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Prevalence of *Salmonella* and their antibiotic susceptibility patterns in the District of Abidjan, Côte d'Ivoire

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ABSTRACT

A study was conducted to estimate the prevalence, antibiotic susceptibility and distribution of *Salmonella* strains in raw chicken gizzards in 11 municipalities (Abobo, Adjamé, Anyama, Attécoubé, Bingerville, Cocody, Koumassi, Marcory, Port-Bouët, Treichville and Yopougon) in the District of Abidjan. Sixty six (66) samples of chicken gizzards were collected between April and September 2012. From the total of 66 samples tested, *Salmonella* was isolated in 77.27% (51/66). One hundred and four strains (104) were isolated and fifteen distinct serotypes identified: Derby (18.9 %), Budapest (17%), Essen and S. Kentucky (11.3%), Hadar (9.4%), Agona (7.5%), Chester, Ruiru, Schwarzengrund (3.8%), Aoto, Bargny, Elisabethville, Fortune, Poeslderf and Santiago (1.9%). Serogroup O:4 was the most prevalent (67.3 %). Prevalence of other serogroups were 18.3% for O:8, 10.6% for O:3,10, 1.9% for O:21, 1% for O:1,3,19 and O:13. Antibiotic profiles of *Salmonella* spp. showed 93.37 % of resistance to cotrimoxazole, 73.08% to tetracycline, 46.15% to ticarcilline, 35.58% to nalidixic acid and resistance rates ≤ 30% to chloramphenicol and ciprofloxacin. Strains were found susceptible to cefoxitim (100%), cefotaxim (99.04 %), cephalotin (90.38%), amoxicillin and amoxicillin/clavulanic acid (92.31%) and gentamycin (89.42%).

Keywords: *Salmonella*, raw chicken gizzards, serogroups, antibiotic susceptibility, Côte d'Ivoire.

INTRODUCTION

Salmonella enterica are one of the most important foodborne pathogens, leading to millions of cases of enteric diseases, thousands of hospitalizations and deaths worldwide each year (Callaway et al., 2010). *Salmonella* includes more than 2500 different serotypes (Popoff et al., 2001). *Salmonella* species can cause systemic infection especially in children and immuno-compromised individuals, while healthy individuals suffer from symptoms such fever, diarrhea, nausea, abdominal pain and septicemia (Coburn et al., 2007; Willford et al., 2007).

The majority of the infections are associated with ingestion of contaminated foods such as eggs, chicken, beef, pork, milk products, fruits, and vegetables (Brands et al., 2005; Zhao et al., 2008). Poultry and poultry

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products are usually incriminated in human salmonellosis outbreaks cross-contamination of workers hands, working equipment and utensils, subsequent handling of the raw poultry carcasses and organs, and products together with the consumption of undercooked poultry meat could serve as the most frequent causes of infection by *Salmonella* reported in humans (Yildirim et al., 2011).

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 (Forshell and Wierup, 2006). *Salmonella* species are becoming increasingly resistant to antibiotics, making it more difficult to treat patients with severe infections. This makes *Salmonella* serovars that are resistant to multiple antibiotics a continuous and an important subject area of research, and a major concern for food safety (Adzitey et al., 2011).

Isolates of *Salmonella* that are resistant to antimicrobial agents have become a worldwide health problem (Butaye et al., 2006; Parry and Threlfall, 2008).

The objective of this work was to determine the prevalence of *Salmonella* isolated from chicken gizzards in the District of Abidjan and the antibiotic resistance profiles of strains.

MATERIALS AND METHODS

Sampling

A total of 66 batches of 20 raw chicken gizzards samples were collected from April to September 2012 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 once per week in sterile plastic bags and transported to the laboratory within one hour after collection, in an ice chest for analysis upon arrival.

Culture and identification of strains

All strains were isolated according to the protocol described by standard ISO 6579 (ISO-6579, 2002). Ten grams of chicken gizzard were pre enriched in 90 mL of BPW (Buffered Peptone Water) (BIO-RAD) in a stomacher bag (AJ seward, London) and incubated at 37 °C for 24 hours. After incubation, 0.1 mL and 1 mL of pre enriched broth was added respectively to 10 ml of Rappaport Vassilliadis broth (BIO-RAD) and to 10 ml of Müller-Kauffman Tetrationate broth in tubes to provide enrichment. Tubes were incubated at 42 °C and 37 °C for 24 hours respectively for Rappaport Vassilliadis (RV10) broth and Müller-Kauffman Tetrationate (MKT) broth. From each of the two tubes (RV10 and MKT), Hektoen agar and Xylose-Lysin-Deoxycholate (XLD) agar in Petri dishes were streaked and incubated at 37 °C for 24 hours. The suspected colonies were tested for catalase and oxidase test, Gram control; and then identified on the reduced rack of LEMINOR constituted of four culture media (Kligler-Hajna/Mannitol-Mobility/Urea indole/Simmons Citrate) (BIO-RAD).

Serotyping

Serotypes were determined by agglutination tests slide with sera group and immune antisera directed against antigens O, H and Vi of *Salmonella* (BIO-RAD). Reading the results was made according to the Kauffmann White scheme (Grimont and Weill, 2007). *Salmonella* strains serogrouping was done by slide agglutination tests using poly O and groups O:4; O:8; O:3,10; O:21…. Antisera.

Antimicrobial susceptibility testing

Salmonella strains were tested for their susceptibility to different antibiotics using the

agar diffusion method as described by the Clinical Laboratory Standards Institute (CLSI, 2005) . An inoculum was prepared with 3 or 4 colonies of pure culture onto nutrient agar (Mueller Hinton 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. A sterile swab was moistened in the bacterial suspension, and Miller-Hinton agar previously dried was seeded by swabbing the entire surface of 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 for 18 to 24 hours. After incubation, agar plats were read by measuring the diameters of inhibition zones around each antibiotic disk with a graduated ruler. A reference Strain of *E. coli* ATCC 14028 was tested as a quality control for culture and susceptibility testing. According to the Clinical and Laboratory Standard Institute standard (CLSI, 2005), results were interpreted and transcribed by the terms: Susceptible (S), intermediated (I) and resistant (R). Strains resistant to 3 or more antimicrobials from different classes were considered as Multidrug-Resistant (MDR). The following antibiotics were tested: amoxicillin, amoxicillin/clavulanique acid, tircacilline, cephalothin, cefoxitin, cefotaxim, gentamicin, chloramphenicol, cotrimoxazole, nalidixic acid, ciprofloxacin and tetracycline.

RESULTS

Serogrouping

Salmonella was isolated from 77.27% of samples (n=51). Further analysis of *Salmonella* positive samples led to identification of 104 isolates: 20 were from Marcory municipality, 13 were from Koumassi municipality, 11 were from Abobo and Treichville, 10 were from Bingerville, 9 were from Cocody, 8 were from Adjamé and Attécoubé, 6 were from Port-Bouët and Yopougon and 2 from Anyama. *Salmonella* isolates was distributed in six (6) serogroups: O: 4 (B), O: 8 (C1), O: 21 (L), O: 13 (G), O: 3,10 (E1) and O: 1,3,19 (E2) (Table 1 and Figure 1). Serogroup O: 4 was the most commonly isolated (67.31%), followed by serogroup O: 8 (18.27%), serogroup O: 3,10 (10.58%), serogroup O: 1,3,19 (0.96%), serogroup O: 13 (0.96%) and serogroup O: 21 (1.92%). Majority of *Salmonella* isolates in the 11 municiplities had antigen O:4 (67.31%) and antigen O:8 (18,27%). These two antigens represent most of 85% of *Salmonella* isolates in the district of Abidjan.

Antimicrobial susceptibility testing

Among 104 *Salmonella enterica* isolates, 103 were resistant to 1-11 categories of antimicrobial agents that include β-lactams, Sulfonamides, Aminosides, Phenicols, Quinolons and Cyclines. Strain showed resistance to tetracycline (73.08%), sulfonamide (93.37%), nalidixic acid (35.76%), ciprofloxacin (28.85%), chloramphenicol (29.80%) and gentamycin (10.58%). Among the β-lactams, resistance to Tircacillin (n=48) was found highest, followed by Amoxicillin or/and Amoxicillin-clavulanic acid, and Cefalotin (n=6). Contrariwise, one resistance was observed to the third generation of cephalosporins (cefotaxime (n=1)) (Table 2). 99.04% of isolates (103/104) presented resistance to all antimicrobials tested, 7.77% (8/103) presented a single type of resistance. 28.16% (29/103) showed resistance to two classes of antimicrobials and 64.07% (66/103) were multidrug-resistant (Resistance to 3 or more antimicrobials). Twenty strains of the 66 multidrug-resistant strains were selected for the tests of antimicrobials resistance genes (Table 3).

Table 1: Distribution of Serogroups of *Salmonella* isolated from raw chicken gizzards in the District of Abidjan.

Municipalities	**Strains isolated**	**OMA**			**OMB**	
		B (O:4)	**E (O:3,10 and O:1,3,19)**	**L (O: 21)**	**C (O:8)**	**G (O:13)**
Abobo	12	8	0	0	4	0
Adjamé	8	5	1	0	2	0
Anyama	2	2	0	0	0	0
Attécoubé	8	8	0	0	0	0
Bingerville	10	6	0	0	4	0
Cocody	8	3	1	0	3	1
Koumassi	13	7	3	0	3	0
Marcory	20	16	1	2	1	0
Port-Bouët	6	2	2	0	2	0
Treichville	11	7	4	0	0	0
Yopougon	6	6	0	0	0	0
Total	104	70	12	2	19	1
(%) agglutinates	100	67,31	11,54	1,92	18,27	0,96

Table 2: Antimicrobial susceptibility patterns of *Salmonella* isolates of chicken gizzards in the District of Abidjan.

Famillies		**Drugs**	***Salmonella* (%)/ N=104**	
			R	**S**
Bêta-lactams	Penicillins	amoxicillin	8 (7,69%)	96 (92,31%)
		amoxicillin/ clavulanic acid	8 (7,69%)	96 (92,31%)
		Ticarcilline	48 (46,15%)	56 (53,85%)
	Cephalosporins	Cefalotin	10 (9,62%)	94 (90,38%)
		cefoxitim	0 (0%)	104 (100%)
		cefotaxim	1 (0,96%)	103 (99,04%)
Aminosides		gentamycin	11 (10,58%)	93 (89,42%)
Phenicols		chloramphenicol	31 (29,81%)	73 (70,19%)
Sulfonamides		cotrimoxazole	97 (93,37%)	7 (6,73%)
Quinolones		nalidixic acid	37 (35,58%)	67 (64,42%)
		ciprofloxacin	32 (30,77%)	73 (69,23%)
Cyclines		tetracycline	76 (73,08%)	28 (26,92%)

N: Number of strains isolated; R: Resistant; S: Susceptible.

Table 3: Profile of resistance of 20 strains of *Salmonella* isolated from chicken gizzard in the District of Abidjan.

Strains	Municipalities	Serogroups	Serovars	Profile of resistance
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	AAMCTicCfTeCip
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

ND: Non determinated. A: amoxicillin; AMC: amoxicillin/clavulanic acid; Tic: ticarcillin; Cef: cephalotin; Ctx: cefotaxime; G: gentamicin; C: chloramphenicol; Sxt: cotrimoxazol; Nal: nalidixic acid; Cip: ciprofloxacin; Te: tetracycline

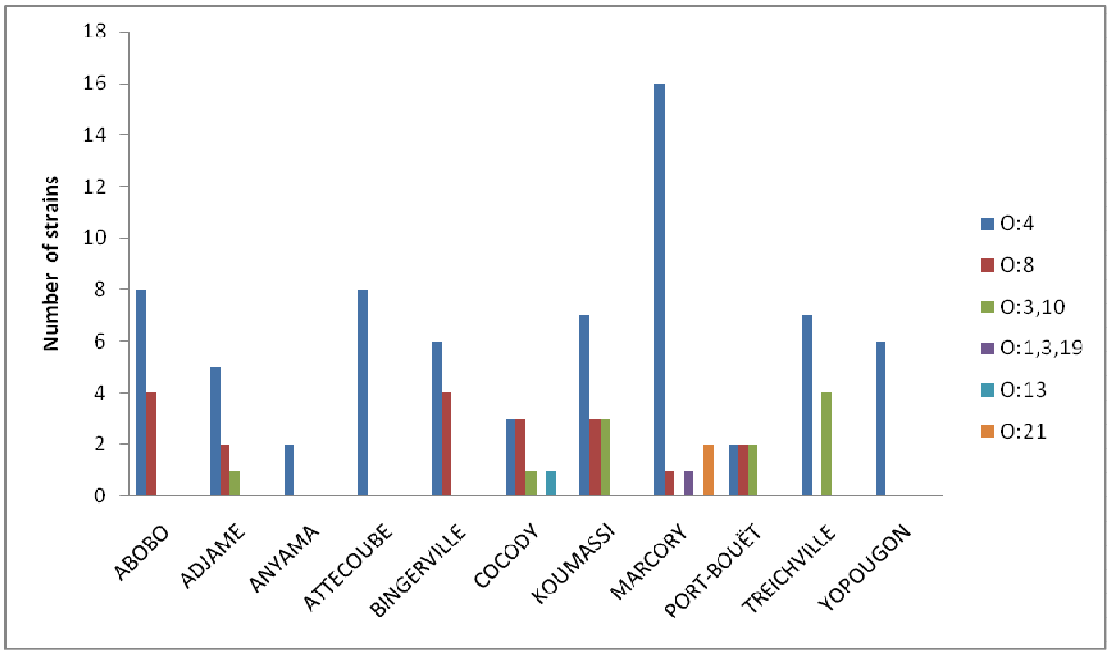

Figure 1: Distribution of *Salmonella* serogroups in the District of Abidjan, Côte d'Ivoire.

DISCUSSION

The majority of strains of *Salmonella* isolated in our study possessed antigen O:4 with a proportion of 67.31%, and antigen O:8 with proportion of 18.27%. These two antigens (O:4 and O:8), represent most of 85% of *Salmonella* isolated. These rates presented similarly with studies conducted in some countries. A studies conducted in Turkey, revealed that *Salmonella* isolated from chicken carcasses also showed a high prevalence (69.05%) for antigens O:4 and O:8 (Yildirim et al., 2011). Likewise in Algeria, an important presence of *Salmonella* strains isolated from chickens contained antigens O:4 and O:8, with proportion of 61.55% (Elgroud et al., 2008). In Malaysia, *Salmonella* strains isolated from ducks, showed also high proportion (80%) of strains with antigens O:4 and O:8 (Adzitey et al., 2011).

Salmonella are among the main pathogens involved in several reported cases of food toxi-infection. This is particularly the cas of salmonellosis due to *Salmonella* O:4, such as *Salmonella* Typhimurium linked to the consumption of undercooked beef (Dencos, 2000) and *Salmonella* Coeln linked to the consumption of poultry meat and beef in France (Haeghebaert et al., 1998). Several outbreaks of salmonellosis due to *Salmonella* Agona described in other countries, were linked to the consumption of powdered milk in the Czech Republic (Sramova et al., 1991) and France (Brouard et al., 2005) and herbal tea anise and fennel, in Germany (Koch et al., 2005). These cases of Salmonellosis were accompanied by nausea, vomiting and diarrhea. These observations indicate that, there is a probable link between the antigen O:4 and pathogenicity of the strain.

Other *Salmonella* infections were noted, particularly for strains with antigen O:8. Salmonella Kentucky for example, involved in an explosion of salmonellosis since 2002 in Europe and Africa, with poultry as primary vector (Le Hello et al., 2011). *Salmonella* Manhattan has also been involved in a large outbreak of salmonellosis associated with meat products of porcine origin in France (Noel et al., 2006). Similarly an outbreak of salmonellosis due to *Salmonella* Hadar, linked to consumption of undercooked poultry and roasted poultry in France (Decludt et al., 1995). These cases were also accompanied by diarrhea and gastroenteritis. These observations also indicate that, there is a probable link between antigen O:8 and pathogenicity of the strain.

The study of the antibiotic susceptibility of *Salmonella* strains isolated showed high rates of resistance to cotrimoxazole and tetracycline. With tircacilline, nalidixic acid, ciprofloxacin and chloramphenicol, rates are relatively average compared to gentamycin, amoxicillin and amoxicillin/clavulanic acid. The level of antibiotic resistance observed in this study is comparable to those observed in Malaysia (Chen et al., 2004; Thong et al., 2002), and in Senegal (Toko, 2010).

The relatively high resistance obtained with fluoroquinolones in this study is comparable to those observed in Senegal (Fofana, 2004). The emergence of resistant strains to fluoroquinolones is due to their unreasonable use in the poultry field. Long-term treatment using antibiotic as Sulfonamides or Tetracyclines, and the use of criticism families for human medicine (cephalosporins and fluoroquinolones), promote the emergence of resistance in these molecules, especially with increasing use of enrofloxacin in poultry (Chauvin et al., 2005).

The emergence of strains resistant to multiple antibiotics has been reported in Salmonella serotypes associated with poultry (Antunes et al., 2003; Zhao et al., 2005).

The excessive and uncontrolled use of antibiotics may lead to the selection of resistant bacteria (Chaslus-Dancla, 2003). The development and emergence of resistance in pathogenic bacteria to humans and animals are the result of use of these molecules with poor understanding of the environmental impact of their use on the bacterial microflora (Sanders, 2005).

Salmonella strains isolated in Abidjan, with their two types of antigens (O:4 and O:8), could be considered as potential pathogens.

Conclusion

This study confirmed that the chickens' gizzards might act as the reservoirs for antimicrobial resistant bacteria. Therefore, to control the further emergence of antimicrobial resistance, monitoring the food processing and the careful use of antibiotics in animal husbandry is essential.

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