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SHORT COMMUNICATION
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Antimicrobial Resistance in *Salmonella* spp. Isolated from Poultry Waste in Abidjan District, Côte d'ivoire

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ABSTRACT

Poor management of poultry waste can lead to major public health problems, particularly if this waste is contaminated by antibiotic-resistant pathogenic bacteria. The purpose of our study was to assess the potential risks associated with the presence of *Salmonella* in poultry waste. Thus, twenty-eight (28) samples of poultry manure from farm and 30 samples of poultry slaughterhouse waste were analyzed in accordance with ISO 6579 for the isolation of *Salmonella*. Among the 200 strains obtained after biochemical characterization, 150 were positive for the *inva* virulence gene. The antibiotics susceptibility evaluated by the disk diffusion method indicate that all the 150 Salmonella strains were resistant to at least one of the drugs tested in this study. The resistance rate ranged from 8.67 to 66.67% for betalactam, from 10 to 56.67% for fluoroquinolones and from 23.33 to 31.33% for Aminoglycosides. However, high resistance rates were detected for Ampicillin (66.67%), Ticarcillin (56.67%), Piperacillin (36.67%) and for Perfloxacin (56.67%). Moreover, multidrug resistance (MDR) including these three antibiotics families was also detected in 43.33% of the *Salmonella* tested isolates. These results show the need to set up a biological system for treating the poultry waste in order to promote the reduction of its negative effect on human and animal health.

Keywords: *Salmonella*, Virulence gene, MDR, Poultry waste.

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INTRODUCTION

The Ivorian poultry sector has experienced spectacular growth, with production increasing from 18,000 tons in 2011 to 68,360 tons in 2020 and could reach 200,000 tons in 2030. However, the intensification of poultry farming is allowing the production of a high amount of waste that is still poorly managed.

In addition, this intensification of poultry farming in Côte d'Ivoire is also accompanied by insufficient application of biosecurity measures and also uncontrolled use of antibiotics (Kone and Danho, 2008). These poor practices during breeding create a favorable environment for the emergence, development, and spread of resistant and multidrug-resistant pathogenic bacteria against to many drugs. The resistance of these bacteria to antibiotics today constitutes one of the most threats to global health, food security, and development, as it reduces the effectiveness of available antibiotics and increases the risks of therapeutic failure in avian diseases and the direct transmission of this resistance from livestock to humans (OMS, 2022; Nadi et al., 2024).

Among these pathogenic bacteria, infections due to Salmonella species remain a major public health concern worldwide and contribute to a high economic burden for both industrialized and developing countries through the costs associated with treating these diseases. Indeed, it is estimated that in most developing countries, human salmonellosis causes between 20 and 25% of deaths per year (de Melo et al., 2021; He et al., 2023). In addition, the emergence of multi-drug resistant *Salmonella* strains reduces all efforts to combat this threat worldwide. Most contaminations by this bacterium occur through the handling and consumption of poultry meat, eggs, as well as any products derived from farms, from production sites

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to slaughterhouses (Cox et al., 2000; Dunkley et al., 2009). Due to its presence in the digestive tract of poultry in general, poultry waste could be a reservoir for a large number of *Salmonella* species. In this context, poor management of these waste could contribute to a rapid spread of *Salmonella* in environment and thus increases the risk of its transmission to human and animal (Tacconelli et al., 2018; Schar et al., 2018; OMS, 2022; Iqbal et al., 2024). So, the objective of our study was to assess the potential risks associated with the presence of *Salmonella* in poultry waste by virulence gene detection and antibiotic resistance level determination.

MATERIALS & METHODS

Study Sites

In this study, two types of poultry waste were targeted for *Salmonella* genus. Thus, 28 poultry manure samples were collected from 16 farms of four municipalities (Anyama, Bingerville, Songon and Yopougon) of the District of Abidjan (Côte d'Ivoire) because of their high poultry production capacity in this aera. In each poultry farm, the manure was collected directly from stored bags which are intending for use in vegetables crop.

Concerning The poultry slaughterhouses waste they were sampled from the artisanal slaughterhouses located in the large markets of ten municipalities of the District of Abidjan, including Abobo, Adjamé, Attécoubé, Bingerville, Cocody, Koumassi, Marcory, Port-Bouët, Treichville and Yopougon. In each municipality, three samples consisting of a mixture of feathers, intestinal contents, and the other offcut (such as nails, intestines, etc.) were collected for a total of 30 samples. Each sample was packed in a Stomacher® bag and transported to the laboratory for microbiological analyses.

Isolation and Biochemical Identification of *Salmonella* Strains

All collected samples were analyzed for the isolation of *Salmonella* strains according with NF-EN ISO 6579 in four-step process: pre-enrichment, enrichment, isolation, and biochemical identification. Thus, for each sample of manure or slaughterhouse waste, 25g were taken and suspended in 225mL of sterile BPW (buffered peptone water) (Scharlau, Spain) and the mixture was incubated at 37°C during 18 hours after homogenization. Then, 100µL of the broth was added to 10mL of Rappaport Vassiliadis medium (Scharlau, Spain) and then incubated at 42°C for 24 hours. The isolation was performed on the selective Hektoen medium (Scharlau, Spain) by streaked of enrichment broth on the medium using a sterile Pasteur pipette. The plates were incubated at 37°C for 24 hours and five presumptive colonies of *Salmonella* were selected on each plate for biochemical and molecular identification. All the presumptive colonies selected were identified based on their morphological (motility observation and Gram staining) and the biochemical characteristics. The biochemical tests were carried out using Le Minor method. The API®20E gallery (BIOMERIEUX, France) was also used to confirm the strains corresponding to *Salmonella* genus with Le Minor method. All the *Salmonella* isolates were then stored at -20°C in nutritive buffer (Bio Rad, France) supplemented with glycerol (25%) for further tests.

Determination of Prevalence of the *invA* Gene in *Salmonella* isolates

The extraction of DNA was performed using E.Z.N.A.® Food DNA Kit extraction (Omega BIO-TEK, USA) according to the manufacturer's instructions. The sequences of primers used for virulence *invA* gene PCR including INVA-1: 5'ACAGTGCTCGTTTACGACCTGAAT3' and INVA-2: 5'AGACGACTGGTACTGATCGATAAT3' (Chiu & Ou, 1996). The PCR was performed in a 25µL amplification mix containing 12.5µL of Master Mix (FIREPol®, EU), 10.5µL of nuclease-free water, and 2µL of DNA. The PCR was conducted using a thermocycler (Thermoplus, France) and the amplification program consisted of an initial denaturation at 94°C/2min, followed by 35 cycles at 94°C/30s, 56°C/30s, 72°C/2min, and a final amplification at 72°C/10min. The PCR products were stained with a 0.3% solution of SIBR Safe GREEN (Invitrogen, USA) and were visualized under UV light on gel DOC system imager (Biorad, France) after gel electrophoresis on 1.5% agarose at 120volts/cm for 30-45min. In this study, a reference strain of *Salmonella typhimurium DT104 from the National Food Institute (Lyngby, Denmark) was used for the validation of the results*.

Antibiotics Susceptibility Testing of *Salmonella* spp. Strains

The determination of antibiotic susceptibility level of *Salmonella* spp. strains isolated from poultry manure and slaughterhouse waste samples were carried out using the agar diffusion method on Mueller-Hinton solid medium (MHA) (Bauer et al., 1966), and all drugs tested were selected according to CA-SFM/EUCAST (2022) guidelines. Thus, Amoxicillin/Clavulanic acid (AMG), Ampicillin (AMP), Imipenem / Meropenem (IMI), Cefoxitin (FOX), Cephalothin (KF), Piperacillin (PRL), Ticarcillin (TC), Ceftazidime (CAZ), Cefepime (CPM), Cefotaxime/Ceftriaxone (CRO) Perfloxacin (PEF), Norfloxacin (NOR), Levofloxacin (LEV), Kanamycin (K), Tobramycin (TN), Amikacin (AK) and), Gentamicin (GEN), were tested.

Thus, from a 24-hour culture, two to three bacterial colonies were taken and emulsified in 10 mL of 0.85% NaCl saline water to obtain a MacFarland turbidity of 0.5, corresponding approximately to 10^6 CFU/mL. Then, sterile swabs soaked in the bacterial inoculum were used to inoculate the entire surface of the MHA in tight streaks, rotating the dish each time. After inoculation, the antibiotic discs were put on the agar surface and the plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones were measured and the isolates were classified according to the CA-SFM/EUCAST (2022) guidelines.

RESULTS

Prevalence of *Salmonella* spp. Strains Isolated from Poultry Waste

All 30(100%) poultry slaughterhouse waste samples analyzed in this study were positive for Salmonella sp. This bacterium was detected in 21(75%) of the poultry manure

samples among the 28 analyzed. Based on the morphological (macroscopic and microscopic) characteristics and the biochemical identification, a total of 200 strains, 110 from manure waste and 90 from slaughterhouse samples, were isolated.

Prevalence of Virulence *invA* Gene in *Salmonella* spp. Isolates

The investigation of the *InvA* virulence gene from the 200 strains of *Salmonella* isolates revealed that 150(75%) were positive for this gene because of the presence of specific bands referencing molecular weights of the virulence gene InvA-244bp. Moreover, 98(65.33%) strains from poultry manure were positive for this virulence gene while *invA* gene was detected in 52(34.64%) isolates from poultry slaughterhouses waste. These 150 strains positive for virulence gene *invA* were tested for their susceptibility to various antibiotics.

Antibiotic Profile of *Salmonella* Strains Isolated from Poultry Manure and Slaughterhouse Waste

The 150 *Salmonella* strains were tested for 17 molecules belonging to three different antibiotic families, including beta-lactam, fluoroquinolones, and aminoglycosides. The proportions of simple resistance of all tested isolates are shown in Table 1. All the *Salmonella spp*. strains analyzed were resistant to one or more antibiotics tested in this study. The resistance rate ranged from 8.67 to 66.67% for beta-lactam, from 10 to 56.67% for fluoroquinolones, and from 23.33 to 31.33% for aminoglycosides. In general, high resistance rates were observed for Ampicillin, Ticarcillin, and Perfloxacin, with rates above 50% (Table 1). While, low resistance rates were recorded for Amoxicillin-clavulanic Acid (8.67%), Imipenem/Meropenem (14.67%) and Norfloxacin (10%). Finally, antibiotics belonging to aminoglycosides family were the most effective on the isolates tested in this study.

Table 1: Antibiotic profile of isolated *Salmonella* spp. strains

Antibiotic family	Antibiotics used	Resistance (%)	Number of strains
Beta-lactam	Ampicilin (AP)	66.67	100
	Piperacillin (PRL)	36.67	55
	Ticarcillin (TC)	56.67	85
	Cefoxitin (FOX)	22	33
	Cefotaxime / Ceftriaxone (CRO)	28	42
	Ceftazidine (CAZ)	28	42
	Cefepime (CPM)	23.33	35
	Cephalotin (KF)	22	33
	Amoxicillin - clavulanic Acid (AMC)	8.67	13
	Imipenem / Meropenem (IMI)	14.67	22
Quinolones/	Norfloxacin (NOR)	10	15
Fluoroquinolones	Levofloxacin (LEV)	42	63
	Perfloxacin (PEF)	56.67	85
Aminoglycosides	Tobramycin (TN)	23.33	35
	Kanamycin (K)	31.33	47
	Amikacin (AK)	30	45
	Gentamicin (GEN)	26.67	40

Cross and Multiple Resistance in *Salmonella* spp. Tested Strains

In this study, cross-resistance corresponding to simultaneous resistance to antibiotics belonging to the same family was observed for 2 to 7; 2 to 3 and for 2 drugs respectively in the beta-lactam, fluoroquinolones and aminoglycosides families. The resistance rates ranged between 2 to 37% and the highest cross-resistance was detected in the aminoglycosides family (Table 2).

Table 2: Cross-resistance profile of *Salmonella* strains isolated (%) from poultry waste

Antibiotic families	Number of antibiotics					
	2	3	4	5	6	7
Beta-Lactam	18.67	30.00	28.67	8.67	2	5.33
Fluoroquinolones	24.44	17.78	0	0	0	0
Aminoglycosides	36.67	0	0	0	0	0

Moreover, among the 150 *Salmonella* tested strains, 65(43.33%) showed simultaneous resistance to various drugs belonging to the three antibiotics families targeted in this study. However, the majority of these (53.84%) strains are from waste collected in poultry slaughterhouses (Table 3). This multiple drug resistance concerned at least four antibiotics and antibiotics molecules most implicated in this resistance were Ampicilin, Levofloxacin, Perfloxacin and Kanamycin. Moreover, two strains isolated from poultry slaughterhouse waste exhibited resistance to ten antibiotics and these strains were resistant to all antibiotics of the aminoglycoside's family.

Table 3: Resistance profile of *Salmonella* spp. strains by number of antibiotics

Number of antibiotics	Number of resistant strains	Percentage of multiple resistances (%)
3	0	0
4	3	4.61
5	2	3.08
6	15	23.08
7	22	33.85
8	18	27.69
9	3	4.61
10	2	3.08

DISCUSSION

In this study, 150 *Salmonella* strains were obtained from poultry farm and slaughterhouse waste based on biochemical and molecular analysis. In general, the results revealed a high level of *Salmonella* contamination in both types of waste. The findings aligned with other reports that indicate the high prevalence of this bacterium in poultry waste (Hubbard et al., 2020; Ngogang et al., 2021). The high contamination by this pathogenic genus could be linked to the presence of Salmonella generally in digestive tract as reported by many authors (Poppe, 2000; Ghoddusi et al., 2015; Abba et al., 2017). However, the detection of virulence gene *invA* in 75% of *Salmonella spp.* isolated could indicate the pathogenicity capacity of these strains. Indeed, in addition to its importance in the detection of the *Salmonella* genus by PCR, the chromosomal *invA* gene is essential for host epithelial cell invasion (Malmarugan et al., 2011; Turki et al., 2014). In any case, due to the poor management or poor usage of this waste, these strains could contaminate soil, water and even the vegetables intended for human consumption (Kipré et al., 2023).

Thus, these results suggesting existence of a public health threat because of the absence of a decontamination prior to use of manure as fertilizer for poultry farm waste or to dumping in the bin for the slaughterhouse waste

(Kipré et al., 2023). Indeed, *Salmonella* can survive during a long time in water, soil and on surfaces and can also persist on plants and in infected animal waste slurry after it is spread into the environment (Hubbard et al., 2020).

In addition to these risks to human and animal health, high levels of single, cross and multiple resistance involving betalactam, fluoroquinolones and aminoglycosides were detected in the potentially pathogenic strains analyzed in this study. The high resistance observed in this study is similar to those reported by other authors (Bodering et al., 2017; Kashosi et al., 2018).

These results are due to the uncontrolled use of antibiotics during breeding. These drugs are generally use as feed additives as a growth factor or to preserve general health and prevent diseases caused by bacterial infections (Goualié et al., 2020; Maman et al., 2022). These multiple and cross-resistances could indicate regular use of several antibiotic families and several antibiotics belonging to the same family in poultry farming in Côte d'Ivoire. In addition, these drug resistant bacteria in this waste could be disseminated in soil, water and the environment. Due to possibility of transmission of these bacteria to human and animals, detection of these profile resistance in *Samonella* isolated in this study could suggest the increasing of the risks of therapeutic failure, relapse, and mortality associated with infections induced by this bacterium (de Melo et al., 2021; Maman et al., 2022).

Conclusion

This study highlighted the high prevalence of *Salmonella* strains with the virulence *inva* gene in poultry waste from farms and slaughterhouses. Moreover, many strains of *Salmonella* tested were multidrug resistant against the three classes of antibiotic evaluated in this study. Since no treatment is generally performed for this waste, they could be a source of the environment, water, and also vegetable crop contamination with these pathogenic bacteria. Therefore, it is urgent to improve the surveillance system of antibiotic use in the poultry sector in Côte d'Ivoire to reduce the emergence of Multidrug resistance bacteria. Above all, we need urgently to develop biological methods for poultry waste treatment in view to reduce their negative effect on health.

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Competing Interests: The authors declare that they have no competing interests.

Author's Contribution: Romuald Christ Kipré, Monique Aiza Ainyakou-Sanga, Aboubacar Sylla, Bernadette Gblossi Goualie and Daniel kouamé Kra designed the study and conducted all the analyses. Romuald Christ Kipré managed the literature searches and wrote the first draft of the manuscript. Bernadette Gblossi Goualie corrected the article and produced the final version of the manuscript. Germain Alfred Karou and Solange KAKOU-NGazoa supervised the research team. Finally, all authors read and approved the final manuscript.

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