

# Contamination of chicken gizzards by *Salmonella* sp.: Impact on consumer health in Abidjan, Côte d'Ivoire

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## 1 ABSTRACT

Côte d'Ivoire is contributing to the globally flight in poultry farming, thus responding to the strong demand of the local populations, particularly the population of the District of Abidjan. Consumed by all in the Abidjan society, the chicken is obtained in slaughtered form  $(39.03 \pm 23.34^{\circ})$  or live  $(29.38 \pm 21.3^{\circ})$ . However, they contain in their gizzards, strains of Salmonella sp. The microbiological analysis of 66 batches of raw gizzards taken from slaughter sites in 11 communes of the District of Abidjan (Côte d'Ivoire) shows a portage rate of 57 %. The risks to the consumer are increased by: (i) unhygienic methods of slaughtering and eviscerating chicken; (Ii) the presence of multidrug-resistant *Salmonella* serotypes (Agona, Derby, Hadar, Budapest, Riuru, Essen, Kentucky, Chester) to the antibiotics tested. The Ivorian authorities will have to put in place sanitary control and surveillance systems for antibiotics to raise awareness among operators in the aviculture sector.

## 2 INTRODUCTION

Poisoning from food infections is one of the major causes of death worldwide. According to the World Health Organization, they record 2 million annual deaths caused by the diarrhea. (Anonymous 1, 2006). Among the causes of diarrhea, salmonellosis occupies this an important place. Indeed, according to an estimate made in 2010, salmonellae were involved in more than 80 million cases of foodborne gastroenteritis every year worldwide, of which 155,000 were fatal. The origin of the food of these pathologies was suspected for 80.3 million cases (Majowicz et al., 2010). Thus, due to its impact on the health and productivity of animals, salmonellosis is a concern for the veterinary and medical fields (Elgroud, 2009). Controlling the contamination of poultry meat by Salmonella becomes an essential prerequisite

for the consumer and an economic argument for the industrialists (Carlier & Lagrange, 2001). humans, salmonellosis causes variable In symptoms in severity, ranging from mild abdominal pain to varying degrees of enteritis, to septicemia and even death in extreme cases (Anonymous 2, 2002). Salmonella enterica, by its ubiquitous serotypes, is the main pathogen of contamination of food products for human consumption (Fablet et al., 2003). Salmonella infection is also very commonly associated with the consumption of meat and meat products, especially poultry. Indeed, poultry plays a predominant role as a vector of transmission in the human cases of salmonellosis, involving hazards including many bacterial Campylobacter, Salmonella enterica and Listeria (Fosse, 2004). Consumption of poultry meat is

growing considerably on all continents with an increase in volumes marketed worldwide, by 10% per year (Prin *et al.*, 2001). In Côte d'Ivoire, poultry constitutes also an important source of animal protein for the Ivorian population. Indeed, its consumption increased from 0.39 to 1.05 Kg / inhabitant / year of the period from 2004 to 2011 (Anonymous 3, 2012). Given the level of consumption of poultry meat in Côte d'Ivoire and its role as a reservoir potential of Salmonella strains, the necessity of health surveillance to ensure origin avian food safety is primordial. The general

# 3 MATERIAL AND METHODS

3.1 Poultry consumption survey in the District of Abidjan: In order to assess the risks linked with the consumption and handling of poultry, a survey was carried out using a questionnaire in the 13 communes comprising the District of Abidjan (Côte d'Ivoire), based on the existence of one or more slaughter sites. The communes of Abobo, Adjamé, Anyama, Attécoubé, Bingerville, Cocody, Koumassi, Port-Bouët, Treichville Marcory, and Yopougon were visited. This questionnaire had allowed us to collect information on the mode of consumption of chickens and to identify the likely origins of public health risks, due to the handling of poultry.

3.2. Samples collected: A probability method unistrastrified to sampling the municipality was adopted as part of this study (Le Maux, 2012). A probability Sampling method unistrastified in this study is sampling technique for a stratum (pulling by pooling).It is to draw a pool on a single site listed, batches of samples to be analyzed. It consisted of selecting a poultry slaughter site within each of the targeted communes of the District of Abidjan. Sampling of raw chicken gizzards was carried out at large chicken slaughter sites (150 chickens slaughtered per day) over a 6-month period (April to September 2012). At each sampling site, a batch of 20 gizzards was collected each month, i.e. a total of 6 samples per commune and 120 raw chicken gizzards per commune. The number of samples was 66 and

objective of this work is to be part of a food safety and public health program linked to Salmonella, which will lead to public awareness of the existence of Salmonella strains in the poultry sector, in order to guarantee the food security of Ivorian populations. Salmonella enters the chicken consumed by crosscontamination or contaminated hand. All strains of Salmonella are pathogenic potential, thus causing salmonellosis in the host. Some strains have virulence genes (inv, spv, SPI, SGI) them the conferring on pathogenecity character.

the total number of raw gizzards to be analyzed were7920.

3.3 Research and identification of Salmonella: From the collected samples, the isolation of the Salmonella strains was carried out according to the standard NF EN ISO 6579 (ISO-6579, 2002), Comprising 4 steps: The Pre-enrichment of the test portion (10 g) in buffered peptone water (BioRAD, France). (ii) The enrichment of 0.1 mL and 1 mL of the pre-enrichment culture, respectively in 10 ml of Rappaport deVassiliadisbroth (BioRAD, France) and 10 ml of Müller-Kauffmann broth (BioRAD, France). (iii) The isolation of presumptive Salmonella strains by the stripping seeding technique, two selective Hektoenagar (BioRAD, France) Xylose-Lysineand Deoxycholate(BioRAD, France)), from the previous enriched broths. (iv) The morphological and biochemical identification by inoculation of a reduced Leminor rack consisting of 5 culture media, namely the Kligler-Hajna media (BioRAD, France), ureaindole (BioRAD, France), Mannitolmotility(BioRAD, France), Iron Lysine (BioRAD, France) and Simmons Citrate (BioRAD, France).

**3.4.** Serotyping of *Salmonella* strains isolated: The serotyping of *Salmonella* sp. strains was carried according to the method described by Kauffmann & White (1934), consisting in successively demonstrating somatic antigens (Ag O), flagellar H (Ag H) and



/ or Ag (Vi) capsules (Vi). The antigenic formula obtained were compared with those contained in the table of the Kauffmann-White scheme (Popoff & Le Minor, 2001), to determine the corresponding serotype of *Salmonella* strain.

3.5 antibiotic Determination of resistance: Serotyped Salmonella strains were tested for resistance to some antibiotic molecules used in both veterinary medicine and human medicine during Salmonella sp. infections. The antibiogram was performed on all isolated strains is carried out by diffusion in an agar medium, according to CLSI standard (Clinical Laboratory Standard Institute) on Müller-Hinton agar (BioRad, France) (CLSI, 2005). The antibiotic discs (BioRad, France) used to demonstrate the resistance profile of isolated Salmonella strains were: amoxicillin

### 4 **RESULTS AND DISCUSSION**

4.1 Chicken Consumption Survey: A total of 796 people were interviewed, i.e. 630 consumers and 126 chicken sellers. Analysis of the responses revealed a high consumption of chicken during the holidays (39.11±19.81<sup>a</sup>), especially in slaughtered form  $(39.09\pm23.34^{\circ})$ and live chicken once bought by the consumers but slaughtered at home (129.38±21,30<sup>a</sup>). The mode of preferred cooking, regardless of the place of consumption, is the braised form  $(35.11\pm15.94^{a})$ , with the thigh as a preferred portion (23,00±12,82<sup>a</sup>) followed by chicken  $(8.34\pm5.83^{\text{b}})$ gizzards (Table 1). The prospecting study carried out in the various municipalities also noted the existence of cases of gastroenteritis, mentioned by consumers in certain municipalities. This is particularly the case in the commune of Marcory and Abobo where three (3) cases were registered; Cocody (AMX,  $10\mu g$ ), amoxicillin / clavulanic acid (AMC,  $10 / 20\mu g$ ), Ticarcillin (TIC, 75  $\mu g$ ), cefalotin (CF, 10  $\mu g$ ), cefoxitin (FOX, 10  $\mu g$ ), cefotaxime (CTX, 10  $\mu g$ ), gentamicin (GM, 10  $\mu g$ ), nalidixic acid (Nal,  $10\mu g$ ), ciprofloxacin (Cip,  $10\mu g$ ), cotrimoxazole (SXT,  $10 / 20\mu g$ ), tetracycline (TE,  $10\mu g$ ) and chloramphenicol (C,  $10\mu g$ ). The reference of *Salmonella* strains named ATCC 14028 and IPCI 8297 were used to validate the antibiogram test.

**3.6** Statistical analysis: The information obtained from the survey was synthesized and subjected to a descriptive analysis using the SAS version 6 software. The different responses collected were coded into quantitative data. For the analysis, the comparison between the different characteristics was made using an analysis of variance (ANOVA) to one factor.

with two (2) cases; Treichville and Koumassi, one (1) case. All these cases would have occurred after ingestion of cooked dishes based on braised chickens or "chouconya", outside their homes. The most common symptoms were abdominal pain with diarrhea. The occurrence of these different cases could be related to poor handling of the cooked food (cross contamination) and not poor cooking of chicken cooked dishes. In addition, these sellers use unhygienic utensils and cutting boards. All these factors could contribute to the emergence and spread of pathogenic strains such as Salmonella in cooked dishes. Indeed, the works conducted by Cardinal et al. (2000), indicates that ingestion of raw food and not cooked food put in contact with sources of contamination, contributes to consumer exposure to public health risks related to cross-contamination.

Variables		Percentage ±	F	P value
		Ecart type		
	Slaughtered	$39.03 \pm 23.34^{a}$		
Status of purchase	Supermarket	$0.8 \pm 2.2^{\rm b}$	30.71	< 0.0001
	Living	29.38± 21.3ª		
	Weekly	9.07±13.65°		
Frequency of consumption	Feasts	39.11±19.81 <sup>a</sup>	21.96	< 0.0001
	Occasion	21±15.27 <sup>b</sup>		
Preference of consumption	Male	$62.46 \pm 23.73^{a}$	1.84	0.1877
	Female	$76 \pm 27.06^{a}$		
Preference of cooking forms	Braised	$35.11 \pm 15.94^{a}$		
	Braised and fried	$5.8 \pm 4.31^{\circ}$		
	Fried	$6.73 \pm 5.73^{\circ}$		
	Soup	$17.34 \pm 7.99^{\text{b}}$	72.40	< 0.0001
	Soup and braised	$2.84 \pm 4.3^{\rm dc}$		
	Soup and braised and	$1.11 \pm 2^{d}$		
	fried			
	Soup and fried	$0.23 \pm 0.99^{d}$		
Organs preference	Wing	$6.73 \pm 6.68^{\rm cb}$		
	Others	8.88± 7.21 <sup>b</sup>		
	Thigh	$23 \pm 12.82^{a}$		
	Thigh and wing	$3.30 \pm 7.55^{cd}$		
	Thigh and others	$7.07 \pm 6.19^{b}$		
	Thigh and gizzard	$8.96 \pm 6.49^{b}$	28.52	< 0.0001
	Thigh and gizzard and	$1.21 \pm 2.33^{d}$		
	others			
	gizzard	$8.34 \pm 5.83^{b}$		
	gizzard and others	$0.38 \pm 1.38^{d}$		
Consumption outside the	Male	$62.38 \pm 23.70^{a}$	1.86	0.1851
living environment	Female	$76 \pm 27.06^{a}$		
Infection related to chicken	Male	$0.23 \pm 0.43^{a}$	0.84	0.3679
consumption	Female	$0.53 \pm 1.12^{a}$	1	
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Table 1: Results of the chicken consumption survey in Abidjan District

The letters a, b, c... different on a same column indicate a difference statistic ( $p \le 5\%$ ).

4.2 of Research and identification Salmonella sp. In raw chicken gizzards: Bacterial strains with the following biochemical characteristics: bg-Mobility<sup>+</sup> oxidasecatalase<sup>+</sup>Glc<sup>+</sup> lac<sup>-</sup> H<sub>2</sub>S<sup>+</sup> Gas<sup>+</sup> Urea-Indole<sup>-</sup> Citrate<sup>+</sup> Mannitol<sup>+</sup> ONPG<sup>-</sup> LDA<sup>-</sup> LDC<sup>+</sup> were identified as strains of Salmonella sp. Microbiological analysis of the 66 lots of raw gizzards analyzed revealed 51 batches of raw gizzards on which one or more strains of Salmonella sp. Of these lots of raw gizzards

analyzed, 104 strains of *Salmonella* sp. were isolated, with an isolation rate of 157.57 % and a percentage of contaminated lots of 77.27 %. The distribution of the strains isolated as well as the different isolation rates observed are recorded in Table 2. Overall, batches contaminated range from 33 % to 100 %. The lowest level of contaminated lots was observed in the commune of Anyama with only 2 strains isolated, while the highest contaminated lots (> 83%) were observed in the communes of:



Marcory (20 strains isolated), Cocody (9 strains), Attecoubé and Adjamé (8 strains), Abobo (11 strains), and Koumassi(13 strains). The appearance of Salmonella sp. strains at the raw chicken gizzard level would be linked to the slaughter process at the various sites. These large-scale slaughter sites would have a considerable effect on the appearance of Salmonella sp. strains at the carcass level and on chicken offal. In fact, the massive influx of populations and restorers is causing eagerness among slaughterers who, under the pressure, are transgressing hygienic practices throughout the slaughter process. In addition, the deplorable hygiene conditions observed at these sites would also be the cause of the contamination of chicken carcasses by Salmonella sp. The intrusion of Salmonella sp. on the carcass and offal of the chicken can be done at different levels (moments) of the slaughter process, down to our plates. Human contamination by nontyphoidal Salmonella is mainly caused by the consumption of contaminated food and raw or undercooked poultry preparations. Salmonella is on skin, feathers and droppings of a small proportion of broilers at slaughter, resulting in relatively high levels of contamination (Bell & Kyriakides, 2002). According to Salvat et al. (1995), the poor scalding, animal plumage contamination of and contamination by of animals droppings released after death, promote the proliferation of bacteria .In fact, the pressure exerted by the plumbers during the plucking and on the skin of the animals, promotes transfer of the contamination of the feathers containing scalding water loaded with microorganisms, towards the feathery follicles and at skin surface. In addition, the progressive cooling of the skin causes a closure of the feathery follicles, badly dilated, hence the imprisonment of the bacteria present. Finally, the manual removal of the intestinal cluster at the time of evisceration by foul-contaminated hands and the washing stage are also a secondary source of contamination by intestinal bacteria, thereby water is contaminated with pre-existing bacteria (Salvat et al., 1995). The high level (77.27 %) of contamination of raw chicken gizzards by Salmonella sp. observed on raw chicken gizzards after slaughter reflects the need to improve the quality of hygiene in the chicken environment.

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Communes	Batches Contaminated/	Rate of contamination	Number of Salmonella strains	Number of strains	Number of serovars	Types of sérovars	
	batches analysed	(%)	isolated	serotyped			
Anyama	2/6	33.33	2	-	-	-	
Yopougon	3/6	50	6	5	4	Derby, Chester, Santiago, Schwarzengrund	
Port-Bouët	4/6	66.66	6	3	2	Essen, Kentucky	
Bingerville	4/6	66.66	10	4	3	Fortune, Schwarzengrund, Kentucky	
Treichville	4/6	66.66	11	3	1	Essen	
Abobo	5/6	83.33	11	10	4	Agona, Derby, Essen, Hadar	
Koumassi	5/6	83.33	13	5	4	Budapest, Chester, Kentucky, Poeselderf	
Adjamé	6/6	100	8	4	3	Budapest, Essen, Bargny	
Attécoubé	6/6	100	8	2	2	Budapest, Derby	
Cocody	6/6	100	9	3	3	Derby, Kentucky, Elisabethville	
Marcory	6/6	100	20	13	6	Aoto, Budapest, Ruiru, Kentucky, Derby, Essen	
TOTAL	51/56	77,27	104	52	-		

**Table 2**: Diversity of *Salmonella* sp train isolated in Abidjan District

NB: The rate of contamination is the ratio of the number of batches contaminated on all the batches analyzed



4.3 Serotyping of Salmonella strains isolated: The results of the serotyping show that the 52 strains completely serotyped are divided into 15 different serotypes throughout the District. These are distributed as follows: 1 serotype (Essen) observed at Treichville; 2 different serotypes observed in the communes of Attécoubé (Budapest and Derby), and Port-Bouët (Essen and Kentucky); 3 different serotypes observed in the municipalities of Adjamé (Budapest, Essen and Bargny), Bingerville (Fortune, Schwarzengrund and Kentucky) and Cocody (Derby, Elisabethville and Kentucky); 4 different serotypes were observed in the municipalities of Abobo (Agona, Derby, Essen and Hadar), Koumassi (Budapest, Chester, Kentucky and Poeselderf) and Yopougon (Chester, Derby, Santiago and Schwarzengrund); different 6 serotypes observed in the commune of Marcory (Aoto, Budapest, Derby, Essen, Ruiru and Kentucky) (Figure 1). The greatest diversity of serotypes is found in the municipality of Marcory (Table 2). However, taking into account the ratio of the number of strains isolated and the number of serotypes obtained, the Yopougon commune appears to have a relatively high diversity. All these observations show the diversity of the serotypes and their distribution at the level of the 11 communes of Abidjan District, enable us to consider carrying out a traceability study of probable infectious foci of Salmonella sp. The dominant serotypes are Derby (18.9 %), Budapest (17 %), Essen and Kentucky (11.3 %). Of these 4 serotypes, Derby and Kentucky are the most widely distributed serotypes in France and Belgium (Weill & Le Hello, 2011; Bertrand et al., 2010). Indeed, these two serotypes (Derby and Kentucky) are most often isolated from a variety of sources including poultry (Tao et al., 2014; Turki et al., 2011), and also have some profiles of resistance to several antibiotic families such as Betalactams and Fluoroquinolons (Ciprofloxacin) (Weill & Le Hello, 2011). The presence of these two serotypes in the raw chicken gizzards harvested from different slaughter sites in Abidjan District, suggests an indication of a risk to public health. Given the lack of a monitoring plan, involving the establishment of а surveillance network that could minimize the occurrence of salmonellosis epidemics, it would still all the same difficult for the Ivorian authorities to prevent as well the poultry industry actors as consumers, the danger posed by Salmonella sp. To support this hypothesis, an assessment of the antibiotic resistance of Salmonella sp. strains was carried out.

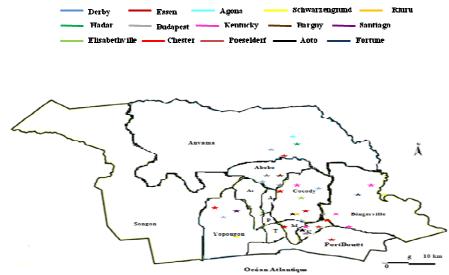


Figure 1: Distribution of Salmonella sp train isolated in Abidjan District



4.4 Evaluation of antibiotic resistance of serotyped Salmonella strains: The study of the resistance to antibiotics of Salmonella strains, carried out on all the strains, allowed to classify them according to CLSI standards (CLSI, 2005), in: resistant, intermediate or sensitive. resistant Considering as а strain, an intermediate and categorized non-sensitive strain, resistance studies show that strains of Salmonella sp. isolated in Abidjan District, present resistance profiles ranging from the wild phenotype to the multiresistant strains (Table 3). The antibiogram revealed Salmonella strains resistant to 6, 8, 9 and even 11 molecules of antibiotics including the following families of antibiotics: β-lactams, sulphonamides, aminoglycosides, phenicols, quinolones and tetracyclines; and resistance profiles ranging from a single resistance to a multiple resistance. Of the 104 strains of Salmonella isolated, 103 (99.04 %) strains showed resistance to at least one antibiotic tested. On the other hand, 8 strains (7.77 %) showed a mono-resistance (resistant to 1 family of antibiotics) and 29 strains (28.16 %) a biresistance (2 families of antibiotics). Sixty-six (66) strains (64.07 %) showed resistance to more than 3 molecules of antibiotics belonging to at least 3 families of antibiotics (Table 3). These are multidrug resistant strains (resistant to at least 3 families of antibiotics). Of the 52 strains of Salmonella sp. completely serotyped, 8 serotypes (Agona, Derby, Hadar, Budapest, Riuru, Essen, Kentucky and Chester) showed multidrug resistance phenotypes for the antibiotics tested (Table 4). The spread of pathogenic bacteria resistant to antibiotics is one of the most serious threats to effective treatment of a disease. The threat exists in developed countries and particularly in developing countries where self-medication is

common. The multiple resistances observed in this study relates to antibiotics commonly used in human and veterinary medicine. These include beta-lactams (Ticarcillin), Tetracyclins, sulfonamides (Cotrimoxazol), Fluoroquinolones (nalidixic acid and Ciprofloxacin). The high prevalence of strains resistant to these families of antibiotics could be due to their non-rational use in the poultry sector, as evidenced by a field survey conducted in Abidjan (Ouattara et al., 2013). The abusive and uncontrolled use of antibiotics may lead to the selection of resistant bacteria (Chaslus-Dancla, 2003).. From this point of view, the development and emergence of resistance in pathogenic bacteria for humans and animals could translate a use of these molecules with a poor understanding of the ecological impact of use on microflora their bacteria. The occurrence of multidrug resistant strains has been reported in Salmonella serovars associated with poultry (Antunes et al., 2005; Zao et al., 2005 ; Musgrove et al., 2006). According to (Threlfall, 2002), Derby, Agona and Kentucky are commonly associated with the phenomenon of multiresistance to antibiotics, as evidenced by this study. Multidrug resistance remains a problem in Salmonella sp. Since it requires the use of third-generation cephalosporins (C3G) or fluoroquinolones to treat salmonellosis, which may tend to favor the emergence of resistances to these two molecules. Resistance to C3G and fluoroquinolones remains uncommon in Salmonella, but they could become a real public health problem if they spread. Since most of the cases of human salmonellosis are foodborne (e.g. through poultry or egg products), monitoring of resistance in livestock is an essential precaution to anticipate the occurrence of such strains.

Profiles	Number of drugs	Number of strains isolated
Savage Strains	0	1
SXT/Te	1	8
CSXT/ NalTe/ TeSXT/ SXTTic/ SXTNal	2	26
CSXTNal/CSXTCip/SXTNalTe/	3	30
SXTNalCip/TicCSXT/CTeSXT/TicTeSXT		
CTeSXTNal/TicCTeSXT/TicGTeSXT/	4	13
CSXTNalCip/TicSXTNalCip/SXTNalCipTic		
CTeSXTNalCip/TicCSXTNalCipTe/	5	16
AAMCTicSXTTe/GSXTNalCipTeTic		
AAMCTicCfTeCip/TicCTeSXTNalCip	6	2
AAMCTicGSXTNalCipTe	8	1
AAMCTicCfGSXTNalCipTe	9	4
AAMCTicCfCTXGCSXTTeNalCip	11	1

Table 3: Antibiotic resistance profile of Salmonella strains isolated

A (Amoxicillin), AMC (Amoxicillin/ Clavulanic acid), Tic (Tircacillin), Cf (Cefalotin), CTX (Cefotaxim), C (Chloramphenicol), G (Gentamycin), Nal (Nalidixic acid), Cip (Ciprofloxacin), SXT (Cotrimoxazol), Te (Tetracyclin).

Table 4: Antibiotic resistance profiles of *Salmonella* serotypes isolated from raw chicken gizzards

Serotypes	Multidrug resistance profiles	Number of multidrug resistant strains
Budapest	TicTeSXT/ TicCSXT/ TicCTeSXT/ CTeSXT	5
Agona	TicTeSXT/ TicCSXT/ TicSXTTe	4
Essen	TicCTeSXT/ AAMCTicSXTTe/SXTNalTe/ TicGTeSXT	4
Kentucky	GSXTNalCipTe/ TicGTeSXT	4
Derby	TicCSXT/ TicCTeSXT	2
Riuru	CTeSXTNal/ CTeSXT	2
Hadar	TicSXTNalCipTe	1
Chester	TicSXTTe	1

A (Amoxicillin), AMC (Amoxicillin/ Clavulanic acid), Tic (Tircacillin), Cf (Cefalotin), CTX (Cefotaxim), C (Chloramphenicol), G (Gentamycin), Nal (Nalidixic acid), Cip (Ciprofloxacin), SXT (Cotrimoxazol), Te (Tetracyclin).

#### 5. CONCLUSION

The presence of *Salmonella* sp. in the raw chicken gizzards sold in the communes of the Abidjan District, as well as serotypes implicated in the epidemics of salmonellosis in the world, reflect the existence of a risk of salmonellosis linked to the consumption of chicken gizzards. Also, the (multiple) resistance observed at critical drugs such as Nalidixic acid and Ciprofloxacin, indicate a therapeutic stalemate with antibiotics. All these observations should prompt the Ivorian authorities to implement a full epidemiological study and to control the contamination by *Salmonella* sp. , at different

levels of production of chicken and outlets in Abidjan District (Côte d'Ivoire); Which should minimize the occurrence of salmonellosis for the welfare of populations on the one hand and ensure food safety on the other.



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