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Sources of contamination of poultry by *Salmonella* Spp. multi-resistant to antibiotics (SMR) in Côte D'ivoire

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Abstract
The objective of this work is to highlight the plausible ways of contamination of chicken in Côte d'Ivoire and their impact on public health. For this purpose, the isolation and antibiotic sensitivity tests carried out on 24 strains of *Salmonella* isolated from poultry (farmed chickens and native chickens) were performed. The agar diffusion method, carried out on Muller-Hinton agar, was use to reveal the antibiotic profiles of these strains. The results obtained show a level of resistance: high to Amoxicillin / Clavulanic Acid (88.23%), to Tetracycline (88.23%), to cotrimoxazole (64.70%), to Nalidixic Acid (76.47%), Ticarcillin (70, 59%), Amoxicillin (64.70%) and Cefoxitim (64.70%); a relatively average level of resistance to Chloramphenicol (47.06%) and Cefotaxim (35, 29%) in *Salmonella* isolated from farm chickens. As for *Salmonella* isolated from native chickens, a very high resistance to Tetracycline and Amoxicillin / Clavulanic Acid (100%), as well as a relatively average level of resistance to Cotrimoxazole (42.86%), Nalidixic Acid (57.14%), Ticarcillin and Amoxcillin (42.85%) were observed. Sulfonamides, β-Lactams, Quinolones and Tetracyclines, are the antibiotic families most involved in the (multi) resistance of isolated strains of *Salmonella* spp. In view of these data, the environment could play an important role in the transmission pathway of antibiotic resistance in poultry, apart from the way in which antibiotic molecules are used. This could constitute a real public health problem.

Keywords: environment, *Salmonella*, poultry, antibiotic molecules, public health

Introduction
Salmonellosis is one of the main causes of foodborne gastroenteritis in humans [1]. They cause symptoms of a wide range of severity, from mild abdominal pain and varying degrees of enteritis, to septicaemia and in extreme cases, death. Infections may remain inapparent, but asymptomatic latent carriage with intermittent spread of *Salmonella* is common [2]. In developed countries, *Salmonella* is one of the main causes of foodborne bacterial disease [3]. *Salmonella* enterica, with its ubiquitous serotypes, is the main pathogen of food contamination for human consumption [4]. *Salmonella* infection is very commonly associated with the consumption of meat and meat products, especially poultry products. Indeed, poultry plays a major role as vector of transmission in human cases of salmonellosis [1]. For many years, *Salmonella* has been the major cause of human digestive tract infections associated with the consumption of food of animal origin, such as poultry meat products and, in particular, eggs [5]. In addition, the consumption of poultry meat has grown considerably on all continents, with volumes trated worldwide increasing, by 10% per year [6]. Poultry is an important source of protein of animal origin for the Ivorian population. However, breeding and slaughtering practices lag considerably behind those of industrialized countries, not only in terms of the productivity of poultry farms, but also and above all in terms of public health. Indeed, the health problems of the poultry industry still depend on farming conditions in general, and more particularly on the hygiene of buildings [7]. The increase and accumulation of antibiotics resistance by *Salmonella* is another aspect of the public health problem, as it is currently accepted in developed countries, but also in developing countries, that some of multidrug-resistant strains found in humans are of animal origin and have acquired resistance determinants on farms before being transmitted to humans through food [8]. Because of this ability to resist antibiotics, bacteria are a constant threat to consumers.

Material and methods
The animal material used in this studied consisted of chicken viscera (broilers and natives) (Figure 1), collated from farms sites and private homes in the commune of Bingerville. *Salmonella* bacterial strains ATCC 14028 and IPCI 8297 were used as controls for antibiotic susceptibility testing. Ten (10) grams of chicken viscera are taken from 30 broilers and 15 native chickens, respectively. The test samples thus obtained were subjected to microbiological analysis for the isolation of the *Salmonella* strains, in accordance to the standard NF EN ISO

6579 [9] comprising 4 stages: pre-enrichment, enrichment, isolation and l biochemical identification on a reduced rack Le Minor.

The presumptive *Salmonella* strains obtained after microbiological analysis were subjected to an antibiogram to test their resistance to certain antibiotic molecules used in both veterinary and human medicine during *Salmonella* infections. The antibiogram carried out on all the isolated strains was performed by agar diffusion according to the CLSI (Clinical Laboratory Standard Institute) on Müller-Hinton agar [10]. The following antibiotic discs are tested: amoxicillin (AMX, 10µg), amoxicillin/clavulanic acid combination (AMC, 10 / 20µg), ticarcillin (TIC, 75 µg), cefalotin (CF, 10µg), cefoxitin (FOX, 10µg), cefotaxime (CTX, 10µg), gentamicin (GM, 10µg), nalidixic acid (Nal, 10µg), ciprofloxacin (Cip, 10µg), Cotrimoxazole (SXT, 10 / 20µg), tetracycline (TE, 10µg) and chloramphenicol (C, 10 µg).

Results

Microbiological analysis of the various chicken viscera revealed the presence of 17 strains of *Salmonella* spp in the viscera of broilers against 7 strains of *Salmonella* in the viscera of native chickens, i.e. respective carriage rates of 56.66 and 46.66 %.

The study of antibiotic resistance of *Salmonella* strains, carried out on all the strains, made it possible to be classify them according to the CLSI standard as resistant, intermediate or susceptible. Considering a resistant strain, an intermediate strain categorized as non-susceptible, it emerges from the study of the overall resistance of the *Salmonella* strains isolated from broiler viscera shows that the strains show a high level of resistance to β-lactams [Amoxicillin / Clavulanic acid (88.23%), Ticarcillin (70, 59%), Amoxicillin (64.70%) and Cefoxitin (64.70%)], Cyclins [tetracycline (88.23%)], Sulfonamides [cotrimoxazole (64.70%] and Quinolones [Nalidixic Acid (76.47%)]. However, the strains show a relatively average level resistance to phenicol [Chloramphenicol (47.06%)]. As for *Salmonella* isolated from indigenous chickens, total resistance was observed to the Cyclins [Tetracycline (100%)] and β-lactams [Amoxicillin / Clavulanic acid (100%)], as well as a relatively average level of resistance to Sulfonamides [Cotrimoxazole (42.86%)], Quinolones [Nalidixic Acid (57.14%)], and to certain molecules of the β-lactam family such as Ticarcillin and Amoxicillin (42.85%) (Figure 2). Taken together, Sulfonamides, β-Lactams, Quinolones and Tetracyclines, are the antibiotics families most involved in the (multi) resistance of *Salmonella* spp strains isolated from chicken viscera. The *Salmonella* strains isolated in this study also show resistance to several antibiotic molecules, i.e 2 to 6 antibiotic molecules for the isolates obtained from native chicken viscera, and 1 to 9 molecules for isolates obtained from broilers (Table I). The multidrug resistance profiles observed were : AMC - NAL - TET (1 strain (14.28 %)) ; SXT - AMC - CTX - TET (1 strain (14.28 %)) ; AMC -AMX -TIC - NAL– TET (1 strain (14.28 %)) ; SXT - AMC - AMX - TIC - NAL -TET (2 strains (28.57 %)), for the isolates obtained from the native chicken viscera, and the profiles SXT - AMC - TET profiles (1 strain (5.88 %)) ; SXT - FOX - NAL - TET (5.88 %) ; SXT - AMC - AMX - TIC -TET (5.88 %) ; SXT - AMC - FOX - NAL - TET (5.88 %) ; SXT - AMC - AMX - CHL - TET (5.88 %) ; SXT - AMX - CEF - FOX - NAL - TET (5.88 %) ; SXT - AMC - AMX - CHL - TIC - TET (5.88%) ; SXT - AMC - AMX - FOX - CHL - TIC - NAL - TET (5.88 %) ; SXT - AMC - AMX - CTX - FOX - CEF - TIC - TET (5.88 %) ; AMC - AMX - FOX - CTX - CHL - TIC - NAL - TET (5.88 %) ; SXT - AMC - AMX - CEF - FOX -CHL - TIC - NAL - TET (5.88 %) (Table 2). Figure 3 shows the various plausible pathways at the origin of the contamination of the two types of chickens and their impact on human health. The immediate environment of the chicken (feeds troughs, hatcheries, feed, faeces) and that of humans (human waste, faeces from farmed chickens used as fertiliser for agricultural soil enrichment, open sewage water) are the key factors involved in the spread and dissemination (multi) antibiotic resistant pathogenic strains such as *Salmonella* spp.

Table 1: Antibiotic resistance profiles of *Salmonella* strains isolated from the viscera of broilers and native chickens

Isolation matrices	Antibiotic resistance profiles	of molecules	of strains
	AMC-TET	2	2
Native chickens	AMC-NAL-TET	3	1
	SXT-AMC-CTX-TET	4	1
	AMC-AMX-TIC-NAL-TET	5	1
	SXT-AMC-AMX-TIC-NAL-TET	6	2
Total strains			7
	NAL	1	1
	NAL – TET	1	1
	AMC – TET	2	2
	SXT – AMC – TET	3	1
	AMC-FOX-CTX-TIC	4	1
	SXT – FOX – NAL – TET	4	1
	SXT-AMC-AMX-TIC-TET	5	1
Broilers	SXT – AMC – FOX – NAL – TET	5	1
	AMC – CTX – FOX – TIC – NAL	5	1

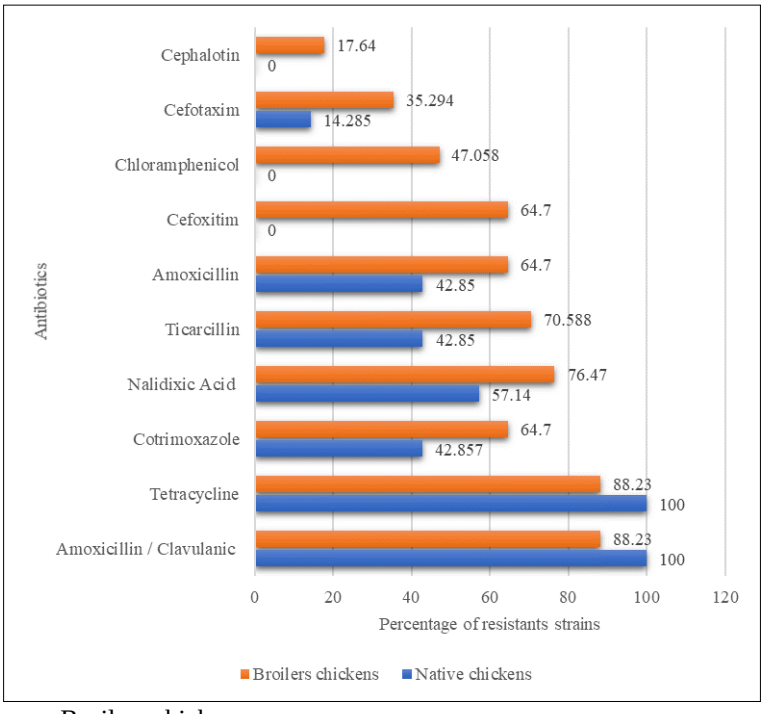
	SXT – AMC – AMX – CHL – TET	5	1
	SXT-AMX-CEF-FOX-NAL-TET	6	1
	SXT – AMC – AMX – CHL – TIC – TET	6	1
	SXT-AMC-AMX-FOX-CHL-TIC-NAL-TET	8	1
	SXT – AMC – AMX – CTX – FOX – CEF – TIC-TET	8	1
	AMC – AMX – FOX – CTX – CHL – TIC – NAL – TET	8	1
	SXT-AMC-AMX-CEF-FOX-CHL-TIC – NAL-TET	9	1
Total strains			17

SXT : Cotrimoxazole; AMC: Amoxicillin / clavulanic acid; AMX: Amoxicillin; CEF: Cephalotin; FOX: Cefoxitin; CTX: Cefotaxim; CHL: Chloramphenicol; TIC: Ticarcillin; NAL: Nalidixic acid; TET: Tetracycline

Table 2: Multidrug resistance profiles of *Salmonella* strains isolated from the viscera of broilers and native chickens

Matrices	Antibiotic resistance profiles	ATB families	Strains (%)
	AMC-NAL-TET	3	1/7 (14.28 %)
Native chickens	SXT-AMC-CTX-TET	3	1/7 (14.28 %)
	AMC-AMX-TIC-NAL-TET	3	1/7 (14.28 %)
	SXT-AMC-AMX-TIC-NAL-TET	4	2/7 (28.57 %)
Total strains			5/7 (71.42 %)
	SXT – AMC – TET	3	1/17 (5.88 %)
	SXT – FOX – NAL – TET	4	1/17 (5.88 %)
	SXT-AMC-AMX-TIC-TET	3	1/17 (5.88 %)
	SXT –AMC–FOX–NAL–TET	4	1/17 (5.88 %)
	SXT–AMC–AMX–CHL–TET	4	1/17 (5.88 %)
Broilers chickens	SXT-AMX-CEF-FOX-NAL-TET	4	1/17 (5.88 %)
	SXT–AMC–AMX–CHL–TIC–TET	4	1/17 (5.88 %)
	SXT-AMC-AMX-FOX-CHL-TIC-NAL-TET	5	1/17 (5.88 %)
	SXT–AMC–AMX–CTX–FOX–CEF–TIC-TET	3	1/17 (5.88 %)
	AMC–AMX–FOX–CTX–CHL–TIC–NAL–TET	4	1/17 (5.88 %)
	SXT-AMC-AMX-CEF-FOX-CHL-TIC–NAL-TET	5	1/17 (5.88 %)
Total strains			11/17 (64.70 %)

SXT : Cotrimoxazole; AMC: Amoxicillin / clavulanic acid; AMX: Amoxicillin; CEF: Cephalotin; FOX: Cefoxitin; CTX: Cefotaxim; CHL: Chloramphenicol; TIC : Ticarcillin; NAL: Nalidixic acid; TET: Tetracycline; ATB: Antibiotics



: Broilers chickens;
: Native chickens

Fig 1: Resistance of *Salmonella* Spp. isolated from the viscera of native and broilers chickens

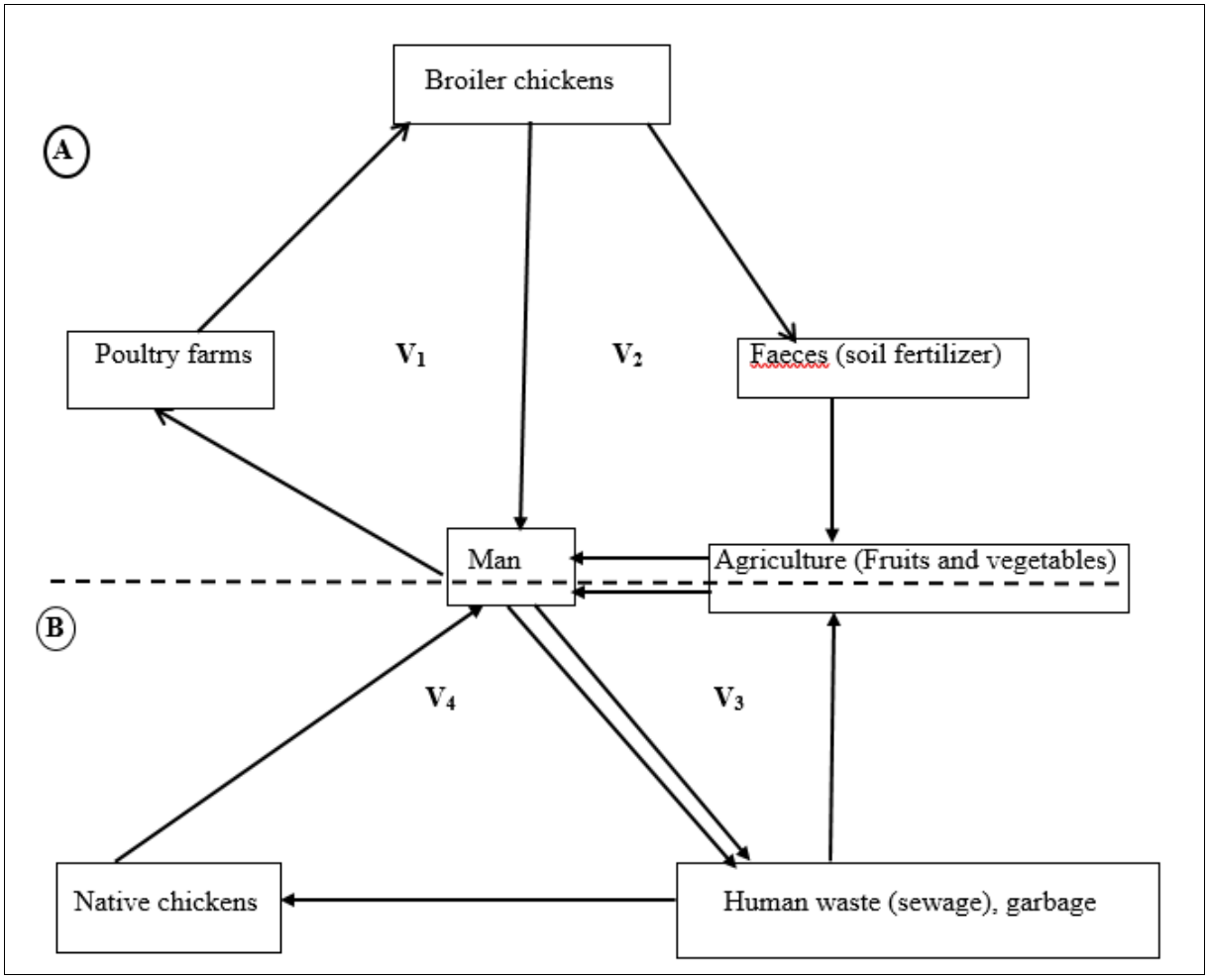


Fig 2: Plausible routes of contamination of chickens and their impact on human health A: Immediate environment of the farmed chicken; B: Immediate human environment

Discussion

Antibiotics, used for variety of purposes and their role is to cure or prevent infections of bacterial origin. The spread of antibiotic resistance in bacterial to is due to a number of factors. As we can see, various reasons could explain the mode of circulation and the origin of the contamination of poultry with *Salmonella* spp. (multi) resistant to antibiotics, in this present study. The overuse of antibiotics for different purposes (curative and preventive and as a growth activator) by humans on Ivorian poultry farms is the primary factor in the spread of multiply antibiotic resistant *Salmonella* in broilers. Indeed, some studies testify to the appearance of multiresistant *Salmonella* strains in connection with the use of antibiotics [11, 12, 13, 14, 15].

These and other studies have also shown that the use of antibiotics in the animal sector constitutes selection pressure and promotes the transmission of resistant mutants to humans either through direct contact or through food [16]. In poultry, antibiotic resistance in zoonotic enteropathogens is particularly dangerous in terms of human health as these bacteria can be transmitted to humans through the food chain [17]. Therefore, the exposure of the human population to antibiotics and the inter-individual and environmental transmission of (multi) resistant strains to antibiotics remains a crucial public health problem [18].

Indeed, the emergence of multi-antibiotic resistant *Salmonella* strains would be linked to the environment in both broiler and native chicken, following four plausible routes of contamination with an impact on consumer health.

The first pathway would highlight the impact of the abusive use of antibiotic molecules for different purposes (growth promoter, prophylaxis and care of broilers) on poultry farms. This favours the selection of antibiotics resistant microorganisms in chickens intended for human consumption and their dissemination in their immediate environment by means of released droppings. The second pathway addresses the potential hazard posed by droppings loaded with multi-antibiotic resistant *Salmonella* spp. used in agriculture for soil enrichment and the production of fruit and vegetables for human consumption. The third pathway considers human waste (human faeces, sewage and household waste), loaded with *Salmonella* spp. and released into the wild, used as agricultural soil fertiliser for the production of fruit and vegetables for human consumption. The fourth pathway considers the exposure of indigenous chickens to potential sources of contamination by the multi-antibiotic resistant *Salmonella* pathogen, and their impact on consumer health. Indeed, the ingestion of human waste and/or household refuse represents a source of contamination of indigenous chickens intended for human consumption.

From all these observations, antibiotic resistance in bacteria responsible for infectious diseases such as Salmonella spp. isolated from poultry appears to be a major public health problem that could become critical if it

continues to increase. From this point of view, it is clear that strategies to contain the emergence of antibiotic resistance are needed to limit the spread of such strains.

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Competing Interests
Authors have declared that no competing interests exist.

Conclusion
The use of antibiotics as feed additives in livestock, long banned by the European Union, is common in Africa and especially in Côte d'Ivoire, and is unfortunately at the origin of the appearance of multidrug resistance. The overuse of molecules in humans and their presence in the environment has contributed to the spread of antibiotic resistance. It is a growing threat to public health and development, requiring urgent action in multiple sectors to ensure food security, improve nutrition and promote sustainable agriculture.

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