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Diversity of bla Ctx-m type genes in *Salmonella* serovars isolated from raw chicken gizzards in Côte d'Ivoire by sequencing of universal sequence tagged PCR-amplicons

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ABSTRACT

Multidrug resistance is emerging in many Gram-negative bacteria like Salmonella spp. Plasmid encoding bla-Ctx-m enzymes represent an important sub-group of class A β-lactamases responsible for ESBL (Extended-spectrum β-lactamases) phenotype which is increasingly found in *Enterobacteriaceae* such as *Salmonella* spp. Molecular typing of ESBL-isolates has become more important for prevention of ESBL-producers dissemination in environment. bla Ctx-m genes were targeted using degenerated bla Ctx-m consensus primers and PCR amplified from *Salmonella* strains presenting particular features of multidrug resistance, isolated from chicken gizzards. *bla* Ctx-m sequences analysis in Salmonella isolates (Kentucky and Muenster), revealed similarities of 96 to 100 % of homology with nucleotides fragments encoding to enzymes type such as bla CTX-M-2,-5,-44,-59,-92,-97 and -131; bla NDM-1 and *bla* OXY. This identification could only been achieved by sequencing of the PCR-amplicons in other *Enterobacteriaceae*. PCR-based molecular typing method described here, enables a rapid and reliable molecular identification of bla genes. The principles used in this study could also be applied to any situation in which antimicrobial resistance genes would need to be sequenced.

INTRODUCTION

Salmonella spp. as Gram-negative bacteria belonging to the family of *Enterobacteriaceae*, are implicated in a number of severe infectious diseases which cause high rate of morbidity among children less than 5 years of age (Ryan and Ray, 2004; Krauss et al., 2003). To make a response to bacterial infection, antimicrobial drugs such as β-lactams are widely used in therapeutic treatment against most of Gram negative bacteria notably *Salmonella* spp. (Kassis-Chikhani, 2012). However, it is well known that Salmonella strains have ability to successfully acquire resistance to antimicrobial drugs particularly to β-lactams (Cloeckaert and Schwarz, 2001; Daly and Fanning, 2000). The ability of the bacteria to acquire resistance to β-lactams is due to the synthesis of β-lactams degradative enzymes called β-lactamases. β-lactamases are bacterial enzymes that catalyze the hydrolysis of the amide bond of the lactam cycle of the antibiotic of the family of β-lactams. Genes that encode these enzymes are chromosomal or plasmid origin. The production of β-lactamases is the most widespread resistance mechanism and the most important at bacteria β-lactams (Livermore, 1995). β-lactamases catalyze effective and irreversible hydrolysis of the amide bond of the beta-lactam cycle giving a biologically inactive product that completely loses its antimicrobial activity (Matagne et al., 1998). Moreover, certain strains present particular characteristics by yielding β-lactamase with capacity to deactivate a wide range of antimicrobial drugs. Multidrug resistance is emerging in many Gram-negative pathogens like *Salmonella* strains, which is an important cause of severe infections such as diarrhea, septicemia. Increasing antimicrobial resistance among extended-spectrum β-lactamase (ESBL)-producing such as bla TEM-1,-2, bla SHV-1, bla PER, bla VEB, bla GES and bla CTX-M, Salmonella spp is a growing concern (Bush et al., 1995). Cefotaximases (Ctx-m) are class A β-lactamases that in general present higher levels of hydrolytic activity against cefotaxim than against Ceftazidim (Batchelor et al., 2005). Ctx-m enzymes comprise a rapidly growing family distributed both overwide geographic areas and among a wide range of bacteria of clinical significance. There are five major Ctx-m groups such as Ctx-m- 1, Ctx-m- 2, Ctx-m- 8, Ctx-m- 9 and Ctx-m- 25 (Bonnet, 2004). These genes encoding these enzymes have mainly been found on plasmids and are currently considered the most prevalent type of ESBLs in many European countries (Cantón and Coque, 2006; Livermore et al., 2007).

Various methods using microbiologic and molecular technique have been developed to detect presence of ESBL. These methods include: (i) inhibitor of β-lactamases such as clavulanic acid in combination with oxyimino- β-lactamins (ceftazidim or cefotaxim) (Bradford, 2001); (ii) double synergy test (Jarlier et al., 1988) between disk of cephalosporin of third or aztreonam and inhibitor of β-lactamases such as clavulanic acid, judged inapt to inhibit all β-lactamases and ESBL; (iii) three dimension test (Thomson and Sanders, 1992) giving an information on β-lactamases profile have a high sensibility, but not easy to perform; (iv) the disc method, (v) the E-test strips (Biodisk AB, Solna, Sweden) and (vi) the Vitek test (bioMerieux Vitek, Hazelton, Missouri) are performed to identify ESBL production by *E. Coli*, *Klebsiella* and *Proteus mirabilis*. The detection of these enzymes in producing strains of cephalosporinases such as *Salmonella* remains a problem. Thus, it is necessary to use appropriate methods such as molecular methods for presumptively identification of ESBLs in any clinical isolate. The use of technical such as the deoxyribonucleic acid probes, polymerase chain reaction (PCR), the oligotyping, ligase chain reaction and the nucleotide sequencing, in addition to subserve the detection of ESBL are also promoting their characterization (Bradford, 2001).

In this study, we have use a simple, accurate and universal primer named bla CTX-Mconsensus to high range able to detect and characterize various types of bla genes in *Salmonella serovars*, after sequencing of the PCR amplicons. By using these method, clinical isolates of ESBL-producing members of the family *Enterobacteriaceae* possessing bla genes (example bla CTX-M), could be characterized at molecular level.

MATERIALS AND METHODS

Isolation, serotyping of *Salmonella* and determination of resistance phenotypes

Salmonella strains were isolated from 66 batches of raw chicken gizzards, obtained from poultry markets in 11 municipalities (Abobo, Adjamé, Anyama, Attécoubé, Bingerville, Cocody, Koumassi, Marcory, Port-Bouët, Treichville and Yopougon) of the District of Abidjan (Côte d'Ivoire). The isolation was conducted following the ISO 6579 standard protocols (ISO 6579, 2002), using Buffered Peptone Water (Bio-Rad, Marnes-la-Coquette, France), as pre-enrichment medium, Rappaport Vassilliadis and Müller-Kauffmann Tetrationate (Difco, Rhône-Alpes, France) as selective enrichment medium, and Hektoen agar and XLD agar (Bio-Rad, Marnes-la-Coquette, France) as selective medium. All isolates were identified as *Salmonella* sp using a minimal biochemical gallery. These tests concerned urea and tryptophan degradation, lactose and citrate utilization as carbon source, glucose fermentation and strains motility. *Salmonella* isolates were serotyped from each positive sample according to the Kauffmann–White scheme in the Pasteur institute of Côte d'Ivoire. Antimicrobial susceptibility testing such as β-lactam resistance of Salmonella strains isolated was evaluated by the disc diffusion method on Mueller-Hinton agar (MH) (Bio-Rad SA), and interpreted according to the CLSI (Clinical and Laboratory Standards Institute) criteria (CLSI, 2008). Antibiotic discs used for this test were sulfonamide (cotrimoxazole (SXT, 10/20 µg)), phenicol (chloramphenicol (C, 10 µg), aminoside (gentamycin (GM, 10 µg), cycline (tetracycline (TE, 10 µg), quinolon (ciprofloxacin (Cip, 10 µg) and nalidixic acid (Nal, 10 µg) and β-lactams (Bio-Rad SA, Marnes-la-Coquette, France). The β-lactams antibiotics discs were: amoxicillin

(AMX, 10g), amoxicillin/clavulanic acid (AMC, 10/20 µg), ticarcillin (TIC, 75 µg), cephalotin (CF, 10 µg), cefoxitin (FOX, 10µg) and cefotaxim (CTX, 10 µg). ATCC 14028 and IPCI 8297 Salmonella strains were used as positive control.

DNA extraction

Plasmid DNA was extracted from some multidrug resistance strains of Salmonella, using alkaline lyses method described by Rozilla et al. (2007) with modification. Pure Salmonella cultures were added into 2 mL of sterile water. The suspension was centrifuged for 10 min at 14,000 rpm (revolution per minute) and pelleted cells were suspended in 100 µL of solution containing: Tris pH8 25mM, Glucose 50 mM, EDTA 10 mM. To the suspension obtained, 200 µL of a second solution containing 10 % SDS, 1 M NaOH was added and mixed gently two or three times, then incubated on ice for 2 minutes. A third solution with a final volume of 150 µL was prepared by adding 5 M of Acetate of potassium, 28.5 mL of glacial acetic acid and incubated on ice for 5 minutes. The final solution obtained was centrifuged at 4°C for 10 minutes at 14,000 rpm. The supernatant of the final solution was transferred into a new tube. Further extraction of the plasmid DNA was carried out with 450 µL of phenol-chloroform-iso amyl alcohol (25:24:1) by vortexing and centrifuging at 14,000 rpm for 10 minute and the supernatant was transferred into a new tube. The nucleic acid was precipated by adding 1 mL of ethanol (100 %) and the tube containing the plasmid DNA was precipated on ice for 5 minutes and centrifuged for 10 minutes at 14000 rpm. The supernatant obtained was pipetted and discarded. A solution of 500 µL of 70 % ethanol was transferred to the tube containing the pellet. The mixture was centrifuged at 14000 rpm for 10 minutes. The supernatant was pipetted and discarded and the pellet was dried between 2 to 5 minutes using a speedvac (DNA Savant, Japan). The plasmid DNA at the bottom of the tube was suspended once again in 50 µL of sterile water.

Genetic characterization of *bla* Ctx-m genes

Confirmation of the presence of β-lactamase gene, that confered resistance to β-lactam, was performed by PCR amplification. A specific primer Forward and Reverse (F: 5'-GACGATGTCACTGGCTGAGC-3' and R: 5'-AGCCGCCGACGCTAATACA-3') named *bla* Ctx-m-1 and a degenerated primers bla Ctx-m consensus Forward and Reverse (F: 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' and R:5'-TGGGTRAARTARGTSAACCAGAAYCAGCGG-3') (Eurogentec), were used to targeting the CTX-M enzymes genes in a final reaction volume of 50 µL, using an Applied Biosystems Thermocycleur Gene Amp PCR system type 9700 (Applied Biosystem, Villebon-sur-yvette, France). The specific primers bla Ctx-m-1 were designed from the aligment of bla Ctx-m sequences representive of group 1. The degenerate primers were designed from the alignment of *bla* Ctx-m sequences representative of each group. PCR amplification conditions were as following: initial denaturation step at 95°C for 5 min; 40 cycles of denaturation at 94°C for 30s; annealing at 55°C for a degenerated primers and 60°C for a specific primers for 30s; extension at 72°C for 1 min, and final extension step at 72°C for 10 min was used, resulting in respectively 499 bp and 593 bp amplicons (Kiiru et al., 2012). *E. coli* Y10278 and *E. coli* X92506 (Institute Pasteur of Côte d'Ivoire) were used as positive control strains for detection of bla Ctx-m consensus and *bla* Ctx-m-1 (groupe 1). Detection and identification of another *bla* Ctx-m genes group was carried out initially by sequencing short fragment obtained with degenerated primers. These were sequenced by automated PCR cycle sequencing using the Applied Biosystems 3730xL96 Big Dye Terminator V 3.1 (Applied Biosystems, GATC Biotech, Germany). Generated DNA sequences were aligned, edited and compared with those in GenBank using the BLAST program. *bla* Ctx-m DNA and bla-like DNA sequences using the bla Ctx-m DNA sequences were retrieved from the NCBI Enter Nucleotide database, available on the site www.blast.ncbi.nlm.gov/Blast.cgi.

RESULTS AND DISCUSSION

Isolation performed in raw chicken gizzards revealed 104 strains of Salmonella. From that number, 62.5 % (65/104) were completely serotyped. They were distributed in 19 serovars (Tableau 1). S. Kentucky (18.75%) and S. Derby (17.19 %) are the most dominants. Those are the main serotypes of Salmonella that are implicated in cases of salmonellosis, and isolated from various sources including poultry meat (Tao et al, 2014. Turkey et al., 2011). The presence of these strains including S. Kentucky in raw chicken gizzard in Abidjan (Ivory Coast) could constitute a public health risk indicator for the Abidjan population.
Sensitivity tests performed on all isolated strains (104) revealed 99.03 % (103) with resistance for β-lactams. Among them, 7.69 % presented resistance to Amoxicillin and Amoxicillin/ Clavulanic acid, 46.15 % to Ticarcillin, 9.62 % to cefalotin and 0.96 % to cefotaxim (Table 1). The double synergy test performed, revealed double synergy between the disk of cephalosporin of third generation (cefotaxim) and an inhibitor of β-lactamases such as clavulanic (amoxicillin/ clavulanic acid) in one strain (Salmonella Kentucky) producer of an ESBL (Figure 1). By cons, PCR amplicons performed with specific bla Ctx-m-1 primers revealed presence of 13 strains producers of ESBL type bla Ctx-m-1 (Figure 2). This reflects the efficiency of the PCR. Still remember that the gene bla CTX-M1 is one of the genes that give to enterobacteria like Salmonella, capacity to resist to beta-lactams (Ergovora et al., 2008). This resistance is the fact of an enzymatic mechanism of resistance to antibiotic by production of betalactamase (Bush, 2003; Foley et Lynne, 2008).

However sequencing performed on amplicons obtained by PCR with a degenerated primer bla Ctx-mconsensus confirmed presence of bla Ctx-m-1 in these strains, but also revealed different types of ESBL in two strains (Salmonella Muenster and Salmonella Kentucky).The analysis of nucleotides sequences obtained after sequencing reveals 96 to 100 % of similarities with fragments of nucleotides sequences encoding enzymes type bla Ctx-m-2, -5, -44, -59, -92, -97 and-131; bla NDM-1 and bla OXY (Table 3).

Table 1: Antimicrobial resistance percentages of *Salmonella* strains isolated from raw chicken gizzards in the District of Abidjan, Côte d'Ivoire.

Families		Antibiotics	*Salmonella* (%)/ N=104	
			R	S
B-lactams	Penicillin	Amoxicillin	8 (7.7 %)	96 (92.3 %°)
		Amoxicillin + clavulanic acid	8 (7.7 %)	96 (92.3 %)
		Tircacillin	48(46.15 %)	56 (53.85 %)
	Cephalosporin	Cefalotin	10 (9.7 %)	98 (94.23 %)
		Cefoxitim	0 (0 %)	104 (100 %)
		Cefotaxim	1 (0.96 %)	103 (99.04 %)
Aminoside		Gentamycin	11 (10.58 %)	93 (89.42 %)
Phenicol		Chloramphenicol	31 (29.8 %)	73 (70.2 %)
Sulfonamide		Cotrimoxazol	97 (93.37 %)	7 (6.73 %)
Quinolon		Nalidixic acid	37 (35.76 %)	67 (64.42 %)
		Ciprofloxacin	30 (28.85 %)	74 (71.15 %)
Cyclin		Tetracyclin	76 (73.08 %)	28 (26.92 %)

Table 2: *Salmonella* serovars isolated from raw chickens gizzards in Abidjan (Côte d'Ivoire).

Serums	Group	Serovars	Percentage (%)	Antigens O	Antigens H		
					Phase 1	Phase 2	Other
OMA	B	Agona	7.81	1,4,12	f, g, s		
		Aoto	1.56	1,4,12	g,m,s		
		Budapest	12.5	1,4,12,[27]	g,t		
		Chester	3.13	1,4,[5],12	e,h	e,n,x	
		Derby	17.19	1,4,[5],12	f,g	[1,2]	[Z_{27}], [Z_{45}]
		Essen	12.5	4,12	g,m		
		Fortune	1.56	1,4,12,[27]	Z_{10}	Z_6	
		Schwarzengrund	3.13	1,4,12,27	d	1,7	
		II	1.56	4,2	b	1,5	
	E (E1)	Elisabethville	1.56	3,{10}{15}	r	1,7	
		Muenster	3.13	3,{10}{15}{15,34}	e,h	1,5	[Z_{48}]
		London	1.56	3,{10}{15}	l,v	1,6	
	L	Ruiru	3.13	21	y	e,n,x	
OMB	C (C2-C3)	Bargny	1.56	8,20	i	1,5	
		Hadar	4.69	6,8	Z_{10}	e,n,x	
		Kentucky	18.75	8,20	i	Z_6	
		Poeselderf	1.56	8,20,54	y	e,n,x	
		Newport	11.56	6,8,20	e,h	1,2	[Z_{67}], [Z_{78}]
		Santiago	1.56	8,20	c	e,n,x	

Table 3: List of bacterial strains of the NCBI database whose *bla* genes are similar to *Salmonella* strains isolated from raw chicken gizzards in Côte d'Ivoire.

***Salmonella* isolated**	**Data base strains**	**Type of *bla*genes**	**Identity (%)**	**Number of accession**
Salmonella kentucky	*S.* Schwarzengrund S782	*bla* Ctx-m-2	96	KC633129.1
	*S.*Typhimurium	plasmide pCtx-m 5-1845		JX017309.1
	S. Typhimurium 18-425 -M-5	*bla* Ctx-m-5 (ISEcp1)		JN003855.1
	E. coli KN113	*bla* Ctx-m-2 (ISEcp1)		AB976589.1
	E. coli BR-79	*bla* Ctx-m-2(ISCR1)		AB976586.1
	E coli KUN-9085	*bla* Ctx-m-44		AB976584.1
	E. coli B275	*bla* Ctx-m-97		HM776707.1
	*E. coli*strain E39	*bla* Ctx-m-92 (BLSE)		GU127598.1
	P. mirabilis TUM11514	*bla* Ctx-m-2		AB770487.1
	P. rettgeri 309/05	class I integronCtx-m-131 (BLSE)		JN969893.2
	P. *aeruginosa* PHB 53	*bla* Ctx-m-2		GU929917.1
	K. pneumoniae K6P	*bla* Ctx-m-2		FJ973568.1
	K. pneumoniae HB 99	*bla* Ctx-m-59		FJ815287.1
	K. oxytoca 76C	*bla* OXY	100	GQ433981.1
Salmonella Muenster	*K.pneumoniae*	*bla* NDM-1	100	CP009114.1

NB. The types of bla genes observed, apart from *bla* OXY and NDM-1 belong all to group 2 or bla Ctx-m-2

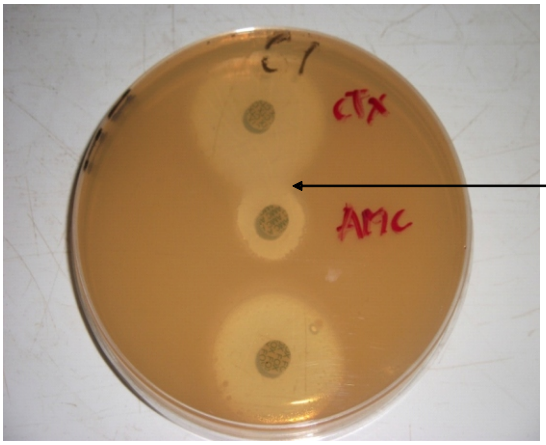

Figure 1: *Salmonella* Kentucky producer of an ESBL

The sequence amplified starting from the amplicons bla Ctx-m consensus of *Salmonella* Muenster of description *bla* Ctx-m/F 14158390, reveals 100 % of homology with the *bla* NDM-1 gene of the strain of *Klebsiella pneumoniae* of NCBI database with the accession number CP009114.1. That amplified starting from the sequence bla Ctx-m consensus of *Salmonella* Kentucky, of description bla Ctx-m/F 14158396 reveals 96 and 100 % of homologies with genes bla Ctx-m and *bla* OXY in bacteria of genus, *Salmonella*, *Escherichia coli*, *Proteus mirabilis*, *Providenci arettgeri*, *Pseudomonas aeruginosa* and *Klebsiella* sp. Sequencing enabled to refine the probable existence of the genes encoding ESBLs in Salmonella strains isolated from raw chicken gizzard in Abidjan, revealing different genetic profiles, specifically at level of two strains (S. Muenster and S. Kentucky) (Table 3).

The classification of these different genes according to the type of enzyme β-lactamase also revealed three distinct groups. The first, bla Ctx-m 1 and 2 group corresponds to the 2be group according to the classification of Bush- Jacoby- Medeiros (Bush et al., 1995) and at to class A according to Ambler molecular classification. The second group bla NDM-1 corresponded to the group of 3a according to the classification of Bush- Jacoby- Medeiros and the Class B according to Ambler molecular classification. The third group (*bla* OXY) corresponded, meanwhile, at the class A. The different types of *bla* Ctx-m genes as well as the percentages of similarity are consigned in table 3.

The resistance observed to the level of beta lactams, could translate the capacity of the *Salmonella* strains (S. *Muenster* and S. *Kentucky*) to develop a mechanism of resistance by enzymatic inactivation by producing beta-lactamases, enzymes able to hydrolyze the beta lactam (Bush, 2003; Foley and Lynne, 2008). Moreover, the resistance observed in Salmonella were mainly due to β-lactamases with Extended spectrum (BLSE) or to cephalosporinases (Egorova et al., 2008).

The *bla* Ctx-m genes are mainly those that confer resistance to third-generation cephalosporins such as cefotaxim (Arlet et al., 2006; Hur et al., 2010). The disk diffusion tests carried out on the isolated strains, revealed the presence of strains producing ESBL (β-lactamase at extended spectrum). By cons, sequencing performed on the set of amplicons of the degenerated primer of *bla* Ctx-m consensus confirmed the presence of *bla* Ctx-m-1 and also revealed, similarities ranging from 96 to 100 % with sequences bla of bacterial strains in the NCBI database. These enzyme sequences are type bla Ctx-m-2, -5, -44, -59, -92, -97, -131, *bla* NDM-1 and bla OXY. These observations explain the probable existence of a diversity of bla gene in *Salmonella* strains isolated. Diversity of *bla* genes is reported by some authors in level of bacterial strains of the genus Klebsiella, *Escherichia coli* and *Salmonella (Lartigue et al., 2004; Mirzaee et al., 2009; Olowe et al., 2013)*. The classification of these different genes depending on the type of beta-lactamase enzyme also revealed three distinct groups: *bla* Ctx-m-2 comprising the genes *bla* Ctx-m-2, -5, -44, -59, -92 , -97, -131; *bla* NDM-1 and *bla* OXY. This classification can allow predict, different resistance mechanisms that may exist in our Salmonella strains isolated from the raw chicken gizzards. In effect, according the molecular classification established by , the beta-lactamases of classes A, C and D have a serine on their active site, which involved in the acylation mechanism during the hydrolysis of the beta-lactam antibiotics. In contrary, class B includes the metallo-β-lactamases whose activity requires the presence of metallic ions. From this point of view, the different types of *bla* genes obtained, contained in the classes A and B translate the capacity of the *Salmonella* strains to resist to antibiotics of the family of beta-lactams, by the mechanism of enzymatic hydrolysis (enzymatic inactivation).

Some fragments of sequences of *bla* Ctx-m genes sequences showed 96 % of identity with fragments of sequences of gene of resistance *bla* Ctx-m, carried by mobile genetic elements. This is particularly the case of integron class I Ctx-m ST 131, the type of insertion sequences *bla* Ctx-m -44 ISEcp1 and *bla* Ctx-m-2 ISCR1, which reflect the possible mobilization of *bla* genes Ctx-m. In effect, the mobilization of *bla* Ctx-m genes is demonstrated experimentally by insertion sequences IS located upstream of genes, such as ISEcp1 . Furthermore, the effect of the promoter of these insertion sequences, increasing the expression of genes *bla* Ctx-m, suggests that these insertion sequences situated upstream of these genes would play a role in the selection and the diffusion of genes *bla* Ctx-m. Moreover, this insertion sequence (ISEcp1) is associated with the expression of all type β-lactamases cefotaximase groups except the group *bla* Ctx-m-8. The insertion sequence ISCR1 as for it, is linked to several members of the Ctx-m-2 group(Barlow et al., 2008).

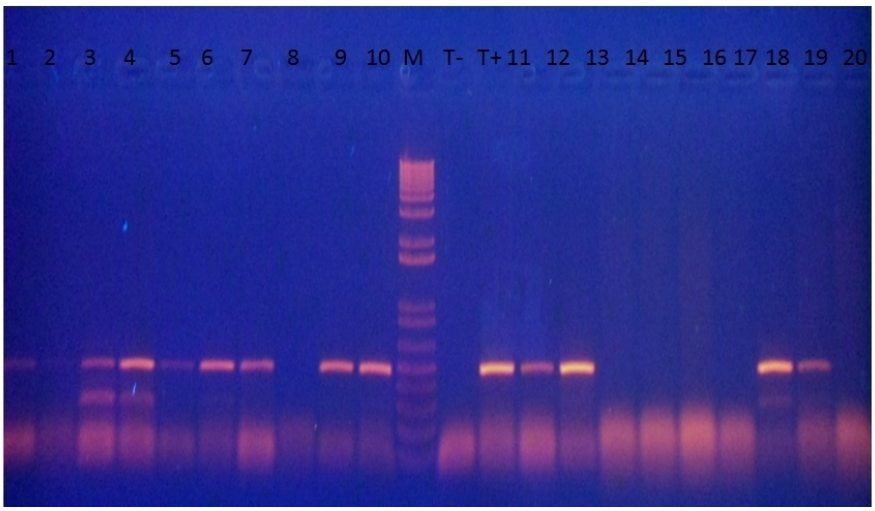

Figure 2: Gel-electrophoresis of Salmonella isolates examined for *bla* Ctx-m group 1 (Specimens 1 >20).

Lane **M**. molecular size marker (Eurogentec, Smart Ladder) depict a 1 kb +; Lane **T**(-).indicates the negative control ; Lane **T**(+) indicates the positive control (*E. coli* X92506). Lanes **1** (*S. Derby*), 2 (S. London), 3 (S. Essen), 4 (S. Kentucky), 5 (S. Kentucky), 6 (S. II), 7 (S. Muenster), 9 (S. Schwarzengrund), 10 (S. Chester), 11 (S. Derby) , 12 (S. Agona), 18 (S. Kentucky) and 19 (S. Kentucky) showed positive results obtained; Lanes 8 (S. Derby), (S. Kentucky), 15 (S. Kentucky), 16 (S. Bargny), 17 (S. Kentucky) and 20 (S. Kentucky) showed no bands, indicating a negative PCR results.

The recent approaches based on genotyping of multi locus showed that despite the existence of a great diversity within strains producing *bla* Ctx-m, some clones or sequence types (ST) grouped into clonals complexes (CC) are most often linked to the production of enzymes *bla* Ctx-m. This suggests that, they would be involved in the diffusion of these enzymes (Woodford et al., 2011), and secondly that the mechanisms of acquisition and diffusion of genetic determinants of resistance would imply the horizontal transfer of DNA between bacteria of animal origin (Carattoli et al., 2001). For this purpose, the existence of these mobile genetic elements could support the transfer of cefotaximase resistance genes between bacteria. From this point of view, the similarity (96 %) observed between the sequences fragments sequences of bla Ctx-m genes in strains of Salmonella isolated in chicken gizzards and those of the different mobile elements bearing the bla genes Ctx-m, could explain their capacity to acquire determinisms of resistance, in order to resist to antibiotics action, such as beta-lactams. These argumentations, suggest that in this study two Salmonella serovars isolated in raw chicken gizzards, in occurrence Kentucky and Muenster, containing various type of ESBL, could be dangerous for the consumer.

CONCLUSION

In conclusion, the use of universal sequence tagged PCR-amplicons by sequencing, in this study, is a quick and reliable method because it allowed the detection of up to resistance genes of ESBL type. The drug resistance in *Salmonella* remains a problem because it leads to the use of C3G or fluoroquinolons to treat salmonellosis, which may tend to support the emergence of resistance to these two molecules. Resistances to C3G and to fluoroquinolons remain low in Salmonella, but could become a real public health problem in Côte d'Ivoire if they were to spread. Most cases of human salmonellosis are foodborne (in particular by the products containing poultries or eggs), from this point of view, the monitoring of resistance in the breeding areas of avian animals are an essential precaution in order to anticipate the emergence and the diffusion of the resistance genes; in summarized to anticipate the appearance of new resistance mechanisms in the field of heath.

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