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Prevalence and antibiotic resistance in *Escherichia coli* strains isolated from poultry farms in Azaguie, Côte d'Ivoire

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Abstract

Poultry farming, a promising sector in Côte d'Ivoire, is faced with a number of infectious diseases that are causing heavy losses at various stages of the value chain. The aim of this study is to determine the antibiotic resistance profile of *Escherichia coli* strains circulating in broiler farms in Azaguié, a locality in the south of Côte d'Ivoire. The methods used are isolation and phenotypic identification, antibiogram for bacterial sensitivity and synergy test for the production of extended-spectrum beta-lactamase. Fifty-seven (57) strains of *Escherichia coli* were isolated from droppings from 14 broiler farms in Azaguié. The study of bacterial sensitivity showed high rates of resistance to amoxicilin + clavulanic acid (48.48%), ceftazidime (21.21%), gentamicin (60.61%), colistin (100%) and nalidixic acid (57.57%). However, low levels of resistance were obtained with Cefotaxime and Ceftriaxone, with a rate of 6.06%. These results show the importance of raising awareness among poultry farmers about the use of antibiotics in order to reduce the level of antimicrobial resistance in this region.

Keywords: *Escherichia Coli*; Broiler Chicken; Resistance; Antibiotics; Azaguié

1. Introduction

Poultry farming plays an important role in the world's food supply. World production amounted to 112.1 million tonnes per year [1]. This is a very promising sector in West Africa, particularly in Côte d'Ivoire, where poultry farming plays an important role in the economic, social and cultural sectors, with an estimated 33 million heads of livestock [1]. It is therefore one of the essential sectors for food security, as it plays an essential role in food production in Côte d'Ivoire, providing a major source of animal protein. However, she is faced with multiple constraints: dietary, financial and, above all, pathological. Poultry diseases remain one of the limiting factors in the development of this type of farming, as they lead to heavy direct and indirect losses of livestock on farms. [2, 3]. Antibiotics are among the most commonly prescribed drugs in hospitals in Africa. [4]. They are most commonly used on livestock farms either as a curative or preventive measure or, in extreme cases, to compensate for inadequate hygiene [5]. A study has also shown that antibiotics are also used as food additives or hormone stimulators [6]. As part of the 'One Health' concept, the use of antibiotics in animal health must also take into account the possible impact on human health and the environment [7]. The two major consequences of the incorrect use of antibiotics are the presence of residues of active molecules in foodstuffs of animal origin and antimicrobial resistance. [5]. Overuse of antibiotics creates selection pressure for resistant pathogenic or commensal bacteria [5].

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Unfortunately, one of the main dangers associated with the use of antibiotics is the selection pressure that reduces the number of sensitive bacteria in the intestinal flora of animals to the level of those that are resistant [8]. In addition, resistance genes are disseminated by mobile genetic elements between bacteria of the same or different species. These mobile genetic elements can be transferred to susceptible bacteria by various mechanisms such as transformation, transduction and conjugation [9]. The WHO maintains that antibiotic resistance is responsible for 700,000 deaths every year and could cause the death of 10 million people a year if no coordinated and effective action is taken by 2050 [10]. The Azaguié sub-prefecture is an agropastoral area with a high level of antibiotic use, as livestock farming is the second most important economic activity in the region. Antibiotic prevalence and resistance must therefore be studied in order to monitor the problem of antibiotic resistance. The aim of this study is to determine the antibiotic resistance profile of *Escherichia coli* circulating in broiler chicken farms in the Azaguié sub-prefecture in Côte d'Ivoire.

2. Materials and methods

2.1. Study area

This study was carried out in Azaguié, a sub-prefecture in the south of Côte d'Ivoire. It is located 45 km north of Abidjan and 35 km from Agboville, the departmental capital, between latitudes 5°35 and 6°15 north and longitudes 3°55 and 4°40 west. Azaguié covers an area of 650 km2. It comprises two districts (Azaguié- Ahoua and Azaguié-Gare) and seven villages. The vegetation consists of dense forests and the climate is humid tropical with four seasons. The town has a population of 38,066 [11]. Azaguié's economy is based mainly on trade, agriculture and livestock, including pigs, broilers, layers and guinea fowl.

2.2. Sampling

2.2.1. Sampling sites

The two districts of the municipality (Azaguié-Ahoua and Azaguié-Gare), as well as the villages of M'bromé and Abbê were targeted in this study. In each locality, the aim was to visit, at random, six broiler farms, all privately owned and volunteering for a survey. All farmers were approached without distinction in order to obtain their signatures authorising access to their farms for the study.

2.2.2. Droppings collection

The droppings were collected from each farm according to the protocol used for this type of study [12]. According to this protocol, 20 freshly emitted droppings were sampled per thousand birds. From 24 to 26 June 2024, freshly emitted droppings were collected along the diagonals and medians of each barn. The droppings collected by axis in each building were collected in sterile pots. The droppings samples obtained were placed on ice before being transported to the laboratory, where they were stored at 4 °C.

2.3. Bacteriological analysis of droppings

2.3.1. Isolation of Escherichia coli strains

The bacteriological analyses were carried out in the bacteriology laboratory of the Genetics Research Unit of the Université Félix Houphouet-Boigny. The standard protocol described in NF EN ISO 6579 (ISO-6579, 2002E) was followed for the isolation of *Escherichia coli*, with the following four steps: pre-enrichment, selective enrichment, selective isolation and biochemical identification.

2.4. Identification of Escherichia coli strains

Characteristic colonies obtained on Hektoen agar were transferred to Müller-Hinton agar (Bio-Rad, Marnes, France) and incubated at 37 °C for 24 hours. Their identification was based firstly on morphological characteristics such as the fresh state and Gram staining, and secondly on biochemical characteristics using Leminor's reduced rack. The Escherichia coli strains were confirmed by Maldi-Tof (Biomerieux, France).

2.5. Bacterial sensitivity

2.5.1. Antibiogram

Antibiotic susceptibility was determined using the Müeller-Hinton agar-impregnated disc diffusion method in accordance with the recommendations of the Antibiogram Committee of the Société Française de Microbiologie [13].

The antibiotic discs tested were gentamicin (10 µg), nalidixic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), colistin (10 µg) and amoxicillin-clavulanic acid (20/10 µg). The reference strain *E. coli* ATCC 25922 was used during the antibiograms in order to carry out the positive control.

2.5.2. Detection of extended-spectrum beta-lactamase (ESBL) production

The double synergy method was used for the detection of *E. coli* ESBL **[**14]. This involved placing 3rd generation cephalosporin discs (cefotaxime, ceftriaxone and ceftazidime) around a central disc of amoxicillin + clavulanic acid in accordance with EUCAST-CASFM recommendations. [13]. The presence of ESBLs is indicated by a distortion of the zone of inhibition in relation to the disc containing clavulanic acid, creating a synergistic image known as a "champagne cork".

2.6. Data analysis

2.6.1. Bacteria identification and prevalence

Prevalences of infection were calculated as a function of locality, age and bacterial strain. A chi-2 test was used to assess the associations between infections and the locality, age and farm studied. The statistical significance threshold for these various tests was $\alpha < 0.05$. The tests were performed using STATA 9.2 software.

2.6.2. Establishment of antibiotic resistance profiles of E. coli strains

The results of the antibiogram were read and interpreted using an ADAGIO® automated system. The results obtained were reported on a form and recorded on a computer file using Excel® software. These values were interpreted as S (sensitive) and R (resistant) according to the critical diameters for Enterobacteriaceae provided by EUCAST-CASFM. [13].

3. Result

3.1. Isolated strains

A total of 16 hen houses were sampled at the four sites in the study area. Sixty-four (64) samples of broiler droppings, four per house, were collected. The search for *E. coli* bacterial strains in these droppings revealed their presence. E. coli strains were obtained with a bacteriological prevalence of 89.06%, i.e. 57 strains (Table I).

Table 1 Distribution of *E. coli* strains isolated by sampling site

Bacterial strains	**Sampling sites**				**Total**
	AHOUA	ABBÊ	GARE	M'BROME	
Escherichia coli	16	20	9	12	57

3.1.1. Prevalence according to sampling sites

Table II shows the results of the effects of the sites on the prevalence of bacterial strains in this study. A significant difference in isolation was observed between sites. In fact, *E. coli* strains were more prevalent in the Azaguié-Ahoua district and the village of Abbê (100 %) than in the Azaguié-Gare district and the village of M'bromé, where prevalence was 75 %.

3.1.2. Prevalence according to age-types of birds

Bacteriological analysis identified 8 strains of *E. coli* in chicks aged 1 to 7 days, 17 strains in those aged 7 to 21 days, and 32 strains in those aged 21 days and over.

Table 2 Prevalence of Escherichia coli strains isolated by sampling site

Variables	Sampling sites	Number of samples examined	Infected samples		p-value
			Nombre	%	
E. coli	AHOUA	16	16	100	0,017
	ABBÊ	20	20	100	
	GARE	12	9	75	
	M'BROME	16	12	75	

3.2. Antibiotic resistance profiles

3.2.1. With regard to beta-lactams

Overall, the profile of Escherichia coli strains against beta-lactams did not reveal any resistance. The molecules amoxicilin+clavulanic acid, ceftriaxone, cefotaxime and ceftazidime were therefore active on the *E. coli* strains tested (Figure 1).

3.3. With regard to Aminosides and Polymyxins

The only aminoglycoside antibiotic used was gentamicin. The antibiogram revealed a resistance rate of 16.67 % in the *E. coli* strains tested. In addition, all the strains tested with colistin showed resistance to this compound, giving a resistance rate of 100% (Figure 1).

3.3.1. With regard to Quinolones

The results showed resistance to nalidixic acid. The rate of resistance of Escherichia coli strains to this molecule was 45.83% (Figure 1).

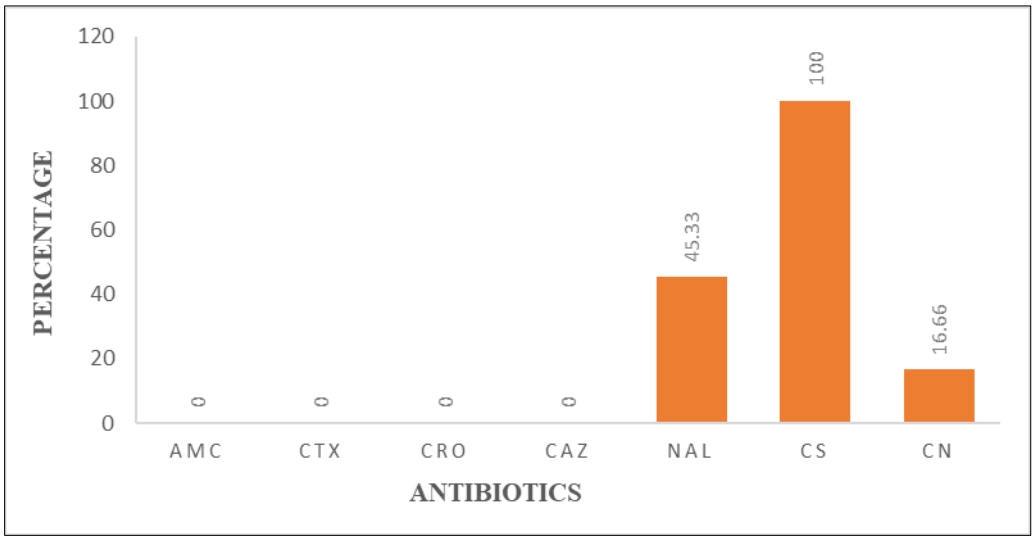

Figure 1 Resistance rates of *E. coli* strains to the antibiotics tested

Gentamicin (CN), nalidixic acid (NAL), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), colistin (CN) and amoxicillin-clavulanic acid (AMC)

3.4. Production of extended-spectrum betalactamase

The synergy test carried out to confirm the production of extended-spectrum beta-lactamases by *E. coli* strains was negative for all strains. The results did not reveal the champagne cork image, which is the synergy observed between the antibiotic-impregnated discs of third-generation cephalosporins (C3G) and the inhibitor amoxicillin and clavulanic acid (AMC).

4. Discussion

The aim of this study was to determine the antimicrobial resistance profile of *Escherichia coli* circulating in broiler chicken farms in the Azaguié sub-prefecture in Côte d'Ivoire.

The prevalence of E. coli strains obtained was 89.06 Our results are in line with those of Berghiche et al. [15] in north-eastern Algeria and Halder et al. [16] in Bangladesh, who report that colibacillosis is the most frequent bacterial infection in poultry. This high rate of infection could be explained by the combination of several factors that can predispose to colibacillosis or influence the expression of the disease: (i) abnormal water stress, (ii) too high humidity level (>50%) and too low temperature, combined with cold litter when the chicks are placed, (iii) the quality of the chicks when they arrive on the farms, (iv) inadequate application of external and internal biosecurity measures [17, 18]. In addition, the E. coli species is the best index of faecal contamination for research into pathogenic Enterobacteriaceae. [19].

Prevalences were also obtained according to farms, sites and age-types, where Chi-squared tests, at the 5 % significance level, revealed that the prevalence of E. coli did not vary significantly according to locality or age-types. These results could be explained by the continuous bacterial exposure in the birds' environment at all the sites and at all times during their different evolutionary periods.

With regard to the antibiotic resistance of E. coli strains, all the strains tested showed no resistance to beta-lactam antibiotics. The absence of resistance to beta-lactam antibiotics in *E. coli* could be explained by a number of factors, including the bacterium's intrinsic susceptibility, the absence of resistance genes, the low selection pressure for the development of antibiotic resistance. However, Meki and Qada [20] obtained a rate of 51.72 % for beta-lactam antibiotics in Algeria.

In the Aminoside family, the results revealed a resistance rate of 16 %. Indeed, the exclusive and intensive use of an antibiotic could select resistant strains [21] in the poultry sector, which would favour the presence of resistant strains in Azaguié. It is important to stress that enterobacteria have a clear capacity to acquire and exchange genes carrying resistance factors, and the intestinal flora provides an extraordinary opportunity for the circulation of genetic information between bacteria [22].

Higher rates have been obtained in various studies. Aberkane et al. [23] and Akkari et al. [24] obtained resistance rates of 32.50 % and 49 % respectively in their work on avian E. coli strains in Algeria.

With colistin, the bacterial strains showed total resistance (100 %). According to Perrin-Guyomard et al. [25], this observed rate is strongly correlated with exposure of poultry to colistin, which could favour dissemination of colistin resistance genes within the bacterial community. Our results do not corroborate those obtained in Morocco by Oubouyahia and Nassik [26], who reported no resistance to this molecule.

The sensitivity of *E. coli* strains was tested with nalidixic acid, which belongs to the quinolone family. The resistance rate obtained was 45.83%. This can be explained by the fact that fluoroquinolones are the drugs most prescribed after β-lactams in Africa, and particularly in Côte d'Ivoire [4]. This result is similar to that obtained in a study of pathogenic avian *E coli* strains in Algeria, where the authors obtained a resistance rate of 49.15% [24].

Resistance can also be determined chromosomally by the occurrence of a genetic mutation leading to a change in the antibiotic binding site, or by active efflux. However, the latter is of plasmid origin, which can be explained by the acquisition or transfer of one or more antibiotic resistance genes via mobile genetic elements such as transposons and integrons [27].

5. Conclusion

This study looked at 57 *Escherichia coli* strains isolated from the 14 broiler farms visited. Bacterial susceptibility showed resistance to nalidixic acid, gentamicin and colistin, in which the rate was particularly high. However, no resistance to beta-lactam antibiotics has been observed. Poultry farmers must therefore ensure the rational use of antibiotics in order to limit or even reduce antibiotic resistance rates.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that there is no conflict of interest.

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