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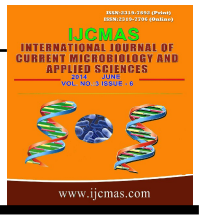

Original Research Article

Serotypes and antibiotic resistance of *Salmonella spp.* isolated from poultry carcass and raw gizzard sold in markets and catering in Abidjan, Côte d'Ivoire

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A B S T R A C T

To determine the serotypes and antibiotic resistance of isolates poultry from products sold in markets and catering, 560 samples, of which 240 gizzards and 360 chicken carcasses were taken. Strains were identified by bacteriological analysis and characterized by serotyping and antibiotypage. The prevalence of *Salmonella* in poultry products was 27 %, with 47.9 % from gizzard and 11.3 % from chicken carcass. The isolation rate was 42.8 %, 32.1 % and 25% respectively for products taken from market, slaughterhouse and catering. Serotypes *S.* Hadar (28.6%), *S.* Virchow (10.7%) and *S.* Kentucky (10.7%) were more frequent and *S.* Gallinarum, *S.* Albany, and *S.* Rissen Montevideo (3.57 %) were rare. Strains are resistant to aminopenicillins (53 to 60.7 %) and cephalothin (53.6 %). The Households and tenants of premises catering must take precautions in handling poultry products purchased on the market.

Keywords

Salmonella, serotype, resistance, food, prevalence

Introduction

Foodborne diseases cause a public health and economic problem in both developed than in developing countries (WHO, 2012). Although billions of people suffer from foodborne diseases yearly, it is difficult to obtain accurate estimates of the incidence of foodborne disease, especially in developing countries such as Côte d'Ivoire (WHO, 2002; Grace *et al*., 2008). In Côte d'Ivoire, people with gastrointestinal symptoms (diarrhea, vomiting, stomach cramps, fever) rarely go to the hospital, because they lack medical coverage; therefore, the rate of food poisoning is underreported (Kouamé-Sina et *al*., 2012). The main cause of

foodborne diseases is bacterial origin. The pathogenic microorganisms most frequently consumed in food in decreasing order are *Salmonella*, *Clostridium*, *Staphylococcus*, and *Escherichia coli* (Kouamé*et al*., 2010; Koffi-Nevry*et al*., 2012)Animal-source foods such as poultry products are important causes of foodborne diseases. In Côte d'Ivoire, poultry products such as eggs, laying hens and broiler chickens as well as chicken carcass and gizzards are flooding the markets and trade area (IPRAVI, 2011). Consumption of local modern poultry carcass increased from 0.46 to 1.05 kg / capita / year from 2005 to 2011 (IPRAVI, 2011).

The raw material used for making meal of poultry products, both in the catering and in households, generally comes from traditional markets. Most farmers, sellers and restorers have little understanding of the hygiene and sanitary aspects of chicken carcass and gizzards handling. The practices of farmers, sellers, and restorers throughout the production and delivery chains do not adequately prevent or reduce contamination by pathogens capable of causing foodborne diseases amongst consumers. *Salmonella* spp., a common poultry product inhabitant, is one such pathogen. *Salmonella* is a ubiquitous and resistant bacteria, which can survive for several weeks in a dry environment and several months in water. It may be responsible in humans, based on their physiological status, of a diarrhea with or not fever and sometimes the infection can be fatal for the consumers.

The aim of this study is to determine the serotypes and antibiotic resistance of *Salmonella* isolated from gizzards and poultry carcass sold in markets and catering.

Materials and Methods

Study area

We conducted a cross-sectional study of production system and traditional markets of chicken carcass and gizzards in four municipalities of Abidjan: Abobo, Cocody, Adjamé and Port-Bouët. Three of these four municipalities (Abobo, Adjamé and Port-Bouët) had traditional slaughtering of poultry and catering. At the market of Cocody, only samples of gizzards were collected. These sites were selected purposively, based on the importance of the market for the traditional poultry sector and the willingness of the actors (famers, sellers, livestock owners) to participate in the study. The sites selected were estimated to incorporate 80% of production system and traditional markets of chicken carcass and gizzards in Abidjan.

Isolation and identification of *Salmonella* spp

Samples were consisted of fresh carcass and raw chicken gizzards (*Gallus gallus).* They were collected on four passages between June and August 2010. During each passage, 60 gizzards samples and 80 chicken carcasses were aseptically collected in stomachers bag. A total of 560 samples, including 240 raw gizzards and 320 chickencarcasses were sampled. Immediately after collection, samples were chilled in an ice cooler box at 4° C and transported to the laboratory CeDReS for analysis within 2h after sampling.

All the samples collected were analyzed for the isolation and identification of*Salmonella* spp. The isolation was carried outaccording to standard NFEN ISO6579(Association Française de Normalisation, 2002).

Salmonella typhimurium (ATCC ® 14028 ™) was used as reference strain in all the tests.

Serotyping of *Salmonella* spp

A study on the serotyping was carried out on 37 *Salmonella* spp strains. Of these strains 9 were isolated from poultry carcass samples, and 28 from gizzards. Self-agglutination test with 1‰ saline was performed with the bacteria strains prepared from a 24 h pure culture on ordinary agar (Bio-Rad, France). Serotyping of *Salmonella* spp. was carried out according to Kauffmann-White scheme (1934). The strains were serotyped with antisera O, H and Vi (BioMérieux, France). Theresults were interpretedaccording to Kauffmann-White-Le Minor scheme (Patrick and François-Xavier, 2007).

Antibiotic susceptibility tests

The antibiogram was carried out on Mueller-Hinton Agar (BioRad, France) according to disk-diffusion method (Bauer *et al.*, 1966). The antibiotics used (BioRad, France) were as following: ampicillin (10 µg), amoxicillin + clavulanic acid (20 µg/10 µg), Imipenem (10 µg), Cephalothin (30 µg), Cefotaxime (30 µg), aztreonam (30 µg), kanamycin (30 µg), Gentamicin (15 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), colistin (50 µg), furans (300 µg), nalidixic acid (30 µg), Pefloxacin (5 µg), ciprofloxacin (5 µg), Sulfamethoxazole + Trimethoprim (1.25 µg/23.75 µg). Inhibition zone was examined and interpreted according to the recommended method by Comité de l'Antibiogramme de la SociétéFrançaise de Microbiologie (CA-SFM, 2010).

Results and Discussion

Prevalence of *Salmonella*

In total, *Salmonella spp*. was found in 151(27%) samples. The highest prevalence was found in chicken gizzards (47.9%). In chicken carcass the prevalence of *Salmonella spp* was 11.3 % (Table 1). The prevalence of market products, slaughter samples and those of catering were presented in Table 2. At the municipality's level, the prevalence is variable; she is highest to Abobo (8.2%) and less elevated in Cocody (3.8%).

Frequency of *Salmonella* serotype

The distribution of the different serotypes of *Salmonella* is shown in table 4. *Salmonella* Enterica was the only specie found in this study. All the 37 strains of *Salmonella* isolated were divided into 13 serotypes (table 2). The highest serotypes found were *S*. Hadar (29.7%) and S. Virchow (13.5%). According to the origin of the isolates, *S*. Hadar and *S*. Virchow are the highest frequency (5.4%) in catering.

However, *Salmonella* Hadar is the predominant serotype isolated from Slaughter house (13.5%) and Poultry market (10.8%). Indeed, in Abobo, 12 strains are distributed among 8 serotypes. In Cocody, 6 strains are divided into 5 serotypes. In Adjamé there are 10 strains for 8 serotypes. Finally in Port-Bouët there are 9 strains divided into 8 serotypes. In all the markets, *Salmonella* serotypes isolated are almost found (Table 3). The distribution of *Salmonella* strains isolated reflects different municipalities selected for the study (Table 3).

Susceptibility to antibiotics

The susceptibility of the serotypes of *Salmonella*to antibiotics is summarized in table 5. The isolates were resistant to ampicillin (48.7%), Amoxicillin + Clavulanic acid (43.2%) and cefalotin (40.5%).On all 13 serotypes, only *S.* Montevideo has a wild profile (Tables 5). The following antibiotics (ATM, IPM, AN, TM, GM and CS) are not resistant to all isolated serotypes of *Salmonella*. Overall, two areas of resistance are observed (Table 5).

The prevalence of *Salmonella* isolated was 27%,the isolation rate was virtually identical in all Abidjan municipalities (table 1). At the municipal level, the prevalence's recorded were the same. They provide 7.5% in 3 municipalities in 4 cases. However, the prevalence of chicken gizzards in isolates was higher (47.9%) than chicken carcasses (11.3%).From chicken carcass and gizzards tested we wereisolated and identified 37 strains of *Salmonella*spp (table 2). This shows the presence of *Salmonella* in these products as required by the work done Brisabois*et al.* 2006. It appears that the isolation rates of *Salmonella* in poultry markets are higher than slaughterhouses and canteens. More bacteria were isolated on Abobo market than in other markets.

In all three sites, the isolation of strains in the markets was more important with 42, 9%. This was the proof that in the markets, the risk of *Salmonella* contamination was high.The important proportion of isolation shows that there was a high risk in poultry products(Modzelewska-Kapituła and Maj-Sobotka, 2014). However, concern appears if markets were riskier than canteens and slaughterhouses that could mean a favorable factor in the emergence of *Salmonella* from these markets. The conditions for the emergence of strains of *Salmonella* were unhygienic (Koffi*et al.* 2012) and especially the handling of food with dirty hands. The emergence or expansions of these problems were largely the result of the change in our agricultural or industrial practices (De Winter *et al.* 2011). At the markets, some traders often coexist with garbage. Flies and other insects commute between garbage and sometimes food was exposed on the floor for sale. This promotes the export of germs from one point to another, and could explain the fact that markets were bacteria tanks, especially *Salmonella* (anonymous, 2014).

Anyway we met in market many germs. The finding in this study was that there were*Salmonella* on each market chosen as research site (Table 4). Another study on raw chicken gizzards displayed for sale was conducted in Yopougon. It revealed a carrier rate of 61.87 % and a presence of 25 *Salmonella* serotypes including *S.* Hadar majority in Yopougon (Bonny *et al.* 2011). These results are in conformity with those of our study. Although, Adjamé market was which had the highest *Salmonella*isolated, it was noted that all markets, the study sites are contaminated with these bacteria. However when the medium was contaminated with *Salmonella*, it was very difficult to remove completely. Our study confirms the presence of *Salmonella* in food of avian origin, mainly in chicken gizzards, fresh carcass and poultry sold in the markets and in specific areas of restoration. The result was similar to that achieved by another study confirms that the gizzards were mostly likely to contain pathogenic bacteria of the genus *Salmonella* (Tibaijuka *et al.* 2003). However, no strain of *Salmonella* was tolerated in 25 g of food (Guillaume, 2006; Wits J. 2009).

Table.1 Prevalence of *Salmonella* spp in Food samples

Food samples (number)	Distribution of *Salmonella* spp in different foods in Abidjan municipalities				
	Abobo (n= 160)	Adjamé (n=160)	Cocody (n= 80)	Port-Bouët (n= 160)	Total (%)
Poultry carcass (320)	9 (2.8)	13 (4.1)	4 (1.3)	10 (3.1)	36 (11.3)
Chicken gizzards (240)	37 (15.4)	29 (12.1)	17 (7.1)	32 (13.3)	115 (47.9)
Total (560)	46 (8.2)	42 (7.5)	21 (3.8)	42 (7.5)	151 (27)

Table.2 Prevalence of *Salmonella spp.* serotypes isolated from chicken gizzards and fresh chicken carcass

Strains	Antigenic formula	Number (%) of chicken carcass isolates	Number (%) of gizzards isolates
S. hadar	6,8 : z10 : e, n, x	2 (5.4)	9 (24.3)
S.virchow	6,7, 14 : r : 1, 2	2 (5.4)	3 (8.1)
S. infantis	6, 7, 14 : r : 1, 5	1 (2.7)	1 (2.7)
S. kentucky	8, 20 : i : z6	0	3 (8.1)
S. montevideo	6, 7, 14 : g, m, p, s : 1, 2, 7	0	1 (2.7)
S. gallinarum	1, 9, 12 : - : -	1 (2.7)	2 (5.4)
S. typhimurium	1, 4, 5,12 : i : 1,2	1 (2.7)	2 (5.4)
S. enteritidis	1, 9,12 : g, m :-	1 (2.7)	2 (5.4)
S. heidelberg	1, 4, 5, 12 : r : 1, 2	0	2 (5.4)
S. rissen	6, 7, 14 : f, g : -	0	1 (2.7)
S. albany	8, 20 : z4, z24 : -	0	1 (2.7)
S. agona	1, 4, [5], 12: e, h : 1,2	1 (2.7)	0
S. saintpaul	1, 4, [5], 12: f, g, s : [1,2]	0	1 (2.7)
Total		9 (24.3)	28 (75.7)

Table.3 Distribution of *Salmonella spp*. serotypes isolated from chicken carcass and gizzards according to municipalities

Strains	Cocody	Abobo	Adjamé	Port-Bouët	Total (%)
S.virchow	1 (2.7)	2 (5.4)	1 (2.7)	1 (2.7)	5 (13.5)
S. infantis	0	1 (2.7)	1 (2.7)	0	2 (5.4)
S. typhimurium	0	1 (2.7)	1 (2.7)	1 (2.7)	3 (8.1)
S. hadar	2 (5.4)	4 (10.8)	3 (8.1)	2 (5.4)	11(29.7)
S. enteritidis	1 (2.7)	1 (2.7)	0	1 (2.7)	3 (8.1)
S. heidelberg	1 (2.7)	0	1 (2.7)	0	2 (5.4)
S. kentucky	0	1 (2.7)	1 (2.7)	1 (2.7)	3 (8.1)
S. montevideo	0	0	0	1 (2.7)	1 (2.7)
S. rissen	0	1 (2.7)	0	0	1 (2.7)
S. albany	0	0	1 (2.7)	0	1 (2.7)
S. gallinarum	1 (2.7)	1 (2.7)	0	1 (2.7)	3 (8.1)
S. agona	0	0	1 (2.7)	0	1 (2.7)
S. saintpaul	0	0	0	1 (2.7)	1 (2.7)
Total	6 (14.2)	12 (32.4)	10 (27.0)	9 (24.3)	37 (100)

Table.4 Distribution of *Salmonella spp*. serotypes isolated from chicken carcass and gizzards according to origin

Strains	Catering	Slaughterhouse	Poultry market	Total (%)
S.virchow	2 (5.4)	1 (2.7)	2 (5.4)	5 (13.5)
S. infantis	0	1 (2.7)	1 (2.7)	2 (5.4)
S. typhimurium	0	2 (5.4)	1 (2.7)	3 (8.1)
S. hadar	2 (5.4)	5 (13.5)	4 (10.8)	11 (29.7)
S. enteritidis	1 (2.7)	0	2 (5.4)	3 (8.1)
S. heidelberg	1 (2.7)	1 (2.7)	0	2 (5.4)
S. kentucky	0	1 (2.7)	2 (5.4)	3 (8.1)
S. montevideo	1 (2.7)	0	0	1 (2.7)
S. rissen	1 (2.7)	0	0	1 (2.7)
S. albany	0	0	1 (2.7)	1 (2.7)
S. gallinarum	1 (2.7)	1 (2.7)	1 (2.7)	3 (8.1)
S. agona	0	1 (2.7)	0	1 (2.7)
S. saintpaul	0	0	1 (2.7)	1 (2.7)
Total (%)	9 (24.3)	13 (35.1)	15 (40.6)	37 (100)

Table 5: Antibiotic resistance of *Salmonella spp*. isolates

Species	AM	AMC	TIC	CF	ATM	IPM	K	AN	TM	GM	NET	TE	CIP	C	CS	SXT
S. hadar	6	5	3	7	0	0	0	0	0	0	0	2	0	1	0	1
S.virchow	1	2	0	1	0	0	0	0	0	0	0	1	0	0	0	0
S. infantis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S. typhimurium	4	3	0	2	0	0	0	0	0	0	0	0	2	0	0	0
S. enteritidis	2	2	0	1	0	0	0	0	0	0	0	0	2	0	0	0
S. heidelberg	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1
S. kentucky	1	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0
S. montevideo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. rissen	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
S. albany	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0
S. gallinarum	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
S. agona	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
S. saintpaul	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Total (%)	48,65	43,24	16,22	40,54	0,00	0,00	2,70	0,00	0,00	0,00	2,70	10,81	13,51	8,11	0,00	13,51

AM = Ampicillin, AMC = Amoxicillin + clavulanic acid, IPM = Imipenem, CF = Cefalotin, TIC = Ticarcillin, ATM = Aztreonam, K = Kanamycin, GM = Gentamicin, TM = Tobramicyn C = Chloramphenicol, TE = Tetracyclin, CS = Colistin, NET = Netilmicyne, AN = Amikacyn, CIP = Ciprofloxacin, SXT = Sulfamethoxazole + Trimethoprim)

After serotyping, 37 strains of *Salmonella spp*, the result was a single species: *Salmonella*Enterica. Serotypes were met: *S.*Virchow, *S.* Infantis, *S.* Typhimurium, *S.* Hadar, *S.* Enteritidis, *S.* Heidelberg, *S.* Kentucky, *S.* Montevideo, *S.* Rissen, *S.* Gallinarum and *S.* Albany. From these serotypes, *S.* Hadar represents 28.57% of the isolates.*S.* Hadar, *S.*Virchow, *S.* Typhimurium and *S.* Enteritidis were isolated in both gizzards in poultry.*S.* Kentucky is found in all the study sites; this presence could be special and give the sign of a serotype expansion. It was this finding led only the CNRS in its Annual Activity Report 2011, as *S.* Kentucky is an emerging serotype (CNRS, 2011). The coexistence of these serotypes in poultry products of different types requires special attention.

This result is consistent with that of Coulibaly e*t al.* in 2010. It is followed *S.*Virchow, *S.* Gallinarum and *S.*Kentucky, in the proportions of 10.72 % (Table 3). Strains isolated from gizzards, and poultry carcass had generally resistance to aminopenicillins carboxypénicillines, with β-lactam inhibitor of β-lactamase (Table 5).

However, *Salmonella* was an enterobacteria, it should be naturally sensitive to β-lactams, aminoglycosides, quinolones, fluoroquinolones and nitrofurantoin. The phenotype displayed by *Salmonella* expresses that it had acquired resistance to these antibiotics. These results were consistent with those showing that fluoroquinolnes resistance had progressed in 2008 (Coulibaly*et al.* 2009). Only one strain *S.* Montevideo was tested sensitive to all antibiotics used in our study. It could be a purely wild strain. This strain came from contamination from the environment or from another source.

In conclusion, the serotype and antibiotic resistance of *Salmonella* in poultry products, markets and catering were concerns. Salmonellosis therefore remained a topical issue. Eradication in poultry was probably a utopia. However, the long-term control programs was undoubtedly the elimination of *Salmonella* infection, if possible, in poultry and consequently in the gizzards; in this order, to reduce greatly salmonellosis in human cases. Protective measures, such as vaccination, might help to reduce the levels of contamination. The question of the level of resistance strains on antibiotics should be answered in a change of behavior in the appropriate use of antibiotics in livestock. Good hygienic slaughter practices were very important in this regard. Finally, we must be promoting integrated risk analysis procedures for characterizing and ensure communication with the consumer, so that it was aware of the level of risk associated with what he eats. Efforts should be concentrated primarily in the areas of distribution of the preparation and consumption, with particular emphasis on safety markets.

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