Molecular characterization of multidrug resistant *Salmonella* from chicken and humans in Yaounde

Authors :

Ariane Nzouankeu[1], Marie-Christine Fonkoua[1], Marguerite Wouafo[1], Thomas Njine[2], Awa Aidara-Kane[3], Antoinette Ngandjio[4]

[1]: Pasteur Centre of Cameroon, Laboratory of bacteriology, Yaounde, Cameroon

[2]: University of Yaounde I, laboratory of General Biology, Yaoundé, Cameroon

[3]: World Health Organization, Headquarters, Geneva

[4]: Pasteur Centre of Cameroon, Laboratory of food and water Analysis, Yaounde, Cameroon

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Corresponding author: Antoinette NGANDJIO, Pasteur Centre of Cameroon

P.O BOX 1274 Yaounde, Cameroon

Email: ngandjio@pasteur-yaounde.org

Tel: +237 22 23 18 03

Fax: +237 22 23 15 64

Abstract

Salmonella is one of the most common foodborne pathogens worldwide and chicken has been recognized as its main reservoir for humans. The aim of this study was to investigate the occurrence of antimicrobial resistance genes in *Salmonella* isolates from chicken and humans, and evaluate their genetic relationship. A total of 200 *Salmonella* strains (103 from Chicken and 97 from humans) were collected from 2006 to 2007 in Yaounde, and characterized for their antimicrobial susceptibility to a panel of 16 antimicrobials. Presence of antimicrobial resistance genes, class1 and class 2 integrons was investigated by PCR in Multidrug Resistant (MDR) isolates. Pulsed-Field Gel Electrophoresis (PFGE) analysis was used to investigate their genetic relatedness. Only serotypes Enteritidis, Hadar, Bareilly and II were recovered from both chicken and humans. Overall, 16 isolates (8%) were susceptible to all antimicrobials including third generation cephalosporins and fluoroquinolons. Resistance was mostly observed to tetracycline (57%) in chicken isolates and to sulfonamides (78.3%) in human isolates. 78 (15.3%) strains were MDR. Class 1 integron was predominant in MDR Hadar and Typhimurium isolates. TEM-1 was the unique Extended Spectrum β-lactamase (ESBL) detected in the β-lactam resistant Typhimurium strain isolated from humans. No *qnr* genes were identified in nalidixic acid resistant isolates. PFGE typing of Enteritidis isolates using *Xba*I restriction enzyme showed close genetic relationship between chicken and human isolates. For Hadar and Typhimurirum, variety of restriction patterns was observed. These results highlight the need for continuous surveillance of antimicrobial resistance in *Salmonella* isolates in Cameroon.

Keywords: *Salmonella*, Antimicrobial resistance, integrons, PFGE, Cameroon

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With considerable economic consequences, about 10 million cases and more than hundred thousand deaths reported each year, food borne diseases are a significant public health concern throughout the world (WHO, 2013). Among the major food borne pathogens, *Salmonella*, the causative agent of salmonellosis remains the leading bacteria and it is responsible for health hazards especially in poultry and humans. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, but as an important food source to man, chicken meat has been recognized as the main reservoir of salmonella for humans (Donado-Godoy et al., 2012; Elgroud et al., 2015; M'ikanatha et al., 2010). For decades, Gallinarum and Pullorum were the predominant *Salmonella* serotypes in poultry where they were responsible of real troubles (Kabir, 2010); measures taken by farmers to destroy these serotypes have led to a decrease of these salmonellosis in poultry. However, while incidence of these 2 serotypes was decreasing, others such as Enteritidis, Hadar, and Typhimurium were emerging (Foley et al., 2011). Nevertheless, not all chicken contaminated with *Salmonella* will have a symptomatic salmonellosis and it is therefore the role of asymptomatic chicken carriers of *Salmonella* which explain the importance of this genus in poultry.

As for humans, they get contaminated either by contact with infected animals or by human to human contact, but mainly through consumption of raw or undercooked food contaminated by *Salmonella*. With a high morbidity rate, human salmonellosis is often associated with gastroenteritis which may require the use of antimicrobials, particularly in children, in elderly people and in immuno-compromised patients, where *Salmonella* infection may also be

linked to severe invasive infections and lead to death.

The discovery of antimicrobials in 1929 aroused a lot of interest in human and veterinary medicine, but the extensive use of these drugs as growth promotor in livestock production, as preventive or curative treatment has led to an increase in bacterial multidrug resistant among several bacterial strains. Resistance of *Salmonella* strains to antimicrobials appeared soon after beginning of use of these drugs in treatment of salmonellosis, favoring therapeutic failure and genetic transfer of resistance. Mobile genetic elements such as plasmids, transposons, and integrons which are able to disseminate antibiotic resistance genes among Gram-negative bacteria are the main support of this horizontal or vertical transfer (Brenner, Butaye, Cloeckaert, & Schwarzt, 2006). Integrons which are the most recently explored mobile genetic elements are able to capture gene cassettes from the environment and incorporate them by using site-specific recombination. Nowadays, 3 classes of integrons have been described but the most widely disseminated ones among the members of the family *Enterobacteriaceae* are class 1 integrons (Ploy, Gassama, Chainier, & Denis, 2005).

In Cameroon, legislation about antimicrobials is very weak and these drugs are misused. Moreover, the link between human salmonellosis and consumption of chicken is not known nor is the resistance rate of multidrug resistant salmonella serotypes in both origins. Thus, this study was initiated to determine the genetic factors of resistance in *Salmonella* serotypes from chickens and humans in Yaounde, between 2006 and 2007 and evaluate the genetic relationship among the isolates

1. Methodology

1.1. Bacterial isolates and serotyping

Strains were collected from chickens and from human biological samples from 2006 to 2007. Chickens

were bought from retail markets in Yaounde and the bacterial strains isolated from chicken neck skin as previously described (Wouafo et al., 2010). Human strains were collected from pleural fluid, blood, urine, pus and stools of patients coming to Pasteur Center of Cameroon (PCC) in 2006 for laboratory analysis. All the human samples were analyzed according to the French REMIC recommendations (REMIC, 2007). Strains were biochemically identified with the API 20E Strips (BioMerieux) and serotyped with the somatic O and flagella H *Salmonella* antisera, according to the Kauffman-White-Le Minor scheme (Grimont & Weill, 2007).

1.2. Antimicrobial susceptibility testing

S. Enteritidis, *S.* Hadar and *S.* Typhimurium isolates were screened for their susceptibility to a panel of 16 antimicrobials on Mueller Hinton agar (Bio-Rad Laboratories) by disk diffusion method.

The following disks (Bio-Rad Laboratories) were used: amoxicillin (AMX: 25 µg), ticarcillin (TIC: 75 µg) amoxicillin-clavulanic acid (AMC:20-10 µg), cephalotin (CF: 30µg), cefoxitin (FOX: 50 µg), cefotaxim (CTX: 30 µg), ceftazidim (CAZ: 30µg), gentamicin (GM: 15 µg), amikacin (AN: 30µg), streptomycin (S: 10 µg), tetracycline (TE: 30 µg), chloramphenicol (C: 30 µg), sulphonamides (SSS:200 µg), trimethoprim/sulfamethoxazole (SXT: 1.25/23.75 µg), nalidixic acid (NA: 30 µg), and ciprofloxacin (CIP: 5 µg). The antibiogram susceptibility testing was performed on Mueller Hinton agar (Bio-Rad Laboratories) by disk diffusion method, as described by the Antibiogram Committee of the French Microbiology Society guidelines (CASFM, 2007). *Escherichia coli* ATCC 25922 was used as the quality control strain. The isolates with intermediate resistance were interpreted as resistant. Multidrug resistant (MDR) strains were those

resistant to three or more antimicrobials from different classes.

1.3. Detection of integrons and resistance genes

Prior to detection of integrons and resistance genes, DNA templates of MDR isolates were obtained using the QIAamp DNA Mini kit (Qiagen).The presence of class1 and class 2 integrons was screened among these strains by PCR using respectively 5'CS-3'CS and hep74 - hep51 primers (table 1). Genes encoding resistance to ß-lactams, tetracycline, streptomycin, sulphonamides, trimethoprim and quinolones in MDR strains were amplified by PCR using the primers listed in table 1. The quality control strains for PCR reactions were obtained from the French National Reference Laboratory of *Salmonella* at Institut Pasteur Paris (table1) PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis.

Table 1: Primers sequences used for PCR

Targets	Genes	Primers	Nucleotide sequences	Target size	Quality control strains	References
Class 1 integrons	*Intl1*	INT-3'-CS INT-5'-CS	GGCATCCAAGCAGCAAGC AAGCAGACTTGACCTGAT	/	*S.* Concord 07-670	(Lévesque, Piché, Larose, & Roy, 1995)
Class 2 integrons	*Intl2*	*hep*51 *hep*74	CGGGATCCCGGACGGCATGCACGATTTGTA GATGCCATCGCAAGTACGAG	/	*S. dysenteriae* 1 CAR 10	(White, Mclver, & Rawlinson, 2001)
β-lactams	OXA-1	OXA1bis-F OXA1bis-R	ATGAAAAACACAATACATATC AATTTAGTGTGTTTAGAATGG	890 bp	*S.* Typhimurium 02-8213	(Olesen, Hasman, & Aarestrup, 2004)
	SHV	SHV-INT-F SHV-INT-R	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	800 bp	*S.* Concord 07-670	(Weill et al., 2004)
	TEM	TEM-F TEM-R	ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATCA	1080 bp	*S.* Concord 07-670	(Weill et al., 2004)
streptomycin	*aad A1*	*aad*A1-F *aad*A1-R	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484 bp	*S. dysenteriae* 1 CAR 10	Ahmed , Furuta, Shimomura, Kasama, & Shimamoto, 2006)
	str A	*str*A-F *str*A-R	TTGATGTGGTGTCCCGCAATGC CCAATCGCAGATAGAAGGCAA	383 bp	/	(Iwanaga et al., 2004)
	str B	*str*B-F *str*B-R	GGCACCCATAAGCGTACGCC TGCCGAGCACGGCGACTACC	459 bp	/	(Iwanaga et al., 2004)
tetracyclines	*tet A*	*tet*A-F *tet*A-R	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	950 bp	*S. dysenteriae* 1 CAR 10	Guardabassi, Dijkshoorn, Collard, Olsen, & Dalsgaard , 2000).
sulphonamids	*sul 1*	*sul*1-F *sul*1-R	CTTCGATGAGAGCCGGCGGC GCAAGGCGGAAACCCGCGCC	285 bp	*S.* Typhimurium 02-8213	Kern, Klemmensen, Frimodt-Moller, & Espersen, 2002).
	sul 2	*sul*2-F	AGGGGGCAGATGTGATCGAC	625 bp	/	(Iwanaga et al., 2004)
		*sul*2-R	TGTGCGGATGAAGTCAGCTCC			
trimethoprim	*dhfr A1*	*dhfr* A1-F *dhfr*A1-R	GTCAAACTATCACTAATGGTA TTAACCCTTTTGCCAGTATTG	474 bp	/	(Turner, Luck, Sakellaris, Rajakumar, & Adler, 2003)
quinolones	*qnrA*	*qnr*A-F *qnr*A-R	TTCTCACGCCAGGATTTGAG TGCCAGGCACAGATCTTGAC	571pb	*S.* Concord 05-5343	(Jacoby, Chow, & Waites, 2003)
	*qnr*B	*qnr*B-F *qnrB*-R	TGGCGAAAAAATT(GA)ACAGAA GAGCAACGAGCCTGGTAG	594pb	S. Havana 07-319	(Jacoby et al., 2003)
	*qnr*S	*qnrS*-F *qnrS*-F	GACGTGCTAACTTGCGTGAT AACACCTCGACTTAAGTCTGA	388pb	*E. cloacae* AME	(Jacoby et al., 2003)

1.4. Pulsed-field gel electrophoresis (PFGE) analysis

Clonal relationship among *S.* Enteritidis, *S.* Hadar and *S.* Typhimurium isolates of human and chicken origin was detected by PFGE using PulseNet protocol (http://www.pulsenetinternational.org/protocols, 2009). Strains were selected on the basis of their resistance profiles, from wildtype to MDR. Briefly, 200 µl of overnight culture cells were lysed, and intact genomic DNA was digested in agarose-embedded plugs with *Xba*I restriction enzyme (Roche, Mannheim, Germany). The restricted plug slices in 1% Seakem Gold agarose (Lonza, Rockland ME, USA) gel were separated by electrophoresis using 0.5X TBE buffer for 19 h at 14°C in a CHEF DR-III (Bio-Rad). Migration conditions were as follows: initial switch time, 2 s; final switch time, 63.8 s at an angle of 120° at 6 V/cm. *Salmonella enterica* serotype Braenderup H9812 was used as molecular reference strain as described by the PulseNet protocol. After electrophoresis, PFGE gels were stained in ethidium bromide, destained in distilled water, and photographed under UV radiations. Images saved in TIFF format were transferred to BioNumerics 5.1 software (Applied Maths, Sint-Martens-Latem, Belgium) for computer analysis of restriction fragments. Clusters analysis was performed using Dice coefficient for band matching with a 1.0% position tolerance; the hierarchic unweighted pair group method with averaging algorithm was used to generate dendrograms describing the relationship among *S.* Enteritidis, *S.* Hadar and *S.* Typhimurium isolates restriction patterns.

2. Results

2.1. Antimicrobial resistance patterns

A panel of 200 *Salmonella* was recovered from our samples; A hundred and three (103) strains were recovered from chickens bought in retail markets in Yaounde as earlier described (Wouafo et

al., 2010). Ninety seven (97) isolates were recovered from patients coming for medical analysis in PCC. 1 isolate was from pus, 2 (2.1%) from pleural fluid, 6 (6.2%) from urine, 14 (14.4%) from blood and 74 (76.3%) from stools. 195 isolates belonged to *Salmonella enterica* subspecies *enterica* (I), 4 isolates to subspecies *salamae* (II) and 1 isolate to subspecies *houtenae* (IV). Serotype prevalence and distribution in chicken and humans are reported in table I. A total of 47 different serotypes were identified among *S. enterica* isolates; the top five included the following serotypes: Enteritidis (36%), Typhimurium (16%), Hadar (15.5%), Tilburg (2%) and Bareilly (2%). Only 4 serotypes were recovered from both chicken and humans, namely Enteritidis, Hadar, Bareilly and II (Table 2).

Table 2: *Salmonella* serotypes in chicken and humans

Serotype	Total		Human		Chicken	
	Number	%	Number	(%)	Number	(%)
Total	200	100,00%	97	100,00%	103	100,00%
Enteritidis	72	36,00%	25	25,80%	47	45,60%
Typhimurium	32	16,00%	32	33,00%		
Hadar	31	15,50%	2	2,10%	29	28,20%
Tilburg	4	2,00%			4	3,90%
Bareilly	4	2,00%	1	1,00%	3	2,90%
undetermined	4	2,00%	1	1,00%	3	2,90%
II	4	2,00%	3	3,10%	1	1,00%
Mikawasima	3	1,50%			3	2,90%
Agona	2	1,00%	2	2,10%		
Essen	2	1,00%	2	2,10%		
Kibusi	2	1,00%	2	2,10%		
Larochelle	2	1,00%	2	2,10%		
Limete	2	1,00%	2	2,10%		
Stanleyville	2	1,00%	2	2,10%		
Muenchen	1	0,50%	1	1,00%	1	1,00%
Cleveland	1	0,50%			1	1,00%
Colindale	1	0,50%			1	1,00%
Duesseldorf	1	0,50%			1	1,00%
Eko	1	0,50%			1	1,00%
Gwosa	1	0,50%			1	1,00%
Harburg	1	0,50%			1	1,00%
Hato	1	0,50%			1	1,00%
Hiddudiffy	1	0,50%			1	1,00%
Liverpool	1	0,50%			1	1,00%
Manhattan	1	0,50%			1	1,00%
Reading	1	0,50%			1	1,00%
Saintpaul	1	0,50%			1	1,00%
Adjame	1	0,50%	1	1,00%		
Bellevue	1	0,50%	1	1,00%		
Brive	1	0,50%	1	1,00%		
Chester	1	0,50%	1	1,00%		
Dublin	1	0,50%	1	1,00%		
Durham	1	0,50%	1	1,00%		
Eboko	1	0,50%	1	1,00%		
Eppendorf	1	0,50%	1	1,00%		
Eschberg	1	0,50%	1	1,00%		
Georgia	1	0,50%	1	1,00%		
Havana	1	0,50%	1	1,00%		
IV	1	0,50%	1	1,00%		
Koessen	1	0,50%	1	1,00%		
Onireke	1	0,50%	1	1,00%		
Sanjuan	1	0,50%	1	1,00%		
Schleisschem	1	0,50%	1	1,00%		
Sipane	1	0,50%	1	1,00%		
Strasbourg	1	0,50%	1	1,00%		
Tanzania	1	0,50%	1	1,00%		
Zanzibar	1	0,50%	1	1,00%		

Drug susceptibility assay revealed that only 8% (16/200) of all investigated isolates were fully susceptible to all 16 antimicrobials tested, that is 4.9% (5/103) from chicken and 11.3% (11/97) from humans. No resistance to cephalotin, cefoxitin, cefotaxime, ceftazidime, gentamicin, amikacin and ciprofloxacin was observed. Chicken strains were mainly resistant to tetracyline, streptomycine and nalidixic acid, whereas human strains were resistant to amoxicillin, chloramphenicol and cotrimoxazole (figure 1).

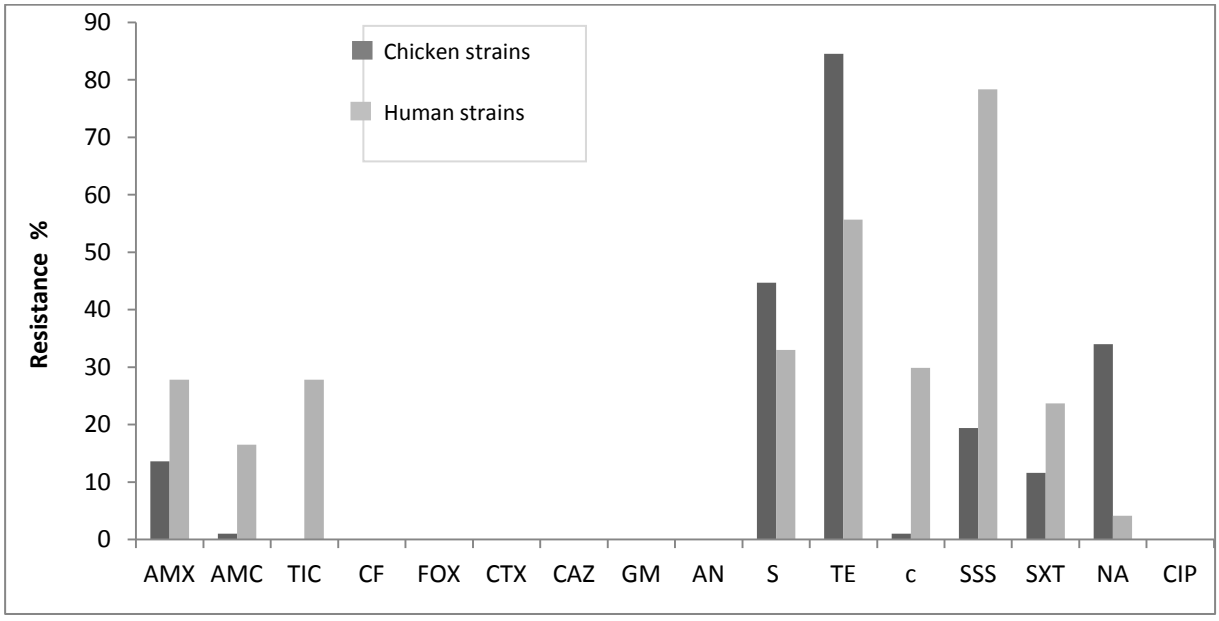

Figure 1: Antimicrobial resistance profile of *Salmonella* isolates

Fifty two isolates (26%) exhibited resistance to at least one antimicrobial, 51 (25.5%) to at least two antimicrobials, while 97 (48.5 %) were resistant to three or more tested drugs. MDR isolates were identified mostly in serotypes Typhimurium (20/38) for human strains and Hadar (28/40) for chicken strains (table 3).

Resistance patterns	Total number of strains	source	Number of strains (%)	Serotypes
Wild type	**16**	**Chicken**	**11(10.37%)**	Enteritidis (9), Muenster (1), Reading (1)
		human	**5 (5.15)**	Typhimurium
Resistance to 1 antimicrobial	**57**	**Chicken**	**35 (34)**	
		human	**22 (22.7)**	
AMX	1	Chicken	1	Enteritidis
NA	1	Chicken	1	Enteritidis
S	1	Chicken	1	sp
TE	36	Chicken	32	Enteritidis (20), Tilburg (3), Bareilly (1), Mikawasima (1), Duesseldorf (1), Gwoza (1), Harburg (1), Hato (1), Hiduddify (1), sp (2)
		human	4	Typhimurium (2), Enteritidis (1), Essen (1)
C	3	human	3	Enteritidis (3), Eboko (1)
SSS	13	human	13	Typhimurium (2), Enteritidis (9), Chester (1), Eppendorf (1)
SXT	1	human	1	Enteritidis
AMX-AMC-TIC	1	human	1	Typhimurium
Resistance to 2 antimicrobials	**49**	**human**	**32 (33)**	
		Chicken	**17 (16.5)**	
AMX-TE	6	Chicken	6	Enteritidis (3), Hadar (1), Cleveland (1), Eko (1)
NA-TE	2	Chicken	2	Enteritidis
S-TE	8	human	2	Brive (1), Strasbourg (1)
		Chicken	6	Bareilly (2), Mikawasima (2), Saintpaul (1), II (1)
TE-SSS	22	human	21	Typhimurium (3), Enteritidis (3), Agona (1), Essen (1), kibusi (2), Limete (1), Stanleyville (2), Adjame (1), Durham (1), Georgia (1), Schleisschem (1), Tanzania (1), Zanzibar(1), IV (1), sp (1)
		Chicken	1	Enteritidis
S-SSS	2	human	1	Koessen
		Chicken	1	Enteritidis
AMX-AMC-S	1	Chicken	1	Enteritidis
S-NA	1	human	1	Hadar
C-SSS	4	human	4	Typhimurium (2), Enteritidis (1), Muenchen (1)
AMX-TIC-TE	2	human	2	Enteritidis
AMX-AMC-TIC-SSS	1	human	1	Typhimurium
MDR	**78**	**human**	**38**	
		Chicken	**40**	
AMX-S-TE-SSS	1	Chicken	1	Enteritidis
AMX-S-TE-SSS-SXT	1	Chicken	1	Enteritidis
AMX-S-TE-NA	2	Chicken	2	Hadar
S-TE- SSS-SXT-NA	10	Chicken	10	Enteritidis (1), Hadar (9)
S-TE-SSS-NA	2	Chicken	1	Hadar
		Human	1	Enteritidis
AMX-TE-SSS	1	Chicken	1	Enteritidis
S-TE-C	1	Chicken	1	Tilburg
S-TE-NA	19	human	1	Hadar
		Chicken	18	Enteritidis (1), Hadar (16), Manhattan (1)
S-TE-SSS	7	human	5	Typhimurium (1), Bareilly (1), Bellevue (1), II (1), Onireke (1)
		Chicken	2	Enteritidis (1), Colindale (1)
AMX-S-TE	1	Chicken	1	Liverpool
TE-SSS-SXT	1	Chicken	1	Enteritidis
TE-SSS-NA	2	human	1	Typhimurium
		Chicken	1	Enteritidis
TE-C-SSS	5	human	5	Agona(1), Larochelle (1), II (1), Limete (1), Dublin (1)
S-TE-C-SSS	2	human	2	Typhimurium (1), II(1)
AMX-AMC-TIC-TE-C	1	human	1	Typhimurium
AMX-TIC-S-C-SXT	1	human	1	Typhimurium
AMX-TIC-S-SSS-SXT	1	human	1	Typhimurium
AMX-TIC-TE-SSS-SXT	1	human	1	Sanjuan
AMX-AMC-TIC-C-SSS-SXT	1	human	1	Enteritidis

AMX-AMC-TIC-S-SSS-SXT	5	human	5	Typhimurium (4), Enteritidis (1)
AMX-TIC-S-C-SSS-SXT	1	human	1	Typhimurium
AMX-TIC-S-TE-SSS-SXT	5	human	5	Typhimurium (2), Eschberg (1), Havana (1), Sipane (1)
AMX-AMC-TIC-S-C-SSS-SXT	3	human	3	Typhimurium
AMX-AMC-TIC-S-TE-SSS-SXT	2	human	2	Typhimurium
AMX-AMC-TIC-S-TE-C-SSS-SXT	2	human	2	Typhimurium
TOTAL	200	human	97	
		Chicken	103	

Table 3: Resistance profiles of *Salmonella* isolates

2.2. Distribution of integrons and resistance genes in MDR isolates

Twenty one (26.9%) of the multidrug-resistant *Salmonella* isolates were found to carry class 1 integrons, and 2 (2.56%) to carry class 2 integrons. Among these isolates, only 1 carried a class 1 integron and a class 2 integron simultaneously. Results indicated that integron-borne multidrug resistance was solely associated with serotype Hadar in chicken strains, of which 10 strains carrying class 1 integron and two carrying class 2 integrons. In human isolates, class 1 integron was detected in 7 different *Salmonella* serotypes, namely Agona, Bareilly, Dublin, Havana, Larochelle, Limete and Typhimurium (5).

Presence of known resistance genes was studied among MDR strains. We identified 9 different antimicrobial resistance genes conferring resistance to 6 classes of antimicrobials. Occurrence of *sul*1 and *sul*2 gene was investigated in all the MDR isolates and these genes were respectively detected in 11 isolates. Only 4 of the integrons positive isolates contain simultaneously *sul*1 gene.

Among the 30 MDR β-lactam resistant isolates, neither *bla*OXA nor *bla*SHV gene was recovered; and only 9 strains originating from humans showed positive amplications, only for *bla*TEM gene (table 4). Sequencing revealed a 100% identity to the sequence of *bla*TEM1. Five (5) human strains out of the 17 chloramphenicol-resistant MDR isolates contained the *catA1* gene. Among all 66 MDR strains exhibiting resistance to streptomycin, 26 harbored *str*A gene, 34 harbored *str*B and only 6 *aad*A1gene. *str*A and *str*B were found together in 19 strains and the 3 genes were detected simultaneously in 3 isolates. 26 out of the 66 tetracycline-resistant strains tested PCR positive for the *tet*A gene.

Table 4: Distribution of integrons and resistance genes in multidrug resistant *Salmonella* isolates

Resistance patterns	Serotypes	Number of isolates	Class 1 integron	Class 2 integron	*sul1*	*sul 2*	*TEM*	*OXA 1*	*SHV*	*catA1*	*str A*	*str B*	*aadA1*	*dhfr A1*	*tetA*	*qnr A*	*qnr B*	*qnr S*
Chicken isolates																		
AMX-S-TE-SSS	Enteritidis	1	0	0	0	0	0	0	0		1	1	0	1	1			
AMX-S-TE-SSS-SXT	Enteritidis	1	0	0	0	0	0	0	0		0	0	0	2	0			
AMX-S-TE-NA	Hadar	2	0	1			0	0	0		1	2	0		2	0	0	0
S-TE-SSS-SXT-NA	Hadar	9	5	1	0	4					2	6	1	1	9	0	0	0
	Enteritidis	1	0		0	1					1	1	0	0	1	0	0	0
S-TE-SSS-NA	Hadar	1	0	0	0	0					1	1	1	0	1	0	0	0
AMX-TE-SSS	Enteritidis	1	0	0	0	0	0	0	0					0	0			
S-TE-C	Tilburg	1	0	0						0	0	0	0		0			
S-TE-NA	Hadar	16	5	0	0	0					8	13	1		7	0	0	0
	Enteritidis	1	0	0							1	1	1		1	0	0	0
	Manhatthan	1	0	0							1	0	0		0	0	0	0
S-TE-SSS	Enteritidis	1	0	0	0	0					1	1	0	0	1			
	Colindale	1	0	0	0	0					0	0	0	1	0			
AMX-S-TE	Liverpool	1	0	0			0	0	0		1	1			0			
TE-SSS-SXT	Enteritidis	1	0	0	0	0								1	0			
TE-SSS-NA	Enteritidis	1	0	0	0	0								0	0	0	0	0
Total		**40**	**10**	**2**	**0**	**5**	**0**	**0**	**0**	**0**	**18**	**27**	**4**	**6**	**23**	**0**	**0**	**0**
Human isolates																		
S-TE-SSS	Onireke	1	0	0	0	0					0	0	0	0	0			
	Bareilly	1	1	0	0	0					0	0	0	0	0			
	Bellevue	1	0	0	0	0					0	1	0	0	0			
	II	1	0	0	0	0					0	0	0	0	0			
	Typhimurium	1	0	0	0	0					0	1	0	0	0			
S-TE-NA	Hadar	1	0	0	0	0					0	0	0		0	0	0	0
TE-C-SSS	Agona	1	1	0	1	0				1				0	0			
	Larochelle	1	1	0	0	1				0				0	0			
	Dublin	1	1	0	0	1				1				0	0			
	Limete	1	1	0	0	0				0				0	0			
	II	1	0	0	0	0				0				0	1			
TE-SSS-NA	Typhimurium	1	0	0	0	0									1	0	0	0
S-TE-C-SSS	Typhimurium	1	0	0	0	0				0	0	0	1	0	1			
	II	1	0	0	0	0				0	0	0	0	0	0			
S-TE-SSS-NA	Enteritidis	1	0	0	0	1					0	0	0		0	0	0	0
AMX-AMC-TIC-TE-C	Typhimurium	1	1	0	0	0	1	0	0	1					0			
AMX-TIC-S-C-SXT	Typhimurium	1	0	0	1	0	0	0	0	1	1	1	0	0				
AMX-TIC-S-SSS-SXT	Typhimurium	1	0	0	0	0	1	0	0		0	1	0	0				
AMX-TIC-TE-SSS-SXT	Sanjuan	1	0	0	1	0	1	0	0					1	0			

AMX-AMC-TIC-C-SSS-SXT	Enteritidis	1	0	0	1	0	0	0	0	0				0				
AMX-AMC-TIC-S-SSS-SXT	Typhimurium	4	0	0	1	0	0	0	0		1	0	0	0				
	Enteritidis	1	0	0	0	1	0	0	0		0	0	0	1				
AMX-TIC-S-C-SSS-SXT	Typhimurium	1	1	0	1	0	1	0	0	0	0	0	0	0				
AMX-TIC-S-TE-SSS-SXT	Eschberg	1	0	0	0	0	1	0	0		1	1	0	0	0			
	Typhimurium	2	0	0	1	1	1	0	0		1	1	0	0	0			
	Sipane	1	0	0	0	0	0	0	0		0	0	0	1	0			
	Havana	1	1	0	1	0	0	0	0		0	0	0	0	0			
AMX-AMC-TIC-S-C-SSS-SXT	Typhimurium	3	1	0	2	0	2	0	0	1	1	0	0	1	0			
AMX-AMC-TIC-S-TE-SSS-SXT	Typhimurium	2	1	0	1	0	0	0	0		1	0	1	1	0			
AMX-AMC-TIC-S-TE-C-SSS-SXT	Typhimurium	2	1	0	0	1	1	0	0	0	2	1	0	0	0			
Total		**38**	**11**	**0**	**11**	**6**	**9**	**0**	**0**	**5**	**8**	**7**	**2**	**5**	**3**	**0**	**0**	**0**

2.3. PFGE restriction patterns

Salmonella Enteritidis

Restriction of 18 *S.* Enteritidis strains (7 from human and 11 from chicken) with *Xba*I enzyme yielded 11 different patterns consisting of 12–15 fragments. The genetic relatedness of these PFGE profiles, as demonstrated by the dendrogram, showed two main clusters (A and B) with 52.8% similarities (figure 2). Most patterns were found in cluster A which was subdivided in many groups. Patterns X1 and X2 shared by both chicken and human strains were predominant, accounting each for 4 of the 18 strains. X3, X4 and X5 were closely related to X1 and X2 with more than 90% similarities. Cluster B was represented by only one human's strain with less than 60% similarities with others.

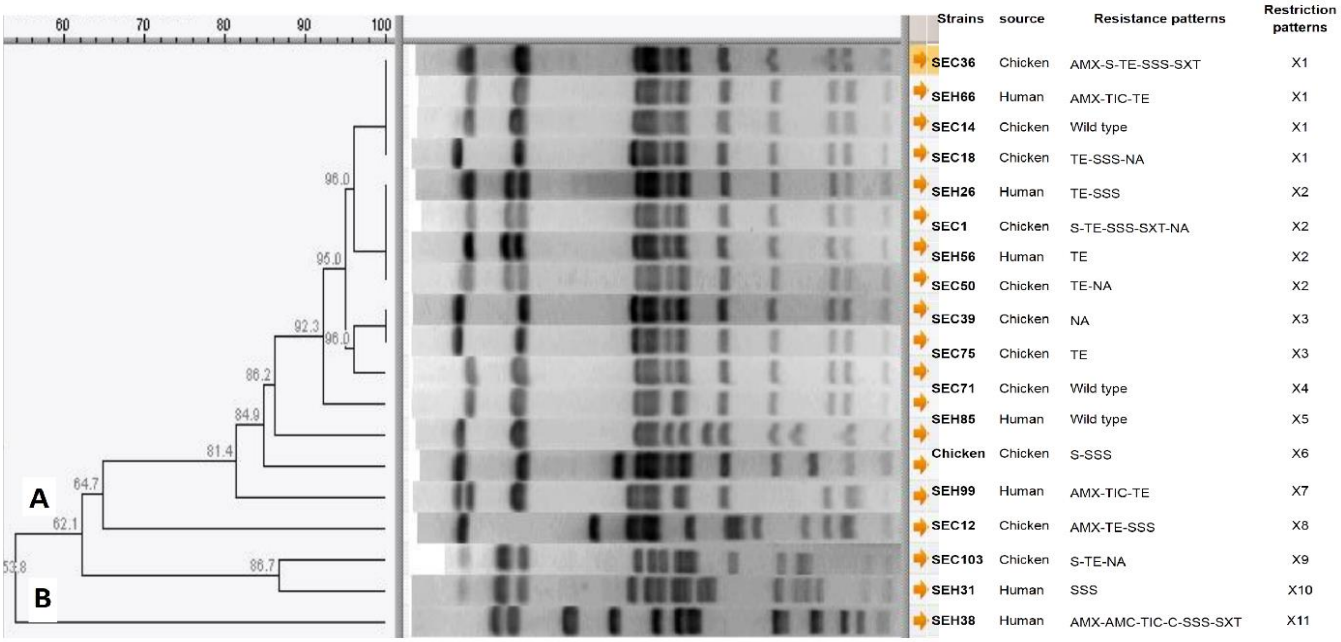

Figure 2: PFGE restriction patterns of *S.* Enteritidis isolates

Salmonella Hadar

The fourteen Hadar isolates (12 from chicken and 2 from humans) selected for PFGE analysis exhibited 4 different resistance Patterns. The similarity dendrogram generated for these isolates yielded two clusters (A and B) with more than 80% similarities accounting both for

11 restrictions patterns (Figure 3). Cluster A was exclusively made up of 4 strains isolated from chicken, sharing the same resistance patterns, but 4 distinct restriction patterns (X1, X2, X3, and X4) were with 82.7% similarities. On the other hand, cluster B gathered the 2 strains isolated from human which yielded 2 different restriction patterns (X5 and X9: 88.8% similarities), and 8 strains from chicken. Among the 8 chicken isolates found in cluster B, 3 shared restriction pattern X7 and 2 restriction pattern X8.

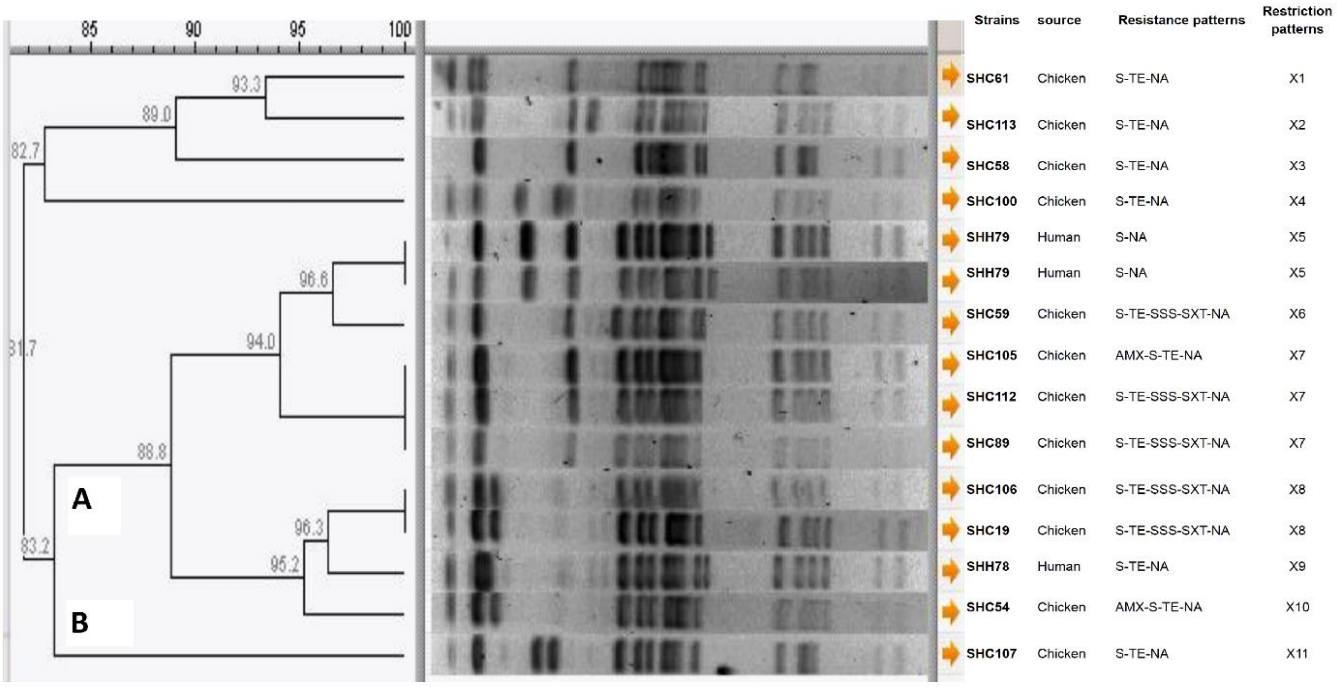

Figure 3: PFGE restriction patterns of *S.* Hadar isolates

Salmonella Typhimurium

The seventeen *S.* Typhimurium isolates exhibiting 15 different resistance patterns yielded 13 distinct restriction patterns on the similarity dendogram built after PFGE analysis (Figure 4). The two main clusters (A and B) on this dendrogram shared more than 75% similarities. Only strains with restriction patterns X1 and X2 (80% similarities) belonged to cluster A, whereas cluster B is mainly made up of 3 clones sharing above 87% similarities and belonging to patterns X5, X10 and X11.

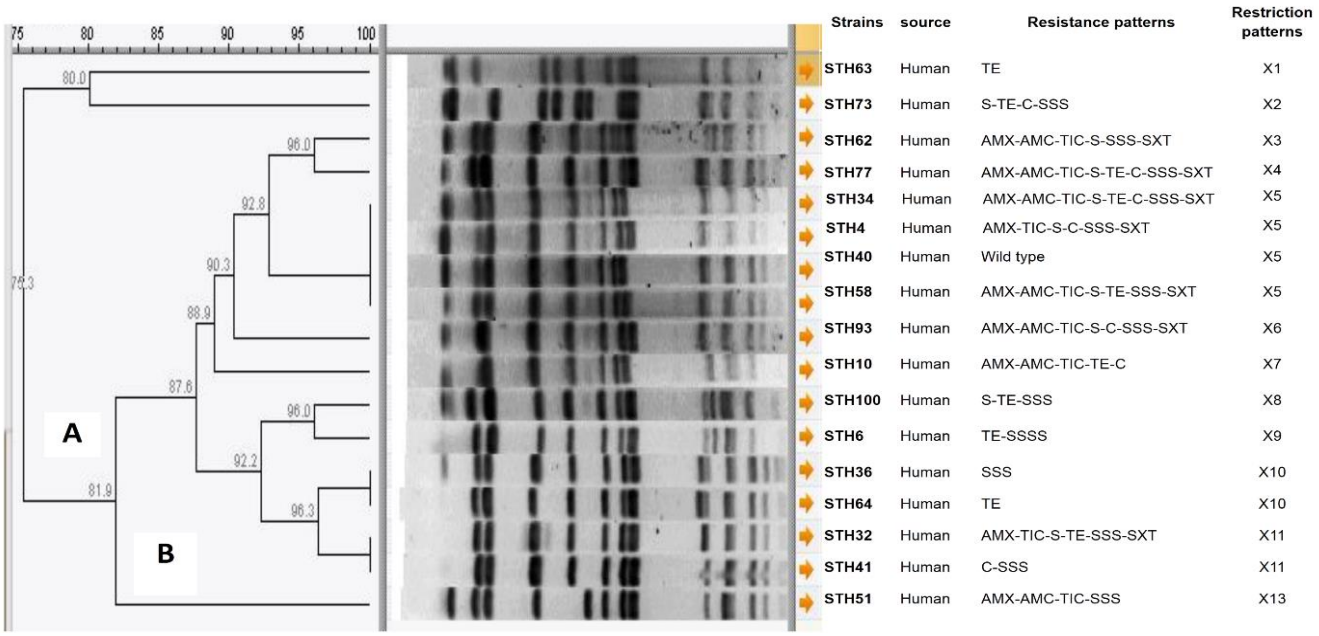

Figure 4: PFGE restriction patterns of *S.* Typhimurium isolates

3. Discussion

Chicken is recognized as a common reservoir of *Salmonella* for humans and the detection of this bacterium in poultry is of great concern worldwide (Aarestrup et al., 1998; Angulo & Swerdlow, 1998; Baümler, Hargis, & Tsolis, 2000). In this study, only 4 *Salmonella* serotypes were common to chicken and humans, namely Enteritidis, Hadar, Bareilly and serotype II (*S. salamae*), suggesting a possible transmission of these bacterium to human through consumption of poultry products. Others Salmonella serotypes isolated in humans could therefore originate either from consumption of other food sources or from the environment. It may be the case of S. Dublin, a serotype normally found only in cattle, which was isolated in humans (Grimont & Weill, 2007). Typhimurium and Enteritidis were the most prevalent *Salmonella* serotype in humans whereas in chicken Enteritidis and Hadar were predominant. The high frequency of these serotypes in humans was observed by Moperth (2009) who concluded that these 2 serotypes were the most prevalent in humans in Africa. On the other hand, the low occurrence of Hadar isolates in human is not surprising,

as it was noticed years ago by Cruchaga et al. that this serotype was becoming poultry-related serotype (Cruchaga et al., 2001). Moreover, since 2006 Hadar isolates are responsible for only 1% of human salmonellosis throughout the world (Zhao et al., 2006). Wildtype strains of *Salmonella* are susceptible to all antimicrobials active on *Enterobacteriaceae.* In this study, only 4.9% (5/103) of isolates from chicken and 11.3% (11/97) of human isolates remained wild type. Globally, resistance patterns of chicken strains were different from those of humans. Strains from chicken were mostly resistant to tetracycline, the antimicrobial commonly used in Cameroon as growth promoter in farms, while in human strains resistance was mostly observed to amoxicillin, chloramphenicol, and cotrimoxazale, antimicrobials used as first line drugs to fight against many other diseases in Cameroon and sold in market's streets. This highlights a potential risk of therapeutic failure in human. These results are similar to observations made earlier in sub-Saharan Africa (Morpeth, Ramadhani, & Crump, 2009).

62.5% of Enteritidis strains in this study were resistant to only 1 antimicrobial. This finding is in agreement with previous reports that have described *S.* Enteritidis to exhibit a lower frequency of multiple resistances to antimicrobials compare to other *Salmonella* serotypes. In this study, 39% of MDR strains (78/200) were obtained mostly among Hadar (28) and Typhimurium (20) isolates; as described by Wouafo *et al.* (2010). The main resistance pattern in Hadar isolates has been identified elsewhere in Africa. In Typhimurium, 3 resistance patterns were prevalent, but our concern is the isolation of 2 strains exhibiting resistance pattern similar to that of lysotype DT 104 of S. Typhimurium, with resistance to 5 classes of antimicrobials namely amoxicillin,-chloramphenicol-Streptomycine, sulphonamides and tetracycline (ACSSuTe). Since lysotyping was not

performed in this study, we were unable to confirm the belonging of these isolates to this DT 104 clone.

Resistance to nalidixic acid in this study was mainly associated to chicken isolates suggesting that although this antimicrobial is not recommended in poultry in Cameroon, it is unfortunately unduly used. Consequently, resistance observed in chicken strains may be due to its informal use. Though all strains were still susceptible to ciprofloxacin, this should be monitored because cross reaction between nalidixic acid and ciprofloxacin resistances have previously been described (Ruiz et al., 2000; Walker et al., 2000); and in such situations an impaired response to ciprofloxacin treatment in severe salmonellosis is predictive. These results suggested plasmid mediated resistance to quinolones, which was investigated in this work by looking at the presence of *qnr*A, *qnr*B and *qnr*S genes. These genes were not detected, but they have already been described in avian and human strains from many African countries such as South Africa, Nigeria and Senegal (Fortini, Fashae, Garcia-Fernandez, Villa, & Carattoli, 2010; Garnier, Raked, Gassama, Denis, & Ploy, 2006; Govender, Smith, Karstaedt, & Keddy, 2009); meaning that in Cameroon, resistance of *Salmonella* isolates is either due to chromosomal mutations in topoisomerases (DNA gyrase (gyrA / gyrB) and topoisomerase IV (parC / parE) or to active efflux pomp. Further investigations need to be performed to detect these mutations.

Because they carry up to 10 genes encoding resistance to antimicrobials, class 1 and class 2 integrons are usually called resistance integrons (An, Duijkeren, Fluit, & Gaastra, 2006; Gassama et al., 2006). In this study, class 1 integron were detected exclusively in Hadar isolates in chicken and mostly in Typhimurirum isolates in humans; whereas class 2 integron was found only in 2 Hadar strains from chicken; According to Okamoto *et al.* (2009), strains carrying integrons usually exhibit resistance to a large panel

of antimicrobials. This may explained the detection of these integrons in the above mentioned MDR serotypes. The low occurrence of class 2 integron in this study is in agreement with the findings of Fluit (2005) and Antunes *et al.* (2009) which mention that class 1 integron is the most common integron in *Salmonella* isolates. Knowing that integrons play an important role in the dissemination of antimicrobial resistance, these results should be of great concern.

Resistance to sulphonamides is usually due to *sul*1, *sul*2, *sul*3 genes. S*ul*1 gene has been described as part of the 3' conserved region of this class I integron (Ploy et al., 2005), but it has not been found in any of the Hadar isolates carrying this integron. A high frequency of these atypical class 1 integron was previously described in United Kingdom (Byrne-Bailey et al., 2009), in Portugal (Antunes et al., 2009) and in United States of America (Aarestrup et al., 2003).

*aadA*1 and *dhfrA*1 genes have also been described as usually being part of gene cassettes carried by class 1 and class 2 integrons. The presence of these genes was investigated using *S.* Typhimurium 02-8213 as positive control strain and they were all detected in some strains despite the absence of integrons suggesting that their presence could be associated to another genetic element.

It was interesting to note that some strains harbored simultaneously 2 or 3 resistance genes conferring resistance to the same antimicrobial and located or not on the same resistance genetic support . It was the case of resistance to streptomycin which was encoded by *str*A and *str*B genes for 19 isolates and which are usually located either on Tn5393 or on a plasmid (Brenner et al., 2006).

S. Typhimurium 02-8213 and *S.* Concord 07-670 were respectively used as positive control strains for search of *bla*OXA, *bla*TEM and *bla*SHV in MDR human isolates exhibiting resistance to β-lactams antimicrobials. Only Tem1 gene was harbored by some of these isolates. Similar findings have been reported

worldwide with TEM-1 gene being recognized among the most common Extended Spectrum β-lactamases in clinical isolates (Phillipa, Jesudason, Thomson, & Amyes, 1998). The presence of this gene in human isolates is worrisome, because it is usually carried by a plasmid and can therefore be transferred from a bacterium, thus causing hospital acquired infections (Carattoli et al., 2002); moreover, four TEM-1 positive isolates were also positive for class1 integron, suggesting those strains possess at least 2 mobile genetic element involved in transfer of antimicrobial resistance.

Mainly 5 main genes (*tet*A, *tet*B, *tet*C, *tet*D, *tet*G) coding for a membrane-associated efflux pump, have been reported to be involved in resistance of *Salmonella* to tetracycline. However,

*tet*A, usually located on Tn*1721*transposon has been widely described (Brenner et al., 2006; Pezzella, Ricci, DiGiannatale, Luzzi, & Carattoli, 2004). That is why its presence was checked in this study. It was identified in 57.5% of MDR chicken isolates and 11.5 % of MDR human isolates suggesting once again the presence of a mobile genetic element in *Salmonella* isolates in this study.

The genetic relationship between strains was investigated by PFGE, the method recognized as the gold standard for genotyping bacterial food-borne pathogens and source tracking in outbreak investigations; therefore, it has been widely recommended for differentiation of *Salmonella*. In this study, PFGE dendrogram of the 18 Enteriridis analyzed yielded 5 restriction patterns (X1 to X5: 100% - > 90% similarities) shared by human and chicken isolates . Isolates with 100% similarities should be considered as indistinguishable and those between 99-90% are probably related (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009), thus, indicating a close genetic relationship between some chicken and human Enteritidis isolates. This suggests a cross-contamination between chickens and humans. It will be useful to confirm

relationship among Enteritidis isolates by screening more strains from chicken and humans. No correlation was established between antimicrobial resistance pattern and restriction pattern for Hadar and Typhimurium isolates; consequently, heterogeneity of restriction patterns was observed for these isolates. None of the patterns obtained in this study has been detected before in PulseNet Africa data base.

Conclusion

This study was intended to compare AMR profiles and genetic relationship between *Salmonella* isolates collected from chickens and from humans. The data obtained reveals that chicken and human shared few salmonella serotypes and as such resistance patterns of chicken strains were different from those of humans. Antimicrobial resistance of *Salmonella* isolates in Yaounde is encoded by a variety of genes previously widespread in *Enterobacteriaceae* and carried mainly on mobile genetic elements. PFGE analysis showed a variety of restriction patterns for Hadar and Typhimurium isolates, but a close relationship was established between chicken and human Enteritidis strains. Consequently, continuous surveillance of antimicrobial resistance in *Salmonella* isolates needs to be implemented in Cameroon to rapidly detect emergence of multidrug resistant isolates.

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