online:  
10 January 2017

Enteropathogenic *Escherichia coli* (EPEC) was a leading cause of infantile diarrhea in many countries. But this bacteria had never research in flours and porridges consume by infant in Côte d'Ivoire. The aim of this study was to determine the prevalence of EPEC in maize and millet flours and porridges made traditionally. EPEC were research in 996 samples of traditional cereals products, with 404 flours and 592 porridges. These products were taken in five municipalities of Abidjan. The strains were characterized by detection of virulence factors and phylogenetic method. The results revealed that the prevalence of EPEC was 4.1% in millet porridge and 3.4% in maize porridge. This prevalence was 6.4% and 5.4% respectively in millet and maize flours. Most of EPEC; 68.2% isolated was atypical. EPEC detected belong to phylogenetic group A (47.2%), D (28.3%), B1 (13.2%) and B2 (11.3%). In short, traditional flours and porridges from maize and millet used in infant food were risk factors by EPEC contamination, which was potentially pathogen for children.

### Introduction

Nowadays, foods based on complete cereals are increasingly recognized as health and wellness factor, as well as of interest because of the different nutrients they contain (carbohydrates, proteins, lipids, vitamins, and minerals) as related by Wrigley (2004); Fletcher (2004); Corsetti and Settanni (2007). Cereals go into the formulation of complementary foods throughout the world (FAO, 2007; Kohajdová and Karovičová, 2007). If cereals are the staple food of the

population, it should be noted that local production does not satisfy the consumption requirement. According to PAM (2008), cereal products accounted for 56% of food imports, in other words 1.5 million tonnes. Throughout Africa, (Abegaz *et al.*, 2002; Traore *et al.*, 2003; Glidja, 2004; Tou, 2007; Elenga *et al.*, 2009; Amankwah *et al.*, 2009; Nout, 2009; Jideani, 2012; Olorode *et al.*, 2013), instant flour porridges are prepared and used as complementary foods during the weaning or post-weaning period for children

over 6 months (Tou, 2007). Industrial cereal-based flours used as complementary foods are those recommended by international bodies in rural and urban areas for their guaranteed nutritional and health qualities (Trèche, 2002; Hervé et Mouquet-Rivier, 2004). However, these foods, although rich in nutrients (Trèche, 2002; Nguyen, 2008), are most often imported and are difficult to access for the majority of the population because of their high cost (TPA, 1998).

The alternative for some developing countries is the formulation of complementary foods or porridges made from simple, fermented, or enriched cereal flours and made traditionally (Tou, 2007, Oumarou *et al.*, 2012). But the sanitary quality of traditional flours and porridge is often questioned. Thus, pathogenic bacteria such as Salmonella, Campylobacter, Listeria and Shigella have been isolated from porridges based on cereal-flour (Gadaga *et al.*, 2004). It was the same for EPEC, *Bacillus cereus* and *E. coli* isolated from children's meals made from sorghum and complementary foods (Gadaga *et al.*, 2004, Islam *et al.*, 2012). In addition, Oluwafemi and Nnanna Ibeh (2011) have found aflatoxins in foods for the consumption of children in Nigeria.

Among these germs, enteropathogenic *E. coli* (EPEC) has a prominent position in the etiology of gastroenteritis in children under five years of age (Yousef *et al.*, 2007). Indeed, the pathovars of *E. coli* persist and develop well in various foods and especially in traditionally formulated foods (Berche, 2001). EPEC are raged in Latin America (Adriana *et al.*, 2009), Asia (Ogata *et al.*, 2002), Europe (Afset *et al.* 2004) and Africa (Rappelli *et al.*, 2005).

In Côte d'Ivoire, the studies of Brou *et al.* (2009, 2013) and Soro-yao *et al.* (2013, 2014) demonstrated the use of cereal meal-based porridge in the same context and in several

forms. The process of flour and porridge production remains partially or wholly traditional, while the instant flours recommended by the international authorities are industrial production. As a result, the health status of traditionally produced products is poorly understood.

In addition, recent studies (Dadie *et al.*, 2010, Dadie, 2013) in Côte d'Ivoire showed the presence of EPECs in cooked minced meat, raw milk, dairy products and their involvement in infant diarrhea (Dadie *et al.*, 2014). In this context, the evaluation and the risk management of EPEC infection related to the consumption of complementary foods is urgently necessity.

The objective of the study was to determine the rate of EPEC contamination of flours and local complement porridges by identifying the virulence genes as well as the phylogenetic grouping of EPECs.

## **Materials and Methods**

### **Study area and selection criteria**

The material of study was consisted of millet (*Pennisetum glaucum*) and maize (*Zea mays*) flours and porridges. The flours and porridge concerned are those intended for human consumption, in particular in infant food. They are sold near certain schools, markets or other public places in the municipalities of Abobo, Adjamé, Attécoubé, Koumassi and Yopougon. These municipalities were chosen for their popular character and especially for the multiplicity of flours and porridge selling places. The sales sites selected are those located near the schools attended by children and those located near certain markets.

### **Flours and porridges samples**

A sample of porridge has been setting up about 300 ml of porridge packaged in a non-

food plastic material. On the other hand, a sample of flour was made up of 200 g of flour. Flours were taken from a set of flours packaged in bulk in plastic or aluminum bowls. The removal of the porridge was carried out by the vendors who used a ladle which they introduced into non-food plastic. For flours, the sellers were used a plastic cup or sometimes their hands to pick up the flours that was packed in paper. Immediately after collection, samples were chilled in an ice cooler box at 4 °C and transported to the laboratory for analysis within 2 h after sampling.

### Identification of *Escherichia coli* strains

The identification process was performed based on the standard biochemical tests. The oxidase negative Gram negative bacilli were identified as *E. coli* based upon the following test reactions; acid on acid reaction with gas and no H<sub>2</sub>S production on Kligler iron agar, catalase positive, indole positive, citrate negative, methyl-red positive, urease negative, and Voges-Proskauer negative (Mahon *et al.* 2007)

### Detection of EPEC by PCR

All strains were examined by polymerase chain reaction (PCR) with the specific primers for the presence of the virulence genes; *eaeA*, *bfpA*, *stx*, Prototype EPEC strain 2348/69 (serotype O127: H6), which expressed intimin, BFP, and EAF and *E. coli* EDL933 (*stx*+) strain was used as positive control in PCR assays, and *E. coli* HB101 was included in the experiment as a negative control.

PCR assays were performed using the protocol described by Beij *et al.* (1991) for detection of *uidA* gene and Yatsuyanagi *et al.* (2002) for detection of the following virulence genes: *eaeA* (structural gene for

intimin of EHEC and EPEC), *bfpA* (structural gene for the bundle-forming pilus of EPEC). The minimum criteria for determination of EPEC were defined as follows: the absence of *stx1* and *stx2* for EHEC (the presence of *stx1* and *stx2* and additional presence of *eaeA* confirmed the detection of a typical EHEC isolate), the presence of *bfpA* and *eaeA* for typical EPEC (but the presence of only *eaeA* for atypical EPEC).

Template DNA was extracted from whole organisms by boiling. Bacteria were harvested from an overnight broth culture, suspended in 500 ml sterile deionized water (Analar NORMAPUR, PROLABO) and boiled at 100 °C for 10 min and then centrifuged at 12 000 g for 15 min to pellet the cell debris (Theron *et al.*, 2000; Momba *et al.*, 2006). A 5 ml aliquot of supernatant was used for PCR. The oligonucleotide primers used in this study are listed in Table 1.

Single PCR: It was used to detect gene *uidA*. PCR mixtures with a final volume of 25 µL consisted of 10.73 µL sterile deionized water (Analar NORMAPUR, PROLABO), 5 µL template DNA, 1.25 mM each dNTP (4 µL); 0.4 µL each primer; 2.5 µL template 10X (EUROBIO), 1.5 mM MgCl<sub>2</sub> (25mM) (EUROBIO) and 0.5 µL Taq DNA polymerase (5 UI/µL; EUROBIOTAQ ADN POLYMERASE).

Multiplex PCR: It was used to detect gene *stx1*, *stx2*, *eaeA* and *bfpA* from *E. coli uidA*<sup>+</sup>. PCR mixtures with a final volume of 25 µL consisted of 9.05 µL sterile deionized water (Analar NORMAPUR, PROLABO), 5 µL template DNA, 1.25 mM each dNTP (4 µL) (SIGMA); 0.8 µL each primer; 2.5 µL template 10X (EUROBIO), 1.5 mM MgCl<sub>2</sub> (25mM) (EUROBIO) and 0.25 µL Taq DNA polymerase (5 UI/µL; EUROBIOTAQ ADN POLYMERASE).

Each PCR was performed using thermocycleur type Biometra UNO II, Thermoblock version 3.300, biotron 1998, série 1712243. The reaction was started with a 3 min denaturation step at 94 °C. The temperature cycles consisted of 1 min at 94 °C, followed by 1 min at 56 °C and 1 min 30 s at 72 °C. Each cycle was repeated 30 times, and the final cycle was followed by incubation of the reaction mixture for 6 min at 72 °C. The amplified DNA was separated by submarine gel electrophoresis in 1.5% agarose, stained with ethidium bromide and visualized under UV transillumination.

### Determination of phylogenetic groups

A triplex PCR were performed using the protocol described by Clermont *et al.* (2000) for detection of the genes *Chua yjaA* and TSPE4.C2 fragment. The oligonucleotide primers used in the phylogenetic groups study are listed in Table 1.

PCR mixtures with a final volume of 25 µL consisted of, 5 µL template DNA, 1.5 mM each dNTP; 25 mM MgCl<sub>2</sub> 20 pmol each primer; a buffer 10X (EUROBIO), and Taq DNA polymerase. The reaction was started with a 4min denaturation step at 94 °C. The temperature cycles consisted of 20 s at 94 °C, followed by 15 s at 59 °C and 30 s at 72 °C. Each cycle was repeated 30 times, and the final cycle was followed by incubation of the reaction mixture for 6 min at 72 °C. The amplified DNA was separated by submarine gel electrophoresis in 1.5% agarose, stained with ethidium bromide and visualized under UV transillumination.

### Statistical analysis

The data were analyzed with SSPS version 20.0 software (SPSS). A  $\chi^2$  test was used to determine the statistical significance of the data. A P value of 0.05 was considered significant.

## Results and Discussion

### Virulence factors for *E coli* strains

All *E. coli* strains study with PCR were *uidA+*. Any *stx* (*stx1 et stx2*) gene have been shown in this study. All *E coli* strains were identified based on the detection of the *eaeA+*, *bfpA-* or *eaeA-*, *bfpA+* profiles and the absence of *stx* gene as atypical EPEC.

Electrophoretic analysis of *E. coli* isolates has been showed in figure 1. Based on the differences in the number of fragments, the isolates showed 2 differentes bands. These bands showed the presence of EPEC.

All strains showed a positive reaction with one or more primers for *eaeA* (367 pb) and *bfpA* (324 pb) genes. On the basis of these positive reactions, two different combinations of virulence factor genes were identified: *eaeA+*, *bfpA-*, *stx-*, *eaeA-*, *bfpA+*, *stx-*. These profiles were recognized as the definitive combination for atypical EPEC. And *eaeA+*, *bfpA+*, *stx-* was recognized as the definitive combination for typical EPEC. The distribution of these virulence genes according to the PCR types identified is shown in Table II.

Out of the 46 strains, 22 (47.8%) showed the characteristic atypical EPEC combination of *eaeA+*, *bfpA-*, *stx-*, 7 (15.2%) *eaeA-*, *bfpA+*, *stx-*, and 17 (37%) two profile combinations. In flours, combination of *eaeA+*, *bfpA-*, *stx-* is 12 (50%), *eaeA-*, *bfpA+*, *stx-* is 2 (8.3%) and the two profiles combination were 10 (41.7%). But in porridges, these combination were 10 (45.5%) for *eaeA+*, *bfpA-*, *stx-*, 5 (22.7%) for *eaeA-*, *bfpA+*, *stx-* and 7 (31.8%) for two profiles combination.

### Prevalence of EPEC

A prevalence of 3.7% of EPEC were found in porridges and 5.9% in flours. There was

statistically significant correlation regarding the prevalence of EPEC (4.6%) isolates in relation to flours and porridges ( $p < 0.05$ ). EPEC strains were mostly isolated in millet product than maize product. Thus, in porridge, 12 (4.1%) EPEC were detected in millet porridge and 13 (6.4%) were also detected in millet flour (Table III).

### **Compliant rate**

The virulence of EPEC strains for children was very study. So the presence of EPEC in children food was considered that this food is not safety for them. The analysis of sample considering the presence of EPEC showed 950 samples out of 996 (95.4%) were non-compliant according of normative values. A rate of 4.6% of products is non-compliant. However, there was no significant difference ( $p \geq 0.05$ ) between the proportions of compliant products. In addition, non-compliance was higher in millet flours with 6.4% (Table IV).

### **Typical and atypical EPEC**

Table V showed all of EPEC detected; it showed that 22 EPEC were found in porridges and 24 in flours. Among the 46 EPEC isolated in flours and porridges, 29 (63%) were atypical EPEC (aEPEC) with 15 (68.2%) aEPEC in porridges and 14 (58.3%) aEPEC in flours. Otherwise, 17 (37%) of EPEC were typical EPEC (tEPEC) with 07 (31.8%) aEPEC in porridges and 10 (41.7%) aEPEC in flours.

### **EPEC phylogenetic group**

Electrophoretic analysis of enteropathogenic *E. coli* phylogenetic group has been showed in figure 2. This figure shows different bands of sizes 279 bp, 211 bp and 152 bp. These sizes correspond respectively to the ChuA, Yja and Tspec gene.

As shown in Table VI, EPEC strains are phylogenetic group A (47.2%). The strains of group D represent 28.3% of all EPEC pathovars. Out of the 19 pathotypes in the porridges, 9 (47.4%) EPEC were to the phylogenetic group A and were the most frequent. Five (5) pathotypes were to phylogenetic group D (26.3%) and 3 to group B1 (15.8%). *E. coli* strains belonging to the phylogenetic group B2 (10.5%) are the weakest represented. Otherwise, in flours, 16 (47.1%) strains were to group A and it is the group which contains more pathotypes. It was followed by group D (29.4%).

The results presented here showed that enteropathogenic *E. coli* were found in a wide range in porridges and flour prepared at home and sold near certain schools, markets or other public places. Our study shows a prevalence of 4.6% in both flours and porridges but, EPEC were most isolated in flours than in porridges ( $p < 0.05$ ). The results were similar to the report of Norazah *et al.* (1998) in Malaysia.

Many studies lead in flours have shown presence of *Escherichia coli* (Sperber, 2007) and others such as *E. coli*, *coliforms*, *Bacillus cereus*, *Bacillus* spp (Berghofer *et al.* 2003) and many other microorganisms in flours in Australia to a lesser extent. Other studies have shown the absence of pathogenic germs in flours (Olorunfemi *et al.*, 2005) and in the porridges (Soro-yao *et al.*, 2013). In Africa, cereals flours are used for porridges and porridges are used as traditional African weaning food. The flour (maize and millet) was the most contaminated commodity in the products. In their study of pathogenic *E. coli* in traditional African weaning food, Nyatoti *et al.* (1997) found *E. coli* in 36% of the food samples used for weaning foods, most of which are prepared from maize flour. Kunene *et al.* (1999) found an *E. coli* incidence of 53% with a range of 3.20–5.02 log<sub>10</sub> CFU/g

in sorghum flour and fermented sorghum porridge, commodities that have similar characteristics to maize flour. Home-made street-vended foods have also been reported to contain *E. coli* incidence of between 7 and 32% (Cardinale *et al.*, 2005; Hanashiro *et al.*, 2005). There was a high overall incidence of enteropathogenic *E. coli* in this study (3.7% of the porridge samples and 5.9% of the flour samples), with the highest incidence in millet flour (6.4%) and millet porridge (4.1%). Previous studies carried out in Zimbabwe have reported finding pathogenic *E. coli* (EPEC) in 16% of all food samples (Simango *et al.*, 1992) while Nyatoti *et al.* (1997) detected pathogenic strains of *E. coli* in 15% of their food samples.

Also, pathogenic bacteria of Salmonella, Campylobacter, Listeria and Shigella have been isolated from various infant foods, including gruels based cereal-flour (Gadaga *et al.*, 2004). The same was true for EPEC, *Bacillus cereus* and *E. coli* isolated from children's meals made from sorghum and complementary foods (Kunene *et al.*, 1999, Islam *et al.*, 2012). Furthermore, Motarjemi *et al.* (1993) detected *Aeromonas* Spp, *Staphylococcus aureus*, *Bacillus cereus*, Salmonella spp. and *Vibrio cholerae* in children's porridge from maize in Ghana.

Hygiene during handling and cooking food is very important. Human beings represent the largest contamination sources of food. Health and personal hygienic knowledge of the street food vendors surveyed are also important. The contamination of flour and porridge could be explained by the fact that, according to many authors pathogenic *E. coli* can survive on the hands, tools at large number and contaminated food (Moore *et al.*, 2003; Dawson *et al.*, 2006; Taalo *et al.*, 2009).

According to WHO (1989), hand-washing is very important because the hands are

considered the most important vehicle for transfer of micro-organisms from faeces, nose, skin and other parts of the body into food. Studies in Epidemiology have further confirmed that bacteria such as *Salmonella typhi*, *Escherichia coli* can survive for varying periods on the fingers and other parts of the body (Pether and Gilbert, 1971). Mensah *et al.* (2002) reported in a study in Accra that out of 511 street food items examined in Accra, 69.7% contained mesophilic bacteria and 33.7% contained Enterobacteriaceae. Also in Côte d'Ivoire Dadié *et al.* (2010, 2013, 2014) reported the presence of EPEC and other *Escherichia coli* in food and their implication in infant diarrheara. From these studies, it is evident that flour and porridges which are manipulated by the hand contain EPEC.

Domestic practices of food handling and preparation are one of the numbers of reasons that explain the increasing incidence of gastrointestinal infection associated with domestic environments (Scott *et al.*, 1982, Daniels, 1998; Beumer and Kusumaningram, 2003). The study has demonstrated that the incidence of EPEC was high in manipulated food.

Thus, this contamination by EPEC could also be justified by environment as shown in Ghana during studies of Feglo and Sakyi (2012).

On the other hand, the contamination of the porridge sold near certain schools, markets or other public places and traditional flours sold in open markets, analyzed during this study, could be favored by an exposure temperature such as temperatures between 20 °C. and 40 °C which are favorable to the proliferation of EPEC. However, the studies of Yeboah-Manu *et al.* (2010) have shown that this contamination is due to excessive handling of food products. In addition, the study of WHO

(2003) showed that non-protection against aerosols is a cause of EPEC contamination.

Temperature has an impact on the transmission of pathogenic germs. Taulo *et al.* (2009) showed in their study that samples collected at temperatures less than 30 °C contained significantly more strains of *Escherichia coli* and *Staphylococcus aureus* followed by samples collected at temperatures between 30 °C and 40 °C and finally Samples collected at temperatures above 40 °C which contained fewer germs.

The presence of EPEC in food is associated with contamination that has been directly introduced into the food by food handlers through coughing and sneezing as well as storage of food at high temperature (Kaneko

*et al.*, 1999; Sandel and McKillip, 2004). In this study, it was observed that most porridge was prepared in advance and stored at ambient temperature for 1– 4 h before finishing selling and this practice could have allowed the pathogens to grow to large numbers. The high number of pathogens and the ambient food storage temperature reported in some households may be a contributing factor with regards to EPEC food intoxication in children population.

One of the major factors contributing to the deterioration of the microbiological quality of foods (street) in developing countries is their maintenance at room temperature for several hours of sale. This explains the frequent association of street foods with gastroenteritis disorders (Barro *et al.*, 2007).

**Table.1** Primers used

Genes	Primers	Size (pb)	References/ Gen bank EMBL, no
<i>uidA</i>	UAL : 5' AAA ACG GCA AGA AAA AGC AG -3' UAR : 5' ACG CGT GGT TAC AGT CTT GCG -3'	147	<b>Beij <i>et al.</i>, 1991</b>
<i>eaeA</i>	Eaef 5'-CACACGAATAAACTGACTAAAATG-3' Eaer 5'-AAAAACGCTGACCCGCACCTAAAT-3'	367	<b>Yatsuyanagi <i>et al.</i>, 2002</b>
<i>bfpA</i>	Y bfp fp-5'-AATGGTGCTTGCGCTTGCTGC-3' Y bfp bp-5'-GCCGCTTTATCCAACCTGGTA-3'	324	<b>Yatsuyanagi <i>et al.</i>, 2002</b>
<i>stx1</i>	stx1f-5'-GAAGAGTCCGTGGGATTACG-3' stx1r-5'-AGCGATGCAGCTATTAATAA-3'	130	AF461172
<i>stx2</i>	stx2-5'-ACCGTTTTTCAGATTTTACACATA-3' stx2-5'TACACAGGAGCAGTTTCAGACAGT-3'	298	AY143337
<i>ChuA</i>	ChuA.1 -5'-GACGAACCA ACGGTCAGGAT-3' ChuA.2 5'-TGCCGCCAGTACCAAAGACA-3'	279	
<i>YjaA</i>	YjaA.1- 5'-TGAAGTGTCAGGAGACGCTG-3' YjaA.2 5'-ATGGAGAATGCGTTCCTCAAC-3'	211	<b>Clermont <i>et al.</i>, 2000</b>
TspE4C2	TspE4C2.1.5'-GAGTAATGTCCGGGCATTCA-3' TspE4C2.2 5'-CGCGCCAACAAAGTATTACG-3	152	

*eaeA* : *E. coli* attaching-effacing

*bfpA* : bundle-forming pilus

*stx1* : shiga toxin 1

*stx2* : shiga toxin 2

**Table.2** Distribution of virulence genes

Virulence genes	Porridges		Flour		Total (%)
	Presence	Frequency (%)	Presence	Frequency(%)	
<i>eaeA</i> +	+	45.5	+	50	22 (47.8)
<i>bfpA</i> +	+	22.7	+	8.3	7 (15.2)
<i>Eae</i> <sup>+</sup> - <i>bfp</i> <sup>+</sup>	+	31.8	+	41.7	17 (37)
<i>Stx</i> <sup>+</sup>	0	0	0	0	0
<b>Total</b>	<b>22</b>	<b>100</b>	<b>24</b>	<b>100</b>	<b>46 (100)</b>

*Eae*: *E. coli* attaching-effacing

*Bfp*: bundle-forming pilus

**Table.3** Prevalence of EPEC

Type of food	Samples (Effective)	EPEC	
		Effective	Prevalence (%)
Porridge of millet	296	12	4.1
Porridge of maize	296	10	3.4
<b>Total</b>	<b>592</b>	<b>22</b>	<b>3.7</b>
Flour of millet	202	13	6.4
Flour of maize	202	11	5.4
<b>Total</b>	<b>404</b>	<b>24</b>	<b>5.9</b>
<b>General Total</b>	<b>996</b>	<b>46</b>	<b>4.6</b>

**Table.4** Compliant rate of analyzed product following the presence of EPEC

Type of food	Samples	Food product qualities	
		Conforme	Non conforme
Porridge of millet	296	284 (95.9%)	12 (4.1%)
Porridge of maize	296	286 (96.6%)	10 (3.4%)
Flour of millet	202	189 (93.6%)	13 (6.4%)
Flour of maize	202	191 (94.6%)	11 (5.4%)
<b>Total</b>	<b>996</b>	<b>950 (95.4%)</b>	<b>46 (4.6%)</b>

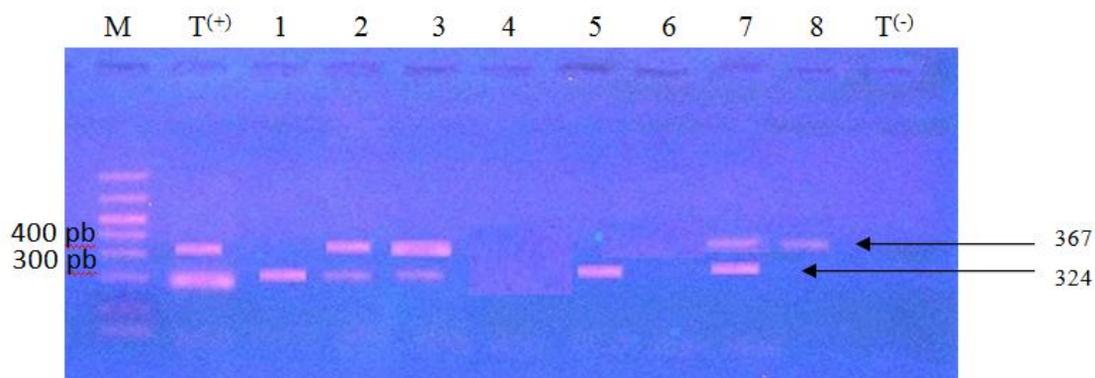
**Table.5** Distribution of typical and atypical EPEC

EPEC	Porridges N (%)	Flours N (%)	Total (%)
<b>Typicals</b>	7 (31.8 %)	10 (41.7 %)	17 (37 %)
<b>Atypicals</b>	15 (68.2 %)	14 (58.3 %)	29 (63 %)
<b>Total</b>	<b>22 (100 %)</b>	<b>24 (100 %)</b>	<b>46 (100 %)</b>

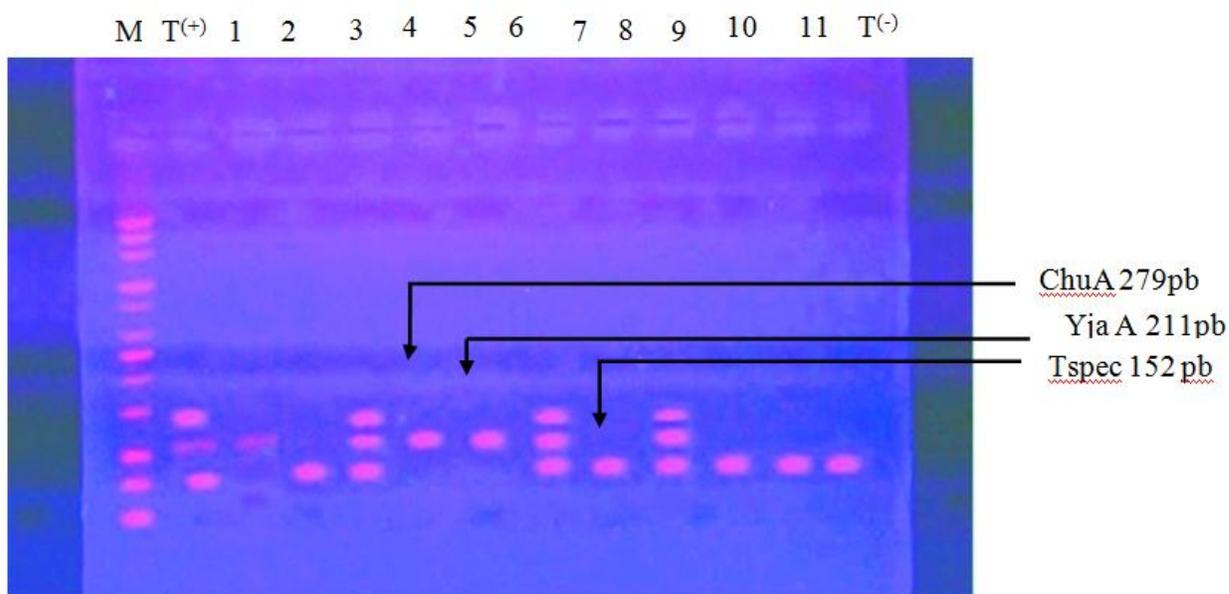
**Table.6** Distribution of EPEC following the phylogenetic group

Origin	Group A	Group B1	Group D	Group B2
Porridge of millet	5	2	3	1
Porridge of maize	4	1	2	1
Flour of millet	7	2	4	2
Flour of maize	9	2	6	2
<b>Total (%)</b>	25 (47.2 %)	07 (13.2 %)	15 (28.3 %)	6 (11.3 %)

**Fig.1** Multiplex PCR of virulence genes in EPEC isolates from porridges and flour samples. Lane 1, 50 à 1000 pb molecular weight marker in base pairs; lane 2, positive control; lane 11, negative control and lane 3 to lane 10, PCR products obtained with DNA of EPEC isolates



**Fig.2** PCR products for phylogenetic group. Lane 1, 50 à 1000 pb molecular weight marker in base pairs; lane 2, positive control; lane 14, negative control and lane 3 to lane 13, PCR products obtained with DNA of EPEC isolates for phylogenetic group



The study showed that there was no significant difference between typical and atypical EPECs ( $p > 0.05$ ). The typical EPEC rate obtained may be due to the difficulty of detecting the *bfp* gene, which is controlled by the plasmid EAF and which according to Nataro and Kaper (1998) would only be expressed if the EPECs were not stressed during cultivation.

According to the TPA (1998) standard, more than half of the porridges and flours analyzed are non-compliant. This result can be explained by the lack of formal surveillance for food prepared and sold on public roads and open markets. Barro *et al.* (2002) considered that this situation could directly threaten the health of consumers.

Clermont *et al.* (2000) developed a simple and rapid method (triplex), allowing the classification of pathovars into 4 phylogenetic groups. The application of this technique to the strains gives a diversified phylogenetic distribution, with the majority (47.2%) of the pathovars belonging to group A. The pathovars of group B2 represent 11.3% and those of the group D, 28.3%. The distribution obtained during our study is different from that obtained, following an analysis of 64 strains, by Clermont *et al.* (2000) who found 58% of B2, 18.7% of A and 17.1% of D. This difference with our results could be due to the fact that Clermont worked on human strains and it is established that most potentially pathogenic extra-intestinal strains belong to group B2 and to a lesser extent group D (Bonacorsi *et al.*, 2000).

In conclusion, the detection of pathogenic *E. coli* in flours and in porridge sold and consumed by children shows that they are sources of pathogens. These germs being etiologic agents of human diarrhea, rules of hygiene must be applied to ensure the safety of porridges and child consumers.

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**How to cite this article:**

Kouame N'zebo, D., A. Dadie, O.L. Anin-Atchibri, N. Kouassi and Dje, K.M. 2017. Prevalence of Enteropathogenic *Escherichia coli* in Maize (*Zea mays*) or Millet (*Pennisetum glaucum*) Flours and Porridges. *Int.J.Curr.Microbiol.App.Sci*. 6(1): 819-833.

doi: <http://dx.doi.org/10.20546/ijcmas.2017.601.097>