International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 11 No. 3 Jun. 2015, pp. 567-578

http://www.ijias.issr-journals.org/

Distribution of serovars and antibiotic resistance genes of *Salmonella* isolated from chicken gizzards in Abidjan, Côte d'Ivoire

A. Carole BONNY[1], T. Germain KAROU[1], M. D. Francine AKE[2], A. Thomas DADIE[2], L. Guichard BOHOUA[2], and Sébastien L. Niamké[1]

[1]Department of Biochimistry,
University Félix Houphouët Boigny, UFR Biosciences, Laboratory of Biotechnology,
22 P.O. Box 582 Abidjan 22, Côte d'Ivoire

[2]Department of Food Sciences,
University of Nangui-Abrogoua, UFR of Food and Technology Sciences, Laboratory of Biotechnology,
02 P.O. Box 801 Abidjan 02, Côte d'Ivoire

ABSTRACT: *Salmonella* enterica is a pathogen of humans and animals, and is the most frequent causes of foodborne illness worldwide. The increasing prevalence of multidrug resistance among *Salmonella* isolates from retail meat such as chicken gizzards has been an emerging problem in Côte d'Ivoire. 66 batches of raw chicken gizzard were collected to determine *Salmonella* prevalence. Serotyping, antimicrobial susceptibility, presence of *tet*(A), *qnr*(A, B and S), *Sul1*, *bla CTX-M*$_1$ genes were researched. *Salmonella* strains obtained by the method of culture were 104 (77.27 %). Distinct serovars obtained was: Derby (18.9 %), Budapest (17 %), Essen and Kentucky (11.3 %), Hadar (9.4 %), Agona (7.5 %), Chester, Riuru and Schwarzengrund (3.8 %), Aoto, Bargny, Elisabethville, Fortune, Poeselderf and Santiago (1.9 %). All isolates, exhibited resistance to one or more antimicrobial agents used. The antibiotic resistance was mainly detected against, Cefotaxim (0.96 %), Cefalotin (9.62 %), Amoxicillin/Clavulanic acid (7.69 %), Amoxicillin (7.69 %), Gentamycin (10.58 %), Ciprofloxacin (30.77 %), Chloramphenicol (29.81 %), Nalidixic acid (35.58 %), Tircacillin (46.15 %), Tetracyclin (73.08 %) and Cotrimoxazol (93.37 %). 65 isolates (63.1 %) were resistant to more than 3 antibiotic families (ß-lactams, quinolones, cyclines, sulfonamides). The detection by PCR of 20 isolates highlighted the presence of 3 different genes (*tet* (A), *bla CTX-Mgroup1* and *sul*$_1$). Our findings showed that raw chicken gizzards could be considered as an important source of transmission of salmonellosis to human. Furthermore, the presence of resistance genes exhibited by the strains observed in this study could be a public health problem.

KEYWORDS: *Salmonella*, resistance genes, chicken gizzards, public health, Côte d'Ivoire.

1 INTRODUCTION

Food borne illness in human is mainly caused by *Salmonella*. *Salmonella* infections are mainly transmitted by animals bred in farms and foods. *Salmonella* Typhimurium and Enteritidis have been found to cause human food-borne illnesses [1]. Mead et *al* [2] put stressed that food borne illness cause essentially by *Salmonella. Salmonella* species are involved in the infections of children and people with weak immune system. The symptoms of food borne disease include fever, diarrhea, nausea, abdominal upset, vomiting and sometimes septicemia [3],[4]. These illnesses require antimicrobial therapy as treatment. *Salmonella* is also a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistant strains, due to indiscriminate use of antibiotics in animal feeds as growth promoters and therapeutic agents is a further threat to human and animal health [5].

Corresponding Author: A. Carole BONNY

Salmonella serotypes isolated from foods of animal origin have shown that the bacteria exhibited a wide range of drugs resistance in many countries [6], [7], [8]. In addition the strains of *Salmonella* found in infected human and the food consumed by the animals present resistant to numerous antimicrobial agents, such as fluoroquinolones drugs and third generation of cephalosporin drugs [9].

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND *SALMONELLA* IDENTIFICATION

A total of 66 batches of 20 retailed raw chicken gizzard samples were collected in different poultry market in 11 municipalities (Abobo, Adjamé, Anyama, Attécoubé, Bingerville, Cocody, Koumassi, Marcory, Port-Bouët, Treichville and Yopougon) of the District of Abidjan Côte d'Ivoire. Samples were collected from April to September 2012. The collection was conducted once per week. The samples were transported to the laboratory in ice chest and processed immediately for the isolation of *Salmonella* strains.

All strains were isolated following the protocols described by standard ISO 6579: 2002 [10]. Using the Buffered Peptone Water (Bio-Rad), as pre-enrichment medium, Rappaport Vassilliadis and Müller-Kauffmann Tetrationate (DIFCO) as selective enrichment medium, and Hektoen agar and XLD agar (Bio-Rad) as selective medium. All isolates were biochemically identified with reduced gallery of LEMINOR, constituted of 4 medium (Mannitol Motility, Kligler –Hajna medium, urea-tryptophane medium and Citrate of Simmons medium) (Bio-Rad).

2.2 SEROTYPING

The strains were serotyped at Pasteur institute of Abidjan, Côte d'Ivoire (IPCI), on the basis of somatic O, phase 1 flagellar, and phase 2 flagellar antigen agglutination using commercial antisera (Bio-rad) according to the White-Kauffman-Leminor schem [11].

2.3 ANTIMICROBIAL SENSITIVITY TESTING

Sensitivity patterns of *Salmonella* serotypes to 12 antibiotics were determined using the agar-diffusion method described by the National Committee for Clinical Laboratory Standards (NCCLS) [12]. A bacterial suspension of 10^4 *Salmonella*/mL was prepared with 3 to 4 colonies of pure culture from Müller-Hinton (M.H; BioRad) agar in slope. These colonies were emulsified in a tube of 5 mL of physiological water in order to obtain a homogenous suspension of density equivalent to 0.5 Mc Farland standards. Using a sterile swab moistened in the suspension, a M.H agar previously dried was seeded by swabbing the entire surface of the M.H agar by scoring tightened. The operation was repeated twice. The antibiotic disks were disposed onto the surface of the dried agar medium with a distance of 3 cm between disk and the agar was incubated 24 hours. After incubation, agar plats were read by measuring inhibition zones around each antibiotic disk with a graduated ruler. A reference strain of *E.coli* ATCC 14028 was used as a quality control for culture. The results were interpreted and transcribed by the terms: Susceptible (S), intermediate (I) and resistant (R) [13]. Strains resistant to 3 or more antimicrobials from different classes were considered as Multidrug-Resistant (MDR).

Antimicrobial disk (BioRad) with the following antibiotics were tested for *Salmonella* susceptibility: amoxicillin (AMX) 10 µg, amoxicillin/alavulanic acid (AMC) 10/20 µg, Ticarcilline (TIC) 75µg. Gentamicin (GM)10 µg, tetracycline (TE) 10 µg, ciprofloxacin (Cip)10 µg, nalidixic acid (Nal) 10 µg, cefoxitin (FOX) 10µg, cefotaxim (CTX) 10µg, cefalotin (CF)10µg, chloramphenicol (C) 10 µg and trimethoprim/sulfametoxazole (SXT) 10/20 µg. *Salmonella* ATCC 14028 and IPCI 8297 were used as a reference strains. *Salmonella* isolates resistant to three or more classes of antimicrobials were defined as multidrugs resistant (MDR) isolates.

2.4 DNA EXTRACTION

Plasmid DNA was extracted using the alkaline lyses method with modifications [14]. Six or seven colonies of pure *Salmonella* culture were added into 2 mL of sterile water. The suspension was centrifuged for 10 min at 14,000 rpm (revolution per minute) and a pellet was obtained. The Pellets were suspended in 100 µL of solution containing: Tris pH8 25mM, Glucose 50 mM, EDTA 10 mM. The first mixture obtained was vortexed and stored on ice. After, 200 µL of a second solution containing 10% SDS, 1 M NaOH was added to the first solution. The micro tube containing both solutions was mixed

by inverting the tube rapidly two or three times, and incubated on ice for 2 minutes. A third solution with a final volume of 150 µL was prepared by adding 5M 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 novel 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 14,000 rpm. The supernatant obtained was pipetted and discarded. A solution of 500 µL of 70% ethanol was transfered 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. The plasmid DNA at the bottom of the tube was suspended once again in 50 µL of sterile water.

2.5 POLYMERASE CHAIN REACTION (PCR) DETECTION OF RESISTANCE GENES

PCR conditions to detect 6 antimicrobial resistance genes that confer to the β-lactams, Tetracycline, Ciprofloxacine and Sulfonamides using published primers are listed in Table 1. All PCR amplification was performed in a 50 µL final volume, with a reaction mixture containing 10 µL plasmid DNA; Gotaq buffer (1X) (Proméga®, France); 0.4 mM each deoxynucleoside Triphosphates (dNTPs) (BioRad® France); 1.5 mM of MgCl2 (Quiagen®); 0.2 µM each primers; 1 U GoTaq DNA polymerase (Proméga®, USA). The amplification products were loaded onto the agarose gel and left to run in a 1% (w/v) gel electrophoresis, and visualized under UV light after Ethidium bromide staining. Six strains of *Escherichia coli* (*E. coli* PSL 18X61367- *E. coli* Y10278- *E. coli* X92506- *E. coli* DJ21-15- *E* Coli J53 PMG252 et *E. coli* 57) were used as positive control for detection of *tet* (A), *bla* CTX-M consensus, *bla* CTX-M-1 (groupe 1), *sul* 1, *qnr* (A) and *qnr* (S) genes, respectively. A *Klebsiella pneumoniae* B1 strain was used as positive control for *qnr* (B) gene. Sterile RNAse- free water (10 µL) was used as negative control for all PCR detection. PCR primers and gene targets for antimicrobial resistance were summarized in table 1.

3 RESULTS

3.1 SALMONELLA SEROVARS

The microbiologic analysis of the 66 batches of the raw chicken gizzards collected in the district of Abidjan has shown that 51 (77.27 %) batches were found to harbor *Salmonella*. Further analysis of the 51 batches led to identification of 104 isolates: 20 isolates were detected in the raw chicken gizzards of Marcory, 13 isolates were found in the raw chicken gizzards of Koumassi, 11 isolates were found in the raw chicken gizzards of Abobo and Treichville, 10 isolates were detected in the raw chicken gizzards of Bingerville, 9 isolates were found in the raw chicken gizzards of Cocody, 8 isolates were found in the raw chicken gizzards of Adjamé and Attécoubé, 6 isolates were detected in the raw chicken gizzards of Port-Bouët and Yopougon , 2 isolates were detected in the raw chicken gizzards of Anyama. The strains were subdivided into fifteen different sérovars: Derby (18.9 %), Budapest (17 %), Essen and Kentucky (11.3 %), Hadar (9.4 %), Agona (7.5 %), Chester, Ruiru and Schwarzengrund (3.8 %), Aoto, Bargny, Elisabethville, Fortune, Poeselderf and Santiago (11.9 %) (Figure 1).

3.2 ANTIMICROBIAL SENSITIVITY PROFILE OF *SALMONELLA* ISOLATES

Out of the 104 *Salmonella* enterica isolates, 103 were resistant to 1-11 categories of antimicrobial agents that include β-lactams, sulfonamides, aminosides, phenicols, Quinolones and Tetracycline. Among the β-lactams, 46.15 % isolates exhibited resistance to tircacillin, 7.69 % isolates showed resistance to amoxicillin and amoxicillin-clavulanate, and finally 9.62 % isolates exhibited resistance to cefalotin. In addition, resistance to the third generation of cephalosporins such as cefotaxim, 0.96 % isolates was also observed (Figure 2). The antibiotic resistance testing has highlighted the presence of 103 strains of *Salmonella*. Intermediated resistance strains has considered as resistant. Among the 104 strains isolated, 103 (99.04 %) strains were found to be susceptible to all antimicrobials. Furthermore a total of 103 strains were also tested and the results showed that 8 (7.77 %) exhibited a single type of resistance, 29.12 % (30/103) showed resistance to two classes of antimicrobials and 64.07 % (65/103) were multidrug-resistant (Resistance to 3 or more antimicrobials). The profile of multidrug *Salmonella* resistant strains is summarized in table 2. The antimicrobial resistance genes were examined by using 20 strains of the 65 multidrug-resistant strains (Table 3).

3.3 ANTIMICROBIALS RESISTANCE GENES

The results of the analysis of the resistance genes (6) investigated, showed 3 genes (*tet(A)*, *Sul1*, *bla CTX-M1*). Among the sulfonamide resistant isolates, 8 (40%) were positive for *Sul1* (figure 3). Out of Tétracycline-resistant isolates, 8 (40 %) were positive to *tet(A*) (figure 4). None of the Quinolone gene (*qnr* (A, B and S) was detected in the Ciprofloxacine and Nalidixic acid resistant *Salmonella* isolates. In contrast, the β-lactams-resistant isolates showed: 13 (65 %) positive to *bla CTX-M1* (figure 3). Furthermore 3 strains (2 *Salmonella* serogroup O:8 and 1 *Salmonella* serogroup O: 4) of *Salmonella* showed resistance to 3 genes and 8 strains (4 *Salmonella* O:8, 2 *Salmonella* serogroup 0:4, 2 *Salmonella* serogroup O: 3, 10) showed resistance to 2 genes. In addition, 7 strains (3 *Salmonella* serogroup O: 3, 10, 2 *Salmonella* serogroup O: 8 and 2 *Salmonella* serogroup O: 4) showed resistance to 1 gene. Whereas 2 strains of *Salmonella* O: 4 (Derby and Essen) did not exhibited any resistance genes. The prevalence of resistance genes was summarized in Table 4.

4 DISCUSSION

The prevalence rates of *Salmonella* strains in poultry and poultry products have been previously highlighted by numerous scientists [15], [16], [17], [18], [19], [20]. The contamination rates of raw chicken gizzards by *Salmonella*, obtained in this study are high (77.27 %) indicating that poultry meat could be a factor of *Salmonella* strains transmission. The cross-contamination of *Salmonella* could have been arisen while the meat was handled, processed, packed and distributed. Meat sellers could be responsible of the spreading of salmonella [21]. In addition the carcasses could also be infected with *Salmonella* as the meat intestines containing the bacteria are disrupted during the processing of the poultry. The results of our investigation has been found to be higher compared to the results (53%) of Tibaijuka et *al* [22] and (38.2%) of Uyttendaele et *al* [23]. Moreover, our results has been found to be greater than the prevalence of *Salmonella* in retail poultry meat in Malaysia 35% [24], Spain 35.8 % [25], Belgium 36.7 % [26] and USA 35 % [27]. The higher result obtained in our study could be explained mainly by the poor standard of hygiene observed during the slaughtering of the animals. Indeed during the slaughter process, the animals are butchered on the ground and the same water is used to wash all the chickens. Besides as the meats are processed, the butchers do not wear protective gloves and hygienic clothing. As in Ivory Coast the training of the butchers in term of meat processing is practically nonexistent leading to the cross-contamination of bacteria.

In term of public health worldwide, *Salmonella* enterica resistance to antimicrobial has been found to be a serious cause of concerned [28]. The present study demonstrated that raw chicken gizzards were highly contaminated by *Salmonella* (51/66 batches) and 64.08% (66/103) were multidrug resistant (MDR). These percentages obtained in our investigation are slightly close to the contamination rate of 67 % of Multidrug resistant *Salmonella* obtained by Thong and Modarressi in Malaysia [2]. Concerning the multidrug resistant of *Salmonella*, our data were higher than that obtained by Bouchrif et *al* in Morocco (44%) [29] and Van et *al* in Vietnam (34 %) [30] Multidrug resistant phenotypes were observed in 7 *Salmonella* serovars (Budapest, Ruiru, Kentucky, Essen, Derby, Bargny and Hadar) in our study. High resistant rates to Tetracycline (73.08 %), sulfonamides (93.37 %) were also observed in our results. These finding concurred with previous report that *Salmonella* isolates in the retail meats were resistant to multiple antimicrobials, including Tetracycline, Sulfonamides and/or Streptomycin [25], [26], [28], [29], [30], [31]. Resistance of *Salmonella* isolates to Nalidixic acid (35.76%) and Ciprofloxacin (28.85%) was significant. Our finding showed that *Salmonella* isolates exhibited resistance to a wide range of antibiotics. These results could be explained by the indiscriminate use of antibiotics in the different farms. The shortage of specialist veterinarians in the country, Côte d'Ivoire and the lack of training of the farmers could be the main factors of the improper use of the antibiotics.

We tested 6 gènes (*tet* (A), *qnr* (A, B and S), *sul* 1, *bla CTX-M group 1*) and found 3 genes: *tet* (A), *sul* 1, *bla CTX-M group 1*, with respectively rates of 45 %, 45 % and 65 %. But none of our strains presented *Qnr(B)* genes.

The *tet* (*tet* A and B) tetracycline resistance determinants have been reported to be the most common genetic determinant in members of the Enterobacteriaceae [32]. In our study we tested *tet* (A) resistance gene in *Salmonella* bacteria which are also genus of Enterobacteriaceae, and we obtained tetracycline resistance strains from a samples. Of the tetracycline-resistant's strains, 45 % tested positive for the *tet* (A) gene, indicating that this gene is diffused in *Salmonella* strains. This suggests that *tet* (A) tetracycline resistance circulating *Salmonella* strains in animals. Study conducted by Pezzella *et al* [33] in both northern and southern Italy, was also confirmed an epidemiologically unrelated *Salmonella* strains of animal origin isolated.

Sulfonamide resistance in gram-negative bacilli generally arises from the acquisition of either of the two genes *sul*1 and *sul*2, encoding forms of dihydropteroate synthase that are not inhibited by the drug [34]. Study conducted in Portugal by Antunes *et al* [35], revealed predominant *Salmonella* isolates (76 %). In our study *Salmonella* isolates showed 75 % of the *sul* 1 gene. This suggests that, the *sul*1 gene could be the mechanism of resistance to sulfonamides. The consumption of sulfonamides for veterinary use is generally widespread. So, the appearance of the presence of *sul*1 genes may explain, its high usage in food-producing animals, such as poultry as proved in our country. The persistence of several sulfonamide resistance genes may be the result of the successive pressure exerted by sulfonamides that are also commonly used for animals and human.

Since 1998, Plasmid-mediated quinolone resistance encoded by *qnr* genes A, B, and S that confer low-level resistance to nalidixic acid and reduced susceptibility to ciprofloxacin has been identified in several enterobacterial species, including *Salmonella* [36].

Plasmids carrying *qnr* genes have been found to transmit Quinolone resistance. The major genetic mechanisms of resistance to fluoroquinolones in *Enterobactericeae* could be (i) the mutations in the QRDR region of the gyrase subnit A (*gyrA*) gene of the topoisomérase II, (ii) the mutations in *par*C and *par*E genes of topoisomerase IV, (iii) alterations of the bacterial membrane permeability, and (iv) the presence of *qnr* gene [37]. Since the aim of our study was to detect the presence of *qnr* genes by PCR in plasmids, we not obtained the presence of the *qnr* gene, which has been currently found in the plasmids. In this regard, none of the strains that presented phenotypic resistance to ciprofloxacin amplified the gene *qnr*, could be indicating that other molecular mechanisms of resistance could be present.

Disk diffusion tests detected ESBL production of 5 % (1/20) for cefotaxim, a member of third generation beta lactams. PCR used to identify *bla* CTX-M gene showed several samples (70 % for *bla* CTX-M groupe 1, 10 % expressing more than one gene for the test primers, which is in accordance with the literature that reports that Enterobacteria may carry several genes that encode for ESBL [38]. This gene has previously been described several times for *Salmonella* species [39]. Our study, showed the presence of ESBL genes in 13 strains for which the phenotypic not revealed its. Thus, there was interference in the phenotypic expression of ESBL by the presence of the *bla* CTX-M gene.

Table 1. PCR primers and gene targets for antimicrobial resistance

Gene	Nucleotide sequence (5' to 3')	Amplicion size (pb)	PCR conditions	Resistance mechanism	References
bla CTX-Mconsensus	F:ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSAACCAGAAYCAGCGG	593	5 min at 94°C; 40 cycles of 30s at 94°C, 45s at 55°C and 1 min at 72°C; 10 min at 72°C	β-Lactamase	[40]
bla CTX-M1	F:GACGATGTCACTGGCTGAGC R:AGCCGCCGACGCTAATACA	499	5 min at 94°C; 40 cycles of 30s at 94°C, 45s at 60°C and 1 min at 72°C; 10 min at 72°C	β-Lactamase	[40]
tet (A)	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	210	5 min at 94°C; 40 cycles of 30s at 94°C, 45s at 60°C and 1 min at 72°C; 10 min at 72°C	Efflux	[41]
sul 1	F:CTTCGATGAGAGCCGGCGGC R:GCAAGGCGGAAACCCGCGCC	417	5 min at 94°C; 40 cycles of 30s at 94°C, 45s at 69°C and 1 min at 72°C; 10 min at 72°C	Dihydropteroate Synthase Inhibitor	[42]
qnr (A)	F:ATTTCTCACGCCAGGATTTG R:GATCGGCAAAGGTTAGGTCA	516	5 min at 94°C; 40 cycles of 30s at 94°C, 45s à 60°C and 1 min à 72°C; 10 min at 72°C	Efflux	[43]
qnr (B)	F:GATCGTGAAAGCCAGAAAGG R:ACGATGCCTGGTAGTTGTCC	469	5 min at 94°C; 40 cycles of 30s at 94°C, 45s at 60°C and 1 min at 72°C; 10 min at 72°C	Efflux	[43]
qnr (S)	F:ACGACATTCGTCAACTGCAA R:AAATTGGCACCCTGTAGGC	417	5 min at 94°C ; 40 of cycles de 30 s à 94°C, 45s à 60°C et 1 min à 72°C; 10 min à 72°C	Efflux	[43]

Table 2. Resistance profiles of multidrug Salmonella strains (MDR) isolated from raw retailed chicken gizzards in Abidjan, Côte d'Ivoire

Resistance level	Profils SMR	Drugs (n)	Number of SMR (%)	Number of famillies
LEVEL I	CSXTNal	3	1 (1,53)	3
	CSXTCip	3	1 (1,53)	3
	SXTNalTe	3	2 (3,07)	3
	TicCSXT	3	3 (4,61)	3
	CTeSXT	3	7 (10,76)	3
	TicTeSXT	3	14 (21,21)	3
	AAMCTicSXTTe	5	1 (1,53)	3
	AAMCTicCfTe	5	1 (1,53)	3
LEVEL II	CTeSXTNal	4	2 (3,07)	4
	TicCTeSXT	4	4 (6,15)	4
	TicGTeSXT	4	1 (1,53)	4
	CSXTNalCip	4	2 (3,07)	4
	SXTNalCipTic	4	3 (4,61)	4
	TicSXTNalCipTe	5	7 (10,76)	4
LEVEL III	CTeSXTNalCip	5	3 (4,61)	5
	TicCSXTNalCip	5	1 (1,53)	5
	GSXTNalCipTe	5	3 (4,61)	5
	TicCTeSXTNalCip	6	1 (1,53)	5
	GSXTNalCipTeTic	6	1 (1,53)	5
	AAMCTicGSXTNalCipTe	8	1 (1,53)	5
	AAMCTicCfGSXTNalCipTe	9	4 (6,15)	5
LEVEL IV	AAMCTicCfCTXGCSXTTeNalCip	11	1 (1,53)	6

MDR (multidrugs resistance), n (number of strains), **A** (Amoxicillin), **AMC** (Amoxicillin/ clavulanic Acid), **Tic** (Tircacillin), **Cf** (Cefalotine), **CTX** (Cefotaxim), **C** (Chloramphenicol), **G** (Gentamycin), **Nal** (Nalidixique acid), **Cip** (Ciprofloxacin), **SXT** (Cotrimoxazole), **Te** (Tétracycline).

Table 3. Resistance profiles of the different strains (20) of Salmonella serovars

Strains	**Municiplaties**	**Serogroups**	**Serovars**	**Resistance Profile**
R 211/A	Marcory	O:4 (B)	ND	**CfCSXTNalCip**
D 311/A	Adjamé	O:3,10(E1)	ND	**CTeSXTNal**
E 111/A	Port-bouët	O:4 (B)	Essen	**AAMCTicSXTTe**
E 212/A	Port-bouët	O:8(C2-C3)	Kentucky	**CfGSXTNalCipTe**
E 221/A	Port-bouët	O:8(C2-C3)	Kentucky	**CfGSXTNalCipTe**
H 411 /B	Treichville	O:3,10(E1)	ND	**CfCTeSXTNalCip**
H 421/F	Treichville	O:3,10(E1)	ND	**CTeSXTNalCip**
P 412/B	Yopougon	O:4 (B)	Derby	**AAMCTicCfTe Cip**
H 512/A	Treichville	O:4 (B)	ND	**TeSXTNalCipTic**
H 611/A	Treichville	O:4 (B)	ND	**TicTeSXTNalCip**
H 611/B	Treichville	O:4 (B)	ND	**TicTeSXTNalCip**
H 612/B	Treichville	O:4 (B)	ND	**TicTeSXTNalCip**
H 412/A	Treichville	O:3,10(E1)	ND	**TicCTeSXTNalCip**
D 412/A	Adjamé	O:8(C2-C3)	ND	**GSXTNalCipTeTic**
G 221/A	Bingerville	O:8(C2-C3)	ND	**AAMCTicCfGSXTNalCipTe**
D 512/A	Adjamé	O:8(C2-C3)	Bargny	**AAMCTicCfGSXTNalCipTe**
G121/D	Bingerville	O:8(C2-C3)	Kentucky	**AAMCTicCfGTeSXTNaCip**
G 212/A	Bingerville	O:8(C2-C3)	ND	**AAMCTicCfGSXTNalCipTe**
C 322/A	Cocody	O:8(C2-C3)	Kentucky	**AAMCTicCfGSXTNalCipTe**
C 211/A	Cocody	O:8(C2-C3)	ND	**AAMCTicCfCTXGCTeSXTNalCip**

A: amoxicilline; **AMC**: amoxicillin/clavulanic acid; **Tic**: ticarcillin; **Cf**: cefalotin; **CTX**: cefotaxim; **G**: gentamycin; **C**: Chloramphenicol; **SXT**: cotrimoxazole; **Nal**:nalidixic acid; **Cip**: ciprofloxacin; **Te**: tetracycline**.**

Table 4. Genetic profile of the different strains (20) of Salmonella serovars

Strains	Serovars/ serogroups	Genetic profils
1. R 211/A	O:4(B)	*tet* (A), *bla* CTX-M-1
2. D 311/A	O:3,10(E1)	*tet* (A), *bla* CTX-M-1
3. E 111/A	Essen	*bla* CTX-M-1
4. E 212/A	Kentucky	*sul* 1, *bla* CTX-M-1
5. E 221/A	Kentucky	*tet* (A), *sul* 1, *bla* CTX-M-1
6. H 411/B	O:3,10(E1)	*tet* (A), *bla* CTX-M-1
7. H 421/F	O:3,10(E1)	*tet* (A), *bla* CTX-M-1
8. P 412/F	Derby	ND
9. H 512/A	O:4(B)	*bla* CTX-M-1
10. H 611/A	O:4(B)	*tet* (A), *bla* CTX-M-1
11. H 611/B	O:4(B)	*bla* CTX-M-1
12. H 612/B	O:4(B)	*tet* (A), *bla* CTX-M-1
13. H 412/A	O:3,10(E1)	*bla* CTX-M-1
14. D 412/A	O:8(C2-C3)	*sul* 1
15. G 221/A	O:8(C2-C3)	*sul* 1
16. D 512/A	Bargny	*sul* 1
17. G 121/D	Kentucky	*tet* (A), *sul* 1, *bla* CTX-M-1
18. G 212/A	O:8(C2-C3)	*sul 1*, *bla* CTX-M-1
19. C322/A	Kentucky	*sul 1*, *bla* CTX-M-1
20. C211/A	O:8(C2-C3)	*tet* (A), *sul* 1

ND: None determinated. ***tet*(A)** resistant gene of tetracyclin, ***Sul1*** resistant gene of sulfonamides, ***bla* CTX-M-1** resistant gene of β-lactams

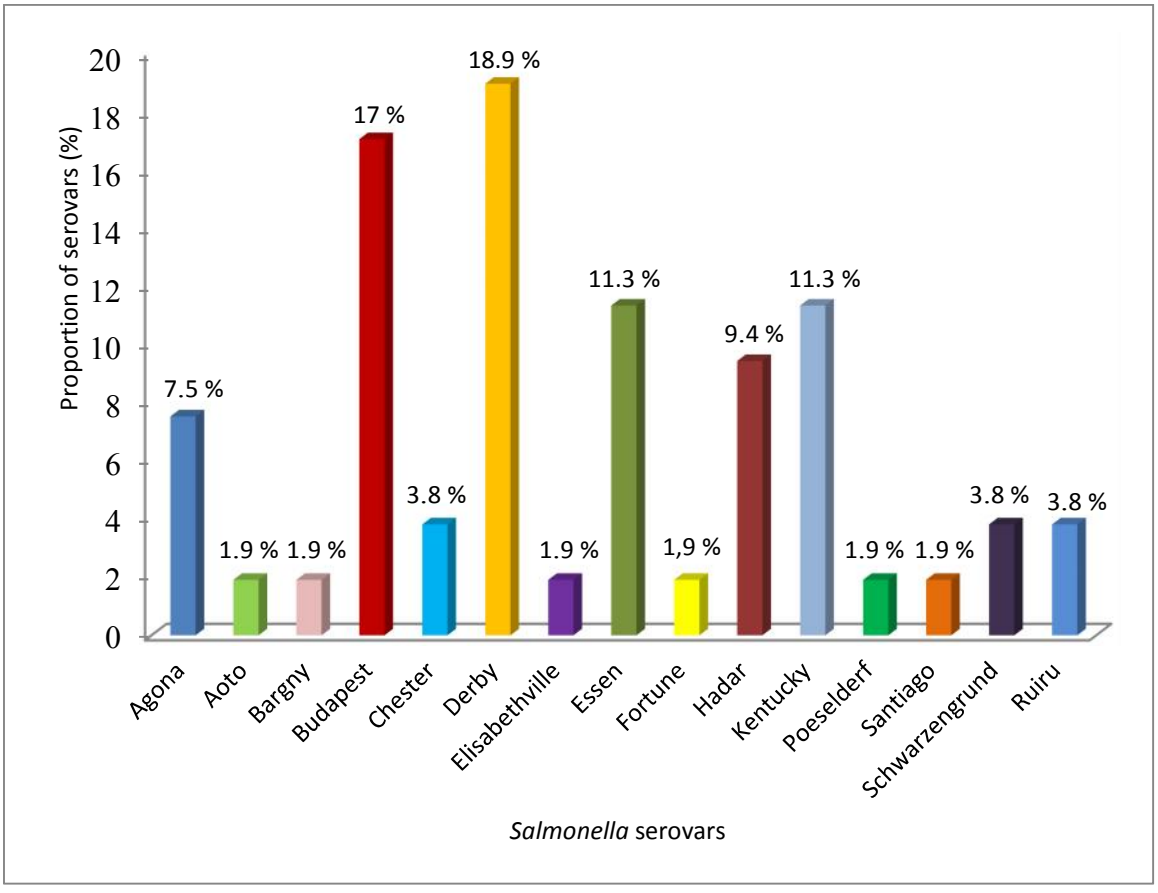

Figure 1: Proportion of Salmonella serovars isolated from raw retailed chicken gizzards in Abidjan, Côte d'Ivoire.

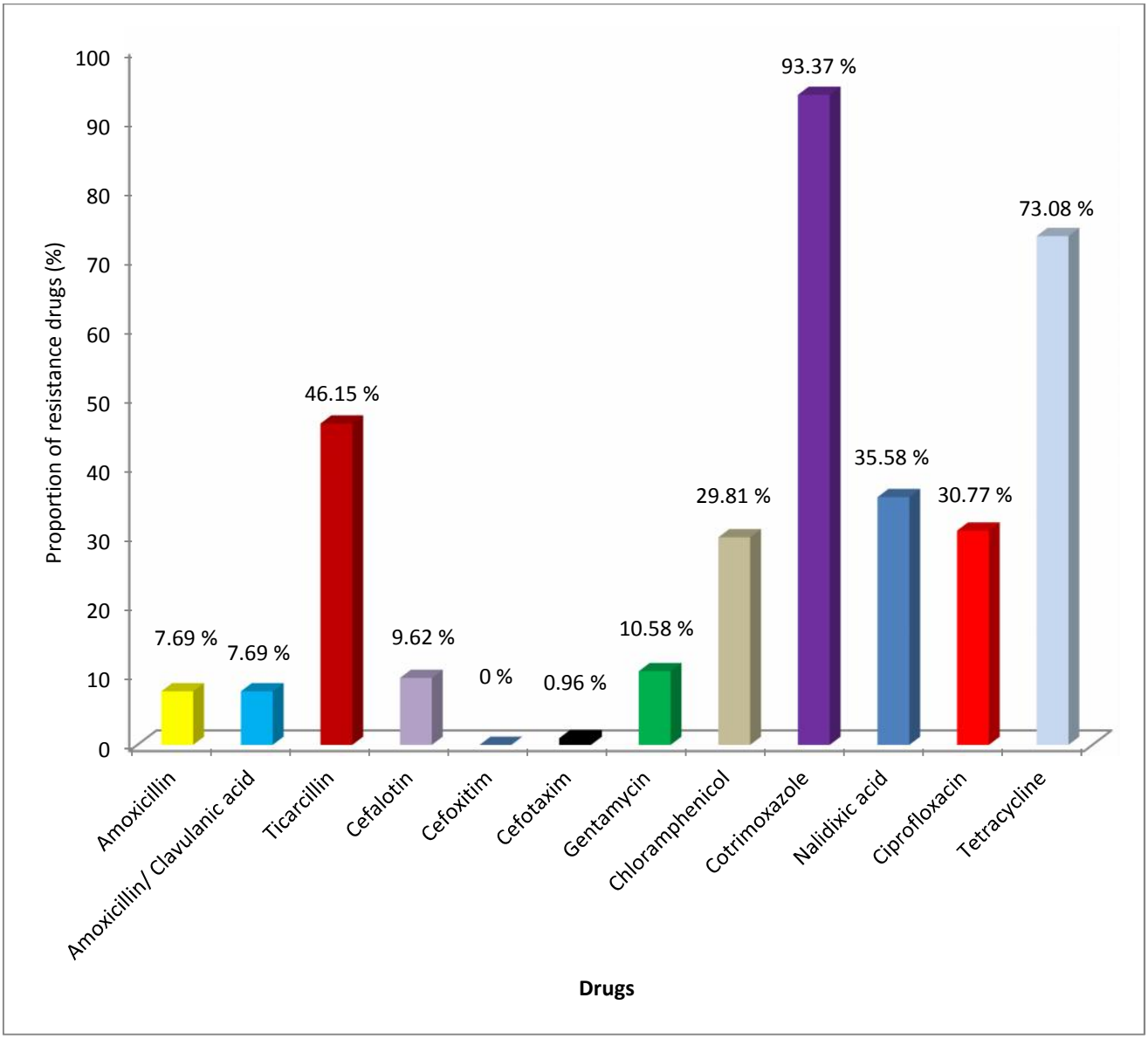

Figure 2: Antimicrobial resistances of Salmonella strains isolated from raw retailed chicken gizzard in Abidjan, Côte d'Ivoire.

Antibiotic famillies: ***β-lactams*** *(amoxicillin, amoxicillin/ clavulanic acid, ticarcillin, cefalotin, cefoxitim, cefotaxim);* ***aminosides*** *(gentamycin);* ***phenicols*** *(chloramphenicol);* ***sulfonamides*** *(cotrimoxazole);* ***quinolones*** *(nalidixic acid, ciprofloxacin);* ***cyclines*** *(tetracycline).*

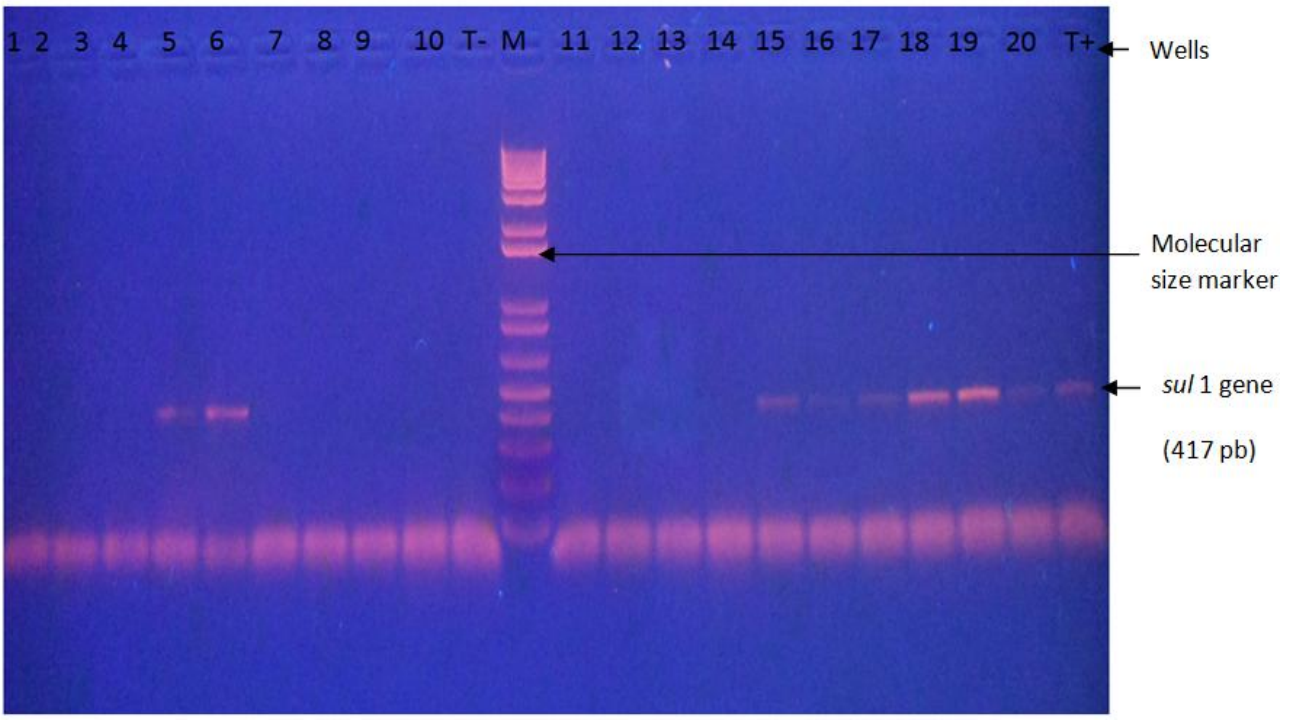

Figure 3: Gel-electropheris of Salmonella isolates examinated for sul 1(specimens 1 → 20).

*Lane **M**. depict a 1 kb + molecular size marker (Eurogentec, Smart Ladder); Lane **T(-)**.indicates the negative control; Lane **T(+)**.indicates the positive control (E. coli DJ21-15). Lanes **5**, **6**, **15**, **16**, **17**, **18**, **19** and **20** showed positive results obtained; Lanes **1**, **2**, **3**, **4**, **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14** showed no bands, indicating a negative PCR results.*

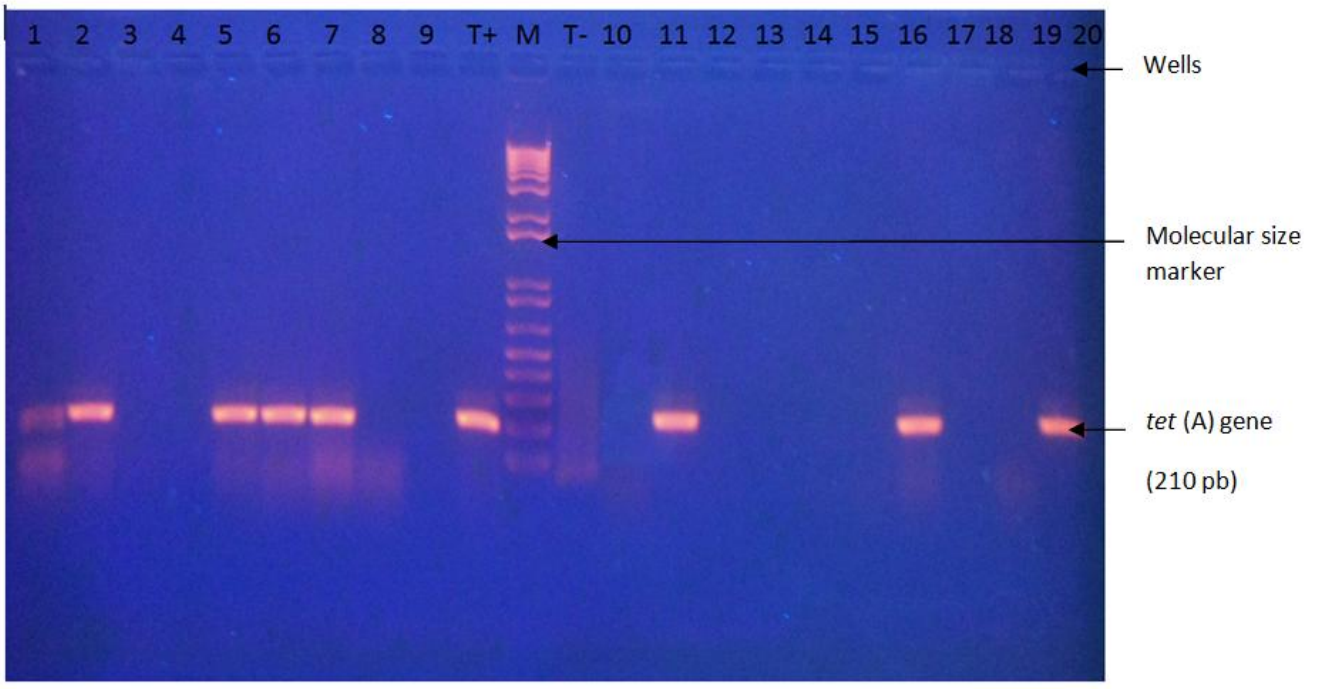

Figure 4: Gel-electrophoresis of Salmonella isolates examined for tet(A) (Specimens 1 → 20).

*Lane **M**. depict a 1 kb + molecular size marker (Eurogentec, Smart Ladder); Lane **T(-)**.indicates the negative control ; Lane **T(+)**.indicates the positive control (E. coli PSL 18X61367). Lanes **1, 2, 5, 6, 7, 9, 11, 16** and **19** showed positive results obtained; Lanes **3, 4, 8, 10, 12, 13, 14, 15, 17, 18** and **20** showed no bands, indicating a negative PCR results.*

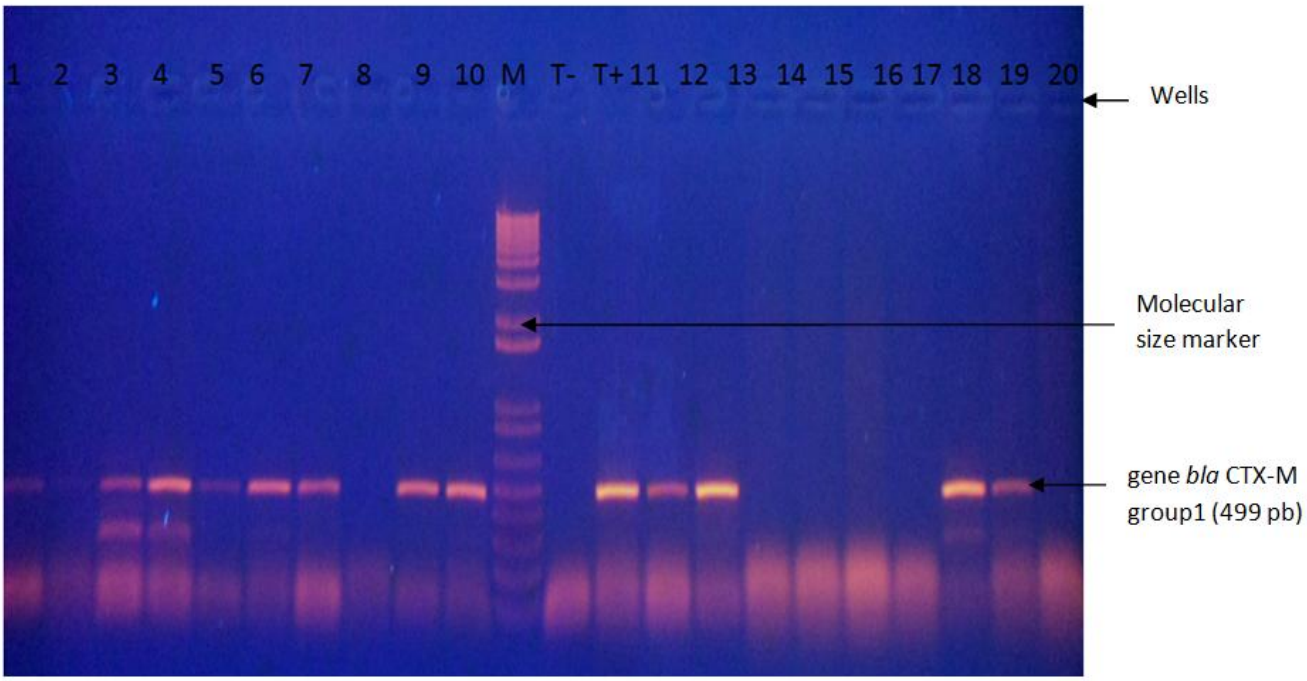

Figure 5: Gel-electrophoresis of Salmonella isolates examined for bla CTX-M group 1 (Specimens 1 ⟶20).

*Lane **M**. depict a 1 kb + molecular size marker (Eurogentec, Smart Ladder); Lane **T(-)**.indicates the negative control ; Lane **T(+)**.indicates the positive control (E. coli X92506). Lanes **1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 18** and **19** showed positive results obtained; Lanes **8, 10, 11, 12, 14, 15, 16, 17** and **20** showed no bands, indicating a negative PCR results.*

5 CONCLUSION

Since β-lactams, fluoroquinolones, sulfonamides and Cyclines, use in veterinary medicine, the extensive use of such drugs may have contributed to the successful spread of these genetic determinants in zoonotic bacteria as *Salmonella* which could be transferred to other bacterial species suggesting an important public health problem. Therefore, it seems highly recommended the development of vigilance programs in veterinary medicine and human medecine at the government level in Côte d'Ivoire, in order to control expending or applying of antimicrobials drugs.

ACKNOWLEDGEMENTS

The authors are thankful to Professeur Dosso Mireille, Director of Pasteur Institute of Abidjan Côte d'Ivoire; to Dr Guessend Nathalie for her disponibility and her contribution to antimicrobials interpretation and Bleoue Joel, Molecular Biologist for his true collaboration.

REFERENCES

[1] T. Suresh, A. A. Hata, D. Sreenivasen, N Sangeetha and P. Lashmanaperumalsamy, "Prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other *Salmonellas* in the eggs and egg-storing trays from retail markets of Coimbatore, South India", *Food Microbiology*, vol 23,pp. 294−299, 2006.

[2] P. S. Mead, L. Slutsker,V. Dietz, L. F. McCaig, Breese J. S and C. Shapiro, "Food related illness and death in the United States", *Emergence of Infectious Disease*, vol 5, pp. 607−625, 1999.

[3] B Coburn, G. A. Grass and B. B. Finlay, "*Salmonella*, the host and disease: A brief review", *Immunology and Cell Biology*, vol 85, pp. 112−118, 2007.

[4] J. Willford, W. Manley, T. Rebelein and L. D. Goodridge, "Pulsed field ribotype and intégron analysis of multidrug resistant isolates of *Salmonella* enterica sérovar Newport"*, Food. Res. Int,* vol 40, pp. 975−981, 2007.

[5] L. P. Forshell and M. Wierup, "*Salmonella* contamination: A significant challenge to the global marketing of animal products". *Revue Scientifique Et Technique De L'office International Des Epizooties*, vol 25, pp. 541−554, 2006.

[6] K. E. Holt, N. R. Thomson, J. Wain, M. D. Phan, S. Nair and R. Hasan, "Multidrug-resistant *Salmonella* enterica sérovar Paratyphi A harbors IncHI1 plasmids similar to those found in serovar Typhi*", Journal of Bacteriology*, vol 189, pp. 4257−4264, 2007.

[7] G. Prats, B Mirabilis, T. Love, C Munoz, E. Miro and F. Navaro, "Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995– 1998 in Barcelona", *Antimicrobial Agents and Chemotherapy*, vol 44, pp. 1140−1145, 2000.

[8] P. L. Winokur, A. Brueggemann, D. L. Desalvo, L. Hoffmann, M. D. Apley and E. K. Uhlenhopp, "Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC ßetalactamase", *Antimicrobial Agents and Chemotheraphy*, vol 44, no. 10, pp. 2777−2783, 2000.

[9] FAD, *National Antimicrobial Resistance Monitoring System-Enteric Bacteria (NARMS) 2003 Executive report.* Rockville, MD: US Food and Drug Administration, 2010.

[10] Anonymous, "*Microbiology of food and animal feeding stuffs-horizontal method for the detection of Salmonella spp.* International Standard (ISO 6579)", 2002.

[11] M. Y. Popoff, *Antigenic formulas of the Salmonella serovars*, I.P. WHO Collaborating Center for Reference and Research on *Salmonella*, Editor, 2001.

[12] NCCLS, *Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards. (7th edn) Wayne, Pennsylvania,* 2000.

[13] CLSI, *Performance standards for antimicrobial susceptibility testing. 15th information supplement, Wayne, Pennsylvania.*, C.a.L.S. Institute, Editor, 2005.

[14] Rozilla A., Nur-Harni A, Tan D and Wee S, "Rapid molecular detection of *Salmonella* isolated from poultry farm. 19 th veterinary Association Congress, Malaysia, pp. 201-203, 2007.

[15] S. Ciu, B. Ge, J. Zhen and J. Meng, "Prevalence and antimicrobial resistance of *Campylobacter spp*. and *Salmonella* sérovars in organic chickens from Maryland retail stores*", Applied and Environmental Microbiology*, vol 71, no. 7, pp. 4108-4111, 2005.

[16] C. Dominguez, L. Gomez, J. Zumalacarregui, "Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain"*, International Journal of Food Microbiology*, vol 72, pp. 165-168, 2002.

[17] S. I . Lestari, F Han, B. Ge, "Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores*, Journal of Food Protection*, vol 72, no. 6, pp. 1165-1172, 2009.

[18] C. Logue, J. S. Sherword, P. A. Ollah, I. M. Elijah and M. R. Docker, "The incidence of antimicrobial-resistant *Salmonella spp.* on freshly processed poultry from US Midwestern processing plants", *Journal of applied Microbiology*, vol 94, pp. 16-24, 2003.

[19] B. Molla, and A. Mesfin, "A survey of *Salmonella* contamination in chicken carcass and giblets in central Ethiopia"*, Revue de Medecine Veterinaire,* vol 154, no**.** 4, pp. 267-270, 2003.

[20] P. Whyte, K. Mcgill, J. D. Collin and E. Gormley, "The prevalence and PCR detection of *Salmonella* contamination in raw poultry"*, Veterinary Microbiology*, vol 89 no. 1,pp. 53-60, 2002.

[21] M. R. Uyttendaele, J. M. Debevere, R. M. Lips and K. D. Neyts, "Prevalence of *Salmonella* in Poultry carcasses and their products in Belgium", *International Journal of Food Microbiol*ogy, vol 40, no. 1, pp. 1-8, 1998.

[22] B. Tibaijuka, B. Molla, G. Hildebrandt and J. Kleer, "Occurrence of Salmonellae in retail raw chicken products in Ethiopia"*, Berl Munch Tierarztl Wochenschr*, vol 116, pp. 55-58, 2003.

[23] M. Uyttendaele, P. De troy, and J. Debevere, "Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on Belgian retail market"*, Journal of Food Protection*, 1999.vol 62, no 7, pp. 735-740.

[24] G. Rusul, J. Khair, S. Radu, C. T. Cheah and R. M. Yasin, "Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia"*, International Journal of Food Microbiology*, vol 33, no. 32, pp. 183-194, 1996.

[25] D. G. White, S. Zaho, R. Sudler, S. Ayers, S. Friedman and S. Chen, "The isolation of antibiotic resistant *Salmonella* from retail ground meats", *New England Journal of Medecine*, vol 16, no 345, pp. 1147-1154, 2001.

[26] K. L. Thong and S. Modarressi, "Antimicrobial resistant genes associated with *Salmonella* from retail meats and street foods"*, Food Research International*, p. 6, 2001.

[27] B. Bouchrif, B Paglieti, M. Murgia, A. Piana, N. Cohen, M. M. Ennaj, S. Rubino and M. Timinoun, "Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco", *Journal of Infections Diseases in Developing Countries*, vol 3, no. 1, pp. 35-40, 2009.

[28] P. Velge, A. Cloeckaert and P. Barrow, "Emergence of *Salmonella* Epidemics: the problems related to *Salmonella* enterica serotype Enteritidis and multiple antibiotic resistance in other major seotypes", Veterinary Research, vol 36, no. 3, pp. 267-288, 2005.

[29] T. T. H Van, G. Moutafis, T. Istivan, T. L. Tran and P. J. Coloe, "Detection of *Salmonella spp* in retail raw food samples from Vietnam and characterization of their antibiotic resistance"*, Applied and Environmental Microbiology*, vol 73, pp. 6885-6890, 2007.

[30] S. Chen, S. H. Zaho, D. G. White, C. M. Schroeder, R. Lur and H. C. Yan, "Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats"*, Appl. Env. Microbiol*, vol 70, pp. 1-7, 2004.

[31] K. L. Thong, Y. L. Goh, S. Radu, S. Noorzaleha, K. Yasin, K. T. Koh and V. K. E. Lim, "Genetic diversity of Clinical and environmental isolates of *Salmonella enterica* serotype Weltevreden isolated in Malaysia"*, Journal of Clinical Microbiology*, vol 40, pp. 2498-2503, 2002.

[32] E. Schnabel and A. Jones, "Distribution of Tetracycline Resistance Genes and Transposons among Phylloplane Bacteria in Michigan Apple Orchards", *Applied Environm. Microbiol*, vol 65, no. 11, pp. 4898–4907, 1999.

[33] C. Pezzella, A. Ricci, E. Digiannatale, I. Luzzi and A. Carattoli, "Tetracycline and Streptomycin Resistance Genes, Transposons, and Plasmids in *Salmonella enterica* Isolates from Animals in Italy", *Antimicrobial agents and chemotherapy*, vol 48, no. 3, 2004.

[34] Hindi A. K. , Shubbar E. E. and Addos S. A, "Molecular Study on Distrbution of *Sul*-1 and *Sul*-2 genes among *Salmonella enterica* causing Enteric Fever" in *Magazin of Al-Kufa University for Biology*, 2013.

[35] P. Antunes, J. Machado, J. Carlos Sousa and L. Peixe, "Dissemination of Sulfonamide Resistance *(sul1 , sul2 and sul3)* Genes in Portuguese *Salmonella enterica* Strains and Relation with Integrons"*, Antimicrob. Agents Chemother*, vol 49, no. 2, pp. 836-839, 2005.

[36] K. L. Hopkins, M. A. E. Day and E. J. Threlfall, "Plasmid-mediated Quinolone Resistance in *Salmonella enterica,* United Kingdom", *Emerging Infectious Diseases*, 2008. vol 14, no. 2, pp. 635-642.

[37] H. Mammeri, L. Poirel and P. Nordmann, "Bacterial activity of Fluoroquinolones against plasmid-mediated Qnr A-producing *Escherichia coli"*, *Clin Microbiol Infect*, 11 no 12 pp. 1048-1049, 2005.

[38] G. A. Jacoby and L. S. Munoz-Price, "The new beta-lactamases", *New England Journal of Medecine*, vol 252, no. 4, pp. 380-391, 2005.

[39] B. Uma, K. Prabhakar, S. Rajendran and S. Y. Lakshmi, "Prevalence of extended spectrum beta lactamases in *Salmonella* species isolated from patients with acute gastroenteritis"*, Indian .J. Gastroenterol*, vol 29, pp. 201–204, 2010.

[40] J. Kiiru, S. Kariuki, B. MGoddeeris, P. Butaye, "Analysis of β-lactamase phenotypes and carriage of selected β-lactamase genes among strains obtained from Kenyan patients during an 18-year period", *BMC Microbiology*, vol 12, no. 155, pp. 302-310, 2012.

[41] L. K. Ng, I. Martin, M. Alfa and M. Mulvey, "Multiplex PCR for the detection of tetracycline resistant genes"*, Molecular and Cellular Probes*, vol 15, pp. 209–215, 2001.

[42] L. Sundström, P. Rädström, G. Swedberg and O. Sköld, "Site-specific recombination promotes linkage between trimethoprim-and sulfonamide resistance genes. Sequence characterization of *dhfrV* and *sulI* and a recombination active locus of Tn21", *Mol. Gen. Genet*, vol 213, pp. 191–201, 1988.

[43] A. Robicsek, J. Strahilevitz, D. F. Sahm, G. A. Jacoby and D. C. Hooper, "*qnr* Prevalence in Ceftazidime-Resistant Enterobacteriaceae Isolates from the United States", *Antimicrobial Agents and Chemotherapy*, vol 50, no. 8, pp. 2872–2874, 2006.