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ORIGINAL ARTICLE

The occurrences of *E. coli* strains with multi-drug resistance profiles and virulence genes from poultry slaughterhouse waste in Abidjan (côte d'ivoire)

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ABSTRACT

Background and Objectives: Poultry production generates huge quantities of waste, mainly from slaughterhouses, which can be major reservoirs of pathogenic microorganisms. *Escherichia coli* is of particular concern due to its ability to acquire antibiotic resistance and virulence factors. This study aimed to characterise *E. coli* contamination in poultry slaughterhouse waste from ten municipalities in the district of Abidjan (Côte d'Ivoire).

Materials and Methods: *E. coli* strains were isolated from poultry slaughterhouse waste and identified using morphological and biochemical methods. Antibiotic susceptibility was assessed by the disk diffusion method, and virulence genes, including *eae* and *stx1*, were detected using a duplex PCR assay.

Results: Between January and April 2023, waste samples were collected, and *E. coli* strains were isolated and identified. Of 90 isolates, high resistance rates were observed against β-lactams (88.88%), aminoglycosides (77%), and fluoroquinolones (88.87%). MDR was detected in 11.11% of isolates, while 20% produced ESBL. The *eae* and *stx1* genes were detected in 14.47 and 6.57% of isolates, respectively.

Conclusion: These results highlight significant antimicrobial resistance and virulence potential in *E. coli* from poultry slaughterhouse waste, underscoring the need to improve management strategies.

Keywords: Poultry; Slaughterhouse; Waste; *Escherichia coli*; Multidrug-resistant; Virulence

INTRODUCTION

Poultry meat is an important source of protein worldwide, but its production generates substantial amounts of waste, especially in slaughterhouses (1).

In many regions, including Côte d'Ivoire, poultry slaughterhouse waste is often disposed of untreated into the environment or dumped in landfills. This practice can be a potential source of contamination that could affect soil and water, and also increase the

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risk of disease transmission between humans, animals and the environment, particularly due to the presence of bacterial strains such as *Escherichia coli* (2, 3). While many *E. coli* strains are commensal and naturally colonize the intestinal tract of healthy hosts (4), others acquire virulence factors that enable them to cause disease. Notably, enterohaemorrhagic *E. coli* (EHEC) can trigger symptoms ranging from mild diarrhea to haemorrhagic colitis (HC) and potentially fatal haemolytic uremic syndrome (HUS) (5-7). EHEC is part of the broader group of Shiga toxin-producing *E. coli* (STEC) (8), frequently found in the intestines of warm-blooded animals and excreted in large quantities in slaughterhouse waste (9, 10).

A particularly alarming feature of pathogenic *E. coli* from animal sources is their increasing resistance to antibiotics. Strains harboring virulence genes often display resistance to multiple antimicrobials used in both human and veterinary medicine (10, 11). Discharging this waste into the environment without adequate treatment facilitates the spread of antimicrobial-resistant pathogens, which complicates the control and treatment of infections in humans and animals.

The objective of this study was to characterise *E. coli* contamination in waste from poultry slaughterhouses in the district of Abidjan (Côte d'Ivoire). This involved determining the antibiotic resistance profile of the isolated *E. coli* strains from poultry slaughterhouse waste, detecting their major virulence genes and evaluating the environmental implications of their presence, with the aim of proposing waste management and treatment measures that minimise these risks.

MATERIALS AND METHODS

Study area and sample collection. Ten small-scale poultry slaughterhouses located in markets were randomly selected, one from each of the following municipalities in the district of Abidjan (Côte d'Ivoire): Abobo, Adjamé, Attécoubé, Bingerville, Cocody, Koumassi, Marcory, Port-Bouët, Treichville, and Yopougon. In each municipality, three waste samples were collected consisting of feathers, intestines, intestinal contents, beaks, and claws. This approach took into account logistical constraints and the need for representative coverage of all municipalities in the district. Samples were placed in sterile zip-lock bags and transported in a cool box to the laboratory for analysis.

Isolation and identification of bacteria. Samples were collected between January and April 2023. From each sample, 10 g of waste was homogenised in 90 mL of Brain Heart Infusion (BHI) Broth and incubated at 37°C for 24 h. After incubation, aliquots were streaked onto TBX agar (Tryptone Bile X-glucuronide Agar). Presumptive *E. coli* colonies were identified by Gram staining, oxidase test, and Leminor's reduced rack method. Isolates were stored in glycerol at -20°C until further analysis.

Antimicrobial susceptibility and ESBL detection. Antimicrobial susceptibility was determined for all isolates using the agar disc diffusion method described by Yao (12) on Mueller–Hinton Agar (MHA). Antibiotics tested included ampicillin (AP, 10 µg), piperacillin (PRL, 30 µg), ticarcillin (TC, 75 µg), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (CPM, 30 µg), cephalothin (KF, 30 µg), amoxicillin- clavulanic acid (AMC, 20/10 µg), imipenem (IMI, 10 µg), nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), levofloxacin (LEV, 5 µg), pefloxacin (PEF, 5 µg), tobramycin (TN, 10 µg), kanamycin (K, 30 µg), amikacin (AK, 30 µg), gentamicin (GEN, 10 µg), and trimethoprim-sulfamethoxazole (TS, 1. 5/23.75 µg).

A 24-h culture on MHA was used to prepare a bacterial suspension adjusted to 0.5 McFarland standard (10^6 CFU/mL). Plates were inoculated with sterile swabs, and antibiotic discs were placed at least 30 mm apart and 15 mm from the plate edge. After 15 minutes at room temperature (25 ± 2°C), plates were incubated at 37°C for 24 h. Inhibition zones were interpreted according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (13).

Extended-spectrum β-lactamase (ESBL) production was assessed using the double-disk synergy test (DDST) (14). Discs of ceftazidime, ceftriaxone, cefepime, and cefoxitin were placed around an amoxicillin–clavulanic acid disc on MHA and incubated at 37°C for 24 h. A "champagne cork" distortion of the inhibition zone indicated ESBL production (15, 16).

PCR detection of virulence genes. 76 *E. coli* isolates showing cross-resistance, multidrug resistance,

or ESBL production were screened for the *eae* and *stx1* genes using multiplex PCR.

DNA was extracted using the EZNA® Food DNA Kit (Omega Bio-Tek, USA). PCR reactions (25 µL) contained 5 µL of FirePol Master Mix (Solis Bio-Dyne, Estonia), 0.5 µL of each primer (10 µM), and 5 µL of DNA template. Primer sequences and annealing temperatures are listed in Table 1. Cycling conditions were: initial denaturation at 94°C for 2 min; 45 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 90 s; and final extension at 72°C for 5 min.

PCR products were resolved on a 1.5% agarose gel in 1× TAE buffer containing SYBR® Safe DNA gel stain (Invitrogen, USA) and visualised with a GelDoc EZ Imager (Bio-Rad, USA). Expected amplicon sizes were 555 bp for *stx1* and 425 bp for *eae*.

RESULTS

Antibiotic resistance profiles by municipality. A total of 90 *Escherichia coli* strains were isolated from poultry slaughterhouse waste. Antibiotic resistance data revealed marked heterogeneity between municipalities in the district of Abidjan.

For β-lactams, amoxicillin-clavulanic acid ranged from 33.33% in Cocody and 66.67% in Koumassi. Piperacillin resistance was highest in Abobo (88.88%), followed by Cocody (77.78%) and Port-Bouët (55.55%).

Within the aminoglycoside class, resistance to kanamycin varied from 11.11% in Attécoubé to 77.78% in both Abobo and Bingerville. Resistance to amikacin remained low overall, ranging between 11.11% and 33.33%.

In the fluoroquinolone group, resistance to pefloxacin was high, with values of 66.67% in Yopougon and 88.87% in Cocody.

Cross-resistance of *E. coli* strains. Cross-resistance results (Table 2) showed that 10% of isolates were resistant to three aminoglycoside molecules, 6.66% were resistant to three fluoroquinolone molecules, and 4.44% exhibited resistance to seven or eight β-lactam molecules.

Multidrug resistance, virulence genes, and ESBL production. A substantial proportion of isolates displayed multidrug resistance, particularly against levofloxacin and pefloxacin. Several strains were confirmed as extended-spectrum β-lactamase (ESBL) producers (Fig. 1).

Table 2. Rates of cross-resistance of *E. coli* strains by antibiotic family (n=90)

No. of molecules	β-lactams n (%)	Fluoroquinolones n (%)	Aminoglycosides n (%)
2	11 (12.22)	41 (45.55)	25 (27.77)
3	17 (18.88)	6 (6.66)	9 (10.00)
4	16 (17.77)	–	–
5	9 (10.00)	–	–
6	7 (7.77)	–	–
7	4 (4.44)	–	–
8	4 (4.44)	–	–

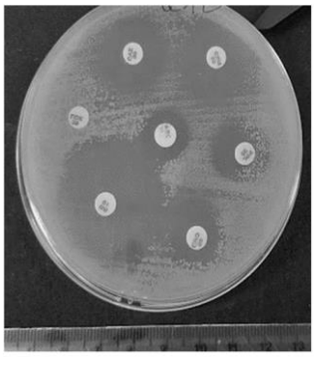

Fig. 1. DDST for confirmation of ESBL-producing *Escherichia coli* isolated from poultry slaughterhouse waste.
Note: ceftazidime (CAZ), cefoxitin (FOX), amoxicillin clavulanic acid (AMC), piperacillin (PRL) ceftriaxone (CRO), imipénèm (IMI) and cefepim (CPM).

Table 1. Primers, PCR amplicon size, annealing temperature and references used in this study

	Primers	Nucleotide sequence (5'-3')	Fragment Size (pb)	References
Enterohaemorrhagic *E. coli* (EHEC)	*stx1-f*	TTCGCTCTGCAATAGGTA	555	
	stx1-r	TTCCCCAGTTCAATGTAAGAT		(12)
Enteropathogenic *E. coli* (EPEC) / (EHEC)	*eae-f*	ATATCCGTTTTAATGGCTATCT	425	
	eae-r	AATCTTCTGCGTACTGTGTTCA		

Molecular analysis detected the eae and stx1 virulence genes in selected isolates, including ATEC13, ATEC22, and PBEC21 (Table 3 and Fig. 2).

DISCUSSION

The presence of antibiotic-resistant pathogenic *Escherichia coli* in poultry slaughterhouse waste is of particular concern, as this waste is often discharged untreated into the environment in the Abidjan district. This study aimed to characterise *E. coli* contamination in waste collected from ten municipalities, assess antibiotic resistance, and evaluate virulence gene profiles to determine the associated public health risks.

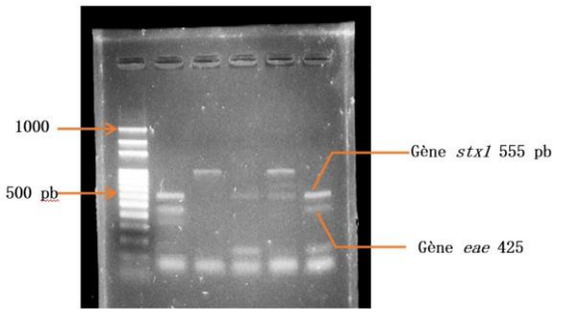

Fig. 2. Multiplex PCR amplification of *stx1* and *eae* genes

All *E. coli* strains were isolated on TBX agar with a prevalence rate of 100%. TBX agar is a selective and differential chromogenic medium compliant with ISO standards, containing the chromogen X-glucuronide to detect β-glucuronidase activity, an enzyme highly specific to *E. coli* (17). Morphological identification (Gram staining) and biochemical confirmation using Leminor's reduced rack allowed the selection of 90 presumptive *E. coli* strains (nine per municipality) for antibiotic resistance testing. The detection of *E. coli* in poultry waste is unsurprising, given its natural habitat in the intestinal tract of animals and its predominance in faecally contaminated environments (18, 19), underscoring the potential of this waste as a contamination source.

Antibiotic susceptibility testing revealed high re-

Table 3. Multi-resistance profile, presence of virulence gene and ESBL

Strains	Multi-resistance profile	*stx 1*	*Eae*	ESBL
BIEC 11	KH,LEV,PEF,K,GEN			*BLSE*
BIEC 22	KH,LEV,PEF,K,GEN			*BLSE*
ABEC 12	AP,PRL,TC,CRO,LEV,PEF,K,GEN		*Eae*	
ABEC13	AP,PRL,TC,KF,LEV,PEF,K,GEN		*Eae*	*BLSE*
ABEC22	AP,PRL,TC,FOX,PEF,AK		*Eae*	
ABEC33	AP,PRL,TC,LEV,PEF,K,AK,GEN		*Eae*	
ADEC 12	TC,NA,LEV,TN,K		*Eae*	*BLSE*
ADEC 13				*BLSE*
ADEC 21				*BLSE*
ADEC 22				*BLSE*
ADEC 31				*BLSE*
YOEC 11				*BLSE*
YOEC 12				*BLSE*
YOEC 13				*BLSE*
YOEC 32				*BLSE*
ATEC 13		*stx 1*	*Eae*	*BLSE*
ATEC 22		*stx 1*	*Eae*	*BLSE*
TREC 22	CAZ,NA,LEV,PEF,TN,AK		*Eae*	
PBEC 21	AP,FOX,CRO,COM,AMC,NA,LEV,PEF,K,GEN	*stx 1*	*Eae*	
COEC 11		*stx 1*		*BLSE*
COEC 12			*Eae*	*BLSE*
COEC 13				*BLSE*
COEC 21			*Eae*	*BLSE*
COEC 31	CAZ,CPM,LEV,PEF,AK	*stx 1*		

sistance rates to multiple antibiotic classes. Within the β-lactam family, resistance patterns varied across municipalities, with notable resistance to cephalothin in Bingerville and to third-generation cephalosporins in Attécoubé. High resistance rates to ampicillin and piperacillin, particularly in Yopougon and Port-Bouët, together with cross-resistance among β-lactams, indicated the spread of extended-spectrum β-lactamases (ESBLs). ESBL production, confirmed in isolates such as ABEC13, ADEC12, and ATEC13, is concerning, as these enzymes confer resistance to a wide range of β-lactam antibiotics, including third-generation cephalosporins (20, 21). Such strains can transfer resistance genes horizontally, increasing the environmental risk (22).

Resistance to aminoglycosides (kanamycin, tobramycin, amikacin, gentamicin) was particularly high in Abobo, which is alarming given their role as last-resort treatments for severe infections (23, 24). Mechanisms such as target site modification and efflux pump activation likely contribute to this resistance (25, 26). Similarly, high resistance rates to fluoroquinolones (pefloxacin, norfloxacin, levofloxacin), especially in Bingerville (88.87%), revealed significant cross-resistance within this class, indicating that overuse of one agent can drive resistance to others.

Some isolates (e.g., ABEC13, BIEC11, PBEC21) showed multidrug resistance to at least three antibiotic families, significantly narrowing treatment options. In Côte d'Ivoire, the extensive use of antibiotics, both therapeutically and as growth promoters in poultry production, has been linked to the emergence of resistant bacteria (16). The resistance levels observed in this study are consistent with previous results obtained in Algeria (27), India (28), and the USA (29).

Virulence gene screening revealed the presence of the *stx1* and *eae* genes in several isolates. The *stx1* gene encodes Shiga toxin, which damages endothelial cells and can cause severe complications such as haemolytic uraemic syndrome (HUS). The *eae* gene encodes intimin, an adhesion protein that enables intimate attachment to intestinal epithelial cells, leading to the characteristic attachment and effacement lesions of enteropathogenic and enterohaemorrhagic *E. coli* (30). From a public health perspective, the coexistence of these virulence genes with multidrug resistance represents a critical threat. Indeed, the *stx1* gene can cause life-threatening systemic effects, while the *eae* gene facilitates persistent intestinal colonisation, making clearance more difficult. In addition, multidrug resistance reduces effective therapeutic options, increasing the likelihood of disease progression to severe outcomes. Moreover, the release of these strains into the environment can facilitate human exposure through contaminated water, crops or direct contact with waste, which can lead to epidemics even in urban areas (31).

The uncontrolled disposal of poultry slaughterhouse waste, particularly waste harbouring multidrug-resistant and virulent *E. coli*, poses a significant and ongoing public health hazard. Accumulation in public dumps, farmlands and informal disposal sites creates reservoirs for the persistence and dissemination of pathogenic strains. This risk necessitates the implementation of strict waste treatment strategies such as controlled composting or thermophilic digestion, alongside rigorous regulation of antibiotic use in poultry farming. Regular surveillance of both antibiotic residues and resistant bacteria should form part of integrated One Health interventions to mitigate environmental and health impacts.

CONCLUSION

Poultry slaughterhouse waste in Abidjan contains strains of *E. coli* that combine multidrug resistance with virulence genes (*stx1, eae*), representing a significant threat to both public health and the environment. To address this issue, urgent and coordinated action is needed to ensure the enforcement of strict regulations on the use of veterinary antibiotics. The implementation of controlled composting processes could also effectively reduce the pathogenic bacterial load and enable the transformation of poultry slaughterhouse waste into biofertilisers free of harmful microorganisms.

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