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Low Prevalence of the Genetic Determinants of OXA-1 and AAC (6') Resistance in *Escherichia coli* Isolated from Broiler Chicken Droppings in Azaguié, Côte d'Ivoire: A Surveillance Study

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Abstract

Background: The rise in avian antibiotic resistance poses a major risk to public health. In Côte d'Ivoire, surveillance studies on the genetic determinants of resistance in avian *Escherichia coli* remain limited. **Objective**: The aim of this surveillance study is to assess the prevalence of the *OXA*-1 and *AAC* (6') resistance genes in *E. coli* strains isolated from broiler chicken droppings in the town of Azaguié, Côte d'Ivoire. **Methods**: A cross-sectional collection of droppings samples was conducted at 14 broiler farms from June 24 to 26, 2024. After isolating and identifying *E. coli* strains, susceptibility testing for seven antibiotics was performed using the antibiotic disk diffusion method on agar medium, followed by testing for extended-spectrum beta-lactamase (ESBL) production using the double synergy method. Conventional PCR was performed to detect the OXA-1 and AAC (6') genetic determinants and class 1 integrons (*Int* 1). **Results:** A total of 57 strains of *E. coli* were isolated. They showed complete susceptibility to beta-lactams (AMC, ceftriaxone, cefotaxime, ceftazidime), 17.54% resistance to gentamicin, 45.61% resistance to nalidixic acid, and 100% resistance to colistin. Molecular analysis of resistant *E. coli* strains revealed the presence of the *oxa*-1 and *aac* (6) genes in 12.50% and 25% of the isolates, respectively, with a significant co-occurrence of the two genes ($p = 0.00988$). Furthermore, molecular analysis revealed the presence of class 1 integrons (*Int* 1) at a rate of 70.83%, with no significant association with the $bla_{OXA\text{-}1}$ gene ($p = 0.47$) or the *aac* (6') gene ($p = 0.321$). **Conclusion:** This study revealed low prevalences of resistance determinants. However, the high prevalence of class 1 integrons (*Int* 1) suggests a potential risk of resistance spreading in poultry

farms in the Azaguié area, hence the need to continue surveillance within a "One Health" framework to prevent the emergence and spread of antibiotic resistance.

Keywords

Broiler Chickens, *Escherichia coli,* OXA-1, AAC (6), Côte d'Ivoire

1. Introduction

Antibiotics are among the most commonly prescribed drugs, particularly in Africa [1]. In animal husbandry, these drugs can be used either as a preventive treatment, as a metaphylactic treatment, or as a curative treatment. They are also used as growth promoters in animal husbandry [2]. The overuse of antibiotics in poultry farming is a major factor in the selection of resistant bacteria, thereby reducing the number of susceptible bacteria in the animals' gut flora [3]. Among these bacteria, *Escherichia coli* is the leading pathogen responsible for deaths related to antibiotic resistance in humans [1].

Furthermore, the intensive and inappropriate use of antibiotics poses a major zoonotic threat. Indeed, since *E. coli* is a commensal bacterium in the digestive tract of animals, it serves as a reservoir of resistance genes that can be disseminated into the environment, contributing to a decline in the effectiveness of these antibiotics and the inability to cure some bacterial diseases in both humans and animals [4] [5].

Antibiotic resistance results from genetic factors (mutations and gene acquisition via plasmids) and biochemical mechanisms (enzymatic inactivation of the antibiotic, target modification, membrane impermeability, and efflux pump activity) [6] [7]. Among these mechanisms, OXA-1-type beta-lactamases, which inhibit penicillins and some cephalosporins, and AAC (6') modification enzymes, which inactivate aminoglycosides, are particularly prevalent [8].

In Côte d'Ivoire, antibiotic resistance in *E. coli* strains isolated from various livestock sectors has reached alarming levels in several livestock-raising areas [9] [10]. Resistance has been documented in poultry to several antibiotics, with rates reaching as high as 100% for colistin in particular [11] [12]. However, despite the alarming rates of antibiotic resistance in poultry, molecular detection of resistance determinants is very limited. This surveillance study aims to address this gap by determining the prevalence of the *OXA*-1 and *AAC*(6') resistance genes in *E. coli* strains isolated from the droppings of broiler chickens in the town of Azaguié, Côte d'Ivoire, in order to assess the health risks associated with this activity.

2. Materials and Methods

2.1. Study Site

Azaguié is a town in southern Côte d'Ivoire, located 40 km north of Abidjan, the

country's economic capital. The area has a subequatorial climate characterized by hot and humid temperatures (25˚C - 28˚C). The town's two neighborhoods (Azaguié-Ahoua and Azaguié-Gare), as well as the villages of M'bromé and Abbê, served as the setting for this cross-sectional study, which took place from June 24 to 26, 2024.

2.2. Collecting Droppings Samples

Sixteen broiler farms housing chickens of all ages were visited throughout the study area. In each poultry house, droppings were collected according to the standard protocol [13], which specifies that 20 droppings must be collected per 1000 birds. Thus, 15 to 104 freshly laid droppings were collected from the floor along the two diagonals and the two medians of each rearing building. The droppings collected along each characteristic line of the building were placed in a polyethylene plastic bag, then stored on ice for transport to the laboratory, where they were kept at +4˚C.

2.3. Microbiological Analyses

2.3.1. Isolation of *Escherichia coli* Strains

E. coli strains were isolated according to the standard protocol described in the NF EN ISO 6579 standard (ISO-6579, 2002E). The process began with pre-enrichment, which consisted of incubating each sample—suspended in buffered peptone water at a 1:10 ratio—in test tubes at 37˚C for 18 to 24 hours. Next, the pre-enriched solutions were used to inoculate pre-labeled TBx agar plates using the quadrant method. These plates were then incubated at 37˚C for 24 hours. The typical (blue-green) colonies obtained after this enrichment process were collected for bacterial identification.

2.3.2. Identification of *Escherichia coli* Strains

Colonies suspected of being *E. coli* strains isolated from TBx agar plates were inoculated into 1.5 mL of brain heart broth (BCC) in labeled hemolysis tubes. The mixtures were incubated at 37˚C for 24 hours. They were then selectively cultured on plates containing EMB agar at 37˚C for 24 hours to identify *E. coli* strains based on their classic morphological and biochemical characteristics.

2.4. Antibiotic Susceptibility Testing

The bacterial susceptibility of *E. coli* strains was determined using the antibiotic-impregnated disk diffusion method on Mueller-Hinton agar, as proposed by Kirby-Bauer, in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology [14]. Seven antibiotics (BIORAD) were used in the tests. These included four compounds or combinations from the beta-lactam family: ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), amoxicillin plus clavulanic acid (30 µg), gentamicin (10 µg), colistin (10 µg), and nalidixic acid (30 µg).

2.5. Screening for the Production of Extended-Spectrum Beta-Lactamase

The double-synergy method was used to identify bacteria that produce extended-spectrum beta-lactamases (ESBL). This technique involves screening Mueller-Hinton agar for a synergy pattern resembling a "Champagne cork" between an antibiotic disc containing a beta-lactamase inhibitor such as amoxicillin plus clavulanic acid and third-generation cephalosporin (3GC) discs, notably ceftazidime, cefotaxime, and ceftriaxone, in accordance with the recommendations provided by CASFM [15].

2.6. Molecular Detection of Antibiotic Resistance Genes

Twenty-four strains of *Escherichia coli* were selected at random from those exhibiting antibiotic resistance profiles for the purpose of identifying resistance genes. DNA extraction was performed using heat shock. A standard polymerase chain reaction (PCR) was used to detect the *oxa*-1 and *aac* (6) antibiotic resistance genes and class 1 integrons (*Int* 1) using specific primer pairs (Table 1). PCR amplification was performed in a 30 µl volume using a thermal cycler (Perkin® Elmer GenAmp Applied Biosystems 9700). The amplification conditions are summarized in Table 2. The reaction mixture consisted of 10 µl of DNA, 1X Master

Table 1. List of primers used for the polymerase chain reaction.

Genes	Primers	Sequences 5'-3'	Amplicon size (bp)	Reference
bla OXA-1	F	GCAGCGCCAGTGCATCAAC	198	Maynard *et al.*, 2003
	R	CCGCATCAAATGCCATAAGTG		
bla aac (6)	F	TTGCGATGCTCTATGAGTGGCTA	482	Park *et al.*, 2006
	R	CTCGAATGCCTGGCGTGTTT		
*Int*1	F	CCTCCCGCACGATGATC	280	Goldstein *et al.*, 2001
	R	TCCACGCATCGTCAGGC		

F: Forward, R: Reverse, bp: base pair.

Table 2. Conditions for amplifying antibiotic resistance genes.

Amplification steps	Conditions		
	OXA-1 gene	*aac* (6) gene	*Int*1
Initial denaturation	95˚C/5 min	95˚C/5 min	95˚C/5 min
Cyclic denaturation	95˚C/30 s	95˚C/30 s	95˚C/30 s
Annealing	58˚C/30 s	60˚C/30 s	60˚C/30 s
Cyclic extension	72˚C/4 min	72˚C/4 min	72˚C/4 min
Final extension	72˚C/10 min	72˚C/10 min	72˚C/10 min
Number of cycles	30	30	30

Mix, and 10 µM of each specific primer for each target. Another reaction mixture without DNA was used as a negative control. The amplified products were analyzed by electrophoresis on a 1.5% agarose gel (Invitrogen) stained with SYBR Safe. The gel was imaged using an ultraviolet imaging system (GelDoc).

2.7. Data Analysis

The data collected during this study were entered and recorded in an Excel 2016 spreadsheet. The frequencies of phenotypic resistance and the prevalences of the *OXA*-1 and *AAA*(6') genes, as well as those of class 1 integrons (*Int* 1), were calculated. Fisher's exact test was used to identify significant associations between the simultaneous presence of the two resistance genes, as well as between carriage of the Int 1 integron and the presence of the resistance genes. The threshold for statistical significance was set at 5%, and the analyses were performed using STATA software, version 9.2.

3. Results

3.1. Isolated *Escherichia coli* Strains

A total of 16 chicken coops were sampled across all four sites in the study area. As a result, sixty-four (64) samples of broiler chicken droppings—four per coop—were collected. Fifty-seven (57) *Escherichia coli* strains were identified in these droppings (Table 3).

Table 3. Distribution of identified E. coli strains by sampling site.

Bacterial strains	Sampling sites				Total
	Ahoua	Abbê	Gare	M'brome	
Escherichia coli.	16	20	9	12	57

3.2. Antibiotic Resistance Profiles of *Escherichia coli* Strains

The results of susceptibility testing of *E. coli* strains to the antibiotics tested indicated complete susceptibility to beta-lactams (AMC, ceftriaxone, cefotaxime, ceftazidime), with 0% resistance. In contrast, resistance was 17.54% for gentamicin (aminoglycoside class), 45.61% for nalidixic acid (quinolone class), and 100% for colistin (polymyxin class) (Table 4).

Table 4. Resistance rates of *Escherichia coli* isolates to the antibiotics tested.

Antibiotics	Number of tested strains	Critical values (mm) * R < S≥	Resistance level	
			Number of resistant strains	Resistance rate (%)
AMC	57	14 - 21	0	0
Ceftriaxone	57	22 - 25	0	0
Cefotaxime	57	15 - 22	0	0

Continued

Ceftazidime	57	19 - 22	0	0
Gentamicin	57	16 - 18	10	17.54
Nalidixic acid	57	15 - 20	26	45.61
Colistin	57	15 - 18	57	100

*Selon CASFM 2023; R= Resistant; S= susceptible; AMC= Amoxicillin + clavulanic acid.

3.3. Production of Broad-Spectrum Beta-Lactamases

The synergy test conducted to confirm the production of extended-spectrum beta-lactamases was negative for all *Escherichia coli* strains tested. Review of the results revealed the absence of a "champagne cork" pattern—which indicates synergy—between the antibiotic-impregnated discs containing third-generation cephalosporins (C3G) and the amoxicillin-clavulanic acid (AMC) inhibitor.

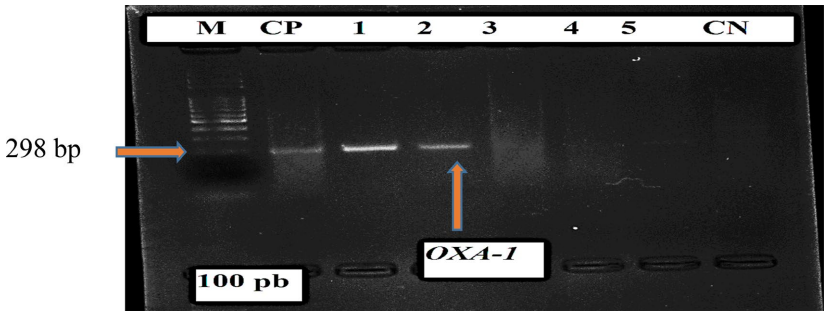
3.4. Molecular Characteristics of Antibiotic-Resistant *E. coli* Strains

3.4.1. Amplification Profile of the bla_{OXA-1} and *aac* (6') Resistance Genes in *E. coli*

PCR analysis of 24 resistant strains of *E. coli* revealed the presence of the *oxa*-1 gene in 3 of them (12.50%) characterized by a 298-bp band (Figure 1). Furthermore, *the aac* (6) gene, identified by a 482-bp band, was detected in 6 of these strains, representing a rate of 25% (Figure 2). The aac (6) resistance determinant was twice as common as the *oxa*-1 gene in this Azaguié location. Furthermore, the search for significant associations revealed a co-occurrence between the *oxa*-1 and *aac* (6) genes ($p = 0.00988$). There is therefore a link between these two biomarkers.

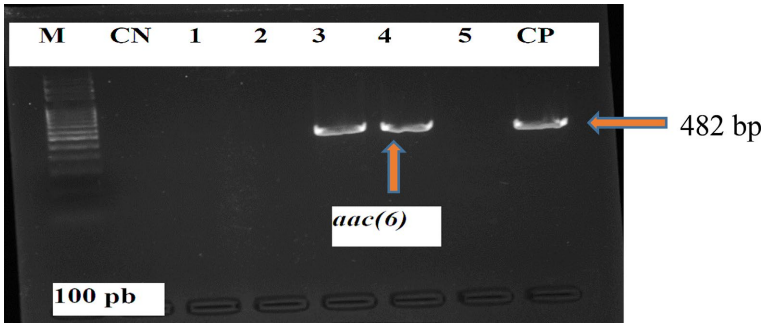
3.4.2. Prevalence of Class 1 Integrons (*Int* 1)

Molecular analysis of strains that were phenotypically resistant to the antibiotics tested revealed the presence of class 1 integrons (*Int* 1). Thus, 17 of the 24 *E. coli* strains analysed carried this integron, representing a rate of 70.83% (Figure 3).



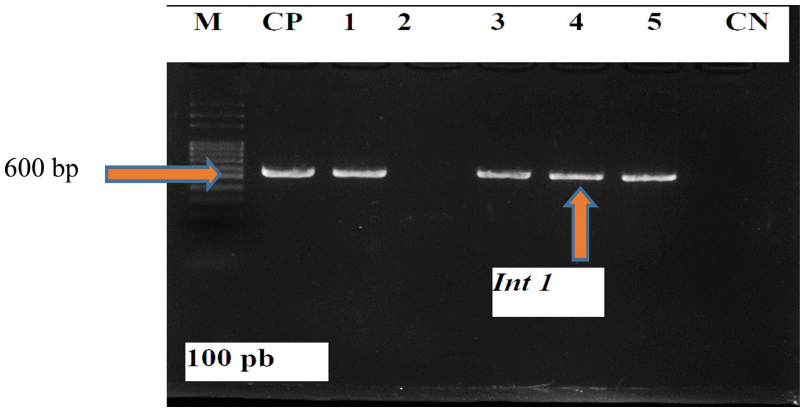
Lane M: molecular weight marker (Invitrogen, 100bp DNA Ladder), Lane CN: Negative Control, Lane CP: Positive Control *OXA*-1 (298 pb), Lanes 3, 4 and 5: Negative samples, Lanes 1 and 2: Positive samples for *OXA*-1.

Figure 1. 1% agarose gel electrophoresis showing a simplex PCR for the detection of the *OXA*-1 gene in *E. coli.*



Lane M: molecular weight marker (Invitrogen, 100 bp DNA Ladder), Lane CN: Negative Control, Lane CP: Positive Control *aac* (6) (482 bp), Lanes 1, 2 and 5: Negative samples, Lanes 3 and 4: Positive samples for *aac* (6).

Figure 2. 1% agarose gel electrophoresis showing a simplex PCR for the detection of the *aac* (6) gene in *E. coli.*



Lane M: molecular weight marker (Invitrogen, 100 bp DNA Ladder), Lane CN: Negative Control, Lane CP: Positive Control *Int* 1 (600 pb), Lane 2: Negative sample, Lanes 1, 3, 4 and 5: Positive samples for *Int* 1.

Figure 3. Agarose gel electrophoresis using 1% agarose, showing a simplex PCR assay for the detection of class 1 integrons in *E. coli.*

Furthermore, an investigation into associations between the presence of class 1 integrons and resistance genes showed that the class 1 integron was not significantly associated with either the $bla_{\text{OXA-1}}$ gene ($p = 0.47$) or the *aac* (6') gene ($p = 0.321$).

4. Discussion

Several studies have shown that *E. coli*, a commensal bacterium found in the digestive tract of animals, acts as a reservoir of resistance genes which can be released into the environment, thereby contributing to a reduction in the efficacy of these antibiotics and the inability to treat some bacterial diseases in both humans and animals [4] [5] [15]. Furthermore, class 1 integrons (*Int* 1) have already been reported in *E. coli* strains isolated from broiler chickens [16].

This study, which was conducted as part of an antibiotic resistance surveillance programme, aimed to determine the prevalence of the *OXA*-1 and *AAC* (6') re-

sistance genes in *E. coli* strains isolated from the droppings of broiler chickens in the town of Azaguié in Côte d'Ivoire, with a view to assessing the health risks associated with this activity.

Our results showed that all strains of *Escherichia coli* isolated from droppings and tested were fully susceptible to beta-lactams (amoxicillin + clavulanic acid, ceftazidime, ceftriaxone and cefotaxime), indicating the absence of extended-spectrum beta-lactamase (ESBL) production. These drugs remain fully effective and could constitute a first-line treatment for infections caused by *E. coli* strains circulating in local poultry farms. This absence of resistance could be explained by the lack of use or limited use of these drugs on farms in the Azaguié area. These results are similar to those of authors, who reported no resistance in broiler chickens in Tunisia [17]. They are very close to those reported by another author in Algeria, who found a very low rate of 1.96% of avian *E. coli* strains exhibiting the ESBL phenotype [18]. However, higher rates of 38.89%, 51.72% and 93.5% were reported by different authors in Togo [19], in Algeria [20] and in Nigeria [21], respectively.

The results of this study also revealed a gentamicin resistance rate (Aminoglycoside family) of 17.54%. This rate, which indicates the active circulation of aminoglycoside resistance determinants, could be explained by the unregulated use of gentamicin in poultry farming.

Similar results were obtained by some authors in Algeria, who, in their study on pathogenic avian *E. coli* strains, found resistance rates of 18.46% [22]. Particularly high rates have been reported by other authors: 32.50% in Algeria [23], 58.09% in China [24] and 91.7% in Nigeria [3].

Testing the susceptibility of *E. coli* strains to colistin, an antibiotic belonging to the polymyxin family, revealed a resistance rate of 100%. This total resistance to colistin may be due to intense selection pressure linked to its historical or recent use for metaphylaxis or as a growth promoter on poultry farms in the study area. Indeed, colistin is widely used in the poultry industry to treat colibacillosis and salmonellosis, even though it is classified by the WHO as a critically important antibiotic of major priority. It should not be used as a first-line antibiotic in the treatment of bacterial infections in animals. Our results are far higher than those obtained by two authors in Morocco, who reported no resistance [25], and by other authors in Senegal, who reported a rate of 2.2% [26].

The susceptibility of *E. coli* strains was tested using nalidixic acid, a member of the quinolone family. The resistance rate of *E. coli* strains to this compound is 45.61%. This high rate could be explained by the widespread prescription of fluoroquinolones following β-lactams in Africa, and particularly in Côte d'Ivoire [27], or by frequent chromosomal mutations in the target genes (*gyrA* and *parC* genes) that encode topoisomerases [28]. This rate is similar to that obtained in a study of pathogenic avian *E. coli* strains in Algeria. Indeed, the authors found a resistance rate of 49.15% for this substance [22]. However, a lower rate of resistance to nalidixic acid (28.57%) was reported in Togo [19].

PCR analysis of resistant *E. coli* strains revealed the presence of the *oxa*-1 and *aac* (6') genes at rates of 12.50% and 25% respectively. The *oxa*-1 gene encodes a class D beta-lactamase. It confers resistance to penicillins (such as amoxicillin) and clavulanic acid inhibitors on *E. coli*, hence the potential failure of Augmentin [8]. The detection of the blaOXA-1 gene in *E. coli* strains that are fully susceptible to beta-lactams (amoxicillin + clavulanic acid, ceftazidime, ceftriaxone and cefotaxime) could be explained by a defect in gene expression due to mutations in the promoter region or in the gene's coding sequence [29]. As for the *aac* (6) gene, it encodes an enzyme that modifies aminoglycosides, thereby leading to resistance to several antibiotics in this class, including gentamicin [30].

The resistance rates obtained in this study relate solely to the group of strains previously identified as resistant. Thus, the majority of strains are more resistant to aminoglycosides than to penicillins. This suggests either frequent use of aminoglycosides or effective clonal or plasmid-mediated spread within the study area.

The 12.50% prevalence of the *oxa*-1 gene reported in this study is identical to that obtained in avian strains of *E. coli* in Ghana [31]. This prevalence is, however, higher than the 4% (1/25) reported on broiler chickens in Tunisia [17]. Our results are, however, considerably lower than those obtained in Senegal (59.37%) in a study screening for TEM, SHV and OXA-1 ESBL genes in *E. coli* strains [32].

Furthermore, the 25% prevalence of the *aac* (6') gene identified in this study differs completely from the results reported by a study, which found no *E. coli* isolates positive for the *aac* (6') gene on broiler farms in the peri-urban area of Dakar, Senegal [33]. Similarly, our findings report clearly higher prevalence rates than the 9.37% and 5.40% rates reported respectively in different studies in Algeria for avian strains of ESBL-producing *E. coli* harbouring the *aac* (6')-*Ib*-*cr* variant [34], and in Nigeria during a study on broiler chickens and the poultry environment [35].

The association between these two resistance biomarkers, *oxa*-1 and *aac* (6), identified in this study reveals a multidrug-resistant (MDR) profile in the *E. coli* strains investigated. This co-occurrence of the two genes suggests co-selection of the two biomarkers. Indeed, these two resistance determinants to beta-lactams and aminoglycosides co-exist on the same plasmids or within genetic cassettes such as class 1 integrons in Enterobacteriaceae [36]. The co-occurrence of antibiotic resistance determinants has been reported in poultry farming by some authors. Indeed, associations involving the *oxa*-1 and *aac* (6) genes in avian *E. coli* isolates have been identified in Ghana [31]. Similarly, a co-presence of the *aac* (6) gene with other resistance markers in a study characterising multi-drug-resistant Enterobacteriaceae strains of avian origin has been found in western Algeria [34].

The co-presence of these two markers could compromise the efficacy of several last-resort antibiotics: the *oxa*-1 gene could confer resistance to combinations such as penicillin and beta-lactamase inhibitors, whilst the gene could neutralise crucial aminoglycosides.

The search for class 1 integrons (*Int* 1) in *E. coli* strains that were phenotypically

resistant to the antibiotics tested revealed a prevalence of 70.83% during this study. This result suggests that 70.83% of the phenotypic resistance observed is attributable to this genetic element, which acts as a system for the capture, expression and dissemination of genes in the form of cassettes. It plays a role in the dissemination of antibiotic resistance genes. Resistance in the other strains (29.17%) could be due to other classes of integrons or genetic mutations leading to a change in the antibiotic binding site, or to active efflux pumps [37] [38]. The high prevalence of class 1 integrons (*Int* 1) could considerably reduce the effectiveness of first-line antibiotic treatments, as these genetic elements promote the rapid spread of resistance not only to bacterial descendants but also to other bacterial species [39] [40].

Similarly high rates of class 1 integrons (*Int* 1) have also been reported in other studies. A prevalence of 49.8% in pathogenic avian strains of *E. coli* has been obtained in northern Italy [41], whereas prevalence rates of up to 72.70% in pathogenic *E. coli* strains isolated from broiler chickens have been found in southern Iran [17].

Furthermore, this study showed no significant association between the presence of class 1 integrons and the $bla_{\text{OXA-1}}$ and *aac* (6') resistance genes. This finding suggests that resistance to beta-lactams and aminoglycosides may be carried by plasmids or free integrons [42].

5. Conclusion

This study revealed that the 57 *E. coli* strains isolated from droppings collected from 14 broiler farms sampled in the Azaguié area were fully susceptible to beta-lactams and completely resistant to colistin (100 per cent), with confirmed carriage of the *oxa*-1 (12.50%) and *aac* (6) (25%). Although the prevalence of resistance determinants was low, the *E. coli* strains exhibited a high prevalence of class 1 integrons (*Int* 1) (70.83%), suggesting a potential risk of resistance spreading within poultry farms in the Azaguié area.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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