

Original Article

## Risks associated with the discharge of poultry slaughterhouse waste in public landfill sites in Abidjan, Côte d'Ivoire

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### Abstract

**Introduction:** The poultry sector in Côte d'Ivoire is expanding and generating huge quantities of waste. This study aimed to analyze the risks associated with the discharge of poultry slaughterhouse waste in public landfill sites in Abidjan.

**Methodology:** The chemical and microbiological analysis of 30 poultry slaughter waste samples from 10 Abidjan communities were evaluated using high performance liquid chromatography and detection of pathogenic bacteria on specific media, respectively. The antibiotic susceptibility of isolates was determined using the agar diffusion method.

**Results:** A total of 4 antibiotic molecules — sulfamethoxazole, oxytetracyclin, ciprofloxacin, and erythromycin — were detected in analyzed samples with levels ranging from  $1500 \pm 22$  to  $16200 \pm 129$  µg/kg. Moreover, 270 bacterial strains including 71 *Salmonella*, 109 *Escherichia coli*, and 90 *Staphylococcus aureus* were isolated. Among *E. coli* isolates, 16.5% and 19.3% were identified as enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *Escherichia coli* (EPEC) respectively. Among the *Salmonella* strains, 45% were positive for the virulence gene *invA*. The levels of antibiotic resistance were between 12 and 67%, with high level of resistance observed for imipenem in *E. coli*, and for amoxicillin/clavulanic and ampicillin in *Salmonella* strains. The highest rates of resistance in *S. aureus* were for fluoroquinolones (23.0–45.0%), fusidic acid (42.2%), and sulphonamides (77.8%). Moreover, multidrug resistance to 3, 4, and 5 antibiotic families was detected in 14.8%, 10%, and 2.2% of the 270 tested strains, respectively.

**Conclusions:** These results indicate the necessity of managing this waste to reduce its negative effect on the environment and public health.

**Key words:** antibiotics; poultry; waste; health; risks.

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### Introduction

The poultry industry in Côte d'Ivoire has experienced a spectacular boom in recent years, and has grown into a prosperous agricultural industry, making this sector an important part of the economy of the country. Poultry meat production has increased from 23,140 tons in 2010 to 85,000 tons in 2019, and is expected to reach 200,000 tons and more than 1.678 billion of eggs/year by 2030, to fully meet the animal protein needs of the population [1]. However, this increase in poultry production and consumption also leads to an increase in the volume of waste, particularly from poultry slaughterhouses [2].

In general, this waste from slaughterhouses is directly disposed of in public landfills, often situated in close proximity to residential areas, consequently generating foul odor and probably causing respiratory problems for the inhabitants [3]. However, data on the chemical and microbiological composition of these

wastes remain poorly documented in our country. This data should help to better assess their environmental and health impact. Yet, some bacteria such as *Escherichia coli* (mainly Shiga toxin-producing *E. coli*), *Salmonella* spp., and *Staphylococcus aureus*, which are among the most dangerous of the life-threatening foodborne pathogens, are generally isolated from the digestive tract of chicken and their environment [4]. Moreover, various studies on the poultry production chain that were conducted in the district of Abidjan have highlighted the presence of potentially pathogenic bacteria, that are resistant to several families of antibiotics, during breeding, during slaughter, and even in poultry carcasses [5,6]. The presence of these bacteria could be attributed to unregulated use of antibiotics during breeding and poor hygiene practices [7–9].

In any case, these bacteria of different origins present in poultry slaughter waste end up in the

environment. Thus, this study aimed to analyze the risks associated with the discharge of poultry slaughterhouse waste in public landfill sites in Abidjan.

## Methodology

### *Samples collection in the slaughterhouses*

Poultry waste was collected from the slaughterhouses located in the large markets of 10 municipalities of the District of Abidjan, including Abobo, Adjamé, Attécoubé, Bingerville, Cocody, Marcory, Koumassi, Treichville, Port-Bouët, and Yopougon. Sampling was carried out once a week in each municipality; 3 samples were collected from each municipality, resulting in a total of 30 samples. The samples, approximately 100 g in weight, consisted of a mixture of feathers, intestinal contents, and the other offcut (such as nails, intestines, etc.). The samples were placed in a sterile stomacher bag and transported to the laboratory at 4 °C in an ice box.

### *Detection of antibiotics residues in poultry slaughterhouse waste samples*

Eleven representative antibiotics which are widely used in poultry production systems [5]; and which belong to five families of antibiotics: (1) sulfonamides, (2) tetracyclines, (3) fluoroquinolones, (4) macrolides, and (5) beta-lactams; were targeted. Each antibiotic was extracted by the ultrasonic method for the classes of macrolides and beta-lactams; or by using a chemical solvent for sulfonamides, tetracyclines, and fluoroquinolones. The targeted antibiotics were analyzed using high-performance liquid chromatography (HPLC) equipment (Waters<sup>TM</sup> Corporation, Milford, USA) which consisted of the UV visible spectrophotometer (Agilent, Santa Clara, USA) systems using a Waters 986 Tunable absorbance detector, a Waters 600 controller, an X-Act degasser, a Waters 717 plus autosampler, a C18 column (4.6 x 150 mm, 4 µm) (Avantor® Genesis, Radnor, USA), a C18 guard column (10 x 4 mm, 4 µm), and a column support (10 mm). For each antibiotic, the standard consisted of a pure solution of the target antibiotic molecule (Merck, Burlington, USA).

### *Bacteria isolation and biochemical identification*

The pre-enrichment suspension was obtained by adding 10 g of previously ground waste into 90 mL of buffered peptone water, and incubated at 37 °C for 16–24 hours. *E. coli* and *S. aureus* were isolated by plating the pre-enrichment suspension on TBX agar (Tryptone Bile X-glucuronide) (Bio-Rad, Hercules, USA) and Baird Parker (Bio-Rad, Hercules, USA) agar,

respectively; supplemented with 5% potassium tellurite egg yolk solution, followed by incubation at 37 °C for 16–24 hours. *Salmonella* spp. was isolated following the ISO 6579-1:2017 recommendations [10]. Thus, 0.1 mL of the pre-enrichment suspension was used to inoculate 10 mL of Rappaport-Vassiliadis broth (Bio-Rad, Marne-la-Coquette, France) and incubated for 19 hours at 42 °C. *Salmonella* was isolated on Hektoen agar medium (Bio-Rad, Hercules, USA) at 37 °C for 24 hours. After incubation, five typical colonies were selected from each medium for morphological (Gram staining); and biochemical tests by using API®20E gallery (Biomerieux, Lyon, France) for *E. coli* and *Salmonella* strains, and API® Staph gallery (Biomerieux, Lyon, France) for *S. aureus* strains.

### *Antimicrobial susceptibility testing*

The susceptibility of the 270 poultry slaughterhouses waste-derived *E. coli*, *Salmonella*, and *S. aureus* strains to a panel of commonly used antibiotics were determined using the Kirby-Bauer method on Mueller–Hinton agar plates (Bio-Rad, Hercules, USA) according to the guidelines and breakpoints of the Antibiotic Susceptibility Testing Committee of the French Society of Microbiology / European Committee on Antimicrobial Susceptibility Testing (CASFM/ EUCAST) [11]. The antimicrobial discs used were obtained from Bio-Rad (Bio-Rad, Hercules, USA) and included: ampicillin (10 µg), amoxicillin/acid clavulanic (20/10 µg), cefalotin (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriazone (30 µg), ceftazidim (10 µg), imipenem (10 µg), piperacillin (30 µg), ticarcillin (75 µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 µg), amikacin (30 µg), nalidixic acid (30 µg), norfloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and pefloxacin (5 µg) for *E. coli* and *Salmonella*; and norfloxacin (10 µg), pefloxacin (5 µg) and levofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 µg), cefoxitin (30 µg), ceftazidim (10 µg), cefalotin (30 µg), ampicillin (10 µg), piperacillin 30µg), ticarcillin (75 µg), imipenem (10 µg), fusidic acid (10 µg), trimethoprim/sulfamethazole, vancomycin (30 µg), rifampicin (5 µg), and fosfomycin (200 µg) for *S. aureus*. *E. coli* ATCC 25922 was used as a control strain. The test results were validated only when the diameters of the inhibition zones of the *E. coli* ATCC 25922 control strain were within the performance ranges.

**Table 1.** Sequences of primers used in this study.

Strains	Target gene	Primer	Sequence (5'-3')	Annealing temperature	Size (bp)	Reference
EHEC	<i>Stx1</i>	stx1-f stx1-r	TTCGCTCTGCAATAGGTA TTCCCCAGTTCAATGTAAGAT	50	555	[12]
EPEC	<i>eae</i>	eae-f eae-r	ATATCCGTTTTAATGGCTATCT AATCTTCTGCGTACTGTGTTCA	50	425	
<i>Salmonella</i>	<i>invA</i>	invA -f invA -r	ACAGTGCTCGTTTACGACCTGAAT AGA CGA CTG GTA CTG ATC GAT AAT	56	244	[13]

EHEC: enterohemorrhagic *Escherichia coli*; EPEC: enteropathogenic *E. coli*.

*Molecular identification of pathogenic E. coli and Salmonella strains*

The DNA of each strain was extracted from freshly grown colonies using the heat shock method followed by purification with phenol-chloroform and precipitation with ethanol [12]. Simplex polymerase chain reaction (PCR) using specific primers was used to detect virulence genes including *eae* and *Stx1* in enteropathogenic *E. coli* (EPEC) and in enterohemorrhagic *E. coli* (EHEC) strains, respectively; and *invA* in pathogenic *Salmonella* strains. All specific primer sequences used in this study and their annealing temperatures are listed in Table 1 [12,13]. Each PCR reaction was conducted in the final volume 25 µL using 2.5 µL *Taq* polymerase buffer 10X (Promega, Madison, USA) containing a final concentration of 1 mM MgCl<sub>2</sub>, 2 µL of 2.5 mM dNTPs, 1 µL of 10 pmol/µL of each specific primer, 0.2 µL *Taq* polymerase (5 U/µL), and 2.5 µL DNA. The PCR program consisted of an initial denaturation at 94 °C for 5 minutes; followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at specific temperature (Table 1) for 30 seconds, and extension at 72 °C for 1 minute; and an additional extension at 72 °C for 10 minutes. Aliquots of amplified products were loaded in 1.5% agarose gel and visualized, and photographed using a gel documentation system (Syngene, Cambridge, England).

**Results**

*Antibiotic residues detected in the waste from slaughterhouses*

Analysis of waste from slaughterhouses located in ten areas of Abidjan district showed that among the 11 targeted antibiotics, 4 (ciprofloxacin, erythromycin, oxytetracyclin, and sulfamethoxazole) were detected in analyzed samples with concentrations ranging between 16,200 ± 129 and 1,500 ± 22 µg/kg (Table 2).

Ciprofloxacin had the highest presence with concentrations between 16,200 ± 129 and 2,900 ± 23µg/kg of waste. However, it was not detected in samples from Koumassi and Treichville. In the case of sulfamethoxazole and oxytetracyclin, the concentrations detected were between 9,400 ± 13 and 2,600 ± 54 µg/kg, and between 9,500 ± 51 and 1,900 ± 33 µg/kg respectively. Erythromycin was detected at levels between 12,700 ± 98 and 1,500 ± 22 µg/kg; however, it was only detected in samples from 5 areas. Furthermore, among the 10 municipalities from which samples were collected, 4 antibiotics were detected in samples from Abobo, while only 2 were detected in samples from Port-Bouët and in Bingerville (Table 2).

*E. coli, Salmonella spp., and S. aureus strains isolated from poultry slaughterhouse waste*

*E. coli, Salmonella spp., and S. aureus* were isolated from 100% of the analyzed samples. Morphological and biochemical identification were used to confirm 270 strains, including 109 *E. coli*, 71 *Salmonella spp.*, and

**Table 2.** Antibiotic residues detected in poultry slaughterhouse waste.

Communes of Abidjan	Antibiotics detected			
	Ciprofloxacin (µg/L)	Sulfamethoxazole (µg/L)	Erythromycin (µg/L)	Oxytetracycline (µg/L)
Abobo	2900 ± 23	3000 ± 27	5100 ± 29	4800 ± 45
Adjamé	3300 ± 31	4600 ± 111	nd	4900 ± 39
Attécoubé	16200 ± 129	nd	3400 ± 32	4400 ± 19
Bingerville	5800 ± 37	8300 ± 87	nd	nd
Cocody	4300 ± 102	nd	12700 ± 98	9500 ± 51
Koumassi	nd	5600 ± 21	9400 ± 117	8100 ± 63
Marcory	3900 ± 91	2600 ± 54	nd	1900 ± 33
Port-Bouët	5500 ± 48	7600 ± 62	nd	nd
Treichville	nd	nd	1500±22	nd
Yopougon	8800 ± 104	9400 ± 13	nd	8600 ± 67

nd: not detected. The values listed in the table correspond to the averages of the three samples for each area. Trimethoprim, doxycylin, spiramycin, amoxicillin, ampicillin, oxacillin, penicillin G were not detected in any sample.

90 *S. aureus*; corresponding to 40.4%, 26.3%, and 33.3% of all isolated strains, respectively.

#### Antibiotic susceptibility and resistance profiles of *E. coli*, *Salmonella* spp., and *S. aureus*

Out of the 19 antibiotics tested, the highest resistance rates in *Salmonella* spp. were observed for ampicillin, amoxicillin/clavulanic acid, ticarcillin, and pefloxacin; with rates between 53 to 66.2%. More than 30% of the *Salmonella* strains that were tested were resistant to piperacillin, levofloxacin, and kanamycin; and 15 to 29.4% of them were resistant to 8 of the antibiotics belonging to the beta-lactam family antibiotics tested in this study. While, low resistance was observed for cefepime, cefotaxime, imipenem, and norfloxacin with rates of 12.7, 12.7, 15.5, and 9.8%. No resistance was observed for ciprofloxacin and nalidixic acid in these *Salmonella* tested strains (Table 3).

In the case of *E. coli* strains the highest resistance rates were observed for ampicillin, piperacillin, ticarcillin, imipenem, levofloxacin, and pefloxacin; and low resistance was observed for norfloxacin. All the *E. coli* strains tested were also susceptible to ciprofloxacin (Table 3).

The *S. aureus* tested in this study had low levels of resistance to cephalosporins (cephalothin, cefoxitin, ceftazidime), carbapenems (imipenem), and penicillins,

particularly to the ticarcillin. The highest level of resistance was observed to trimethoprim/sulfamethoxazole (77.8%), and more than a third of *S. aureus* tested were resistant to norfloxacin, pefloxacin, kanamycin, and fusidic acid. In general, beta-lactam antibiotics were the most effective against the *S. aureus* strains tested, with resistance rates of less than 25% (Table 3).

#### Cross-resistance of *E. coli*, *Salmonella* spp., and *S. aureus*

Cross-resistance refers to the resistance of a strain to several antibiotics of the same family, and was identified in certain strains of *E. coli*, *Salmonella*, and *S. aureus* isolated from poultry slaughterhouse waste.

Concerning the beta-lactam antibiotics, cross-resistance involved 2 to 8 antibiotics in the case of *E. coli*, 2 to 7 antibiotics in the case of *Salmonella*, and up to 5 antibiotics in the case of *S. aureus* strains. Generally speaking, the antibiotics most involved in cross-resistance were amoxicillin/clavulanic acid (AMC), ampicillin, ticarcillin, and ceftazidime in the case of *E. coli* strains; and ampicillin, ticarcillin, and piperacillin in the case of *Salmonella* tested strains.

In addition, of the 109 *E. coli* strains tested in this study, the rates of cross-resistance to 2, 3, 4, 5, 6, 7, and 8 antibiotics were 18.3, 19.3, 9.2, 7.3, 4.5, 4.5, and

**Table 3.** Frequency of antibiotics resistance identified in the bacterial strains isolated from poultry slaughterhouse waste.

	Antibiotic resistance rates (%)		
	<i>Salmonella</i>	<i>E. coli</i>	<i>S. aureus</i>
<b>Betalactam antibiotics</b>			
Cefoxitin	22.53 (16 /71)	22.1 (24/109)	11.1 (10/90)
Ceftazidime	28.2 (20/71)	32.1 (35/109)	6.6 (6/90)
Ceftriazone	28.2 (20/71)	34.8 (38/109)	–
Cefotaxime	12.7 (9/71)	23.8 (26/109)	–
Cefepime	12.7 (9/71)	23.8 (26/109)	–
Cefalotin	19.7 (14/71)	18.3 (20/109)	1.1 (1/90)
Ampicillin	66.2 (47/71)	56.8 (62/109)	21.1 (19/90)
Piperacillin	36.6 (26/71)	42.2 (46/109)	24.4 (22/90)
Tircacillin	53.5 (38/71)	57.8 (63/109)	7.7 (7/90)
Amoxicillin/Clavulanic acid	66.2 (47/71)	22 (24/109)	–
Imipenem	15.5 (11/71)	66 (72/109)	6.6 (6/90)
<b>Fluoroquinolone antibiotics</b>			
Norfloxacin	9.8 (7/71)	7.3 (8/109)	41.1 (37/90)
Pefloxacin	54.3 (40/71)	58.7 (64/109)	44.4 (40/90)
Ciprofloxacin	0	0	–
Nalidixic acid	0	15.6 (17/109)	–
Levofloxacin	42.2 (30/71)	42.2 (46/109)	23.3 (21/90)
<b>Aminoglycoside antibiotics</b>			
Gentamicin	26.7 (19/71)	17.4 (19/109)	16.6 (15/90)
Tobramycin	22.5 (10/71)	17.4 (19/109)	22.2 (20/90)
Amikacin	29.4 (21/71)	14.4 (16/109)	–
Kanamycin	30.9 (22/71)	31.2 (34/109)	36.6 (33/90)
<b>Other antibiotics</b>			
Fusidic acid	–	–	42.2 (38/90)
Trimethoprim/Sulfametazole	–	–	77.8 (70/90)
Vancomycin	–	–	0 (0/90)
Rifampicin	–	–	0 (0/90)
Fosfomycin	–	–	0 (0/90)

– : not tested.

1.8%, respectively. In the case of the 71 *Salmonella* strains that were tested, the cross-resistance rates were 18.3, 11.9, 18.2, 8.4, 5.6 and 2.5% to 2, 3, 4, 5, 6, and 7 antibiotics, respectively.

On the other hand, *S. aureus* resistance involving a maximum of 2 antibiotics, particularly ampicillin and piperacillin, was observed in 11 (12.2%) strains and the cross-resistance rates involving 3, 4, and 5 antibiotics were observed in 3.3; 3.3, and 2.2% strains respectively.

Cross-resistance was also observed in the case of fluoroquinolones and aminoglycosides; and in this case, it involved 2 to 3 antibiotics. The proportions of cross-resistance involving 2 fluoroquinolone antibiotics were 5.5% (6/109), 2.8% (2/71), and 16.6% (15/90) in *E. coli*, *Salmonella*, and *S. aureus*, respectively. Resistance involving 3 antibiotics was identified in 2.7% (3/109) *E. coli*, 0% (0/71) *Salmonella*, and 11.1% (10/90) *S. aureus* strains. Norfloxacin and pefloxacin were the antibiotics most frequently observed in cases of the cross-resistance.

In the case of aminoglycosides, cross-resistance involved 2 to 3 antibiotics. The proportions of strains with cross-resistance involving 2 aminoglycosides were 7.3% (8/109) for *E. coli*, 16.9% (12/71) for *Salmonella*, and 18.9% (17/90) for *S. aureus*; and those involving three antibiotics were 4.5% (5/109) for *E. coli* and 7.7% (7/90) for *S. aureus*.

*Multi-drug resistance (MDR) in E. coli, Salmonella spp., and S. aureus*

Among the 270 *Salmonella* spp., *S. aureus*, and *E. coli* strains, MDR involving 3, 4 and 5 antibiotic classes were observed in 40 (14.8 %), 27 (10 %), and 6 (2.2 %) of the tested strains respectively (Table 4).

In the case *S. aureus*, the proportion of strains with MDR involving 3 and 4 antibiotic families were 16.7% and 10%, respectively (Table 4). In addition, 6 strains from Cocody (2 strains), Port-Bouët (2 strains), Koumassi (1 strain), and Yopougon (1 strain) showed resistance to 5 antibiotic classes.

The *E. coli* and *Salmonella* spp. tested strains did not have MDR involving 5 families of antibiotics. However, 22% and 10% for *E. coli*, and 29% and 9.8% for *Salmonella* exhibited MDR involving 3 and 4 families of antibiotics, respectively (Table 4). In

**Table 5.** Molecular identification of virulence genes in *Salmonella* and *E. coli*.

Virulence genes detected per Strain	Prevalence of virulence genes
<i>E. coli</i>	
<i>Stx1</i>	16.5% (18/109)
<i>eae</i>	19.2% (26/109)
<i>Salmonella</i> spp.	
<i>invA</i>	45% (32/71)

addition, the beta-lactam and fluoroquinolone classes were most involved in the MDRs observed in *E. coli* and *Salmonella* spp. strains, whereas MDR in *S. aureus* involved fluoroquinolones and sulphonamides families.

*Prevalence of virulence genes in E. coli and Salmonella strains*

Among the 109 *E. coli* strains tested in this study, 18 were positive for the *Stx1* gene encoding Shiga toxin from the EHEC pathovar. In addition, the *eae* gene of EPEC, which is needed for intimate attachment to host epithelial cells, was detected in 21 *E. coli* strains; and 32 *Salmonella* strains were positive for the *invA* virulence gene (Table 5).

**Discussion**

The results of this study showed the presence of various antibiotic residues including ciprofloxacin, erythromycin, oxytetracyclin, and sulfamethoxazole; with levels ranging from 16,200 µg/kg to 1,500 µg/kg; in poultry slaughterhouse waste samples from different areas of Abidjan. These results are similar to those reported by Kipré *et al.* who demonstrated the presence of the same antibiotic residues in litter from farms in the Abidjan district [14].

Indeed, preliminary studies have shown that certain antibiotics, including erythromycin and oxytetracyclin, are used during poultry farming, and even during the period of sale of live poultry at sites near slaughterhouses [5]. These results also show that although some antibiotics such as ciprofloxacin are not intended for veterinary use, they are regularly administered to animals during breeding.

Soil and water contamination by these antibiotics can lead to changes in the microbial biodiversity of these habitats. Mu *et al.* reported that antibiotics could alter the diversity, structure, and ecological functions of bacterial communities, stimulating widespread abundance of antibiotic-resistant bacteria and

**Table 4.** Multidrug resistance rates of bacteria isolated from slaughterhouse waste of Abidjan District.

Bacteria	Resistant to three antibiotic families (%)	Resistant to four antibiotic families (%)	Resistant to five antibiotic families (%)
<i>S. aureus</i> (n = 90)	16.7 (15/90)	10 (09/90)	6.6 (06/90)
<i>E. coli</i> (n = 109)	22 (24/109)	10 (11/109)	0
<i>Salmonella</i> (n = 71)	29.6 (21/71)	9.8 (07/71)	0
All strains (n = 270)	14.8 (40/270)	10 (27/270)	2.2 (06/270)

antibiotic-resistant genes in contaminated environments [15]. Furthermore, in this study, high antibiotic concentrations (ranging from 1,500 to 16,500 µg/kg waste) were reported, compared to the work of Proia *et al.* [16] and Bengtsson-Palme and Larsson [17]. Proia *et al.* showed that even an exposure to low levels of antibiotics (concentrations between 0.005 and 1.5 µg/kg), can result in alterations in the composition of the bacterial community, and in particular, an increase in Actinobacteria in the contaminated soil [16].

All these data show that the regular dumping of poultry slaughterhouse wastes in public landfills can have a significant environmental and health impact. In addition, bacteriological analyses carried out on these samples revealed the presence of potentially pathogenic bacteria such as *Salmonella* and *S. aureus*; as well as *E. coli*, which, although an indicator of fecal contamination, can be the cause of severe cases of diarrhea in humans. The sources of contamination of this waste by micro-organisms are diverse and concern all stages of poultry meat production [18]. However, the gastrointestinal tract of animals, which is also the natural habitat of most enteric pathogens, could be the main source of contamination of this waste.

The bacteria present in poultry slaughterhouse waste can be disseminated in the environment and contaminate run-off water, as well as lagoons which, as previously mentioned, are used for fishing and for watering vegetable crops. Under these conditions, these microorganisms can contaminate food intended for human consumption [3].

*E. coli* is a typical component of the intestinal microbiota of poultry, as it is a characteristic occupant of the gastrointestinal tract [19]. However, specific strains of *E. coli*, such as avian pathogenic *Escherichia coli* (APEC) can be the cause of colibacillosis, a common disease of poultry characterized by infection of several bird organs, including the liver, kidneys and spleen [20]. In humans, *E. coli* can be responsible for diarrheal illnesses that can sometimes be serious. Indeed, EHEC strains encode the Shiga-toxin gene, and its product Shiga-toxin causes life-threatening problems in humans following systemic absorption [21]; while EPEC binds to small intestinal enterocytes and destroys the normal microvillar architecture, resulting in inflammatory changes and diarrhea [22]. In this study, 18 (16.5%) and 21 (19.2%) isolates were identified as EHEC and EPEC pathotypes, respectively. This result was consistent with Al-Marri *et al.*, who reported that poultry is reservoir for these *E. coli* pathotypes [23].

Moreover, *Salmonella* constitutes one of the main zoonotic agents threatening public health and animal production worldwide; in particular, the non-typhoidal serovars have been reported to be associated with acute or chronic gastrointestinal disease in humans [24,25]. Indeed, the worldwide incidence of non-typhoidal *Salmonella*-derived gastroenteritis is estimated at around 93.8 million cases per year, resulting in approximately 155,000 deaths per year [24]. According to numerous studies, poultry represents one of the main sources of infection [26,27], indicating the pathogenicity of *Salmonella* species of avian origin. Moreover, the current investigation revealed high frequency (45%) of the virulence *invA* gene in the *Salmonella* spp. strains from poultry waste. The *invA* gene is used for the diagnosis of *Salmonella* spp. at the genus level, and also for detection of pathogenic strains [28]. The *invA* gene encodes proteins in bacterial cell membranes that are needed for invasion into host epithelial cells — the first step in the intracellular pathogenicity cycle of *Salmonella* [29].

*Staphylococcus aureus* is also an important zoonotic pathogen that can infect both humans and animals. *S. aureus* is responsible for several infectious diseases, including tissue and skin infections, pneumonia, septicemia, mastitis, arthritis, and soft tissue infections [30]. Therefore, it has a considerable impact on animal health and welfare, and causes major economic losses in livestock production. In addition, these bacteria are capable of surviving in the environment and being transmitted via contaminated water or food [31]. The studies on the antibiotic susceptibility of these strains, isolated from poultry waste, have shown very high rates of resistance to antibiotics that are commonly used in the treatment of numerous human and animal pathologies; ranging from 0% to 78% for *Salmonella*, *E. coli*, and *S. aureus*. These high rates of resistance to some antibiotics have also been reported in several regions of the world [32].

Generally, antibiotic resistance is related to the uncontrolled use of antibiotics for therapeutic, prophylactic, growth promotion purposes during breeding. Thus, the resistance to these antibiotics families observed in the strains tested, can reflect overuse of these different antibiotics by livestock farmers in the Abidjan district [33]. Another direct consequence of the use of multiple antibiotics is the emergence of multidrug resistant bacteria, as observed in this study. Indeed, multiple resistances involving 3 to 4 families in Enterobacteria, and up to 5 families in *S. aureus* have been reported [34]. These results show that the direct discharge of waste contaminated with these

bacteria into public landfills can contribute to the dissemination of these multi-antibiotic-resistant microorganisms in the environment, soil, and water; thereby posing a danger to human, animal and environmental health [9,33,34]. In addition, broad-spectrum antibiotics such as carbapenems, cephalosporins, and fluoroquinolones are widely used as a last resort to treat various Gram-negative and Gram-positive bacterial infections in humans [35–37]. Sulfonamides and aminoglycosides are frequently administered to chicken for therapeutic, preventive, or growth-promoting purposes, to prevent bacterial proliferation in poultry production [38].

In this study, *Salmonella* and *E. coli* strains showed resistance to various beta-lactam antibiotics, in particular cephalosporins (19–35%), penicillins (19–66%), and carbapenems (15–66%). The same observation was made for the *S. aureus* strains tested in this study, where resistance rates to these three subclasses ranged from 6–24%.

According to the World Health Organization (WHO), broad-spectrum antibiotics such as cephalosporins and carbapenems are molecules of last resort in the treatment of many pathologies caused by multidrug resistant bacteria [35,36]. The high levels of fluoroquinolone and aminoglycoside resistance observed in *Salmonella* and *E. coli* strains tested show that these potentially pathogenic bacteria are resistant to many antibiotics and can be present throughout the poultry production chain in Côte d'Ivoire. Thus, the presence of antibiotic-resistant pathogenic bacteria and antibiotic residues in this waste constitutes an even more serious risk to human and animal health in the Abidjan district. The results of our study underline the need to improve the surveillance system and policies for the responsible use of antimicrobial molecules in the poultry industry in our country; and above all, the need to provide a means of treating and recovering waste from poultry slaughtering.

## Conclusions

This study demonstrated the presence of antibiotic residues and pathogenic bacteria in poultry slaughterhouse waste collected in the Abidjan district. The study also revealed high levels of resistance in these strains to various families of antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides. This study therefore reveals the existence of a high health risk associated with the direct discharge of poultry slaughterhouse waste into public landfill sites in the Abidjan district. Under these conditions, it is necessary to limit the use of antibiotics in the poultry

sector in our country. More importantly, it is necessary to set up an ecological means of recycling this waste in order to minimize these risks.

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## Conflict of interests

No conflict of interests is declared.

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