INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 1, p. 192-199, 2022

RESEARCH PAPER OPEN ACCESS

Detection of virulence genes associated with diarrheagenic pathotypes in *Escherichia coli* isolates from local beverages and ice samples in Ouagadougou

G. Bsadjo Tchamba[1,2*], H. Bawa Ibrahim[2, 3], N. Barro[2]

[1]*Department of Microbiology and Parasitology, University of Buea, P.O. Box 63, Buea, Cameroon*

[2]*Laboratoire de Biologie Moléculaire d'Epidémiologie et de Surveillance des agents Transmissibles par les Aliments (LABESTA), Université Ouaga I Pr Joseph Ki-Zerbo, Ouagadougou, Burkina Faso 09 P.O. Box 903 – Ouagadougou, Cameroon*

[3]*Unité de Formation et de Recherche en Sciences et Technologies, Université de Ouahigouya, Burkina Faso 01 BP 346 Ouahigouya 01, Cameroon*

Key words: *Escherichia coli*, Virulence factor, Local beverages, Ice blocks.

http://dx.doi.org/10.12692/ijb/21.1.192-199 Article published on July 28, 2022

Abstract

Escherichia coli is one of the foodborne pathogens responsible for diarrhea with approximately 2 million deaths per year. The objective of this study was to determine the virulence genes of diarrheal *E. coli* isolated from local beverages and ice blocks. One hundred and twenty-one *Escherichia coli* strains isolated from local beverages and ice blocks were examined for virulence genes associated with diarrheagenic pathotypes. Twenty-one (17.3%) of the 121 *E. coli* strains carried the virulence genes. Nineteen (5.2%) of the isolates harbored the *eae* gene. The *eae* gene present in the EPEC was found in 6 strains isolated from "*gnamakoudjii*", 6 strains isolated from "*zoom-koom*", 6 strains isolated from ice blocks and 1 strain isolated from "*bissap*". Shiga toxin genes, the *stx2* gene present in STEC was observed in 2 strains isolated from "*zoom-koom*" and food ice, respectively. The *stx1* and *estla* genes present in STEC-ETEC were detected only in one strain of *E. coli* isolated from "*zoom-koom*". *E. coli* produces some virulence factors, which may be involved in human disease, constituting a public health concern.

* **Corresponding Author:** G. Bsadjo Tchamba ✉ bsadjotchamba@gmail.com

Introduction

Diarrheagenic *Escherichia coli* is the major bacterial etiological agent of severe diarrhea and a major concern of public health. These pathogens have acquired genetic characteristics from other pathotypes, leading to unusual and singular genetic combinations, known as hybrid strains and may be more virulent due to a set of virulence factors from more than one pathotype O (Munhoz *et al.*, 2021). The polysaccharide portion, called the O-antigen, of the lipopolysaccharide layer of the outer membrane of *Escherichia coli* provides antigenic specificity and is the basis of serogrouping. Of the 187 formally defined O antigens, six (O31, O47, O67, O72, O94 and O122) have since been removed and three (O34, O89 and O144) strains do not produce any O antigen (Liu *et al.*, 2020). Based on the virulence factors, it has been identified that there are six different types of diarrheagenic *E. coli* pathotypes based on their specific virulence traits under the following names: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffuse adherent *E. coli* (DAEC) (Canizalez-Roman *et al.*, 2013).

The current study's main objective was to investigate the frequency of pathogenic *E. coli* by detecting 16 genes belonging to the 5 main pathogenic groups of *E. coli* in local beverages («bissap» *Hibiscus sabdariffa*; «zoom-koom» *Penissetum glaucum* «gnamakoudjii» (*Zingiber officinale*) and ice block by using a single reaction (16-plex PCR): *E. coli* (*uidA*); STEC, EPEC (*eaeA, escV*, *ent*); typical EPEC (*bfpB*); STEC (EHEC-*hly*, *stx*1, *stx*2); EIEC (*ipaH*, *invE*); EAEC (*aggR*, *pic*, *astA*); ETEC (*elt*, *estIa*, *estIb*).

Materials and methods

This cross-sectional study was carried out from January 10th 2015 to January 31st 2015 on *E. coli* strains obtained from local beverages («bissap», «gnamakoudji», «zoom kom») and ice blocks samples at the Laboratory of Molecular Biology of Epidemiology of Waterborne and Foodborne Pathogens at the University of Ouaga I Pr Joseph Ki-Zerbo situated in, Burkina Faso. A total of 121 *E. coli* strains were used to evaluate the specificity of the multiplex PCR assay in this study. The bacterial strains listed in Table 1 were obtained from previous work (Bsadjo Tchamba *et al.*, 2014; Bsadjo Tchamba *et al.*, 2015). The *E. coli* strains previously-stored at -20°C in 30% glycerol were subcultured on Muller Hinton medium (Oxoid, UK) at 37°C. Biochemical tests (indole production and Kligler Hajna tests) were then performed for confirmation.

Chromosomal DNA extraction

Chromosomal DNA extraction was performed using the heating method from 24 h cultures of *E. coli* on Müeller Hinton agar. Using a Pasteur pipette, a small amount of bacterial mass from the 24 h colonies was removed and added to an Eppendorf tube (Hamburg, Germany) containing 500 µl of sterile water. This was placed in a boiling water bath for 10 min. Then the tube was centrifuged (OLE DICH) at 11000 g for 10 min. The presence of bacterial DNA was confirmed by visualizing the presence of bands after performing an agarose gel migration. The supernatant was collected and stored at -20°C and used for PCR (Moyo *et al.*, 2007).

Molecular analysis (characterization of E. coli pathotype by 16-plex-PCR)

16-plex PCR is a method that allows the simultaneous detection of the presence of 16 genes (*uidA*, *pic, bfp*, *invE*, *hlyA*, *elt*, *ent*, *escV*, *eaeA*, *ipaH*, *aggR*, *stx*1, *stx*2, *estIa*, *estIb* and *ast*) belonging to the 5 main pathotypes of *E. coli* in a single reaction. It consists of several chronological steps. The principle of PCR is based on the cyclic and specific amplification of a DNA fragment using a thermostable DNA polymerase (Taq polymerase: *Thermus aquaticus* polymerase) in the presence of specific primers and nucleotides (dNTPs). A multiplex PCR was used in our study called 16-plex PCR, developed by Antikainen *et al.* (2009).

E. coli pathotypes were detected by amplification of 16 genes with specific primers in a single PCR reaction for the detection of virulence genes (Table

2). PCR reactions were conducted according to the proposal developed by Antikainen *et al.* (2009) and slightly modified. The reaction mixture (reagents without DNA), also called MIX, used in our study was AccuPower® PCR PreMix certified ISO 9001 (Bioneer). The reagents used in the lyophilized PreMix are: 1 U of DNA polymerase, 250 mM of dNTPs (dATP, dCTP, dGTP, dTTP), 10 mM of Tris-HCl (pH 9.0), 30 mM of KCL, 1.5 mM of MgCl2 and the loading buffer. The PreMix-DNA mixture was made in another hood previously sterilized with UV radiation for 20 minutes. To the PreMix was added: 1 µl of Mueller-mix (Mix 1) containing 12 pairs of primers (Table 2), 1 µl of Jenni-mix (Mix 2) containing 4 pairs of primers, 1 µl of the supernatant containing the bacterial DNA and 17 µl of sterile water. The final volume of the preMix was 20 µl for each sample. The amplification program in the thermocycler (Perkins Helmer Cetus, USA) was as follows: 35 cycles each consisting of a denaturation step for 30 sec at 98°C, a hybridization step for 60 sec at 62.5°C and elongation for 90 sec at 72°C. These cycles are preceded by a denaturation step for 30 sec at 98°C, then followed by a final elongation step lasting 10 min at 72°C.

Tubes containing the amplification products were transferred to the post-PCR bench for agarose gel electrophoresis development. The agarose gel (SIGMA A-9539 Agarose for gel electrophoresis) concentrated to 2% (w/v) was prepared in 0.5× Tris Borate EDTA (TBE) buffer (100 mM Trizma base, 100 mM boric acid, 2 mM EDTA), the gel was stained by adding 8 µl ethidium bromide (BET, 10%) and cast onto the gel holder for electrophoresis. The electrophoresis vessel (APPELEX) was filled with the 0.5×TBE buffer until the gel was fully immersed. To each well of the gel 10 µl of the PCR reaction product was added using a micropipette. A 100-bp molecular weight marker (Norgen, Biotech Corp) appropriate for the size of the different expected amplicons was used. Electrophoretic migration was performed at 120 volts for 120 min. Visualization was done under a U.V. (Ultra-Violet) lamp (TLUM COVER SYTC 1295) and photos of the gel were taken.

Strains from the bacteriology laboratory of the National Institute of Public Health and Welfare in Helsinki, Finland were used as references: FE 102301 (Burkina Faso, beef [Kagambèga *et al.*, 2012]) for STEC, FE 94725 (Burkina Faso, mutton [Kagambèga *et al.*, 2012]) for ETEC, FE 95562 (Burkina Faso, beef [Kagambèga *et al.*, 2012]) for STEC-ETEC, RHE 6647 (Statens Serum Institute [SSI], Copenhagen, Denmark) for EIEC and IHE 56822 (Finland patient isolate, [Keskimäki *et al.*, 2000]) for EAEC. The negative control was *E. coli* IHE 50246 (Finland patient isolate). The other negative control was performed using sterile water.

The presence of the *eaeA* and *escV* genes and possible additional genes *ent* and *bfpB,* (absence of *bfpB* indicates an atypical EPEC) indicates an EPEC. The presence of *elt* indicates a ETEC. The presence of *stx*1 and/or *stx*2 and possible additional genes *eaeA*, *escV*, *ent* and ECEH-*hly* indicates ETEC. The presence of *invE* and *ipaH* indicates ECEH. The presence of *pic* and/or *aggR* indicates EAEC. The *uidA* gene was used as a general marker for *E. coli*. The *astA* gene is not pathogen-specific, so it is not used for the final analysis (Antikainen *et al.*, 2009).

Results and discussion

The main approach consisted of the simultaneous detection of the 16 virulence genes of the five main *E. coli* pathotypes responsible for different forms of diarrhoea in humans using the multiplex PCR called the "16-plex PCR", developed by Antikainen *et al.* (2009) and slightly modified. This work allowed us to determine whether the different *E. coli* strains isolated belonged to one of the different pathotypes.

Table 1. List of *E. coli* strains isolated in beverage and ice blocks samples.

Samples	*Number of E. coli* strains
«Bissap»	19
«Gnamakoudjii»	31
«Zoom-koom»	36
Ice blocks	35
Total	121

The virulence genes of the different *E. coli* pathotypes were searched for in the strains isolated from

beverages and ice cream. **Fig. 1** below shows the band sizes of the *escV* gene present in two strains isolated from ice block and «zoom-koom». Of the pathotypes tested on all strains isolated from beverages and ice blocks, STEC was determined in two «zoom-koom» samples (2.7%) and in one ice block sample (2.8%). STEC-ETEC was also detected in 1 (2.7%) «zoom-koom» sample. The virulence genes EAEC, EECI and ETEC were not detected in any of the samples analyzed. EPEC was determined in one sample (5.2%) of «bissap», 6 (19.3%) samples of «gnamakoudjii», 6 (16.6%) samples of «zoom-koom» and 6 (17.1%) samples of ice blocks. These results are recorded in Table 3 below.

Table 2. Primers sequences and PCR products sizes for virulence genes used in 16-plex PCR.

Pathotypes	Target gene	Primer sequence (5'-3')	Product size (bp)	[C] (µM)
STEC, EPEC	*eaeA*	F: TCAATGCAGTTCCGTTATCAGTT	482	0.1
		R: GTAAAGTCCGTTACCCCAACCTG		
	escV	F: ATTCTGGCTCTCTTCTTCTTTATGGCTG	544	0.4
		R: CGTCCCCTTTTACAAACTTCATCGC		
	ent	F: TGGGCTAAAAGAAGACACACTG	629	0.4
		R: CAAGCATCCTGATTATCTCACC		
EPEC typique	*bfp B*	F: GACACCTCATTGCTGAAGTCG	910	0,1
		R:CCAGAACACCTCCGTTATGC		
STEC	EHEC-*hly*	F: TTCTGGGAAACAGTGACGCACATA	688	0,1
		R: TCACCGATCTTCTCATCCCAATG		
	stx1	F: CGATGTTACGGTTTGTTACTGTGACAGC	244	0,2
		R: AATGCCACGCTTCCCAGAATTG		
	stx2	F:GTTTTGACCATCTTCGTCTGATTATTGAG	324	0.4
		R: AGCGTAAGGCTTCTGCTGTGAC		
EIEC	*ipaH*	F: GAAAACCCTCCTGGTCCATCAGG	437	0,1
		R: GCCGGTCAGCCACCCTCTGAGAGTAC		
	invE	F: CGATAGATGGCGAGAAATTATATCCCG	766	0,2
		R:CGATCAAGAATCCCTAACAGAAGAATCAC		
EAEC	*aggR*	F: ACGCAGAGTTGCCTGATAAAG	400	0,2
		R: AATACAGAATCGTCAGCATCAGC		
	pic	F: AGCCGTTTCCGCAGAAGCC	1111	0,2
		R: AAATGTCAGTGAACCGACGATTGG		
	astA	F: TGCCATCAACACAGTATATCCG	102	0,4
		R: ACGGCTTTGTAGTCCTTCCAT		
ETEC	*elt*	F: GAACAGGAGGTTTCTGCGTTAGGTG	655	0,1
		R: CTTTCAATGGCTTTTTTTTGGGAGTC		
	estIa	F:CCTCTTTTAGYCAGACARCTGAATCASTTG	157	0,4
		R: CAGGCAGGATTACAACAAAGTTCACAG		
	estIb	F: TGTCTTTTTCACCTTTCGCTC	171	0,2
		R: CGGTACAAGCAGGATTACAACAC		
E. coli	*uidA*	F: ATGCCAGTCCAGCGTTTTTGC	1487	0,2
		R:AAAGTGTGGGTCAATAATCAGGAAGTG		

STEC : Shiga toxine producing *E coli* ; EPEC: Enteropathogenic *E. coli*; EIEC: Enteroinvensive *E. coli*; EAEC: Enteroaggregative *E. coli;* ETEC: Enterotoxigenic *E. coli ;* [C] : Concentration.

The vast majority of *E. coli* belong to the commensal digestive flora and some can acquire specific virulence factors and give either extra-intestinal pathologies (meningitis, urinary tract infections) or intestinal pathologies (Liu *et al.*, 2020).

The *eaeA* gene present in the EPEC was found in 6 strains isolated from «gnamakoudjii", 6 strains isolated from «zoom-koom», 6 strains isolated from ice blocks and 1 strain isolated from «bissap». The *stx*2 gene present in STEC was observed in 2 strains isolated from «zoom-koom» and ice block respectively. The *stx*1 and *estla* genes present in ECST-ETEC were detected only in one strain of *E. coli* isolated from «zoom-koom». The results are shown in Table 4.

Table 3. Pathotypes present in local beverages and ice blocks by detection with multiplex PCR.

Products	Different pathotypes of *E. coli* (%)					
	EPEC	STEC	STEC-ETEC	EAEC	EIEC	ETEC
«Bissap» (n = 19)	1(5,2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
«Gnamakoudjii» (n = 31)	6(19,3%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
«Zoom-koom» (n = 36)	6(16,6%)	1(2,7%)	1(2,7%)	0(0%)	0(0%)	0(0%)
Ice blocks (n = 35)	6(17,1%)	1(2,8%)	0(0%)	0(0%)	0(0%)	0(0%)
Total (n = 121)	19(15,7%)	2(1,6%)	1(0,8%)	0(0%)	0(0%)	0(0%)

Atypical EPEC does not harbor the EPEC adhesion factor (EAF) plasmid, the *bfp*A gene is absent and only the *eaeA* gene is present. Therefore Atypical EPEC was the predominant pathotypes determined in our study with the detection of the *eaeA* gene. Other studies conducted in Burkina Faso reported the presence of *eaeA* gene present in EPEC in stool (Bonkougou *et al.*, 2012), and in dairy products samples in Nigeria (Akhibe *et al.*, 2014). However, no EPEC was detected in recent study conducted on grilled/flamed chicken (Somda *et al.*, 2018).

The presence of atypical EPEC strains (possessing only the *eaeA* gene) has been associated with childhood diarrhea as documented by Hedge *et al.* (2012) in India.

Table 4. Identification of virulence genes of strains isolated from beverages and ice blocks.

Origin of *E. coli* strains	Reference strains/ Isolated strains		Virulence genes			
			eae	*stx1*	*stx2*	*estla*
Burkina Faso, beef	STEC	FE 102301	-	-	+	-
Burkina Faso, beef	STEC-ETEC	FE 95562	-	+	-	+
Finland, patient	*E. coli*	IHE 50246	-	-	-	-
«Bissap» n= 19	EPEC		1	-	-	-
«Gnamakoudjii» n= 31	EPEC		6	-	-	-
«Zoom-koom» n= 36	STEC		-	-	1	-
	STEC-ETEC			1	-	-
	EPEC		6	-	-	1
Ice blocks n= 35	STEC		-	-	1	-
	EPEC		6	-	-	-
Total of virulence genes			19	1	2	1

Legend: +: Presence of gene ; - : Absence of gene ; n=1...6 : number of strains presenting the virulence genes.

Of the pathotypes tested on all strains isolated from beverages and ice blocks, STEC was determined by observation of the *stx*2 gene. STEC-ETEC was also detected by observation of the *stx*1 and *estla* genes. In a study conducted in Mexico, out of 100 samples of beet juice collected, 2% ETEC was found, (Gómez-Aldapa *et al.*, 2014). According to Tobias and Vutukuru, (2012), the primer *stx* was designed for the specific identification of *E. coli* O157: H7 which detects both stx1 or/and *stx2* genes. Shiga toxin *(stx)* is one of the major virulence factors causing hemorrhagic colitis and hemolytic uremic syndrome involved in the pathogenesis of *E. coli* O157:H7 (Melton-Celsa *et al.*, 2012). Indeed, the presence of shiga toxin in local beverages and ice blocks could cause sporadic or epidemic cases of diarrhea, often accompanied by bloody stools.

The EAEC, EIEC and ETEC pathotypes were not detected in any of the samples in our study. Indeed, none of the virulence genes (*aggR*, *pic*, *astA*, *ipaH*, *invE*, *elt*, *estIa*, *estIb*) belonging to these pathotypes could be observed after amplification and gel migration.

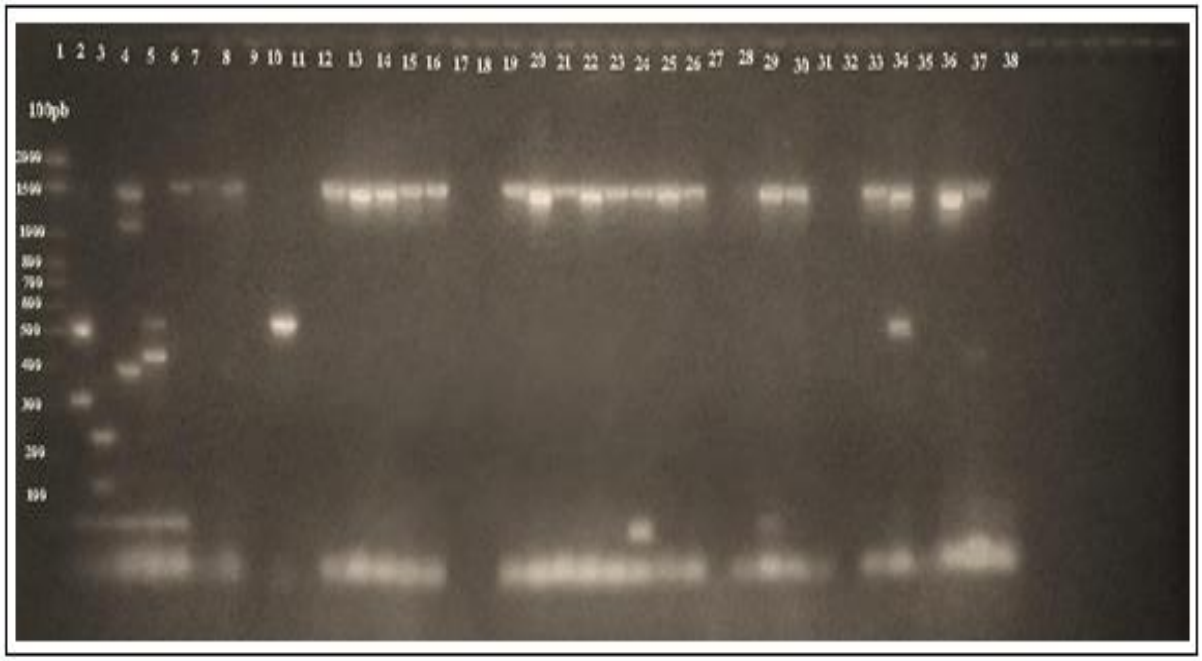

Fig. 1. Amplicons of the different *E. coli* pathotypes (Photo: Gertrude Bsadjo Tchamba, 2015).

Although most cases of illness caused by *E. coli* pathotypes have been associated with undercooked meat or water contaminated with cattle feces, the presence of pathotypes in beverages and ice blocks could be attributed to the various failures during the various stages between production, preservation and distribution. Similar work has shown that the most likely mechanisms of contamination by pathogenic microorganisms of the juices, fruits and dairy products from which they are made are either direct contact with human or animal feces or indirectly through contact with water, soil, processing equipment, or infected workers (Akhibe *et al.*, 2014; Somda *et al.*, 2018).

Conclusion

At the end of this study, diarrheal *E. coli* pathotypes were isolated from local beverages and ice blocks. Atypical strains of EPEC (*eaeA* gene), ETEC (*stx*1 and *estla* genes) were detected. Indeed, the presence of these *E. coli* virulence genes in local beverages and ice blocks could cause sporadic or epidemic cases of diarrhea. Training of vendors on good hygiene and manufacturing practices should be emphasized for the improvement of finished products purchased by consumers.

Acknowledgments

This work was supported by the International Foundation for Science with the grant E-5391-1.

Authors' contributions

Gertrude Bsadjo Tchamba and Hadiza Bawa Ibrahim collected the samples, performed the experiments. Gertrude Bsadjo Tchamba analyzed and interpreted the data and wrote the manuscript. Nicolas Barro supervised the works. Compliance with ethical standards.

Conflict of interest

On behalf of all the authors, the corresponding author states that there is no conflict of interest.

References

Akhibe I, Olufemi EO, Morenike AD. 2014. Shiga toxin-producing *Escherichia coli* O157:H7 in milk and milk products in Ogun State, Nigeria. Veterinaria Italiana **50(3),** 185-91. https://doi.org/10.12834/vetit.129.2187.1

Antikainen J, Tarkka E, Haukka K, Siitonen A, Vaara M, Kirveskari J. 2009. New 16plex PCR method for rapid detection of diarrheagenic

Escherichia coli directly from stool samples. European Journal of Clinical Microbiology & Infectious Diseases **28,** 899–908. https://doi.org/10.1007/s10096-009-0720-x

Bonkoungou IJO, Lienemann T, Martikainen O, Dembelé R, Sanou I, Traoré AS, Siitonen A, Barro N, Haukka K. 2012. Detection of diarrhoeagenic *Escherichia coli* by 16-plex PCR from young children in urban and rural Burkina Faso. Clinical Microbiology and Infection **18,** 901-906. https://doi.org/10.1111/j.1469-0691.2011.03675.x

Bsadjo Tchamba G, Bawa IH, Nzouankeu A, Bagré TS, Dembélé R, Bonkoungou IJO, Zongo C, Savadogo A, Traoré AS, Barro N. 2014. Occurrence and antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from "zoom-koom" beverage and ice in Ouagadougou, Burkina Faso. African Journal of Microbiology Research **8,** 3243-3249. http://dx.doi.org/10.5897/AJMR2014.7014

Bsadjo Tchamba G, Bawa IH, Nzouankeu A, Bagré TS, Traoré AS, Barro N. 2015. Isolation, characterization and antibiotic susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from local beverages (bissap, gnamakoudji) sold in Ouagadougou, Burkina Faso. International Journal of Biosciences **6(2),** 112-119. http://dx.doi.org/10.12692/ijb/6.2.112-119

Canizalez-Roman A, Gonzalez-Nuñez E, Vidal JE, Flores-Villaseñor H, León-Sicairos N. 2013. Prevalence and antibiotic resistance profiles of diarrheagenic *Escherichia coli* strains isolated from food items in north western Mexico. International Journal of Food Microbiology **164,** 36. https://doi.org/10.1016/j.ijfoodmicro.2013.03.020

Gómez-Aldapa CA, Rangel-Vargas E, Bautista-De León H, Castro-Rosas J. 2014. Presence of non-O157 Shiga toxin-producing *Escherichia coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli* and *Salmonella* in fresh beetroot (*Beta vulgaris* L.) juice from public markets in Mexico. Journal of the Science of Food and Agriculture **94,** 2705-2711. https://doi.org/10.1002/jsfa.6614

Hegde A, Balla M, Shenoy S. 2012. Detection of diarrhoeagenic *Escherichia coli* by multiplex PCR. Indian Journal of Medical Microbiology **30(3),** 279–84. https://doi.org/10.4103/0255-0857.99485

Kagambega A, Martikainen O, Lienemann T, Siitonen A, Traore AS, Barro N, Haukka K. 2012. Diarrheagenic *Escherichia coli* detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. International Journal of Food Microbiology **153(1),** 154–158. https://doi.org/10.1016/j.ijfoodmicro.2011.10.032

Keskimäki M, Mattila L, Peltola H, Siitonen A. 2000. Prevalence of diarrheagenic *Escherichia coli* in Finns with or without diarrhea during a round-the-world trip. Journal of Clinical Microbiology **38(12),** 4425–4429. https://doi.org/10.1128%2Fjcm.38.12.44254429.2000

Liu B, Furevi A, Perepelov AV, Guo X, Cao H, Wang Q, Reeves PR, Knirel YA, Wang L, Widmalm G. 2020. Structure and genetics of *Escherichia coli* O antigens. FEMS Microbiology Reviews **44(6),** 655-683. https://doi.org/10.1093/femsre/fuz028

Melton-Celsa A, Mohawk K, Teel L, O'Brien A. 2012. Pathogenesis of Shiga-toxin producing *Escherichia coli*. Current Topics in Microbiology and Immunology **357,** 67–103. https://doi.org/10.1007/82_2011_176

Moyo SJ, Gro N, Matee MI, Kitundu J, Myrmel H, Mylvaganam H, Maselle SY, Langeland N. 2011. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. BMC Pediatrics **11,** 19.

https://doi.org/10.1186/1471-2431-11-19

Munhoz DD, Santos FF, Mitsunari T, Schüroff PA, Elias WP,_Carvalho E, Piazza RMF. 2021. Hybrid Atypical Enteropathogenic and Extraintestinal *Escherichia coli* (aEPEC/ExPEC) BA1250 Strain: A Draft Genome. Pathogens **10(4),** 475.
https://doi.org/10.3390/pathogens1004047

Somda NS, Bonkoungou OJI, Zongo C, Kagambèga A, Bassolé IHN, Traoré Y, Mahillon J, Scippo ML, Hounhouigan JD, Savadogo A. 2018. Safety of ready-to-eat chicken in Burkina Faso: Microbiological quality, antibiotic resistance, and virulence genes in *Escherichia coli* isolated from chicken samples of Ouagadougou. Food Sciences & Nutrition **6(4),** 1077–1084.
https://doi.org/10.1002/fsn3.650

Tobias J, Vutukuru SR. 2012. Simple and rapid multiplex PCR for identification of the main human diarrheagenic *Escherichia coli*. Microbiological Research **167(9),** 564–570.
https://doi.org/10.1016/j.micres.2011.11.006

Legend:

Well 1: Molecular weight marker (100 bp DNA Ladder 0.13µg/µl)
Reference strains: well 2: STEC; well 3: STEC-ETEC; well 4: EAEC; well 5: EIEC ;
Well 6: ETEC;
Wells 7 to 16: ice samples10 STEC (*escV* = 544 bp)
Well 19 to well 26: «gnamakoudjii» samples;
Wells 29 to 30: «bissap» samples;
Wells 33 to 36: «zoom koom» samples; ZK34 STEC (*escV* = 544 bp)
Well 37: *E. coli uidA*; Negative control:
Well 38: Water;