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# Antibiotic resistance profile of pathogenic enterobacteria strains isolated from some poultry farms in the Forécariah prefecture in the Republic of Guinea

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## Abstract

**Introduction:** *Escherichia coli* and *Salmonella spp*. are among the *Enterobacteria* responsible for human and animal diseases causing considerable economic losses in the poultry sector. The uncontrolled use of antibiotics in poultry farming often leads to the selection of antibiotic-resistant bacterial germs and a decrease in productivity.

**Objectives:** This study, conducted from July to September 2024, aimed to determine the prevalence and antibiotic resistance of pathogenic *Enterobacteriaceae* strains isolated from poultry farms in Forécariah prefecture.

**Material and Methods:** A total of 119 samples were collected from 5 breeding sites. *Enterobacteriaceae* strains were isolated using standard microbiology methods and confirmed by the Api 20 E gallery. Antibiotic resistance was determined using the agar diffusion method.

**Results:** *Enterobacteriaceae* contamination was detected in 75.79% of fresh droppings samples and in 79.16% of cloacal swabs. The results of antibiotic resistance showed that: 100% of the pathogenic *Enterobacteriaceae* tested were resistant to at least one antibiotic: *Escherichia coli* strains were resistant to 92.86% to Gentamicin, 77.27% to Ampicillin and 42.86% to Azithromycin. Similarly, *Salmonella spp*. were 100% resistant to Gentamicin, 75% to Ampicillin and Nitrofurantoin. Furthermore, this study highlighted the presence of five genera of Enterobacteriaceae in the Forécariah prefecture. Although the most pathogenic ones are underestimated, it is necessary to strengthen biosecurity measures to stem the spread of these pathogens.

**Keywords:** Resistance; Antibiotics; Pathogenic *Enterobacteriaceae*; Poultry farms; Forécariah

## 1. Introduction

Poultry farming is the animal production sector that has experienced the most remarkable growth in recent decades. Production costs and product prices have contributed to making poultry the preferred meat for producers and consumers worldwide, particularly in developing countries [1].

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However, this sector faces challenges that are hampering its development. These include the hygienic quality of the water used to water exotic chickens, unfair competition from imported poultry meat, and pathological problems [2,3]. These pathologies, often of bacterial, viral, and parasitic origin, remain ever-present.

Among the bacterial species that contaminate meat, some are known to be extremely pathogenic, and their presence makes the food unsafe, such as *Salmonella*, and those that are tolerated at very specific thresholds, such as *Escherichia coli* [1].

The presence of these germs in poultry products, including meat, eggs, and their derivatives, has often made them unsafe for human consumption [4,5]. This leads to gastrointestinal illnesses in humans and animals worldwide.

In Europe, it is estimated that 25,000 deaths are due to infection with multidrug-resistant bacteria (MDR) each year. This cost is €1.5 billion per year [6].

Colibacillosis and salmonellosis are zoonotic diseases caused by Salmonella and Escherichia coli, which currently represent one of the most significant causes of economic losses in the poultry sector and a major concern for the food industry [7].

To counter the spread of these pathogens, poultry farmers resort to the overuse or inappropriate use of antibiotics as a cure or preventative measure to increase their production profitability and meet consumer demand. This inappropriate use of antibiotics can lead to the selection of resistant pathogenic bacteria [8].

In low-income countries, the cost of these antibiotics most often falls on patients, as there are few or no effective health insurance or social security systems [9]. This practice contributes to the emergence and spread of antibiotic-resistant or multi-resistant bacteria, leading to serious risks to human health.

In West Africa, bacterial diseases of avian origin, particularly colibacillosis and salmonellosis, are frequently diagnosed in many countries such as Côte d'Ivoire, Senegal, and Mali [10-12].

In the Republic of Guinea, very little reliable statistical data are available on the prevalence and antibiotic resistance of these strains in poultry farms, particularly in the Forécariah prefecture. It is therefore clear that a better understanding of this issue could allow the implementation of a dynamic approach to the preservation of animal and human health in the Forécariah prefecture. The objective of this study was to determine the prevalence and resistance of pathogenic *Enterobacteriaceae* strains isolated from poultry farms in the Forécariah prefecture.

## 2. Material and methods

### 2.1. Presentation of the Study Area

Forécariah is a prefecture within the administrative region of Kindia and plays an important role due to its geographical location (straddling the capital, Conakry, and the Republic of Sierra Leone).

Forécariah Prefecture is located 99 km from Conakry between 9° 26′ 05″ N latitude and 13° 05′ 07″ W longitude.

It is bordered by Coyah Prefecture to the north, the Republic of Sierra Leone to the south, Kindia Prefecture to the east, and the Atlantic Ocean to the west.

It covers an area of approximately 4,187 $km^2$ with a population of approximately 259,573 inhabitants in 2016, or 62 inhabitants/$km^2$ [13]. It is crossed by the river of the same name which is a tributary of the Mellacorée and plays an important role in the life of the prefecture, particularly for agriculture, livestock farming and fishing.

The climate in this area is sub-Guinean, with temperatures ranging from 21°C to 37°C and alternating seasons (rainy and dry). This seasonal variation favors the development of agriculture and livestock farming.

Agriculture is dominated by the production of rice, peanuts, cassava, and pineapples, most of which are managed by groups/cooperatives that play an important role in the local economy.

Livestock farming, particularly poultry farming, is characterized by a strong presence of farms in the peri-urban area, which were significantly impacted by the avian flu epidemic in 2022.

Despite certain challenging constraints, these two sectors are major providers of employment and income for approximately 70% of the working population [14]. The Institute of Applied Biology Research of Guinea (IRBAG), which served as our study framework, is located 6km northwest of the urban commune of Kindia. It consists of a management, a doctoral school and six scientific departments (Virology, Epidemiological Clinic, Medical Zoology, Venereology, Food Hygiene, Environment and Bacteriology).

### 2.2. Sampling

This cross-sectional study was conducted from July to September 2024. The aim was to collect and analyze samples from the visited poultry farms in the laboratory.

The study was conducted between July and September 2024 on a sample of five (05) laying hen farms located in the peri-urban areas of Forécariah. The selection of these farms was based on the owners' consent to cooperate during the study. Two visits were conducted to each farm: the first to identify the site and raise awareness, and the second to collect samples. The biological materials consisted of fresh droppings and cloacal swabs.

### 2.3. Transport

The samples were transported in a cooler containing ice packs (dry ice) at 4°C and transported to the laboratory for analysis or storage within 4 hours, in accordance with ISO 15189 [15].

### 2.4. Methods

A total of 119 samples, including 95 fresh droppings and 24 cloacal swabs, were collected from five (05) private poultry farms between July and September 2024, with the following distribution by farm (Table 1).

**Table 1** Distribution of samples according to poultry farms

| **Sampling sites** | **Poultry Farm Codes** | **Types of samples** | |
|---|---|---|---|
| | | **Fresh droppings (Y)** | **Cloacal swabs (Z)** |
| Forecariah Center | F1 | 20 | 7 |
| | F2 | 17 | 0 |
| Maferinyah | F3 | 22 | 7 |
| Alassoyah | F4 | 21 | 10 |
| | F5 | 15 | 0 |
| Total | 5 | 95 | 24 |

Legend: Y: Number of fresh droppings collected; Z: Number of cloacal swabs collected

As shown in Table I, in farms F1 to F5, the droppings and swabs collected were as follows, respectively:

- 37 and 7 for the two farms in Forécariah Centre;
- 22 and 7 for the Maferinyah farm alone;
- 36 and 10 for the two farms in Alassoyah.

During the sampling, approximately 20g of fresh droppings were collected from one-third of the total surface area of the building and then placed in a sterile tube containing approximately 5ml of sterile distilled water. Cloacal swabs were also taken from laying hens showing suspicious clinical signs (diarrhea, anorexia, oral discharge, etc.) and which were not in the laying phase. All of these samples were placed in a cooler equipped with dry ice to maintain the temperature around 4°C and then transported within a period not exceeding 4 hours. Upon arrival, the samples were then refrigerated and processed the following day.

Each batch of samples collected from the same farm was accompanied by a tracking sheet including the operator's name, the sampling date, the age and number of birds per flock, and any signs of disease.

*2.4.1. Bacteriological Analysis*

Samples of organic products collected from the Forécariah poultry farms underwent bacteriological analysis. This analysis was carried out at the Medical Bacteriology Laboratory of the Guinean Institute for Applied Biology Research (IRBAG).

Isolation and Characterization

The isolation of Enterobacteriaceae was carried out according to the Enterobacteriaceae research protocol applied at the IRBAG bacteriology laboratory, inspired by the French standard NF U 47-101 (2007), which includes 3 steps:

- **Step 1: Pre-enrichment (for all Enterobacteriaceae):**

After cauterizing the surface, 1g of droppings was immersed in 9ml of buffered peptone water (BPPW) and homogenized using a V-32 Vortex Mixer for 1 minute, then each swab was dipped in buffered peptone water.

All homogenates were left for revitalization at room temperature for 30 minutes and then incubated at 37°C for 18 to 20 hours.

- **Step 2: Enrichment (for Salmonella only)**

The next day, after revival, 1 ml of each type from the pre-enrichment is transferred for enrichment into 20 ml of Rappaport-Vassiliadis (RVs) broth and then incubated at 37°C for 18 to 24 hours.

- **Step 3: Isolation**

The next day, after homogenization, 0.1 ml of each suspension was inoculated onto a Hecktoen agar plate for Salmonella and a MacConkey agar plate for other Enterobacteriaceae, then incubated at 37°C for 18 to 24 hours.

Biochemical Identification

The biochemical identification of Enterobacteriaceae was carried out in two media:

- **-Kligler-Hajna agar**

Kligler medium was used to identify the *Enterobacteriaceae* present in the analyzed sample by rapidly demonstrating the fermentation of lactose and glucose (with or without gas production) as well as the production of hydrogen sulfide.

Using a loop, one to two colonies characteristic of *Salmonella spp.* and/or *Escherichia* coli were collected from previously inoculated Petri dishes and subcultured into Kligler-Hajna slant tubes, incubated at 37°C for 18 to 24 hours.

Typical cultures of *Escherichia coli* and/or *Salmonella spp.* corresponded to an alkaline slant (red) and an acidic pellet (yellow), with gas and hydrogen sulfide formation (blackening of the agar).

API 20 E identification gallery

After inoculation and incubation according to the manufacturer's recommendations, the reactions resulted in spontaneous color changes, revealed by the addition or absence of reagents.

Reading was performed using APIWEB identification software (bio-Mérieux, France).

For all these identification steps, incubation was performed at 37°C for 18 to 24 hours.

Antibiograms

The antibiogram was performed using the Mueller-Hinton agar diffusion method of antibiotic disks (bio-Mérieux).

The results were interpreted according to the rules and recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) [16]. Mueller-Hinton agar plates are flooded with bacterial suspensions scraped from

the slope of Kligler tubes, diluting the inoculum 1/1000, to obtain the equivalent of 106 CFU/ml. The reading was taken after 18 to 20 hours of incubation at 37°C.

Inhibition zone diameters were measured, and the results were interpreted according to the criteria of the French Society of Microbiology's Antibiogram Committee. The following antibiotics were tested: Ampicillin (AMP10), Gentamicin (GN10), Trimethoprim/Sulfamethoxazole (SXT25), Ciprofloxacin (CIP5), Nitrofurantoin (F300), and Azithromycin (AZM30).

## 3. Results

### 3.1. Prevalence of *Enterobacteriaceae* contamination in Poultry Farms in Forécariah

**Table 2** Prevalence of *Enterobacteriaceae* contamination in droppings

| Isolated germs | Number of cases | Percentage (%) |
|---|---|---|
| *Escherichia coli* | 32 | 44.44 |
| *Citrobacter spp.* | 11 | 15.28 |
| *Proteus spp.* | 11 | 15.28 |
| *Serratia spp.* | 8 | 11.11 |
| *Enterobacter spp* | 5 | 6.94 |
| *Klebsiella spp* | 3 | 4.17 |
| *Salmonella spp.* | 2 | 2.78 |
| Total | **72** | **100** |

The microbiological study involved 95 droppings collected from five (05) semi-modern poultry farms in the Forécariah prefecture.

Microbiological analysis of these droppings at the Bacteriology Laboratory of the Guinean Institute for Applied Biology Research (IRBAG) resulted in the isolation of 72 strains of *Enterobacteriaceae*. This represents 75.79% (72/95) of all Gram-negative bacteria isolated during the study period (Table 2). The majority of isolated germs were *Escherichia coli* (32 strains), followed by *Citrobacter spp.* and *Proteus spp*. (11 strains each), then *Serratia spp.* (8 strains), *Enterobacter spp.* (5 strains), *Klebsiella spp.* (3 strains), and *Salmonella spp.* (2 strains).

**Table 3** Prevalence of *Enterobacteriaceae* contamination in cloacal swabs

| Isolated germs | Nomber of cases | Percentage (%) |
|---|---|---|
| *Escherichia coli* | 9 | 47.37 |
| *Proteus spp.* | 4 | 21.05 |
| *Klebsiella spp.* | 3 | 15.79 |
| *Salmonella spp.* | 2 | 10.53 |
| *Enterobacter spp.* | 1 | 5.26 |
| Total | **19** | **100** |

A total of 24 cloacal swabs (Table 3) were collected during the surveys in three (03) poultry farms. Bacteriological analysis of these swabs allowed the isolation and identification of 19 strains of *Enterobacteriaceae* with a contamination prevalence of 79.16% (=19/24) for all Gram-negative bacteria.

The proportion of isolated germs was as follows: *Escherichia coli* (47.37%), *Proteus spp*. (21.05%), *Klebsiella spp.* (15.79%), *Salmonella spp.* (10.53%), and Enterobacter spp. (5.26%).

### 3.2. Antibiotic Resistance Profile of Pathogenic *Enterobacteriaceae* Strains

Due to their pathogenicity, particularly in poultry farms, two species were selected for testing against the six antibiotics mentioned above. These are *Escherichia coli*, which has been considered an opportunistic pathogen, and *Salmonella spp.*, which remains the most pathogenic species in this family for poultry.

**Table 4** Frequency of Antibiotic Resistance in *Escherichia coli* Strains

| Poultry Farms | Antibiotics (µg) | | | | | |
|---|---|---|---|---|---|---|
| | AMP10 n (%) | GN10 n (%) | CIP5 n (%) | F300 n (%) | AZM30 n (%) | SXT25 n (%) |
| **F1 (n=14)** | 14 (100) | 13 (92.86) | 3 (21.43) | 1 (7.14) | 5 (35.71) | 14 (100) |
| **F2 (n=10)** | 8(80.00) | 10 (100) | 3 (70.00) | 00 | 4 (40.00) | 10 (100) |
| **F3 (n=4)** | 2 (50.00) | 3 (75.00) | 00 | 00 | 2 (50.00) | 4 (100) |
| **F4 (n= 6)** | 3 (50.00) | 3 (50.00) | 1 (16.66) | 1 (16.66) | 1 (16.66) | 6 (100) |
| **F5 (n=10)** | 7 (70.00) | 8 (80.00) | 4 (40.00) | 00 | 6 (60.00) | 10 (100) |
| **Total (n=44)** | 34 (77.27) | 37 (84.10) | 11 (25.00) | 2 (4.76) | 18 (42.86) | 44 (100) |

**Legend:** AMP10: Ampicillin; GN10: Gentamicin; CIP5: Ciprofloxacin; F300: Nitrofurantoin; AZM30: Azithromycin; SXT25: Trimethoprim/Sulfamethoxazole.

The results of the antibiograms of the present study carried out in Forécariah (Table 4) revealed that the majority of *Escherichia coli* strains were resistant to the following three antibiotics: Trimethoprim/Sulfamethoxazole (100% = 44/44), Gentamicin (84.10% = 37/44), and Ampicillin (77.27% = 34/44). In contrast, the resistance rate was average for Azithromycin (42.86%). However, the resistance rate was rather low to Ciprofloxacin (25% = 11/44) and Nitrofurantoin (4.76% = 2/44). Thus, of all the antibiotics tested on these *Escherichia coli* strains, Nitrofurantoin was the most active, followed by Ciprofloxacin. In other words, the sensitivity rate of *Escherichia coli* strains was 95.24%, and that of Ciprofloxacin was 75%. This indicates its preferred position among these two antibiotics in the treatment of severe infections in poultry farms in Forécariah.

**Table 5** Frequency of antibiotic resistance of *Salmonella spp*. strains

| Poultry farms | Antibiotics (µg) | | | | | |
|---|---|---|---|---|---|---|
| | **AMP10** | **CN10** | **CIP5** | **F300** | **AZM30** | **SXT30** |
| **F4 (n= 2)** | 2 (100) | 2 (100) | 1 (50) | 1 (50) | 1 (50) | 2 (100) |
| **F5 (n= 2)** | 1 (50) | 2 (100) | 0 | 0 | 0 | 2 (100) |
| **Total (n=4)** | 3 (75) | 4(100) | 1 (25) | 1 (75) | 1 (25) | 4 (100) |

**Legend:** AMP10: Ampicillin; GN10: Gentamicin; CIP5: Ciprofloxacin; F300: Nitrofurantoin; AZM30: Azithromycin; SXT25: Trimethoprim/Sulfamethoxazole.

In Table 5, *Salmonella spp*. strains isolated during this study showed varying resistance to the four antibiotics tested. Indeed, all *Salmonella spp.* strains (100%) were resistant to the combination of trimethoprim/sulfamethoxazole and gentamicin, while 75% of these strains were resistant to both ampicillin and nitrofurantoin (F300, 75%). In contrast, these strains of *Salmonella spp*., had a relatively low frequency of resistance (25%) to both Ciprofloxacin and Azithromycin. In other words, the sensitivity of these strains was 75% to both Ciprofloxacin and Azithromycin, suggesting that among the antibiotics tested, only these two antibiotics could be used in the treatment of infections caused by these strains of *Salmonella spp*.

## 4. Discussion

The objective of this study was to assess the antibiotic resistance of pathogenic *Enterobacteriaceae* strains isolated from several poultry farms in the Forécariah prefecture.

The results of this study showed that the prevalence of *Enterobacteriaceae* contamination in Forécariah poultry farms varied depending on the nature and sampling areas.

The isolation rates of *Enterobacteriaceae* were 75.79% (72/95) in droppings and 79.16% (=19/24) in cloacal swabs, for an overall rate of 76.47% (91/119) for avian samples. This contamination rate is higher than that obtained by Boutaiba in 2023 , which was 54.31% in western Algeria [7].

The results of our study also show that *Escherichia coli* is the most frequently isolated species among *Enterobacteriaceae* in poultry farms. Its prevalence is 44% in droppings and 47.37% in swabs. These rates are lower than those reported by Banameur in 2011 [17], which were 48.33% in the farms of Mostaganen, Mascara, Taret, Relizane, Chlef, and Tissemsilt (Algeria).

The predominance of the *Escherichia coli* species in these types of samples may be due to its direct involvement in various infections and indirectly to colibacillary superinfections, as it is rarely a primary infection agent; it is rather an opportunistic bacterium [7,18].

On the other hand, *Salmonella* is the most important ubiquitous zoonotic pathogen in the *Enterobacteriaceae* family. It is present throughout the food chain, from farm to plate. Their direct involvement in foodborne illnesses, especially in poultry products, is often reported. In this study, the isolation rates in droppings and cloacal swabs were 2.78% and 10.53%, respectively.

These rates are lower than those reported in many studies from other countries. Indeed, in 2013, Alloui et al. (2013) reported a rate of 50% in Algeria , while Bonny et al. (2011) reported a rate of 61.87% in Côte d'Ivoire [19,20]. In Cameroon, Piebeng et al. (2014) reported a rate of 78.46% of non-typhoidal *Salmonella* in a total of 65 birds (Gendarme Weavers) that had been captured and examined for microorganisms [21]. In Morocco, in the Meknes region, Abdellah and Fouzia reported a 24% incidence of *Salmonella* in chicken farming in 2016 [22].

Moreover, our results are higher than those reported in eastern Algeria by Elgroud in 2009 in the broiler and egg-laying sectors, with a frequency of positive *Salmonella* isolations of 1.66% and 0.68%, respectively.

Antibiotic susceptibility testing of bacteria during this study revealed a very high rate of resistance of *Escherichia coli* strains to the Trimethoprim/Sulfamethoxazole combination. Indeed, all *Escherichia coli* strains (100%) were resistant to this antibiotic combination. They also had a high rate of resistance to Gentamicin (92.86%) and Ampicillin (77.27%), thus suggesting a high rate of multidrug resistance in these Escherichia coli strains. These results corroborate those mentioned in 2011 by Benameur [17] who showed a very high rate of *Escherichia coli* isolates (100% resistant to at least one antibiotic) as well as the multi-resistance of the isolates analyzed (96.51% of isolates multi-resistant to at least 2 different antibiotics and 92.20% to at least 3 antibiotics). Boutaiba Benkhlaouz M found during his work in 2023, results which showed that 90.34% of *Escherichia coli* strains were resistant to nalidixic acid and 86.89% of strains were resistant to tetracycline, while for ampicillin it was around 82.75%. An alarming rate of antibiotic resistance of *Escherichia coli* to a single antibiotic was revealed (100%), as well as for multiresistance (97.24% for at least 2 antibiotics and 95.86% for at least 3 antibiotics) [7]. On the other hand, Boutaiba BM in [7] revealed in his results that the resistance rate of *Escherichia coli* isolates to the Trimethoprim/Sulfamethoxazole combination was 56.25% and that of Ciprofloxacin was 81.25%.

These data suggest that bacterial resistance to antibiotics depends on several factors, including the adaptive capacity of these bacteria.

Analysis of the antibiotic resistance profile of *Salmonella spp.* strains showed 100% resistance to at least one antibiotic. The highest resistance rates were found for the combination of Trimethoprim/Sulfamethoxazole (100%), Gentamicin (100%), and Ampicillin (75%). These results are similar to those of Abba et al. in 2017, who reported in a similar study in Chad that the isolated *Salmonella spp.* strains showed complete resistance (100%) to Ampicillin, Erythromycin, and Imipenem. These authors also observed a 34.15% frequency of multidrug resistance in the bacterial strains studied.

Many authors have found different resistance profiles for *Salmonella spp.* strains. For example, Sidibé et al., reported a frequency of resistance of *Salmonella* strains of 21.2% to Gentamicin, while Mzungu I et al. (2016) reported 100% resistance of *Salmonella* strains to Ampicillin[12,25]. finally, Lydia B. and Atika B (2022) reported a resistance rate of 50% of *Salmonella* strains to the combination Trimethoprim/Sulfamethoxazole [26].

These different data show that the choice of antibiotics to be tested could vary depending on the authors; this difference could be explained by the level of antibiotic use which varies from one country to another.

Our observation reveals that in poultry farms in Forécariah, the use of antibiotics is done in a haphazard manner (non-specific treatment), which would favor the development and spread of multi-resistant bacteria. This same finding has been observed in other countries such as Chad and Cameroon [24,27].

Multidrug-resistant strains (resistant to three or more antibiotics) in the Forécariah prefecture have an overall prevalence of 58.33%. This rate is lower than that reported in Mali (69.96%) in the three peri-urban sites of Bamako [12].

## 5. Conclusion

During this study, five genera of *Enterobacteriaceae* were identified from 119 samples (95 droppings and 24 cloacal swabs) collected from five semi-modern poultry farms.

It therefore seems essential to take appropriate measures to reduce contamination in laying hen farms and to appropriately use, in the event of contamination, the antibiotics that proved most active during this study. Looking ahead, we plan to expand the number of antibiotics tested on isolated organisms and conduct serological and molecular studies to determine the genetic underpinnings of these resistances and their modes of propagation in different bacterial populations in poultry farms in Guinea.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare no conflicts of interest.

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