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Research Paper

Virulence profiles of pathogenic *Escherichia coli* strains isolated from street foods in Bénin

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Abstract. This study aimed to evaluate the risk incurred by street foods consumers, through the characterization of *Escherichia coli* strains isolated from food samples collected in two major cities of southern Bénin. After bacterial identification, the sensitivity of isolated *E. coli* strains was determined by the disc diffusion method. The phenotypic and genotypic characterization of *E. coli* strains that produce beta-lactamase and toxins were made respectively by acidimetric and PCR method. This study revealed that about 38.70% of analyzed samples were contaminated by *E. coli*. The phenotypical investigation showed respectively that in dry and rainy season, 100% and 21.43% of *E. coli* strains produced penicillinase. The bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were respectively carried by 80.96%, 4.76% and 14.28% of *E. coli* strains producing penicillinase. 4.35% of isolated *E. coli* strains carried STEC: shigatoxin *E. coli* (SLTI: Shiga-like toxin I) whereas 47.83% carried STEC: shigatoxin *E. coli* (SLTII: Shiga-like toxin II) followed by enterohemorragic *E. coli* (VTEC) (30.43%) and then enterotoxigenic *E. coli* (ETEC) (17.39%).

Keywords: Street food, *E. coli*, β-lactamase, resistance genes, toxins, food safety.

INTRODUCTION

For a well-being, the rural people of developing countries move greatly to major cities. This practice induces many food consequences. Indeed, in the largest cities, people will often work far away of their family. Thus, many people resort to food sold in the street to eat (Lalatiana, 2006). This restoration method is a characteristic of developing countries (Diouf, 1992).

The street foods represent an important part of food consumption in cities of developing countries, and it is practiced by millions of low or average-income people (FAO, 1989; Chauliac et al., 1998). With the disorganization prevailing in this sector, there are certain risks of food intoxication that should not overshadow. Nowadays many sellers do not know the good food hygiene practices and they often expose the food in poor conditions that can cause poisoning (Sansonetti, 1987; Secke, 2007). The sellers use often the raw materials and ingredients that are of very poor microbiological quality, non-potable water, unauthorized food additives of bad quality, plates and packaging material unsuitable or inadequately cleaned (Secke, 2007).

Street foods are reported to cause the food poisoning resulting from the consumption of food contaminated by harmful or pathogenic organisms capable to produce toxins (Edema et al., 2005). Thus, the diseases caused by consumption of foods containing the pathogenic microorganisms are nowadays probably the most widespread health problem in the world and an important cause of the reduction of economic productivity (Edema et al., 2005). Thus, it is not rare to contract the epidemic

and gastrointestinal diseases such as gastroenteritis and diarrhea of microbial origin due to the consumption of street foods (Barro, 2000). The number of food toxi-infections can only be estimated, but cannot be measured because the cases are numerous (millions) in country concerned (Flint et al., 2005). There are over 250 types of toxi-infections caused by dozens of pathogens such as *Salmonella* (Hennessy et al., 2004; Jones et al., 2006), *Staphylococcus* (Sina et al., 2011; Attien et al., 2014), *Clostridium perfringens* and *Vibrio cholerae* (Hanoshiro et al., 2004; Ghosh et al., 2007) and *Escherichia coli* (Vincenot et al., 2008).

In Bénin, very few studies have been carried out to research the pathogenic microorganisms in street foods. The only one conducted by Sina et al. (2011) had focused on *Staphylococcus aureus*. As *E. coli* is a commensal bacterium of the mammals' intestines, rarely pathogenic, it does not focus the attention of Béninese researchers working in food safety. However, some *E. coli* strains can be pathogenic and can cause the infections such as gastroenteritis, urinary tract infections, meningitis or septicemia (Dembélé et al., 2015). The pathogenicity of strains may be due to bacteria density ingested (Dexheimer et al., 2015), immunity of infected person (Attien et al., 2014), antibiotic resistance of the strains and the capacity of strains to produce the toxins. Among the pathogenic bacteria, those producing Expanded Spectrum β-Lactamase (ESBL) are reported to cause serious problems of treatment failure because they are resistance to third and fourth- antibiotics generation (Gulamber et al., 2013). Thus, this study aims to:

1. Assess the street food's degree of *E. coli* contamination;
2. Evaluate the capacity of *E. coli* strains isolated and characterized from their foods in Cotonou and Abomey-Calavi, cities of Bénin;
3. Produce β-lactamase and establish the antibiotic profile of their strains.

MATERIALS AND METHODS

Street foods collection

Three types of street foods (Russian salad, vegetable sauce and cooked rice) were investigated for this study. The sellers considered in this study are 'hawker sellers' preparing at home without a fixed sale point, 'semi-fixed sellers' preparing and selling in outdoor or under tree and 'fixed sellers' with refectory. The composition of collected food is same in all sellers. These targeted foods are ready to eat and warm at time of collection except those hawker sellers. These foods were collected in different areas highly frequented by the people in both Cotonou and Abomey cities (Figure 1). The investigated areas have been grouped into four categories namely: 'student area' very frequented by the students for their restoration, 'market area' where people goes to stock up on food crops, 'residential area' lived by rich people of Cotonou and Abomey and 'administrative area' where are implanted Official services. The samples were collected from 54 sellers divided into 18 fixed sellers, 18 semi-fixed sellers and 18 hawker sellers. Only one type of food was sampled from each seller because they do not sell all three types of street foods. The samples were collected from each seller twice in the day. The first collection was made early morning during the sale that the food is freshly cooked and the second at afternoon or the evening when the sale is almost over, in order to determine when the potential contamination occurs. A total of 108 samples were collected per season. The first food collection was done in dry season (February to May 2014) and repeated again in rainy season (June to September 2014). For these two seasons, each seller was collected four times, resulting in a total of 216 foods samples (72 salads, 72 rice and 72 vegetable sauces).

Microbiological density of street foods sampled

Once sample in the laboratory, 10 g of each sample was aseptically mixed into Erlenmeyer containing 90 ml of distilled water (10^{-1} dilution) and diluted serially up to a 10^{-5} dilution. One millimeter of dilutions (10^{-4} and 10^{-5}) were mixed with 15 ml of Plate Count Agar (~45°C) and poured in sterile Petri Dishes. After complete solidification, a second agar stratum (~4 ml) was added before incubated at 30°C for 24 h. The microbial colonies were counted per dish (30 to 300 colonies) and the densities are estimated as colony forming units (cfu/ml).

Isolation and identification of *E. coli* strains

E. coli strains were isolated and identified on Rapid'*E. coli* according to Bristol Bath Road (BDR) 07/01-07/93 standard (Moini et al., 1996). Indeed, 1 ml of each dilution (10^{-4} and 10^{-5}) has been flooded aseptically in sterile Petri dishes. The Rapid'*E. coli* medium (45°C) was added to the inoculum (~15 ml per dish) and the mixture was homogenized. After solidification, these Petri dishes were incubated at 44°C for 24 h. The purple colonies with diameter ≤ 0.5 mm are characteristics of *E. coli* strains producing both β-D-Glucuronidase and β-D-galactosidase while blue colonies of diameter ≤ 0.5 mm are characteristics of *E. coli* strains producing only β-D-galactosidase. The *E. coli* density per gram of products analyzed is determined by calculation according to the dilution factor. The research of *E. coli* is completed by indol production test (Riegel et al., 2006).

Antibiotic profile of *E. coli* isolates

The antibiotic profile of the isolated *E. coli* was

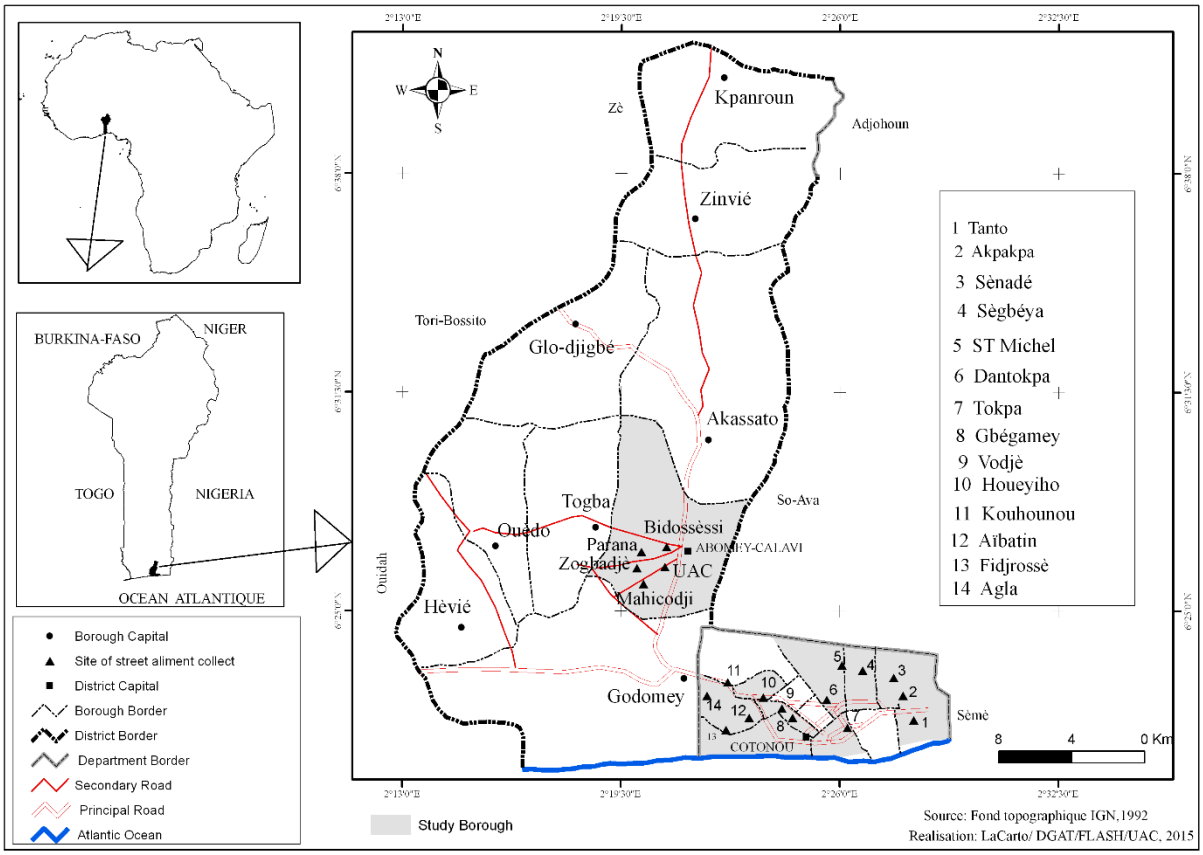

Figure 1. Overview of the garden sites.

established using the disk diffusion method on Mueller-Hinton agar (Oxoid, England). The interpretation of inhibition zone diameter values was done according to the criterion of Antibiogram Committee of the French Society of Microbiology (CASFM, 2012). The 13 antibiotics (BioMérieux, France) used are: amoxicillin/clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), amoxicillin (30 µg), imipenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), penicillin G (6 µg) and cotrimozazol (25 µg).

Screening of *E. coli* strains producing penicillinase

The production of penicillinase by the isolated *E. coli* strains was performed by tube acidimetric method (Koneman, 2006). Benzylpenicillin (600 mg) was added into 400 µl of distilled water and the solution was completed with 300 µl of NaOH (1N) and 300 µl of aqueous phenol red solution (1%, w/v). The pH of this solution was then adjusted to 8 using NaOH (1 N). The final reaction volume was 1 ml. Two young isolated *E. coli* colonies were suspended in 500 µl of distilled water, and were mixed to 150 µl of benzylpenicillin solution. The *E. coli* ATCC 25922 strains was used as a control. The appearance of yellow or orange color within 1 h at 37°C indicates the production of penicillinase.

Phenotypic detection of *E. coli* producing Extended Spectrum Beta-Lactamase (ESBL)

The screening of *E. coli* strains producing ESBL on the isolated *E. coli* strains was performed by double disk synergy test (Jarlier et al., 1988; Thomson and Sanders, 1992). Indeed, the tested strains (10^6 bacteria/ml) were flooded onto Mueller-Hinton according to the recommendations of the French Society of Microbiology (CASFM, 2012). The antibiotic discs used to perform this test are amoxicillin + clavulanic acid and the third generation Cephalosporins namely Cefotaxime (30 µg) and Ceftriaxone (30 µg). The amoxicillin + clavulanic acid disc was placed at the center of the inoculated Mueller-Hinton agar petri dish whereas the cefotaxime and ceftriaxone discs were placed at both sides (about 15 to 20 mm) of the amoxicillin + clavulanic acid disc. After incubation at 37°C for 18 h, the enhancement of the zones of inhibition of any of the cephalosporin disc towards the clavulanic acid disc confirms the strains as an ESBL producer (Allouch et al., 1995).

Table 1. Primers used to search genes in this study.

	Taguets genes	Primers	Primers sequences (5' 3')	Amplicon size (bp)	Reference
E. coli multirestant	bla_{TEM}	OT-F OT-R	5'-TTGGGTGCACGAGTGGGTTA-3' 5'-TAATTGTTGCCGGGAAGCTA-3'	467	Gangoué-Piéboji et al. (2005)
E. coli multirestant	bla_{SHV}	SHV-F SHV-R	5'-CGCCGGGTTATTCTTATTTGTCGC-3' 5'-TCTTTCCGATGCCGCCGCCAGTCA-3'	1017	Nüesch-Inderninen et al. (1996)
E. coli multirestant	bla_{CTX-M}	CTX-F CTX-R	5'-CGCTTTGCGATGTGCAG-3' 5'-ACCGCGATATCGTTGGT-3'	550	Gangoué-Piéboji et al. (2005)
EHEC	VT	VT-F VT-R	5′-GAGCGAAATAATTTATATGTG-3′ 5′-TGATGATGGCAATTCAGTAT-3′	518	Aranda et al. (2007)
STEC	SLTI (stx1)	SLTI-F SLTI-R	5'-GAAGAGTCCGTGGGATTACG-3' 5'-AGCGATGCAGCTATTAATAA-3'	150	Gassama-Sow et al. (2004)
STEC	SLTII (stx2)	SLTII-F SLTII-R	5'-TTAACCACACCCACGGCAGT-3' 5'-GCTCTGCATGCATCTCTGGT-3'	255	Gassama-Sow et al. (2004)
ETEC	LT	LT-F LT-R	5'-GCGACAAATTATACCGTGCT-3' 5'-CCGAATTCTGTTATATATGT-3'	315	Gassama-Sow et al. (2004)

VT: Verotoxin, EHEC: enterohemorragic *E. coli*, LT: heat-labile enterotoxin, ETEC : enterotoxigenic *E. coli*, SLTI: Shiga-like toxin I, SLTII: Shiga-like toxin II, STEC : shigatoxin *E. coli*.

Detection of genes encoding drug resistance and toxins production

Total DNA of all confirmed ESBL producer *E. coli* are extracted. Polymerase Chain Reactions (PCR) was performed on these DNA to detect genes encoding multidrug resistance (TEM, SHV and CTX-M) and some virulence factors such as VT (Verotoxin) encoding to enterohemorragic *E. coli* (VTEC), LT (heat-labile enterotoxin) witch encoding to enterotoxigenic *E. coli* (ETEC), SLTI (Shiga-like toxin I) and SLTII (Shiga-like toxin II) witch encoding to shigatoxin *E. coli* (STEC). For DNA extraction, a loop of *E. coli* colony is suspended into 500 µl of sterile water and boiling during 10 min at 95°C. The suspension was then centrifuged for 5 min at 12,000 rpm, and 10 µl of the supernatant (containing DNA) were used as target DNA. The rest of DNA solution was kept at -20°C for future use.

The primers for bla_{TEM}, bla_{SHV} and bla_{CTX-M} were used for multidrug resistance gene investigation by PCR amplification in 30 µl containing for each: 5 µl of DNA, 0.5 µM of each primer (F and R), 1.5 mM MgCl2, 250 µM dNTPs, 1X PCR buffer (Invitrogen) and 1U Taq DNA polymerase (Invitrogen). The gene amplification has been realized using the following PCR program: i-bla_{TEM} (initial denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 30 s, 52°C for 30 s, 72°C for 1 min and a final elongation step for 10 min at 72°C), ii- bla_{SHV} (initial denaturation at 96°C for 5 min, 30 cycles at 96°C for 15 s, 50°C for 15 s, 72°C for 1 min and a final elongation step for 10 min at 72°C) and iii- bla_{CTX-M} (initial denaturation at 95°C for 5min, 35 cycles at 94°C for 1 min, 54°C for 1 min, 72°C for 2 min and a final elongation step for 10 min at 72°C). Four genes encoding virulence factors (VT, SLTI, SLTII and LT) were searched in 25 ml of reaction solution containing 7.5 µl of DNA, 12.5 µl commercial 2X Master Mix Polymerase (Bio Labs), 0.5 µl of each primer (F and R) at 0.2 µmol/L. The PCR program used for amplification was 5 min at 95 °C of initial denaturation, followed by 40 cycles for 45 s at 95°C, 45 s at 50°C, 45 s at 72°C and 10 min at 72°C for final extension. One control positive for Stx2 and LT was used. The primers sequences and the expected fragments are presented in the Table 1.

PCR products (10 µl) were visualized after electrophoresis at 150 V for 30 min on a 1.5% agarose gel containing ethidium bromide and visualized with an UV trans-illumination. A 100 bp ladder standard was used as molecular weight ladder.

Data analysis

The software Microsoft Office Excel 2010 has been used for statistical processing of the data. The software Epi Info 6 version 6.04cfr January 1999 has helped to make

Table 2. Microbial density of mesophilic flora and *E. coli* in street foods collected from Cotonou and Abomey-Calavi.

Street foods	Mesophilic microflora (CFU/g)	*E. coli* (CFU/g)
Salad	4.76×10^6	0.80×10^6
Rice	1.53×10^6	0.32×10^6
Vegetable sauce	0.27×10^6	2.81×0^6

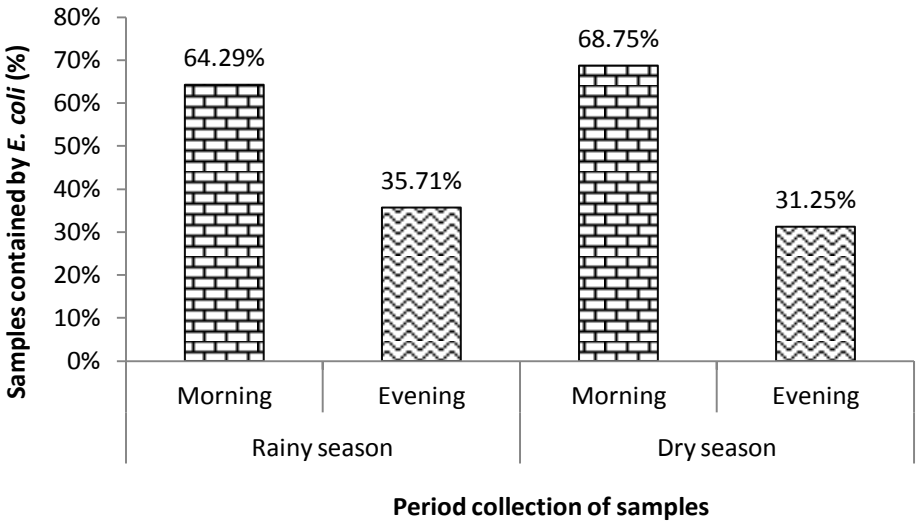

Figure 2. Contamination rate of street food by *E .coli* according to the period collection.

the test of Chi-square. The test is considered statistically significant if $p < 0.05$.

RESULTS

Environment sales

The food preparation starts early in the morning (between 5 am and 6 am) in the houses (hawker and semi-fixed) and sales premises (fixed). Among the three types of sellers, hawker sellers are the first to begin their selling (from 9 am to 12 am), then semi-fixed sellers (from 10 am to 4 pm) and finally fixed sellers (from 12 am to 12 pm). For sale, the foods are deposited on the floor (hawkers) or on makeshift tables (semi-fixed). More elaborate sales executives are noticed in the fixed sellers. In the case of street and semi-fixed, foods are exposed in the vicinity of high human traffic lanes often outdoors or, at best, partially covered with pieces of transparent fabrics.

It is further found that the food is served by hand, without being warmed up and also sales utensils are washed with the same water often drawn from unprotected wells. Prolonged use without renewal of dishwater lets show through of the oil to the surface. The packages used are composed of cement paper bags or moldy old papers and/or inadequate plastic bags by hawker and semi-fixed sellers. It was noticed that the fixed sellers take good care of their dishes, protect better their food and wash frequently the tablecloths.

Mesophylic microflora and *E. coli* density in street foods

The density of mesophilic microflora and *E. coli* isolated are presented in Table 2, it appears that the mesophilic microflora of salad samples was the highest (4.76×10^6 CFU/g) followed by rice's (1.53×10^6 CFU/g) and vegetable sauce (2.7×10^5 CFU/g). The samples of vegetable sauce were most contaminated by *E. coli* (2.8×10^6 CFU/g) followed by the salad samples (8.0×10^5 CFU/g) and rice samples (3.2×10^5 CFU/g).

Identification of *E. coli* strains

62 strains of *E. coli* were isolated from 216 food samples collected both dry and rainy season. In the dry season, 44% of the samples were contaminated by *E. coli* strains whereas in the rainy season 12.96% of the street foods contain *E. coli*.

According to period collection (morning or evening), we found that more than half of the samples collected in the morning were contaminated with *E. coli* (Figure 2) independently to the season. 64.29 and 68.75% of street

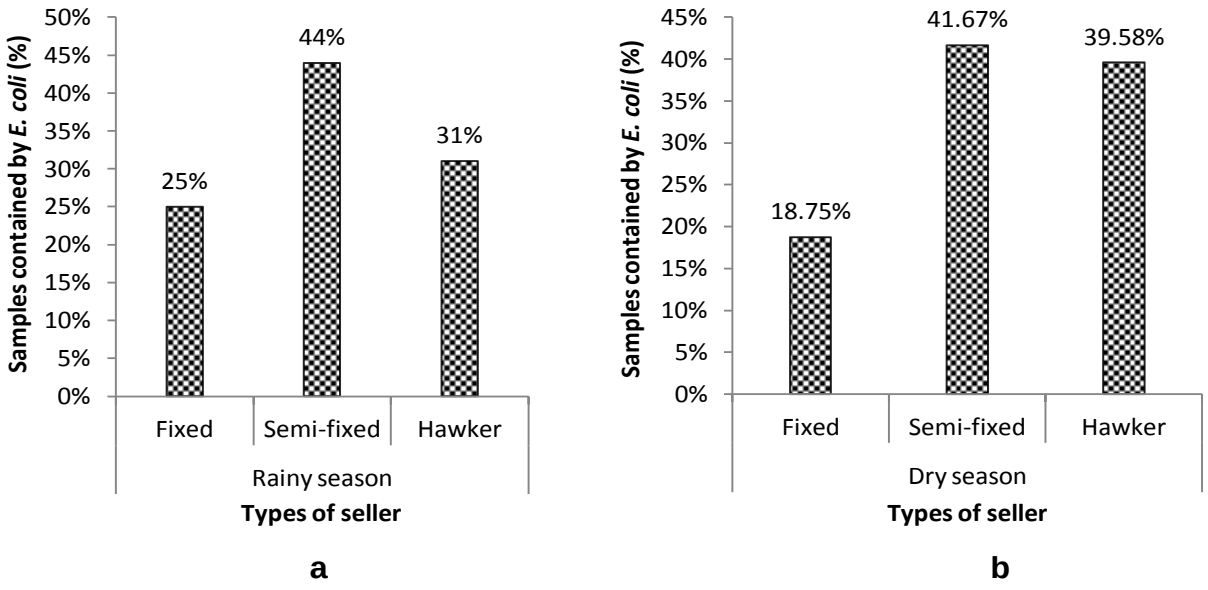

Figure 3. *E. coli* contamination rates of collected samples according to the season and the seller. a: Rainy season. b: Dry season.

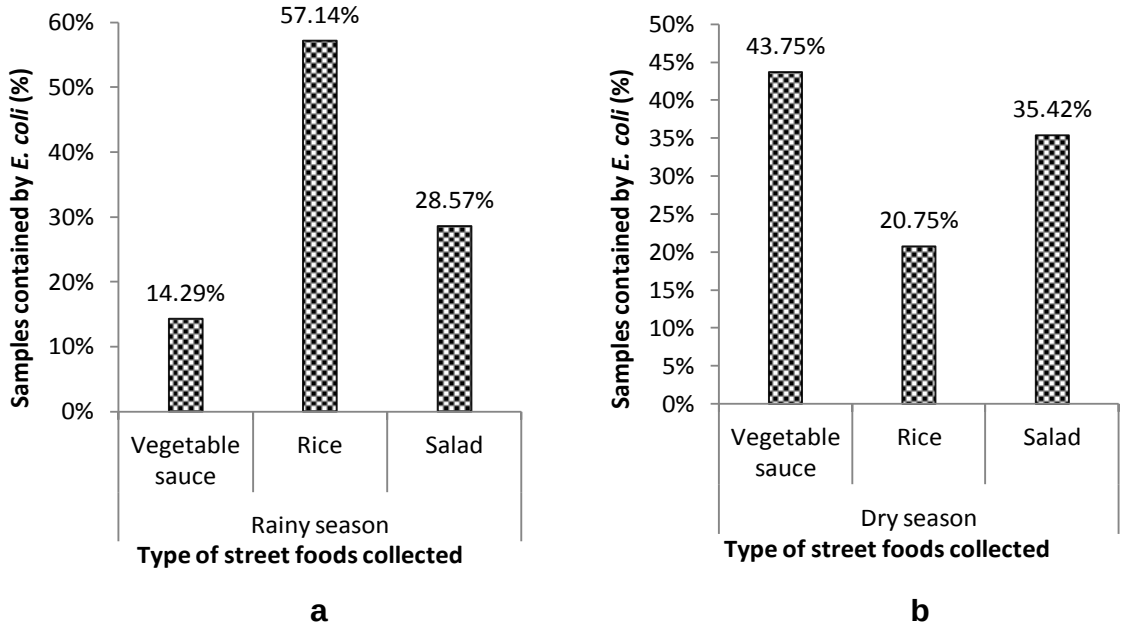

Figure 4. *E. coli* contamination rates of collected samples according to the season and the Street Foods. a: Rainy season. b: Dry season.

foods contain *E. coli* in rainy season and dry season, respectively (Figure 2). The street foods samples collected in the morning of are statistically more contaminated with *E. coli* than those collected in the evening ($p < 0.05$).

The contamination of street foods samples was variable depending on the type of seller ($p < 0.001$) as shown in the Figure 3. Indeed, the samples collected from the semi-fixed sellers were more contaminated both in dry season (41.67%) (Figure 3b) and in rainy season (44%) (Figure 3a) followed by hawkers and then fixed sellers.

Regarding the three type of analyzed samples, *E. coli* strains were isolated in varying proportions (Figure 4). Thus, in rainy season (Figure 4a), rice was most contaminated (57.14%) than salad (28.57%) and vegetable sauce (14.29%) whereas in dry season (Figure 4b) vegetable sauce was the most contaminated (43.75%)

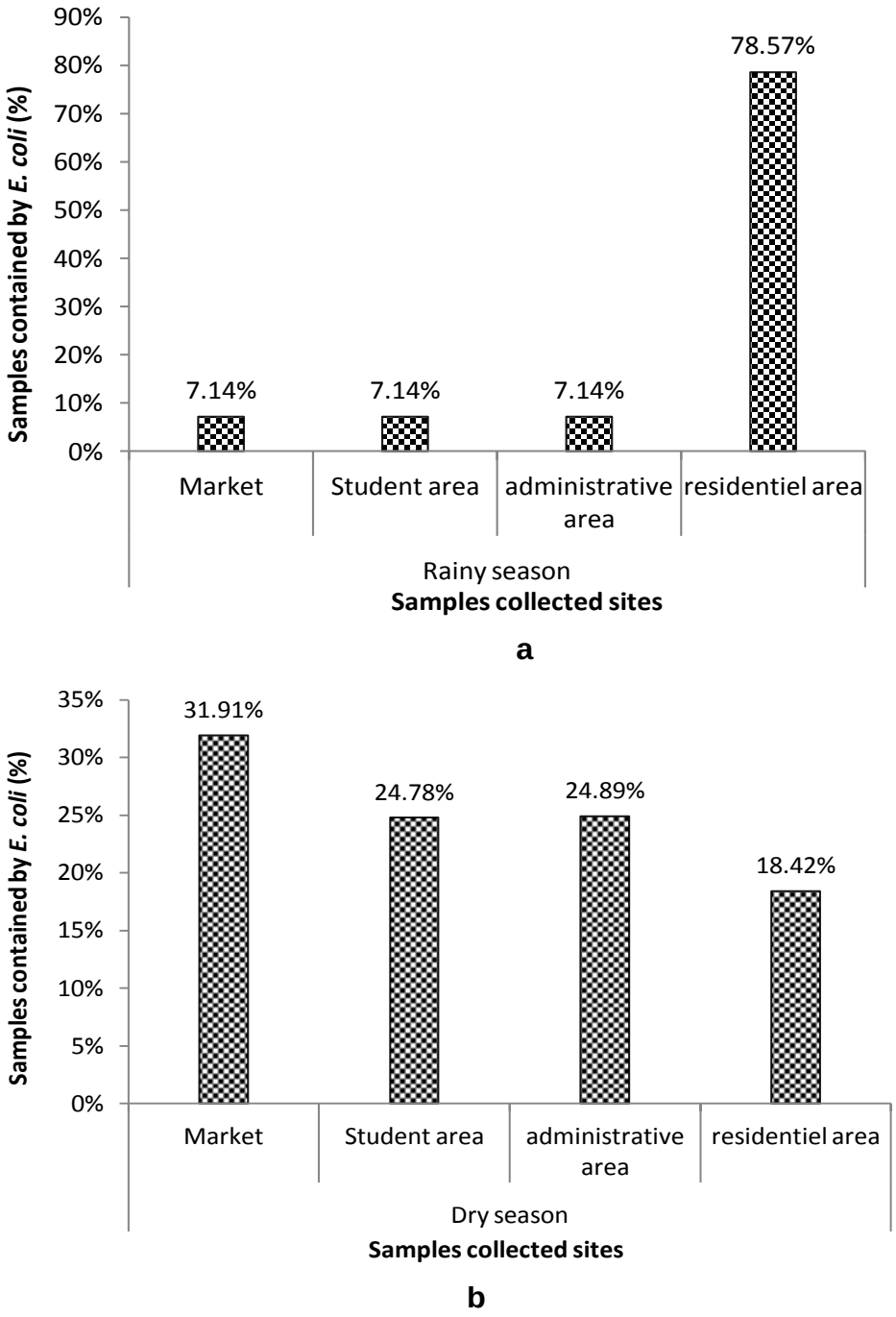

Figure 5. *E. coli* contamination rates of collected samples according to the season and the vendors sites. a: Rainy season. b: Dry season.

followed by salad (35.42%) and rice (20.75%) ($p < 0.001$).

According to their point of sale, our data displays through Figure 5 that the samples collected during the dry season in the markets were the most contaminated (31.91%) by *E. coli* strains followed by those collected in administrative areas (24.89%), student community (24.78%) and finally residential areas (18.42%) ($p < 0.05$) (Figure 5b). The contamination rate by *E. coli* appears highest during the rainy season (78.57%) among food samples collected from the residential areas (Figure 5a) ($p < 0.05$).

Susceptibility of isolated *E. coli* strains to antibiotics

The susceptibility of isolated *E. coli* strains (62) varies depending on the antibiotics tested (Figure 6). Thus, all

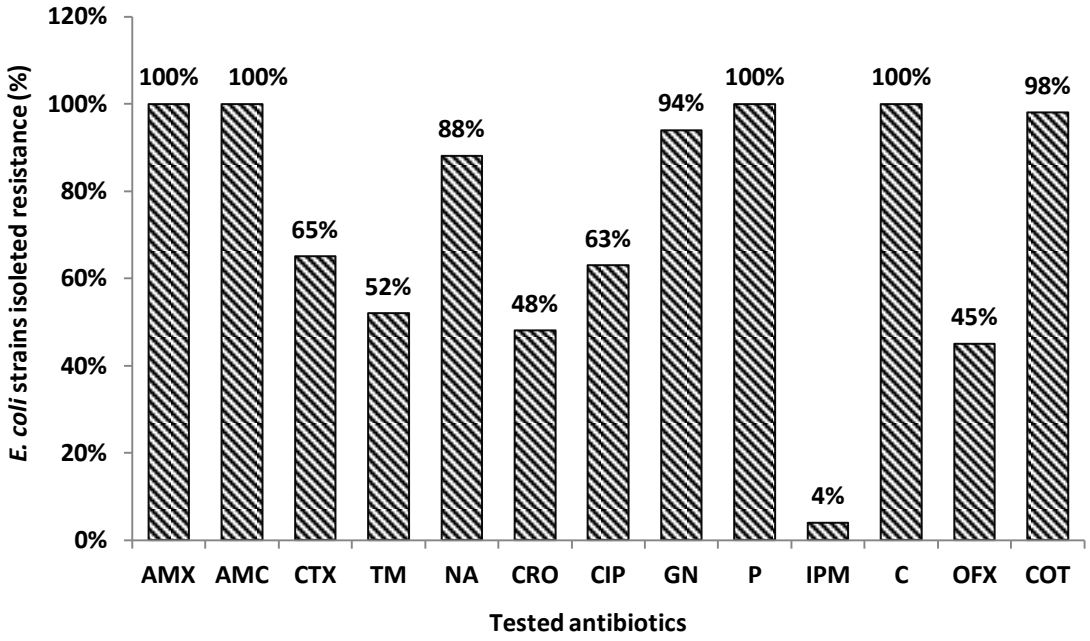

Figure 6. Resistance profile of *E. coli* strains isolated from street foods. Key: Amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX), tobramycin (TM), nalidixic acid (NA), ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (GN), penicillin G (P), imipenem (IPM), chloramphenicol (C), ofloxacin OFX), and cotrimozazol (COT).

Table 3. Distribution of the penicillinase genes carried by *E. coli* strains according to the season.

Genes	Street foods		Total (%)
	Rainy season (%)	Dry season (%)	
Bla_{TEM}	76.20	4.76	80.96
Bla_{SHV}	0	4.76	4.76
Bla_{CTX-M}	9.52	4.76	14.28
Total	85.72	14.28	100

strains were resistant to six antibiotics (Amoxicillin, Amoxicillin + Clavunalic acid, Nadicilique acid, Cloranphenicol, Getamicin and Penicillin) and the most active molecules against the isolated strains was Imipenem ($p < 0.05$). However, it was founded that more than 40% of the strains were resistant to other antibiotics.

Phenotypic and genomic detection of penicillinase and ESBLs

The research of penicillinase and expanded spectrum β-lactamase (ESBLs) strains were made both phenotypically and genotypically. The phenotypical investigation shows that all *E. coli strains* (100%) isolated in dry season produced penicillinase whereas in rainy season, only 21.43% of the isolated strains produced penicillinase. However, no isolated *E. coli* strain produced ESBLs.

The data compiles from the genotypical displays that 80.96% of the tested strains carried the bla_{TEM} genes, 4.76% carried the bla_{SHV} and 14.28 % carried the bla_{CTX-M} (Table 3 and Figure 7). The distribution of the *E. coli* strains considerably varies according to the season ($p< 0.0005$).

Presence of genes encoding to toxins production

The search of 4 toxins produced by pathogenic *E. coli* strains revealed that the tested strains carried Shigatoxin 1 (4.35%) and Shigatoxin 2 (47.83%) while 17.39% carried LT gene and 30.43% carried VT gene (Table 4 and Figure 8).

DISCUSSION

The food selling industry in the streets is of very large

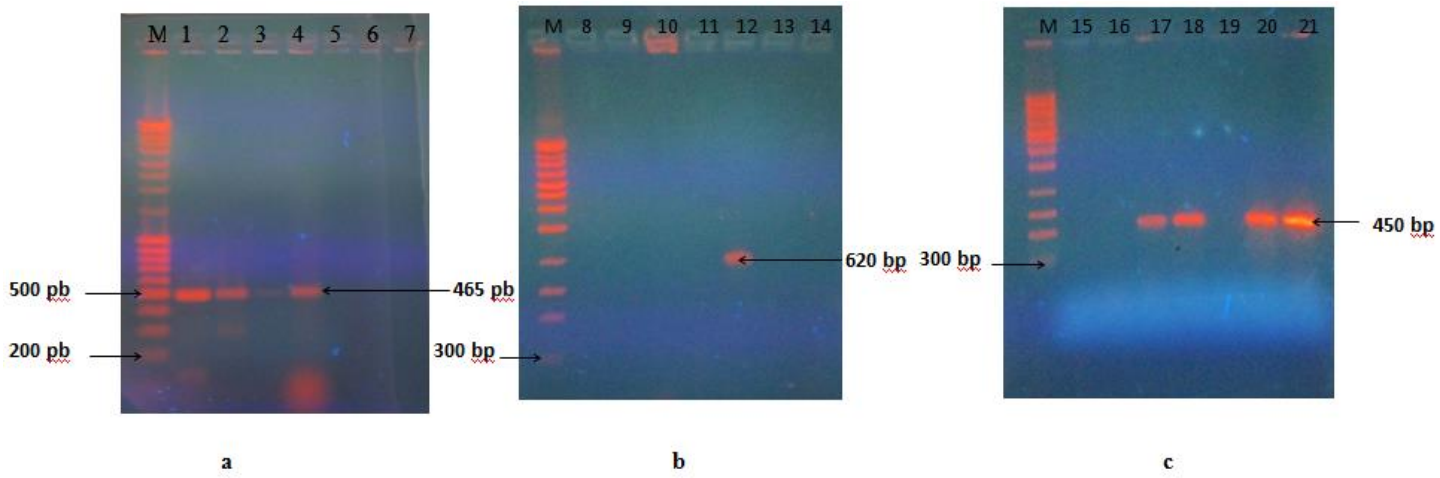

Figure 7. Detection of the presence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes. a: Columns 1, 2, 3, 4: bla_{TEM} positive samples; Column 7: negative control; Columns 5, 6: negative samples bla_{TEM}; Columns M: molecular weight marker. b: Columns 12: bla_{SHV} positive samples; Column 14: negative control; Columns 8, 9, 10, 11, 13: negative samples bla_{SHV}; Columns M: molecular weight marker. **c:** Columns 17, 18, 20, 21: bla_{CTX-M} positive samples; Column 15: negative control; Columns 16, 18 negative samples.

Table 4. Distribution of the toxins genes carried by strains of *E. coli* according to the season.

Genes	Street foods		Total (%)
	Rainy season (%)	Dry season (%)	
STEC (Stx1)	4.35	0	4.35
STEC (Stx2)	30.43	17.40	47.83
ETEC	13.04	4.35	17.39
VTEC	21.74	8.69	30.43
Total	69.56	30.44	100

Key: VT: Verotoxin, VTEC : enterohemorragic *E. coli*; LT: heat-labile enterotoxin, ETEC: enterotoxigenic *E. coli* ; Stx1: Shiga-like toxin I; Stx2: Shiga-like toxin II ; STEC: shigatoxin *E. coli*.

expansion in the cities of Cotonou and Abomey-Calavi. During this study, it was found that street foods are exposed in the vicinity of public roads either on the floor (hawker sellers) or on makeshift tables (fixed and semi-fixed sellers). Moreover, it is not uncommon to find that the food is served near dustbin or open gutters draining sewage. Also, it was observed that foods are mostly prepared with undrinkable water and poor quality condiments. All these may be harmful to consumers because it can be the cause of food poisoning. Indeed, food poisoning are commonly caused by the consumption of unhealthy foods, mainly due to lack of hygiene because those foods are appropriate for microorganisms growth and virulence factors production (Todd et al., 2007). The time that elapses between food preparation and their service was identified as one of the main factors involved in the occurrence of food poisoning cases (Roberts, 1982). Apart from time, one of the most important factors that increased the risk of food poisoning is hands used to serve foods (Mensah et al., 2002).

After foods consumption by customers, utensils are often cleaned using a poor quality of dishwater before re-use to serve other customers. This finding is usually more pronounced with hawker sellers who just use a cloth dampened with a little water for utensils cleaning (Barro et al., 2005). Instead of solving nutritional problem in cities such as Cotonou and Abomey-Calavi, the street foods consumption is able to create additional health problems. This situation keeps peoples of developing countries in the vicious circle of poverty as reported (Algert et al., 2006; Koro et al., 2010; Signs et al., 2011). Then the incomes of such people is often use in disease treatments because diseases related to the consumption of foods contaminated by microorganisms are known to be the most widespread health problem in the contemporary world (FAO, 2007) and an important cause of the reduction in economic productivity (Edema et al., 2005).

Considering the street foods contamination by microorganisms, this study revealed that *E. coli* strains

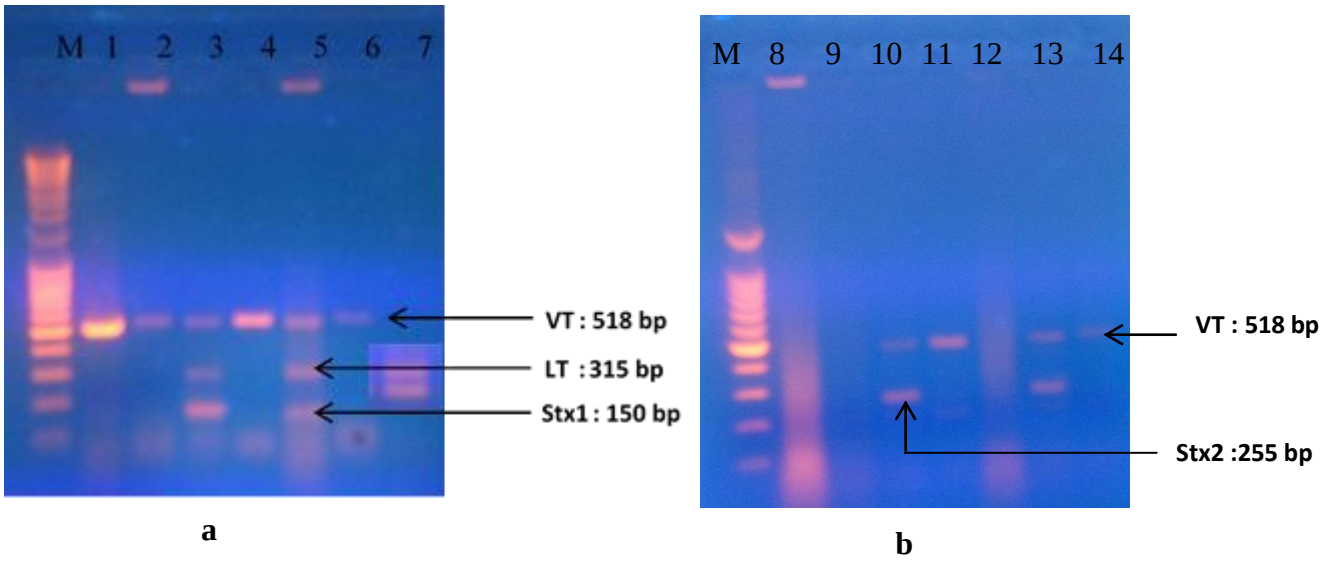

Figure 8. Detection of the presence of toxins genes. a: Columns 1, 2, 3, 4, 5, 6: Samples carried Toxins; Colum 7: positive control. b: Column 9: negative control; Columns 8, 12: negative samples toxins; Columns M: molecular weight marker.

were founded in 28.70% of the collected street foods. It appear that street foods contamination rate regarding mesophilic microflora are higher than the standards as previously published in Nigeria (Nkere et al., 2011). This study showed that the level of foods contamination by *E. coli* overstep the standard. This contamination is variable depending on the samples collection period. Thus, samples collected in the evening in dry season (31.25%) and rainy season (35.71%) were statistically ($p < 0.05$) less contaminated (Figure 1). This observation can be explained by the fact that the food collected in the morning and in the evening do not often come from the same cooking. Indeed, at some sellers the firings in the morning often ending in mid-day (from 12 am to 2 pm) makes the evening cooks fresh, hot and less in contact with potential contaminants. In addition, the contamination rate was highly below (Figure 3) with samples collected from fixed compared with other types of sellers ($p < 0.001$). Fixed sellers put more care in the sale of food than other sellers. These fixed sales premises are mostly located in the residential and administrative areas. It is then logical that the levels of contamination in these two areas are statistically lower ($p < 0.05$) than in the markets and student circles (Figure 5). Our results can find their explanations in the work of Signs et al. (2011) which, through a study conducted in Philadelphia (USA) on the health risk of food available for people of different races and income levels, have shown that people with low socioeconomic status have less access to supermarkets than populations with high socioeconomic status. These people resort to small markets (street and semi-fixed) and financially less access to fixed sites that sell food of better nutritional and health qualities (Koro et al., 2010). It is therefore urgent to take action to sensitize sellers and consumers on the merits of good hygiene.

Samples of vegetable sauce and salad were more contaminated (Figure 4) than rice's in dry season ($p < 0.001$). This high contamination rate could be explained by the fact that these two types of dishes are raw vegetables directly coming from market gardening. Thus, salads and vegetable sauce ingredients could have been contaminated in market sights before cooking operations. Also we should note that the cultural practices adopted by market gardeners favor the permanent faecal contamination of irrigation water either directly or indirectly. On this basis, we can assume that the strong presence of enterobacteria in the two types of dishes could be due to the poor hygienic conditions in which vegetables are grown as already stipulated by Amoah et al. (2007) after several works on vegetables. Furthermore, it is important to consider the fact that growers typically use manure from animals, such as poultry manure as fertilizer for soil fertilization (Ackers et al., 1998; Petterson et al., 2010); which would favor a permanent fecal contamination of irrigation water from shallow wells that are not covered.

The results of antibiotic susceptibility of *E. coli* strains show the resistance to the majority of tested antibiotics at varying proportions (Figure 6). Thus, 100% of *E. coli* strains isolated were resistant to amoxicillin, amoxicillin + clavulanic acid, chlorenphénicol and Penicillin. These rates are higher than those observed (21.73 to 73.3%) for Amoxicillin in some developed countries (Lemort et al., 2006; Dexheimer et al., 2015; Dembélé et al., 2015). The difference of rate may be due to the fact that in a country there are more control of the sale and administration of antibiotics which are more regulated (Golstein, 2000). Thus, the proportion observed in general in this study to this antibiotic would find its explanation in a misuse of the

antibiotic often sold on the street without any medical prescription (Guillemot, 2001). Note that selection pressure is exerted both in the medical and agricultural field (Allen et al., 2010). On cefotaxime, we have 65% of resistant strains. These results are higher than those recorded in USA (Mathai et al., 2001) and at Dakar (Secke, 2007). Also, a very high rate (44%) was observed when compared to studies conducted in Tunisia (Ben Hassen et al., 2003) and in Bénin (Sina et al., 2011) for Ofoxacine. These large resistances may be due firstly to the fact that these antibiotics are widely prescribed by their availability and cost favorable in the local market. Quite high proportions of resistance were also observed with the gentamicin (94%), Nalidixic Acid (88%), ciprofloxacin (63%) and tobramycin (52%). In this study, the Imipenem remains active against *E. coli* strains tested with a resistance rate of 4%. This rate is less than the 19.6% recorded in Tunisia (Abdallah et al., 2008) and the 12.5% in France (Allouch et al., 1995). Meanwhile, some authors founded less than 4% of resistance to Imipenem (Mohammed et al., 2011; Anago et al., 2015). Thus, it clears that there is an emergence of Imipenem resistance formerly considered as miracle antibiotic. This is to confirm the recent review data that brought out increases of multiresistant bacteria cases all over the world (Hawkey, 2008).

For the production of penicillinase, we noticed that all strains were producing in dry season against 21.43% in rainy season. Search for *E. coli* strains producing expanded spectrum β-lactamases (ESBLs) revealed that none of them were producing. This rate is not very far from the 0.4% observed by Mesa et al. (2006) in the food sector. However, this rate is very different to those reported in hospital area such as 16% observed in Cameroon (Lonchel et al., 2012) and 33.33% in America (Dexheimer et al., 2015). Thus, the origin of the strains can have a resistance mechanism speciation because clinical strains are commonly fought by different kind of drugs.

Among the penicillinase producing strains, the genes encoding for bla_{TEM} (80.96%), bla_{SHV} (4.76%) and bla_{CTX-M} (14.28%) were identified at different level. Thus, the presence of genes encoding bla_{TEM}, bla_{SHV} and bla_{CTX-M} confirms the phonotypical resistance of *E. coli* to β-lactams. These results were lower than those reported in hospital isolated strains by Dexheimer et al. (2015) for bla_{SHV} (41.66%) and bla_{CTX-M} (87.5%) on one hand and by Mohammed et al. (2011) for bla_{TEM} (10.9%), bla_{SHV} (13.7%) and bla_{CTX-M} (28.8%) on the other hand. These findings corroborate those of Nijssen et al. (2004) which concludes that the resistance to β-lactam antibiotics significantly increase over the two decades and the presence of bla_{TEM} gene is due to the resistance to third-generation antibiotics that causes secretion of β-lactamases (Galles et al., 2002; Lonchel et al., 2012; Anago et al., 2015; Dexheimer et al., 2015). Originally for the clinic strains, the ß-lactamases of group CTX-M conferred for *Enterobacteriaceae,* the highest level of resistance for céfotaxime (or ceftriaxone), céfépime, aztréonam and ceftazidime (Lagha, 2015). These rates of resistance were highest than the rate obtained in this study. Thus, origin of sample collection may play an important role in the resistance profile of a given strain.

The enterohemorrhagic *Escherichia coli* (EHEC: STEC and VTEC) are reported to be responsible of various infections ranging from watery diarrhea to hemorrhagic colitis, which can progress to hemolytic uremic syndrome in young children or thrombotic microangiopathy in adults (Mariani-Kurkdjian and Bonacorsi, 2014). In this study, the isolated *E. coli* strains were secreting Shiga toxin at levels of 4.35% (Stx1) and 47.83% (Stx2) (Table 4). Stx2 toxin is more virulent than Stx1 (Montet, 2009). In the dry season, the *E. coli* stains isolated carried Stx2 of 0% and 17.40% of Stx1. One study made in Brazil by Oliveira et al. (2008) showed that the prevalence of STEC ranged from 16 to 51.5%, 0 to 50% and 46.7 to 73.3%, respectively, for beef cattle, dairy cattle and goats, depending on the farm. In a study by Nataro et al. (2006) on clinical strains, 0 to 0.2% of *E. coli* strains were respectively STEC and ETEC. Another study in Brazil on samples of infant diarrhea observed rate (1.2%) ETEC and (0.7%) STEC (Araujo et al., 2007). These types of strains are related to the production of cholera toxins (Tozzoli et al., 2010). Therefore, the presence of toxins in food is very dangerous to humans. A rate of 17.39% of isolated *E. coli* strains was secreting heat-labile enterotoxin therefore (ETEC). ETEC in contrast, is pathogenic across all age groups, but it is most common among infants in developing countries, because immunity is acquired from repeated exposure (Nataro et al., 2006). Irino et al. (2005) who conducted a study on dairy cattle in Brazil asserts that the presence of STEC (56.4% for Stx1 and 40.6% for Stx2) may be due to food contamination and water failure during handling by the farmer methods. These different rates observed may be due to the diversity of the origin of strains and geographic variation.

The food safety can help prevent and improve population health, job performance and thus contribute to the fight against poverty through increased income. Hygiene remains a key point in the fight against infectious diseases especially in developing countries. In Bénin, there is a craze for people to eat street foods. Besides being affordable, they are cheap, varied and available everywhere. This sector escaped of health authorities control and is therefore possible sources of poisoning.

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REFERENCES

Abdallah HB, Noomen S, Khélifa ABE, Sahnoun O, Elargoubi A,

Mastouri M (2008). Profil de sensibilité aux antibiotiques des souches de *Pseudomonas aeruginosa* isolées dans la région de Monastir. Med. Maladies. Infect. 38:554-556.

Ackers ML, Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS (1998). An outbreak of *Escherichia coli* O157: H7 infections associated with leaf lettuce consumption. J. Infect. Dis. 177:1588-1593.

Algert SJ, Agrawal A, Lewis DS (2006). Disparities in access to fresh produce in low- income neighborhoods in Los Angeles. Am. J. Prev. Med. 30:365-370.

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010). Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8:251-259.

Allouch PY, Labia R, Pina P, Morin E (1995). Observatoires hospitaliers de la sensibilité d'*Escherichia coli* et de *Klebsiella pneumoniae* à l'association Amoxicilline / Acide clavulanique en 1994. Med. Maladies. Infect. 25:934- 944.

Amoah P, Drechsel P, Henseler M, Abaidoo RC (2007). Irrigated urban vegetable production in Ghana: microbiological contamination in farms and markets and associated consumer risk groups. J. Water. Health. 5:455-466.

Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Tchibozo MA, Bankole HS, Sanni A (2015). Antibiotic resistance and genotype of beta-lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Bénin. Ann. Clin. Microb. Anti. doi: 10.1186/s12941-014-0061-1.

Aranda K, Fabbricotti S, Fagundes-Neto U, Scaletsky I (2007). Single multiplex assay to identify simultaneously enteropathogenic, enteroaggregative, enterotoxigenic, enteroinvasive and Shiga toxin-producing *Escherichia coli* strains in Brazilian children. FEMS. Microbiol. Lett. 267:145-150.

Araujo JM, Tabarelli GF, Aranda KR, Fabbricotti SH, Fagundes-Neto U, Mendes CM, Scaletsky IC (2007). Typical Enteroaggregative and Atypical Enteropathogenic Types of *Escherichia coli* Are the Most Prevalent Diarrhea-Associated Pathotypes among Brazilian Children. J. Clin. Microbiol. 45:3396-3399.

Attien P, Sina H, Moussaoui W, Dadié T, Chabi Sika K, Djéni T, Bankole H, Kotchoni SO, Edoh V, Prévost G, Baba-Moussa L (2014). Prevalence and antibiotic resistance of *Staphylococcus* strains isolated from meat products sold in Abidjan streets (Ivory Coast). Afr. J. Microbiol. Res. 7: 3285-3293.

Barro N, Sangaré L, Tahita MC, Ouattara CAT, Traoré AS (2005). Les principaux agents du péril identifiés dans les aliments de rue et ceux des cantines et leur prévalence en milieu hospitalier. In: Maıtrise des Procédés en vue d'améliorer la qualité et la sécurité des aliments, Utilisation des OGM. Analyse des risques en agroalimentaire Ouagadougou.

Barro N (2000). Aliments de rue au Burkina-Faso: caractéristiques des vendeurs et de consommateurs, salubrité des aliments de rue et santé des consommateurs. Rapports CRSBAN-SADAOC. 2000: 22.

Ben Hassen S, Messadi L, Ben Assen A (2003). Identification et caractérisation des espèces de *Staphylococcus* isolées de lait de vaches atteintes ou non de mammite. In: Annales de médecine vétérinaire 2003, pp. 41-47. Université de Liège, Faculté de médecine vétérinaire.

Chauliac M, Bricas N, Ategbo E, Amoussa W, Zohoun I (1998). Food habits outside the home by school children in Cotonou (Bénin). Santé. 8:101-108.

The Antibiogram Committee of the French Society of Microbiology (ACFSM). (2012). Coordonnateur Soussy. C. J. Recommandations. 2012, pp. 56. In. Edition Janvier 2012.

Dembélé R, Bonkoungou IJO, Konaté A, Tchamba GB, Bawa HI, Bako E. Bagré TS, Kagambèga A, Zongo C, Traoré AS, Barro N (2015). Serotyping and antimicrobial resistance of enteropathogenic *Escherichia coli* and enterohomorrhagic *E. coli* O157 isolated from children under five years of age with diarrhea in rural Burkina Faso. Afr. J. Microbiol. Res. 9:1059-1059.

Dexheimer GM, Prediger J, Weidlich L, Pozzobon A (2015). Prevalence of resistance and molecular characterization of extended spectrum beta-lactamase (ESBL) - producing bacteria isolated in a hospital in Southern Brazil. Afri. J. Microbiol. Res. 9: 294-300.

Diouf F (1992). Contribution à l'étude des aliments vendus sur la voie publique dans la région de Dakar, pp. 36. Thèse médecine Vétérinaire, Dakar.

Edema MO, Omemu AM, Bankole MO (2005). Microbiological safety and quality of ready- to-eat foods in Nigeria. In the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM). University of Agriculture, from 6-10th November; Abeokuta 2005.

FAO (1989). Les aliments vendus sur la voie publique, pp. 96.Rôme FAO.

FAO (2007). Les bonnes pratiques d'hygiène dans la préparation et la vente des aliments de rue en Afrique. 2007;ISBN 92-5-205583-5:188.

Flint JA, Van Duynhoven YT, Fredrick JA, Stephanie MD, Peggy B, Martyn K, B. Peggy, K. Martyn, Elaine Sc, Margaret F, Goutam KA, Paul S, Andrea E, Gillian H, Neyla G, Henry W, Peter B (2005). Estiating the burden of acutegastroenteritis, foodborne disease, and pathogens commonly transmitted by food: Aninternational review. Clin. Infect. Dis. 41:698-704.

Galles AC, Sader HS, Jones RN (2002). Urinary tract infection trends in Latin American hospitals: report from the SENTRY antimicrobial surveillance program (1997-2000). Diagn. micr. infec. dis. 44:289-299.

Gangoué-Piéboji J, Bedenic B, Koulla-Shiro S, Randegger C, Adiogo D, Ngassam P, Adiogo D, Ndumbe P (2005). Extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in Yaounde, Cameroon. J. Clin. Microbiol. 43:3273-3277.

Gassama-Sow A, Sow PS, Guèye M, Guèye-N'diaye A, Perret JL, M'boup S (2004). Characterization of pathogenic *Escherichia coli* in human immunodeficiency virus-related diarrhea in Senegal. J. Infect. Dis. 189:75-78.

Ghosh M, Wahi S, Kumar M, Ganguli A (2007). Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella spp.* in some raw street vended Indian foods. Int. J. Environ. Heal. R.17:151-156.

Golstein F (2000). Antibiotic susceptibility of bacterial strains isolated from patients with community acquired urinary tract infections in France**.** Eur. J. Clin. Microbiol. 19: 112-117.

Guillemot D (2001). Effet de l'usage des antibiotiques sur l'évolution des résistances bactériennes antibiotiques. Eds Maison Paris.

Gulamber C, Altindis M, Kalayci R, Bozdogan B, Aktepe O (2013). Molecular characterization of nosocomial CTX-M type ß-Lactamase producing *Enterobacteriaceae* from University Hospital in Turkey. Afr. J. Microbiol. Res. 6:5552-5557.

Hanoshiro A, Morita M, Matte G, Matte MH, Torres E (2004). Microbiological quality of selected foods from a restricted area of Sao Paulo city. Brazil. 2004: 439-444.

Hawkey P (2008). The growing burden of antimicrobial resistance. J. Antimicrob. Chemoth. 62: i1-i9.

Hennessy TW, Marcus R, Deneen V, Reddy S, Vugia D, Townes J, Bardsley M, Swerdlow D, Angulo FJ, Group EIPFW (2004). Survey of physician diagnostic practices for patients with acute diarrhea: clinical and public health implications. Clin. Infect. Dis. 38: S203-S211.

Irino K, Kato M, Vaz T, Ramos I, Souza M, Cruz A, Gomes T, Vieira M, Guth B (2005). Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in Sao Paulo State, Brazil. Vet. Microbiol. 105:29-36.

Jarlier V, Nicolas M, Fournier G, Philippon A (1988). Extended broad spectrum β- lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 10:867-878.

Jones TF, Ingram LA, Fullerton KE, Marcus R, Anderson BJ, McCarthy PV, Vugia D, Shiferaw B, Haubert N, Wedel S (2006). A case-control study of the epidemiology of sporadic *Salmonella* infection in infants. Pediatrics. 118: 2380-2387.

Koneman E (2006). Test for determining inhibitory. In: Koneman's color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott Williams and Wilkins edn. pp. 1001.

Koro ME, Shivanthi A, Jennifer JQ (2010). Microbial Quality of Food Available to Populations of Differing Socioeconomic Status. Am. J. Prev. Med. 38:478-481.

Lagha N (2015). Etude de la résistance aux antibiotiques des

entérobactéries productrices de β-lactamases à spectre étendu (BLSE) isolées de l'hôpital de Laghouat. Université Abou Bekr Belkaïd Tlemcen.

Lalatiana OR (2006). Contribution à l'étude de la qualité microbiologique d'un aliment de rue dans la ville Talatan'ny Volonondry. (Madagascar : Cas du Koba Ravina).

Lemort M, Neuville S, Medus P, Gueudet M, Saada M, Aumaître H (2006). Evolution comparée de la sensibilité de souches de *Escherichia coli* isolées d'infections urinaires de patients consultant aux urgences et de patients hospitalisés en 2002 et 2004 à l'hôpital Perpignan. Pathol. Biol. 54:427-430.

Lonchel C, Meex C, Gangoué-Piéboji J, Boreux R, Okomo A, Melin P (2012). Proportion of extended-spectrum ß-lactamase producing *Enterobacteriaceae* in community setting in Ngaoundere, Cameroon. BMC. Infect. Dis. 12:53-59.

Mariani-Kurkdjian P, Bonacorsi S (2014). Diagnostic des infections à *Escherichia coli* entéro-hémorragiques. In: Feuillets de Biologie Bactériologie EHEC/STEC. 5:317.

Mathai D, Jones RN, Faller MA (2001). Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). The SENTRY Participant Group North America. Diagn. Micr. Infec. Dis. 40:129-136.

Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A (2002). Street foods in Accra, Ghana: how safe are they?. B. World. Health. Organ. 80:546-554.

Mesa RJ, Blanc V, Blanch AR, Cortés P, González JJ, Lavilla S (2006). Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage), J. Am. C. 58:211-215.

Mohammed S, Anuradha S, Farrukh S, Mohammad R, Abida M, Indu S (2011). blaCTX-M, blaTEM, and blaSHV in Enterobacteriaceae from North-Indian tertiary hospital: high occurrence of combination genes. Asian Pac. J. Trop. Med. 2011:101-105.

Moini R, Grimaldi M, Zorzi P, Mani A, Bordin P (1996). Comparison between selective media "RAPID' *E. coli*" and "VRBA with sodium malonate" for *E. coli* investigation. Ind alimentary. 35:793-796.

Montet M (2009). Contamination des aliments par les *Escherichia coli* producteurs de Shiga-toxines (STEC) en France, et importance de l'acido-résistance des souches. Lyon.

Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, Hirshon JM (2006). Diarrheagenic *Escherichia coli* Infection in Baltimore, Maryland, and New Haven, Connecticut. Clin. Infect. Dis. 43:402-407.

Nijssen S, Florijn A, Bonten M, Schmitz F, Verhoef J, Fluit A (2004). Beta-lactam susceptibilities and prevalence of ESBL-producing isolates among more than 5000 European *Enterobacteriaceae* isolates. Int. J. Antimicrob. Ag. 24:585-591.

Nkere CK, Nnenne II, Christian UI (2011). Bacteriological Quality of Foods and Water Sold by Vendors and in Restaurants in Nsukka, Enugu State, Nigeria: A Comparative Study of Three Microbiological Methods. J. Health. Popul. Nutr. 29:560-566.

Nüesch-Inderninen M, Hächler H, Kayser F (1996). Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. Eur. J. Clin. Microbiol. 15:398-402.

Oliveira MG, Brito JRF, Gomes TAT, Guth BEC, Vieira MAM, Naves ZVF, Vaz TMI, Irino K (2008). Diversity of virulence profiles of Shiga toxin-producing *Escherichia coli* serotypes in food-producing animals in Brazil. Int. J. Food. Microbiol. 127:139-146.

Petterson S, Ashbolt NJ, Sharma A (2010). Microbial risks from waste water irrigation of salad crops: a screening-level risk assessment. J. Food. Sci. 75:283-290.

Riegel P, Archambaud M, Clavé D, Vergnaud M (2006). Bactérie de culture et d'identification difficiles, pp. 93-112.

Roberts D (1982). Factors contributing to outbreaks of food poisoning in England and Wales 1970-1979. J. Hyg. 89:491-498.

Sansonetti P (1987). Facteurs de pathogénicité d'*E. coli*. Med. Maladies. Infect. N° spécial:11-16.

Secke C (2007). Contribution à l'étude de la qualité bactériologique des aliments vendus sur la voie publique de Dakar. Méd. Vét. 20:108-117.

Signs RJ, Darcey VL, Carney TA, Evans AA, Quinlan JJ (2011). Retail food safety risks for populations of different races, ethnicities, and income levels. J. Food. Protect. 74:1717-1723.

Sina H, Baba-Moussa F, Ahoyo TA, Mousse W, Anagonou S, Gbenou JD, Prévost G, Kotchoni SO, Baba-Moussa L (2011). Antibiotic susceptibility and Toxins production of *Staphylococcus aureus* isolated from clinical samples from Bénin. Afr. J. Microbiol. Res. 5: 2797-2808.

Thomson K, Sanders CC (1992). Detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three- dimensional tests. Antimicrobiol. Agents. Chem. 36:1877-1882.

Todd E, Greig J, Bartleson C, Michaels B (2007). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks Location of food consumption and travellers diarrhea. Am. J. Epidemiol. 48:178-183.

Tozzoli R, Caprioli A, Cappannella S, Michelacci V, Marziano ML, Morabito S (2010). Production of the Subtilase AB5 Cytotoxin by Shiga Toxin-Negative *Escherichia coli*. J. Clin. Microbiol. 48:178-183.

Vincenot F, Saleha M, Prévost G (2008). Les facteurs de virulence de *Staphylococcus aureus*. Rev Franc Lab. 407:64-67.