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Isolation and characterization of enteropathogenic and enterotoxinogenic *Escherichia coli* from dairy products consumed in Burkina Faso

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Food-borne diseases represent a public major health problem, and drink-water, juice, meat, and milk products are usually involved. This study aimed to evaluate the antibiotic resistance of diarrheagenic *E. coli* isolated from dairy products consumed in Burkina Faso. Five hundred and twenty-two samples were gathered. *Escherichia coli* were isolated using Standard Microbiological Methods. A 16-plex polymerase chain reaction for virulence associated genes was applied. The standard disc diffusion methods were used to assess the susceptibility to 31 antibiotics. Classes 1, 2, 3 integrons were categorized using PCR. Results showed 1.92% (10/522) of milk products was contaminated by diarrheagenic *E. coli*. Enterotoxinogenic *E. coli* was found in 4.45% (4/89) of curds, 3.4% (3/88) of pasteurized milk, and 1.15% (1/87) of "déguè". Also, "déguè" was contaminated at 2.3% (2/87) by atypical enteropathogenic *E. coli*. Antibiogram susceptibility showed that pathogenic isolated resists mainly to tetracycline, amoxicillin, ticarcillin, nalidixic acid, sulfonamide, and trimethoprim-sulfamethoxazole. Only the class 1 integrons was detected in 80% of diarrheagenic *E. coli*. Among this class 1 integrons, 4 strains contains a variable region, and the subsequent result showed a presence of *dfrA7* gene coding for trimethoprim resistance. It appears in this study that dairy products are contaminated by enteropathogenic and enterotoxinogenic *E. coli*, which are resistant to antibiotics frequently used. This study therefore recommends the training of milk products transformers.

Key words: Dairy products, diarrheagenic *Escherichia coli*, antibiotics resistance, integrons, Burkina Faso.

INTRODUCTION

Food-borne diseases represent a public major health problem, and drink-water, juice, meat, and milk products are usually involved (OMS, 2011). Abdominal cramps, vomiting, diarrhea with/without blood, fever (OMS, 2011)

are illnesses caused by foods contaminated. Diarrhea causes mortality to a fifth of all people and a third to children younger than five years old worldwide (OMS, 2014). Diarrheagenic *Escherichia coli* (DEC) remain the ones mostly associated with endemic and epidemic diarrhea, amongst all the enteropathogenic bacteria worldwide (Nataro and Kaper, 1998). In Burkina Faso, DEC is mainly responsible for diarrhea among infants younger than 5 years often associated with vomiting, fever, and dehydration (Bonkoungou et al., 2013).

Studies showed variable contaminations of milk, pasteurized milk, cheeses, and other milk products by enteropathogenic, enteroaggregative, enterohemorragic and enterotoxinogenic *E. coli* in Iran (Bonyadian et al., 2014), India (Nazir et al., 2013), Ivory Coast (Dadie et al., 2010), Brazil (Paneto et al., 2007), Nigeria (Ivbade et al., 2014), and Saudi Arabia (Al-Zogibi et al., 2015). Studies that were done on diarrheagenic *E. coli* isolated from cheeses, milk, pasteurized milk, and other milk products from Nigeria (Ivbade et al., 2014), Greece (Solomakos et al., 2009), Brazil (Paneto et al., 2007), and India (Nazir et al., 2013) revealed resistance to amoxicillin, amoxicillin-clavulanic acid, ampicillin, nalidixic acid, norfloxacin, gentamicin, streptomycin, tetracycline, doxycycline, erythromycin, cefaclor, cephradine, ceftazidime, chloramphenicol, and sulfamethoxazole-trimethoprim with variable rates. Several mechanisms are involved in antimicrobial resistance. Beyond the efflux system, reduction of the porins structure, and changing of the target of the antibiotics by methylation, plasmids, integrons and transposons also play an important role in antibiotic resistance (Stokes and Hall, 1989; Schwarz and Chaslus-Dancla, 2001; Escudero et al., 2015). These capture systems mobile elements (integrons, plasmids), are responsible for the resistance genes dissemination between the same species and the different species (Escudero et al., 2015). The studies on clinical, food and environmental DEC isolated from Nigeria, Egypt, India, and Kenya revealed that the presence of class 1, 2, and 3 integrons are the cause of the resistance of these pathogens to antibiotics. Class 1, and 2 integrons are connected to several resistance genes (cassettes) encoding antibiotic resistance such as *dfr* for trimethoprim resistance, *aac* and *aad* for aminoglycosides, *sul* for sulfonamides, *tet* for tetracycline's, *cat*, and *cmlA1* for chloramphenicol, *satA1* for streptothricin (Kiiru et al., 2013; Adelowo et al., 2014; Dureja et al., 2014; Ahmed and Shimamoto, 2015). Thus, this study aimed to examine the prevalence and mechanism of antibiotic resistance of diarrheagenic *E. coli* isolated from milk, pasteurized milk, curds, yogurts, and "déguè" (mixture of yogurt and millet lumps) consumed in Burkina Faso.

MATERIALS AND METHODS

Study design and sampling

The study was conducted between October 2011 and June 2015, in ten major cities producing and consuming bovine milk products in Burkina Faso (Figure 1a). Sampling was carried out regularly within three steps of milk production: firstly, 69 farms' milk had been collected in eight cities: "Bobo-Dioulasso" in the Southwest (19), "Dori" in the North (5), "Fada N'Gourma" in the East (12), "Kongoussi" and "Sabcè" in the North Central with respectively 4 and 3 farms, "Koudougou" in the West Central (6), "Léo" in the South (12) and "Ouahigouya" in the North (8). All the farms in an area were connected to dairy transformation units in the same city. Secondly, four yogurts and 13 pasteurized milk products samples were collected from the dairy transformation units associated with the above-cited farms. Thirdly, 436 milk products of consumption from distribution chain were gathered in "Ouagadougou" (Figure 1b) and "Ziniaré". These consisted of 84 sets of milk and 89 curds samples (a traditional production) from open markets, 88 pasteurized milks, 88 yogurts and 87 "dégué" samples from food shop and supermarkets. A total of 125 to 500 ml samples of milk products were gathered and transported at 4°C to the "Laboratoire de Biologie Moléculaire, d'Epidémiologie et de Surveillance des Bactéries et Virus Transmissibles par les Aliments (LaBESTA)/ Université Ouaga I Pr Joseph KI-ZERBO", and examined immediately.

Escherichia coli isolation and identification

The ISO 4832 (ISO, 1991) modified method was used for isolating and identifying *E. coli*. Twenty-five milliliter samples of milk were homogenized into 225 ml of buffered peptone water (Liofilchem, Italy) and incubated at 37°C. Then, after 24 h of incubation, two loopfuls of enriched broth were streaked into violet red bile lactose (VRBL) agar (Liofilchem, Italy) and ChromoCult coliform agar (Merck, Germany), which were incubated at 44.0 ± 0.1°C for 24 h. Suspicious *E. coli* colonies appear small, purple with purple cloud and blue at violet respectively on VRBL and ChromoCult coliform agar. Three to five presumptive colonies were carefully chosen and tested for lactose and glucose metabolism, indole, urea, citrate, and fermentative gas production. *E. coli* colonies were confirmed by API 20E system (BioMerieux, France).

Diarrheagenic *Escherichia coli* characterization

A boiling process was used to obtain the DNA of each strain. This was carried out by homogenizing two loopfuls of each strain into an Eppendorf Tube comprising 250 µl of sterile water. The mixture was boiled afterwards for 10 min and centrifuged for 10 min. The supernatant was collected and used for the PCR reactions.

A multiplex polymerase chain reaction was used for the detection of five major diarrheagenic *E. coli* (DEC). This characterization was carried out for the intensifying of 16 virulence genes of DEC with specific primers (Table 1). The virulence genes that follow were categorized according to Antikainen et al. (2009): For Entero-pathogenic *E. coli* (EPEC), the presence of *eaeA*, *escv* and/or *ent* and *bfpB*. The absence of *bfpB* indicated atypical EPEC; for Shiga Toxin producing *E. coli* (STEC), the presence of stx_1, and/or stx_2 with a possible additional genes as *eaeA*, *escv*, *ent*, and EHEC-*hly*;

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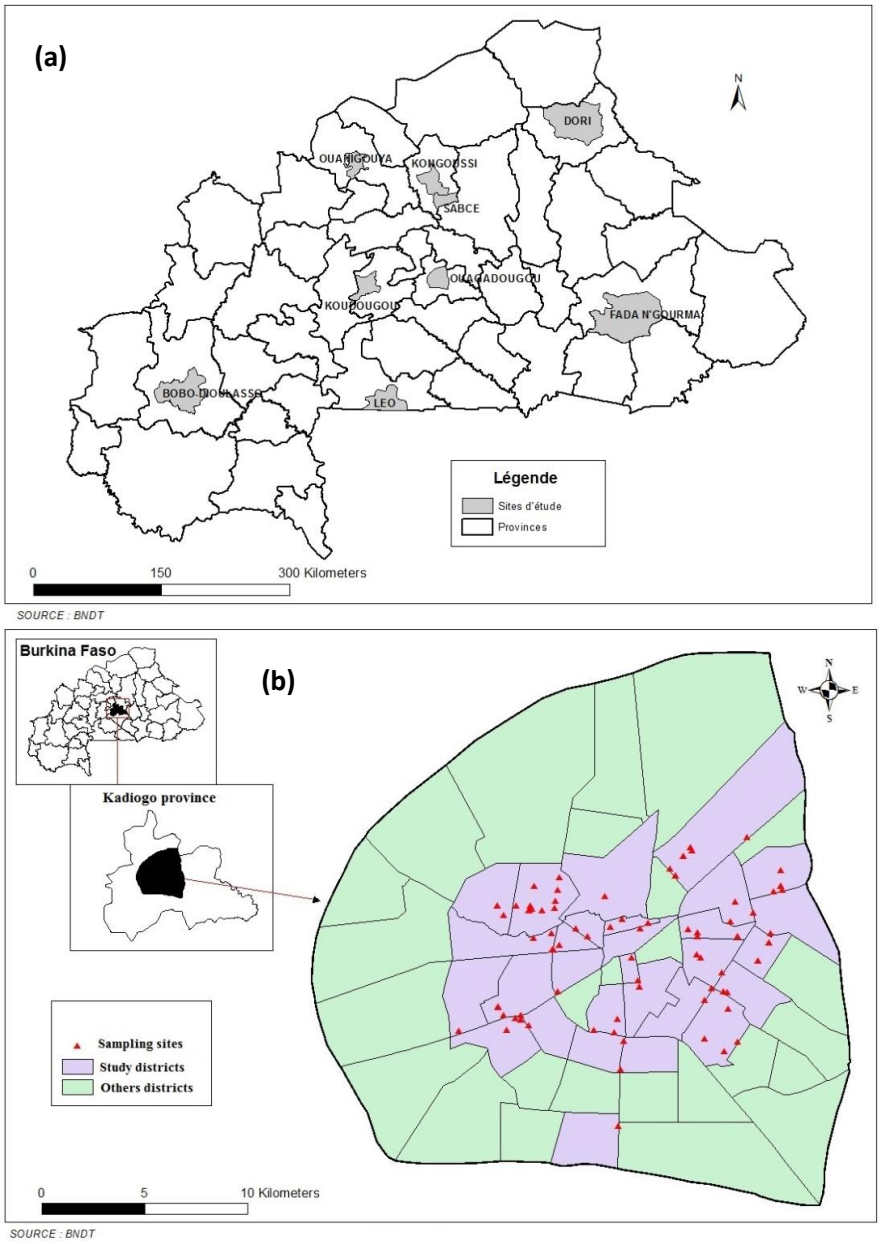

Figure 1. (a): Map of Burkina Faso with sampling sites in the nine (9) majors' cities producing and/or consuming of milk products. (b): Sampling sites in Ouagadougou, Burkina Faso.

for Enteroinvasive *E. coli* (EIEC), the presence of *ipaH*, and *invE* genes*;* for Enteroaggregative *E. coli* (EAEC), the presence of *virF*, and/or *aggR*, and/or *pic*, and/or *astA* genes; for Enterotoxinogenic *E. coli* (ETEC), the presence of *elt*, and/or *sta*, and/or *stb* genes.

A 25 µl reactional mixture was employed to perform the Multiplex. A volume of 2.5 µl of DNA samples were added into 22.5 µl of PCR mixture comprising 5.0 µl of buffer GC, 0.6 µl of $MgCl_2$, 1.0 µl of dNTPs, 0.4 µl of Taq polymerase, 10.5 µl of H_2O, 2.5 µl of Muller mix (*escV, bfpB, stx1, stx2, lt, sta, stb, invE, astA*, *aggR, pic, uidA*), and 2.5 µl of Jenni mix (*eaeA, ent,* EHEC-*hly, ipaH*). Amplification programmes were 98°C for 30 s following to 30 cycles of 98°C for 30 s, 62,5°C for 60 s, 72°C for 90 s, and final extension of 72°C for 10 min. Amplified DNA fragments were divided by agarose gel electrophoresis (1% weight/volume), added ethidium bromide, and visualized under UV light. DNAs of following reference strains were used for positive control: 17.2 for EAEC, E2348-69 for EPEC, EDL 933 for EHEC, M90T for EIEC and EDL 1493 for ETEC. The strain HB101 DNA was also used for negative control.

Antimicrobial susceptibility testing

The agar disc diffusion method (CASFM, 2014) was used to carry out Antimicrobial susceptibility of diarrheagenic *E. coli* isolated. Diameters of inhibition were determined according to « Comité de l'Antibiogramme de la Société Française de Microbiologie » instructions (CASFM, 2014). Thirty-one (31) common antibiotic (BioRad, France) discs used were: amoxicillin 25 µg (AMX),

Table 1. Diarrheagenic *E. coli* primers and the virulence genes detected.

Pathotypes	Target gene	Primer sequence (5'-3')	Product size (bp)	[C] (µM)
STEC, EPEC	*eaeA*	F: TCAATGCAGTTCCGTTATCAGTT R: GTAAAGTCCGTTACCCCAACCTG	482	0.1
	escV	F: ATTCTGGCTCTCTTCTTCTTTATGGCTG R: CGTCCCCTTTTACAAACTTCATCGC	544	0.4
	ent	F: TGGGCTAAAAGAAGACACACTG R: CAAGCATCCTGATTATCTCACC	629	0.4
EPEC typique	*bfp B*	F: GACACCTCATTGCTGAAGTCG R:CCAGAACACCTCCGTTATGC	910	0.1
STEC	EHEC-*hly*	F: TTCTGGGAAACAGTGACGCACATA R: TCACCGATCTTCTCATCCCAATG	688	0.1
	stx1	F: CGATGTTACGGTTTGTTACTGTGACAGC R: AATGCCACGCTTCCCAGAATTG	244	0.2
	stx2	F:GTTTTGACCATCTTCGTCTGATTATTGAG R: AGCGTAAGGCTTCTGCTGTGAC	324	0.4
EIEC	*ipaH*	F: GAAAACCCTCCTGGTCCATCAGG R: GCCGGTCAGCCACCCTCTGAGAGTAC	437	0.1
	invE	F: CGATAGATGGCGAGAAATTATATCCCG R:CGATCAAGAATCCCTAACAGAAGAATCAC	766	0.2
EAEC	*aggR*	F: ACGCAGAGTTGCCTGATAAAG R: AATACAGAATCGTCAGCATCAGC	400	0.2
	pic	F: AGCCGTTTCCGCAGAAGCC R: AAATGTCAGTGAACCGACGATTGG	1111	0.2
	astA	F: TGCCATCAACACAGTATATCCG R: ACGGCTTTGTAGTCCTTCCAT	102	0.4
ETEC	*elt*	F: GAACAGGAGGTTTCTGCGTTAGGTG R: CTTTCAATGGCTTTTTTTTGGGAGTC	655	0.1
	estIa	F:CCTCTTTTAGYCAGACARCTGAATCASTTG R: CAGGCAGGATTACAACAAAGTTCACAG	157	0.4
	estIb	F: TGTCTTTTTCACCTTTCGCTC R: CGGTACAAGCAGGATTACAACAC	171	0.2
E. coli	*uidA*	F: ATGCCAGTCCAGCGTTTTTGC R:AAAGTGTGGGTCAATAATCAGGAAGTG	1487	0.2

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

ticarcillin 75 µg (TIC), piperacillin 75 µg (PIP), piperacillin + tazobactam 85 µg (PPT), amoxicillin + clavulanic acid 30 µg (AMC), ticarcillin + clavulanic acid 85 µg (TCC), cefotaxim 30 µg (CTX), ceftazidim 30 µg (CAZ), cefolatin 30 µg (CEF), cefepim 30 µg (FEP), aztreonam 30 µg (ATM), imipenem 10 µg (IPM), cefuroxim 30 µg (CXM), cefoxitin 30 µg (FOX), imipenem + EDTA 10 µg (EIP), nalidixic acid 30 µg (NAL), norfloxacin 5 µg, ofloxacin 5 µg (OFX), ciprofloxacin 5 µg (CIP), tobramycin 10 µg (TMN), gentamicin 10 µg (GMN), amikacin 30 µg (AKN), chloramphenicol 30 µg (CHL), tetracycline 30 µg (TET), minocyclin 30 µg (MNO), tigecyclin 15 µg (TGC), fosfomycin 50 µg (FSF), sulfonamide 200 µg (SUL), trimethoprim + sulfamethoxazol 25 µg (SXT), nitrofuran 300 µg (FTN), nitroxolin 20 µg (NIT). A multi-drug resistance of strains was examined and defined as being a resistance to at least three families of antibiotics (Dureja et al., 2014). The inhibition zones were evaluated as "resistant", "intermediate sensitive", or "sensitive" according to CASFM (2014) criteria with Antimicrobial susceptibility testing system version 3.0.0.

Integrons detection

For all DEC, the single polymerase chain reaction (Bissonette and Roy, 1992; Ploy et al., 2000; Mazel, 2004) was used to detected classes 1, 2 and 3 integrons. Integrase genes *IntI1*, *IntI2*, and *IntI3*

Table 2. Integrons, and resistance genes primers.

Target gene	Primer sequence (5'-3')	Product size (bp)	Concentration (µM)
IntI1	F: ATTTCTGTCCTGGCTGGCGA R: ACATGTGATGGCGACGCACGA	600	10
IntI2	F: CACGGATATGCGACAAAAAGG T R: GTAGCAAACGACTGACGAAATG	806	10
IntI3	F: GCCCCGGCAGCGACTTTCAG R: ACGGCTCTGCCAAACCTGACT	600	10
36854 *36855*	GGCATGCAAGCAGCAAGCGCGTTA AACCGAACTTGACCTGATAGTTTG		10
Sul1 *Orf4*	GTCCGACATCCACGACGTCTGATC CAAACTATCAGGTCAAGTCTGCTT		10
Sul3 *Orf6*	CCTGGAGATCTGCGAAGCGCAATC GTCGCTGCAACTCGCGACT		10

Table 3. Prevalence of *E. coli* in milk products consumed in Burkina.

Types of dairy products	*Escherichia coli*	
	Number	%
Farm milk (n=69)	68	98.55
Milk (n=84)	29	34.52
Curd (n=89)	29	32.58
Pasteurized milk (n=101)	29	28.71
Yoghourt (n=92)	04	04.35
Déguè (=87)	14	16.09
Total (n=522)	**174**	**33.33**

were carried out with specific primers (Genecust, Luxembourg) (Table 2). Primers 36854 and 36855 were used to categorize the variables regions (VR) of class 1 integrons. Sizes of variables regions were measured, purified, and sequenced to determine resistance genes. For the 3' conserved segment (3'CS), primers sul1-Orf4, and sul3-Orf6 were used by single PCR. Thermocycling conditions were 94°C for 5 min, following to 35 cycles at 94°C for 30 s, 60 s to 60 and 62°C respectively for *IntI1*, VR, 3'CS and *IntI2*/*IntI3*, and 72°C for 60 s. The ultimate extension was 72°C for 10 min. The amplicons were visualized by electrophoresis on 1% (weight / volume) gel agarose in the TAE buffer.

Data analysis

MS Excel 2010 was used to analyze the data. The obtained sequences were compared in GenBank database with the use of BLAST software in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determined resistance genes with a similarity of 98.5%.

RESULTS

Prevalence of *E. coli* in dairy products

The results show that 33.33% of dairy products are contaminated by *E. coli* with 315 strains. High rates were observed in traditional dairy products with 98.55% of farm milk, followed by 34.52% of milk sold in Ouagadougou's open markets, and curds (32.58%). This study also showed that pasteurized milk and "déguè" are contaminated by *E. coli* respectively at 28.71 and 16.09% (Table 3).

Prevalence of diarrheagenic *E. coli*

The study revealed that the milk products consumed in study sites are contaminated by some diarrheagenic *E. coli* with variables rates (Table 4). Atypical enteropathogenic *E. coli* (presence of *eaeA* and *ent* genes) and enterotoxinogenic *E. coli* (presence of *stb* gene) was detected with 0.38 and 1.53% of total milk products respectively. Atypical enteropathogenic *E. coli* (aEPEC) was found in 2.3% of "déguè" while 4.45% of curds, 2.97% of pasteurized milk, and 1.15% of "déguè" were contaminated by enterotoxinogenic *E. coli*.

Antimicrobial resistance of diarrheagenic *E. coli*

Antibiogram results showed diarrheagenic *E. coli* isolated from milk products resists at least to 11 antibiotics used. Atypical enteropathogenic and enterotoxinogenic *E. coli* were resistant to amoxicillin, ticarcillin, piperacillin, nalidixic acid, norfloxacin, ofloxacin, tobramycin, nitroxolin, nitrofuran, tetracycline, sulfonamide, and trimethoprim-sulfamethoxazole (Table 5).

Class 1 integrons, and resistance genes

Class 1 integrons were detected in eight out of the 10 pathogenic *E. coli* isolates (80%). Class 2 or 3 integrons

Table 4. Prevalence of diarrheagenic *Escherichia coli*.

Types of dairy products	Diarrheagenic *Escherichia coli* (DEC) N (%)					
	EPEC	STEC	EHEC	EIEC	EAEC	ETEC
Farm milk (n=69)	-	-	-	-	-	-
Milk (n=84)	-	-	-	-	-	-
Curd (n=89)	-	-	-	-	-	4 (4.45)
Pasteurized milk (n=101)	-	-	-	-	-	3 (2.97)
Yoghourt (n=92)	-	-	-	-	-	-
Déguè (=87)	2 (2.3)	-	-	-	-	1 (1.15)
Total (n=522)	2 (0.38)	-	-	-	-	8 (1.53)

-: None; EPEC: Enteropathogenic *E. coli*; STEC: Shiga toxin producing *E. coli*; EHEC: Enterohemorrhagic *E. coli;* EIEC: Enteroinvasive *E. coli;* EAEC: Enteroaggregative *E. coli;* ETEC: Enterotoxinogenic *E. coli*.

Table 5. Resistance of diarrheagenic *Escherichia coli* to antibiotics used.

Strains	Milk products	Pathovars	Antibiotic resistance	Intermediary resistance
Ld5.4	Déguè	EPEC	TET, SUL	NIT
Ld5.1		EPEC	AMX, TIC, NAL, TET	NIT, PIP
Ld5.2		ETEC	AMX, TIC, NAL, TET	NIT, PIP
Lc37	Curds	ETEC	-	NIT
Lc2.2		ETEC		NIT, PIP, TIC
Lc7		ETEC	FTN	NIT
Lc51.2		ETEC	-	NIT
Lp2.2	Pasteurized milk	ETEC	AMX, TIC, PIP, NAL, NOR, OFX, TMN	NIT
Lp56.4		ETEC	TET	NIT, TIC, PIP
Lp70.2		ETEC	AMX, TIC, PIP, TET, SUL, SXT	NIT

EPEC: Enteropathogenic *E. coli*; ETEC: Enterotoxinogenic *E. coli*, TET: Tetracyclin 30 µg; AMX: Amoxicillin 25 µg; SUL: Sulphonamid 200 µg; TIC: Ticarcillin 75 µg; NAL: Nalidixic acid 30 µg, FTN: Nitrofuran 300 µg; PIP: Piperacillin 75 µg; NOR: Norfloxacin 5 µg; OFX: Ofloxacin 5 µg; TMN: Tobramycin 10 µg; SXT: Trimethoprim-sulfamethoxazol 25 µg; NIT: Nitroxolin 20 µg.

were not detected. Four class 1 integron-containing EPEC, and ETEC strains contained an identical integron harboring a single cassette, *dfrA7*, encoding resistance to trimethoprim. No classes 2 and 3 integrons were detected in this study (Table 6).

DISCUSSION

Prevalence of *E. coli* in dairy products

Investigation showed that the milk products consumed in Burkina Faso are widely contaminated by *E. coli* with variable rates. Traditional milk products, such as farm milk (98.55%), milk (34.5%), and curds (32.58%), mostly sold in open markets, are highly contaminated by *E. coli*. Comparable outcomes have been reported in Ivory Coast and Burkina Faso with slim differences (Katinan et al., 2012; Bagré et al., 2014). These results could be explained by milking conditions in farms, and handling conditions during selling of milk. In fact, Bagré et al. (2015) showed that a majority of farms in Burkina Faso are mainly traditional with unhygienic practices. In these farms, 43.9% do not clean udders before milking, with a calabash being the main collection utensil. These practices, due to poor hygienic training, could explain the traditional milks' contamination during its production. In addition, in this study, fermented and pasteurized milks are contaminated by *E. coli*. The pasteurized milks consumed in Burkina Faso are contaminated by *E. coli* (28.71%). These results are lower than those found in Iran (93.75%) (Nazir et al., 2013). The contamination by *E. coli* could be also explained by a post-contamination during packaging. In unit, packaging is a manual that could cause contamination by workers. The results have shown that the yogurts, which are consumed in Burkina Faso, are less contaminated. In fact, this low contamination could be explained by the acidity of yogurt. Investigations have revealed lactic bacteria produce bacteriocins, which inhibit pathogens as *E. coli, Listeria*

Table 6. Resistance genes associated to class 1 integrons.

Stains	Milk product	Pathovars	Class integrons	Size of cassettes (bp)	Resistance genes
Ld5.4	Déguè	EPEC	*IntI1*	800	*dfrA7*
Ld5.1		EPEC	*IntI1*	800	*dfrA7*
Ld5.2		ETEC	-	-	-
Lc37	Curds	ETEC	*IntI1*	800	*dfrA7*
Lc2.2		ETEC	-	-	-
Lc7		ETEC	*IntI1*	-	-
Lc51.2		ETEC	*IntI1*	800	*dfrA7*
Lp2.2	Pasteurized milk	ETEC	*IntI1*	-	-
Lp56.4		ETEC	*IntI1*	-	-
Lp70.2		ETEC	*IntI1*	-	-

EPEC: Enteropathogenic *E. coli*; ETEC: Enterotoxinogenic *E. coli.*

innocua and reduce bacterial flora (Khay et al., 2011; Yang et al., 2012); but high contamination of milk products by *E. coli* before transformation can still contain these bacteria. About “déguè”, this contamination could be explained by the supplies used to fermented milk with lumps of millet. Lumps, which are not pasteurized and are often exposed to sun, could bring enteropathogens bacteria particularly for the period of the mixture.

Prevalence of diarrheagenic *E. coli*

Our study reveals a contamination of milk products by some pathotypes of *E. coli*. In this study, 522 milk products consumed in Burkina Faso were contaminated characteristically enteropathogenic and enterotoxinogenic *E. coli*. None of enterohemorrhagic, enteroinvasive and enteroaggregative *E. coli* was found in this study. Indeed, “déguè” (semi-modern milk product) is contaminated by atypical enteropathogenic *E. coli* (aEPEC) with the presence of *eaeA* and *ent* genes. A number of studies in some countries revealed that milk products are contaminated by atypical EPEC. For example, several authors noted that milk are contaminated at 1.2 to 1.6% in Ivory Coast (Dadie et al., 2010); 1.56% and 8.25% in Iran (Rahimi et al., 2012; Mohammadi and Abiri, 2013), and 7.03% in Saudi Arabia (Al-Zogibi et al., 2015). Correspondingly, pasteurized milks are contaminated at 22.1 and 28.12% in Brazil (Da Silva et al., 2001) and India (Nazir et al., 2013); milk cheeses in Brazil (2, 4, and 6% respectively by EPEC O125, O111, and O55), and in Iran (19.48% with the serotype O127) (Najand and Ghanbarpour, 2006; Paneto et al., 2007). About these pathovars in “déguè”, none of the studies was carried out in Africa. The contamination of milk products consumed in Burkina Faso may constitute a public health concern particularly for children younger than five years. Studies on diarrhea etiologies in Burkina Faso revealed that atypical EPEC are one of the most typical bacterial causes (Nitiema et al., 2011; Bonkoungou et al., 2013; Dembélé et al., 2015). In addition, Bonkoungou et al. (2013) reported that aEPEC is more predominant than classical EPEC in diarrhea infections from Burkina Faso. In recent times, studies displayed lower prevalence of EPEC in children younger than five years in Burkina Faso (4%) (Bonkoungou et al., 2013) and Senegal (1.16%) (Sambe-Ba et al., 2013). Among DEC found, enterotoxinogenic *E. coli* (ETEC) became predominant in traditional (curd) and semi-modern (“déguè”, and pasteurized milk) milk products. Studies in Germany (Franke et al., 1984), India (Nazir et al., 2013) and Iran (Bonyadian et al., 2014) revealed that milk products (3.2%), pasteurized milk (3.13%), and cheeses (1.66%) are contaminated by ETEC. It gives the impression that ETEC is associated with travelers’ and infantile diarrhea (Nataro and Kaper, 1998). Enterotoxinogenic *E. coli* can create heat-stable toxin (ST) and heat-labile toxin (LT), which are responsible to profuse water diarrhea and others symptoms such as fever, vomiting, abdominal cramps. In this study, heat-stable toxin (*stb*) gene was detected in all strains. In Burkina Faso, ETEC is responsible for infantile watery diarrhea, often associated with dehydration. The consumption of water, foods, unpasteurized milk, raw juice, fruits, vegetables and unheated meals are commonly implicated with ETEC infection (CDC, 2005).

Antimicrobial resistance of diarrheagenic *E. coli*

Antibiogram patterns revealed multidrug resistance of enteropathogenic and enterotoxinogenic *E. coli*. A small number of studies have been carried out on pathogenic *E. coli* isolated from a number of dairy products in the world. Resistance of diarrheagenic *E. coli* to nalidixic acid is comparable to that observed with enterotoxinogenic *E. coli* (ETEC) in Brazil (40%) (Paneto et al., 2007), and STEC in Nigeria (20%) isolated from milk products (Ivbade et al., 2014). However, the tetracycline resistance

is lower than that found in Nigeria (90%) (Ivbade et al., 2014) and Greece (100%) (Solomakos et al., 2009), and higher than that found in Brazil (31%) (Paneto et al., 2007) concerning STEC and ETEC strains. Resistances to norfloxacin and trimethoprim-sulfamethoxazole were observed. Higher results were observed mainly in Nigeria (20%) (Ivbade et al., 2014) and Greece (100%) (Solomakos et al., 2009) for the trimethoprim-sulfamethoxazole on *E. coli* producing Shiga toxin. Additionally, resistances to penicillins (amoxicillin, ticarcillin, and piperacillin), aminoglycosides (tobramycin), sulfonamides and others fluoroquinolones such as ofloxacin were observed. Diarrheagenic *E. coli* isolated from curds, pasteurized milks and "déguè" resist five antibiotics families, such as tetracycline, penicillin, aminoglycoside, sulfonamide, and fluoroquinolone. Such resistance could be clarified by the presence of genes encoding resistance to these antibiotics. Integrons characterization revealed mostly the presence of *dfrA7* genes encoding resistance to trimethoprim. This classification displays resistances to other antibiotics may be encoded by other mechanisms or unwanted genes in this study. Studies carried out on *E. coli* producing shiga toxin in Egypt showed 3' conserved regions of integrons contains *qnrB*, *qnrS*, and *floR* genes encoding resistance to quinolones (Ahmed and Shimamoto, 2015). In this study, 80% (8/10) of diarrheagenic *E. coli* harboured class 1 integrons, with *dfrA7* gene encoding trimethoprim resistance. Previous studies carried out in China on *E. coli* isolated from dairy products revealed the presence of *dfrA17* and *dfrA1* genes encoding trimethoprim resistance (Zhao et al., 2014). In addition, resistance to antibiotics belonging to other families of tetracycline, penicillin, aminoglycoside and quinolone could be due to a selection of resistant strains in dairy products by antibiotic residues. Our earlier data (Bagré et al., 2015) on the same dairy products revealed antibiotics residues belonging to beta-lactam and/or sulfonamides and/or tetracycline and aminoglycosides and/or quinolones and/or macrolides in several magnitudes. For that reason, antibiotic residues could exert selection pressure on pathogenic strains in these dairy products.

This study show that curds, pasteurized milk and “dégué” consumed in Burkina Faso are lowly contaminated by enteropathogenic and enterotoxinogenic *E. coli*. In addition, the results of the Integrons showed that resistance is carried out by plamsids, with risks of a transmission of inter/intra pathogenic species. These mechanisms could help to understand the genetic materials of the DEC resistance isolated from dairy products. Nevertheless, the risk appears low in prevalence terms; special attention should be giving to the dairy products process. Training and awareness should be done with dairy products transformers and farmers, with the view of protecting the health of consumers and avoid the emergence of resistant pathogens.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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