



Prevalence of *Salmonella* and distribution of Serovars Isolated from Retail Raw Chicken Gizzards in Abidjan, Côte D'ivoire

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ABSTRACT

The present study was conducted to determine the prevalence of *Salmonella*, antibiotic susceptibility and distribution of serovars in retail raw chicken gizzards in Abidjan, Côte d'Ivoire. A total of 300 samples of chicken gizzards were collected from a big central retail market in Adjamé from October to March (6 months) and examined for the presence of *Salmonella*. For the isolation and identification of *Salmonella*, the technique recommended by the international organization for standardization (ISO6579, 2002) was used. Serotyping was conducted by slide agglutination test using *Salmonella* polyvalent O and H antisera, a French AFNOR reference method. Determination of antibiotic profiles of *Salmonella* strains was carried out by the CLSI method of disk dissemination in Mueller Hinton (MH) agar medium. *Salmonella* was detected from 156 (52.00%) of the 300 samples examined. No typhoidal *Salmonella* (0.00%) was detected. Out of the 156 *Salmonella* isolated, 9.62% (15/156) of the strains were found lactose positive. Out of the 156 *Salmonella* isolates, four different serovars were identified: *Salmonella* Hadar the most prevalent (71.15%), *Salmonella* Enteritidis (3.85%), *Salmonella* Derby (3.20%), *Salmonella* Essen (3.20%), and non determined serovars (18.60%). The overall profile of antibiotic resistance of the strains showed high rates of resistance to Tetracyclin (94.08%), nalidixic acid (70.39%), ampicillin (67.76%) and amoxicillin + nalidixic acid (63.09%). *S. Hadar* showed a high level of resistance to tetracycline (94.50%), nalidixic acid (89.91%), ampicillin (89.00%) and amoxicillin + nalidixic acid (80.56%).

INTRODUCTION

Salmonella is a leading cause of bacterial foodborne disease outbreaks in developed countries (Bean et al., 1997; Clark, 2005; D'Aoust, 1994; Flint et al., 2005; Hedberg et al., 1999; Poppe, 2000; Roberts, 1988; Voetsch et al., 2004; White et al., 1997). In developing countries, *Salmonella* is of a public health concern (Anyanwu and Jukes, 1990; Dosso et al., 1998; Fonkoua and Wouafo, 2002; Medeiros et al., 2001; Nola et al., 1998; Seydi et al., 2005; Tibaijuka et al., 2003; Wouafo et al., 1996). Diarrhoea, the common enteric disease caused by *Salmonella* causes death of millions and millions of children each year in many countries (Archer and kvenberg, 1985; Flint et al., 2005; Motarjemi et al., 1993; Roberts, 1988; Swartz, 2002; Voetsch et al., 2004). In Africa especially, *Salmonella* is an important opportunistic cause of infection in patients with AIDS (Seydi et al., 2005). A wide range of foods is implicated in foodborne disease due to *Salmonella* (Hedberg et al., 1999). However, foods of animal origin, especially poultry and poultry products, are widely acknowledged to be the reservoir of *Salmonella* due to the ability of *Salmonella* to proliferate in the gastrointestinal tract of chickens (Baeumler et al., 2000; Barrow et al., 2004; Keller et al., 1995; Poppe, 2000). In Côte d'Ivoire, the gizzards of chickens are too much valued by the population who consumes them cooked to the ember and sold in the streets or cooked in sauce. These products of very big consumption remain badly known as for their microbiological quality in particular for their ability to carry pathogens such as *Salmonella*. Traoré (Traoré, 2003) reported 55.66% of contamination level in chicken intestines in Côte d'Ivoire, but no data is available concerning the contamination rates of *Salmonella* in gizzards of chickens. In African countries, data are still nonexistent or very scarce on the topic. We therefore conducted this study to determine the prevalence of *Salmonella* in retail raw chicken gizzards and the distribution of serovars isolated from these products in Côte d'Ivoire.

MATERIALS AND METHODS

Sample Collections

A total of 300 samples of chicken gizzards were collected from a big central retail market (slaughtering more than 650 chickens per day) in Adjamé. Twenty single gizzards of chickens were purchased with the poulterer just after slaughtering and evisceration of the poultry. Each sample (whole gizzard) was placed in a separate sterile plastic bag. 20

samples were collected once a week and placed in an ice chest and immediately transported to the laboratory (less than 1 hour); analysis were realised upon arrival to the laboratory. The study was conducted during 6 months from October to March.

Microbiological analysis

Isolation and identification of *Salmonella* strains were conducted according to the technique recommended by the international standards organization (ISO6579, 2002). A 25 g portion of each sample was taken aseptically by scalpel excision and placed in stomacher bag containing 225 ml of buffered peptone water (AES Laboratoires, Combourg, France). The samples and the buffered peptone water were treated in a stomacher 400 (AJ Seward, London, UK) for 2 min at average speed and incubated together in the bag for 16 - 20h at 37°C. After incubation, the pre-enriched samples were diluted in both 10ml of Rappaport Vassiliadis broth (RVB) and 10ml of Müller Kauffmann tetrathionate broth (MKB) and incubated for 16 - 20h at 42°C and 37°C respectively. A loopful of the overnight enrichment broths (RVB and MKB) was streaked onto Hektoen agar and SS agar plates and incubated for 16 to 20 hours at 37°C. After examination of colonies, one to five green, blue-green or blue colonies with or without black centre (presumptive *Salmonella* colonies) onto Hektoen agar were selected in each sample for subsequent biochemical identification. Onto SS agar, one to five uncoloured colonies with or without black centre (presumptive *Salmonella* colonies) were selected in each sample for the same subsequent identification. The biochemical identification was based on the following tests: Gram stain (Harrigan and McCance, 1976), oxidase reaction, fermentation/oxidation of glucose, motility test, catalase reaction, nitrate reduction (Cowan, 1974), triple sugar iron (Pouillot et al.), gas production, sulphide (H₂S) production, urease production, indole production, glycerol fermentation and lysine decarboxylase (LDC) production. The strains were grouped in biochemical profiles depending on the results of previous tests. Two groups were differentiated. The first group contained typhoidal *Salmonella* (*Salmonella* Typhi and *Salmonella* Paratyphi A) identified according to the biochemical tests. The second group harboured non-typhoidal *Salmonella* and related *Enterobacteriaceae* such as *Citrobacter* sp., *Edwardsiella tarda*, *Proteus mirabilis* and *Proteus vulgaris* characterized by their capacity for gas and H₂S production. Urease production, indole production, glycerol fermentation and lysine decarboxylase (LDC) production allowed identification of non-typhoidal *Salmonella* among the group of the gas and H₂S producers (Table 1).

The non-typhoidal *Salmonella* strains biochemically confirmed were serotyped in the “unité de sérotypage des *Salmonella*” polyvalent O and H antisera (Diagnostic Pasteur, Paris, France). This procedure refers to the French AFNOR (French Agency of Standardization) procedure NF U 47-100 which is the reference method. Some serotyping results were confirmed by the “Centre national de référence des *Salmonella* et centre collaborateur OMS”, Pasteur Institute of Paris. The CLSI (Huang et al., 2012) method of disk dissemination onto a medium agar was performed. An inoculum was prepared with 3 to 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 homogeneous suspension of density equivalent to 0.5 Mc Farland standards. Using a sterile swab moistened in the suspension, a MH agar previously dried was seeded by swabbing the entire surface of the MH 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 disks and the agar was incubated 18 to 24h. After incubation, agar plates were read by measuring inhibition zones around each antibiotic disk with a graduated ruler. According to the CLSI standards, results were interpreted and transcribed by the terms: susceptible (S), intermediate (I) and resistant (R). The strains resistant to three or more antimicrobials from different classes were considered as Multidrug-Resistant (MDR).

RESULTS AND DISCUSSION

Salmonella was detected in 52.00% of the 300 chicken gizzard samples examined (Table 2). Non typhoidal *Salmonella* (00.0%) was detected in the study. 9.62% (15/156) of the strains were found lactose-positive and 90.38% (141/156), lactose-negative. 18.60% of the 156 strains were untyped isolates. We used a pre-enrichment followed by selective isolation method (ISO6579, 2002) which is an effective method for detecting common *Salmonella* in food and environmental samples. The prevalence of *Salmonella* sp. in chicken gizzards (52.00%) we obtained should be taken with caution because the studied sample was not randomised from the overall broiler flocks from Abidjan. Notwithstanding, our results are comparable to some others in developed and developing countries. Even though prevalence depend on the country, we noticed in developed countries: 11.99% in the USA (Roy et al., 2002); 54.38% in Canada (Guerin et al., 2005); 70% in France (Rose et al., 1999); 55% in carcasses and 40% in giblets (gizzards and livers) in Spain (Capita et al.,

2003); 25% in the United Kingdom (Jorgensen et al., 2002); 60% in Portugal (Antunes et al., 2003); 25% in Denmark (Chadfield et al., 2001); 18.6% in Turkey (Carli et al., 2001) and 14.3% in Japan (Limawongpranee et al., 1999).

In developing countries, data are not abundant on the topic: in Thailand, Vadhanasin and al. (2004) reported 24.6% (Vadhanasin et al., 2004); 36.6% was announced in Albania (Goksoy et al., 2004); 41% in Brazil (Fuzihara et al., 2000); 43.3% in Senegal (Cardinale et al., 2005) and 53.1% in Ethiopia (Tibaijuka et al., 2003). Our prevalence (52.00%) is close to the incidence reported in Ethiopia, in Canada and equal to the rate in carcasses in Spain. We deem our prevalence high. Slaughterers (traditional ones in open markets) are provided with chickens that are not inspected by veterinarians. Therefore, chickens could be *Salmonella* asymptomatic carriers or diseased chickens. Nevertheless, it should not be an important risk for consumers since products (raw gizzards) are expected to be heated sufficiently before consumption. But as gizzards are consumed by the whole people, these products may be risky if they were not well heated and consumed.

Out of the 156 *Salmonella* isolates, four different serovars were identified: *Salmonella* Hadar the most prevalent (71.15%), *Salmonella* Enteritidis (3.85%), *Salmonella* Derby (3.20%), *Salmonella* Essen (3.20%), and non-determined serovars (18.60%) were obtained as shown in table 3. The four serovars accounted for 81.40% of the serotyped isolates. With 71.15%, *Salmonella* Hadar was the most prevalent serovar. *Salmonella* Hadar appeared to be an emerging pathogen in our environment especially in poultry. The first isolation of *S. Hadar* in Côte d'Ivoire was reported in 2003 (Traoré, 2003) in retail raw chicken intestines. Until 2003, *Salmonella* Hadar had never been reported in Côte d'Ivoire neither in food, humans nor in the environment. Traoré (2003) reported a contamination level of chicken intestines of 55.60% (Traoré, 2003) with a prevalence of 78.44% for *Salmonella* Hadar in Côte d'Ivoire. *Salmonella* Hadar was also reported in Senegal (western African country) as the most prevalent serovar (Cardinale et al., 2005), in UK (Jorgensen et al., 2002), in the Netherlands (Evers and Nauta, 2001). *Salmonella* Hadar was also isolated frequently from poultry in Canada (Chambers et al., 1998; Guerin et al., 2005), in Spain (Dominguez et al., 2002), in Thailand (Bangtrakulnonth et al., 2004), in Japan (Limawongpranee et al., 1999) *Salmonella* Derby (3.20%) and *Salmonella* Essen (3.20%) are not reported as serovars frequently isolated from poultry and poultry products. The overall profile of antibiotic resistance of the strains showed high rates of resistance to Tetracyclin (94.08%),

nalidixic acid (70.39%), ampicillin (67.76%) and amoxicillin + nalidixic acid (63.09%). Low resistance levels were observed with gentamycin (8.55%) and cotrimoxazole (3.29%).

Almost all of the strains showed a susceptibility to ceftriaxone, chloramphenicol and ciprofloxacin (table 4). *S. Hadar* showed a high level of resistance to tetracycline (94.50%), nalidixic acid (89.91%), ampicillin (89.00%) and amoxicillin + nalidixic acid (80.56%). This resistance was less marked with gentamycin. The resistance levels to cotrimoxazole, ciprofloxacin, ceftriaxone and chloramphenicol remained very weak. The serovar *Hadar* was illustrated particularly by its faculty of simultaneous resistance to several antibiotics (at least 4 antibiotics) such as ampicillin, nalidixic acid, gentamicin and tetracycline (Table 5) The antibiotic resistance of the major serovar (*Hadar*) showed a resistance to beta-lactams (ampicillin) and quinolone (nalidixic acid) with however a decreased susceptibility to ciprofloxacin coupled with a resistance to nalidixic acid. *Salmonella Hadar* was able to withstand to at least four antibiotics (ampicillin, tetracycline, nalidixic acid and gentamicin) thus showing its faculty of multiple resistance. The high proportion of this serovar among strains of intermediate resistance could indicate that the resistance of *Salmonella Hadar* to antibiotics is always in evolution.

The resistance profile of avian strains of *Salmonella Hadar* is comparable to that of the strains of *Salmonella Hadar* of human origin isolated in hospitals in France in 1997 and in 2000 (Breuil et al., 2001). In effect these strains showed 93 per cent of resistance to ampicillin and 90 % to nalidixic acid; however they are all susceptible to cotrimoxazole. In comparison, the *Salmonella Hadar* we isolated from the chicken gizzards were resistant to ampicillin (89.00%) and to nalidixic acid (89.91%) but also susceptible to cotrimoxazole. This correlation can suggest that the avian strains isolated in Côte d'Ivoire could bear the same antimicrobial resistance than those of food origin. The characterization of resistance genes to beta-lactams and their genomic environment coupled with the study of epidemiological markers and genotypic properties brought more elements in favor of a clonal selection of multidrug-resistant strains of a possible transfer of resistance genes to bacteria previously susceptible (Breuil et al., 1998).

The resurgence of salmonella in general and of *Salmonella Hadar* in particular in poultry in Côte d'Ivoire indicated that the expansion of production takes place without any systematic control of hygiene in the poultry sector, while the emergence of resistant clones to antibiotics would result from a non-regulated

use of antibiotics in livestock farms. In these conditions, poultry meats on markets could reach unacceptable levels of contamination by *Salmonella*, without counting the cross-contamination with passage of these bacteria from poultry to other foods eaten raw. Risks of food-borne outbreaks due to *Salmonella Hadar* are real in our country, particularly with the development of the street restoration as for the fast foods in developed countries. Elsewhere in France, in 1994 *Salmonella Hadar* was found responsible for an outbreak affecting 164 people. In 1995 *Salmonella Hadar* (in the same country) was the cause of 15 outbreaks of foodborne illness (Decludt et al., 1996). Many authors suggested a very close link between these infections and the consumption of poultry meat uncooked (Decludt et al., 1996; LOOVEREN et al., 2001). Generally there are in the developed countries, an overlay between the epidemiology of salmonella in food products and those of the human strains both at the level of the distribution of dominant serovars and at the level of the antimicrobial resistance. The reason for this link is the transmission of these strains from foods to humans. In the Côte d'Ivoire, the parallelism concerning the serovar *Hadar* in poultry cannot be established in a formal way in the measure the impact of *Salmonella Hadar* in humans remains unknown.

The reason why the emergence of salmonella in poultry since 2003 is not correlated to an increase of human infection remains unknown. But it could be due to the fact that in Africa we usually eat well cooked; the cooking process constituting a waterproof barrier which allowed avoiding these bacteria. However, the emergence of new dishes with new culinary practices involving new methods of cooking, the more often modeled on those of developed countries who already knows the problem of *Salmonella Hadar*, may result in the survival of the bacteria during cooking and thus can cause an increase of salmonellosis in humans in the Côte d'Ivoire.

Although it is well-known that *Salmonella* is the most important representative bacteria in the microflora of poultry and other domestic animals (Barrow et al., 2004; Keller et al., 1995), we deem the prevalence of 52.00% of *Salmonella* in the gizzards, the prevalence of *S. Hadar* (71.15%), the overall antibiotic resistance of the strains (up to 63%) and this of *S. Hadar* high and then must be of a particular interest. *Salmonella Hadar* appears to be an emerging pathogen in these products. The bacteria (*Salmonella Hadar*) must be awarded to avoid public health problems.

CONCLUSION

In conclusion, raw chicken gizzards are contaminated by *Salmonella* at a high prevalence (52.00%). Only *Salmonella enterica* sub-species enterica is the only one species with four non-typhoidal serovars such as *S. Hadar* the most important (71.0%), *S. Enteritidis* (3.85%), *S. Derby* and *S. Essen* with (3.20%). Strains are multidrug-resistants (MDR) to at least four antibiotics (Tetracyclin, nalidixic-Acid, Ampicillin and Amoxicillin+ nalidixic Acid). The bacteria show an increased resistance to antibiotics.

We did not found typhoidal salmonella strains in the raw chicken gizzards but in the context where Côte d'Ivoire is one of the most affected countries by AIDS in Africa, and the increased resistance to antibiotics observed, *Salmonella* must be of a particular interest in these products. Especial wariness must be taken to prevent public health problems. We recommend especially training for the basics of food hygiene and the prevention of cross-contaminations.

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