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Characterization and evaluation of bacteriological contamination by Salmonella spp. of some poultry farms in the Prefecture of Kindia (Republic of Guinea)

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Abstract

Introduction: Poultry farms are one of the main reservoirs of enterobacteria, especially *Salmonella*, which are often associated with foodborne infections.

Objective: This study aims to characterize and evaluate the contamination of some farms by *Salmonella spp*. strains in the Kindia Prefecture.

Material and Methods: Based on the surveys, two types of farms (Semi-modern and modern) were identified. A total of 66 samples, including 15 cloacal swabs and 41 fresh droppings, were taken between June and September 2024. *Salmonella spp*. strains were isolated according to ISO 6579-1:2017 and confirmed in Api 20E galleries as well as other enterobacteria. The antibiogram was performed on Mueller-Hinton agar diffusion method using antibiotic disks (Bio-Mérieux).

Results: These methods made it possible to isolate 27 germs including 22 Escherichia coli (33.33%) and 4 Salmonella spp. (6.06%). The sensitivity tests showed that all the Salmonella spp (100%) were resistant to Trimethoprim/Sulfamethoxazole, 75% to Ampicillin (AMP10). 50% to gentamicin, and half on them (50%) were resistant to gentamicin. However, the susceptibility of these strains was 100% to nitrofurantoin, 80% to ciprofloxacin, 75% to azithromycin.

Conclusion: This study highlighted the presence of *Salmonella spp*. in some farms in the Kindia prefecture and proved the need for increased surveillance to limit the spread of this germ in the area.

Keywords: Characterization; Bacteria; *Salmonella* Spp.; Resistance; Poultry Farms; Kindia/Guinea

1. Introduction

The growing global human population places a high demand for food to ensure its survival. This puts pressure on several food industries. These industries include poultry production systems, where growth-promoting agents are used to meet

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this growing food demand [1]. However, these activities are not always harmless to human health. Indeed, foodborne diseases are among the major causes of morbidity and mortality worldwide. Previous studies indicate that certain bacterial germs such as Salmonella spp, Clostridium and Escherichia coli are the main pathogenic microorganisms found in poultry products [2,3]. Among these agents, Salmonella are among the most implicated pathogenic bacteria in foodborne illnesses (FBIs) worldwide [4,5].

However, to limit the negative impact of major pathologies encountered in livestock farms, including salmonellosis, livestock farmers resort to the excessive use of veterinary drugs for preventive and curative purposes, particularly antibiotics [6-8]. The misuse of these drugs, such as antibiotics, is often associated with the emergence and spread of antibiotic-resistant or multi-resistant bacteria. While the discovery of antibiotics raised hopes that it would one day be possible to control all infectious diseases, the phenomenon of bacteriological resistance has put an end to this illusion [9].

Guinea has a variety of poultry farms that contribute significantly to poultry and egg production in the country. However, this activity remains informal. This situation could raise concerns about the risk of infections associated with the ingestion of poultry products [10].

The various poultry sectors in Guinea continue to face a number of common constraints that negatively impact its development. These include, among others, poor sanitary conditions leading to frequent epidemics, the low level of organization of breeders, the lack of adequate infrastructure, and low investment capacities [11].

Among the bacterial diseases of avian origin, salmonellosis is a bacterial infection caused by Salmonella strains, mainly affecting the digestive system of poultry. Specific data on the incidence of this pathology in the Kindia prefecture are not available. This is why the choice of this study focused on the characterization and evaluation of bacterial contamination by *Salmonella spp*. of some poultry farms in the Kindia prefecture.

The objective of this study was to characterize and evaluate the bacterial contamination of several poultry farms in the Kindia Prefecture by *Salmonella spp.*

2. Material and methods

2.1. Methodology

2.1.1. Study Area

Kindia is located in the Lower Guinea Natural Region and plays a strategic role due to its proximity to the capital, Conakry. Kindia Prefecture is located 135 km from Conakry between 10° 03′ 29″ North latitude and 12° 52′ 08″ West longitude. It is bordered by the prefectures of Télimélé to the north, Mamou to the east, Forécariah to the south, and Coyah to the west. It covers an area of 9,115 km^2. In 2016, the population was 469,446 inhabitants [12]; or 52 inhabitants/km^2. It is crossed by several important rivers such as the Konkouré, the Samou, the Kilissi, the Kolenté, the Garafiri, etc., which play a crucial role in the local economy, particularly in agriculture, livestock, energy and tourism.

This area is characterized by a sub-Guinean climate with alternating two seasons (rainy and dry). Agriculture and livestock farming are the two main socioeconomic activities, employing approximately 80% of the rural population.

2.1.2. Sampling

This descriptive and analytical study was carried out from June to September 2024. The approach combined, on the one hand, survey investigations and, on the other hand, samples for laboratory analysis.

2.1.3. Transport

The samples were transported in a cooler containing cold accumulators (dry ice) at 4°C and transported to the laboratory for analysis or storage within 4 hours, in accordance with the NF Standard [13].

2.1.4. Biological Material

This material consisted of fresh droppings and cloacal swabs.

2.1.5. Laboratory Équipement

It mainly consisted of flasks, sampling labels, single-use gloves, dry ice, Petri dishes, buffered peptone distilled water, Pasteur pipettes, 70% ethyl alcohol, autoclave, shaker, refrigerator, optical microscope, slides and coverslips, colony counter, Eppendorf tubes, sterile loops, Oxidase Discs, Catalase Discs, Antibiotic Discs (Bio Mérieux), Ordinary Nutrient Agar, Hectoen Agar, MacConkey Agar, XLD Agar, Kligler-Hajna Agar, Muller-Hinton Agar, API 20E Gallery (Bio Mérieux), and survey sheets that were used to assess the health characteristics and practices in poultry farms.

2.2. Methods

2.2.1. Characteristics of Poultry Production Systems

In Kindia Prefecture, the study focused on two types of poultry farms, all privately owned with the owners' consent to the survey.

To characterize the production systems on these farms, a survey form was submitted to the farmers. This form addressed several points, including: farm types, location and environment, infrastructure and equipment, hygiene and sanitation measures, personnel, and poultry feeding.

2.2.2. Samples

A total of sixty-six (66) samples, including 51 fresh droppings and 15 cloacal swabs, were taken between June and September 2024. Thus, 15 cloacal swabs and 19 fresh droppings were taken from Farm A, while 32 fresh droppings were taken from Farm B.

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 taken from laying hens showing suspicious clinical signs (diarrhea, anorexia, oral discharge). 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. The samples were then refrigerated and processed the next 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 animals per flock, and any signs of disease.

2.2.3. Bacteriological Analysis

The swabs and fresh droppings were subjected to bacteriological analysis. This analysis was carried out at the medical bacteriology laboratory of the Guinean Institute for Applied Biology Research (IRBAG).

2.2.4. Salmonella spp. Detection

The *Salmonella* isolation method was carried out according to the *Salmonella-Shigella* detection protocol in force within the IRBAG bacteriology laboratory, inspired by the ISO 6579-1 (2017) standard.

It required four phases in accordance with the above-mentioned standard:

The pre-enrichment phase in a non-selective medium, where 1g of droppings removed from the refrigerator was added to 9ml of buffered peptone water and homogenized using a V-32 Vortex Mixer for 1 minute, and each swab was dipped in buffered peptone water.

All samples were left to revive at room temperature for 30 minutes and then incubated at 37°C for 18 hours ± 2 hours.

2.2.5. Enrichment phase in liquid selective media

The following day, using Pasteur pipettes, 0.1 ml of each previous culture was inoculated into 9 ml of Rappaport-Vassiliadis (RVs) broth and then incubated at 37°C for 18 to 24 hours.

Isolation and Characterization Phase

- The selective agar media Hektoen and Xylose-Lysine-Deoxycholate (XLD) are media characteristic of enteropathogenic bacteria, particularly *Escherichia coli*, *Salmonella*, and *Shigella.*

- These media are inoculated from enrichment products using the quadrant method and incubated in an incubator at 37°C for 24 hours.
- Suspected Salmonella spp. colonies were subjected to characteristic tests, including catalase detection and Gram staining.
- The XLD agar cultures in Petri dishes were then examined; two to three characteristic colonies were picked and re-inoculated onto nutrient agar to obtain pure cultures and incubated at 37°C for 18 to 24 hours.
- The characteristic *Salmonella* isolates were stored at -20°C for the Api 20E gallery test.

2.2.6. Biochemical Identification

The biochemical identification of *Salmonella* spp. and other species belonging to the family of *Enterobacteriaceae*) was performed in two media:

-Kligler-Hajna agar

- Kligler medium was used to identify *Salmonella spp*. and other Enterobacteriaceae present in the analyzed sample by rapidly demonstrating lactose and glucose fermentation (with or without gas production) as well as hydrogen sulfide production.
- Using a loop, one to two characteristic *Salmonella* colonies were collected from previously inoculated Petri dishes and subcultured into Kligler-Hajna slant tubes and incubated at 37°C for 18 to 24 hours.
- Typical *Salmonella spp* cultures consisted of an alkaline slant (red) and an acidic base (yellow), with gas and hydrogen sulfide formation (blackening of the agar);

2.2.7. 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. The reading was performed using APIWEB identification software.

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

Antibiogram Tests

- The antibiogram was performed using the Mueller-Hinton agar diffusion method using 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, 2011).
- Mueller-Hinton agar plates were 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.
- The diameters of the inhibition zones were measured and the results interpreted according to the criteria of the Antibiogram Committee of the French Society of Microbiology. The following antibiotics were tested: Ampicillin (AMP10), Gentamicin (GN30), Trimethoprim-Sulfamethoxazole (SXT25), Ciprofloxacin (CIP5), Nitrofurantoin (F300) and Azitromycin (AZM30).

Data Analysis

Data were entered and analyzed using Microsoft Office Excel 2016.

3. Results

3.1. Characteristics of Poultry Production Systems

The following results were obtained from the analysis of the data collected on these two farms:

3.1.1. Farm A

The poultry facilities were semi-modern, equipped with sheds and demarcated by baked brick walls covered with sheet metal. This system limited the entry of stray animals into the farms but did not allow for humidity or heat control inside the buildings. There were three sheds, two of which housed laying hens and the other housed cockerels. All the birds were imported breeds.

- Each shed had a flock with a population of between 1,500 and 2,000 poultry, aged 3 to 6 months. The density ranged from 4 to 12 animals per square meter.
- The farms were equipped with plastic feeders and waterers, wood shavings bedding and no ventilation system.
- Poultry feed was provided by local companies, and the watering source was borehole water.
- The farms were planted with market garden crops (eggplants, tomatoes, cabbages, etc.).
- Farm hygiene and production monitoring were ensured by two male employees. One of these employees was educated and had received some training in biosecurity.
- Health monitoring was provided by a veterinarian, but difficulties accessing medication were reported during our sampling. This left the farms in a state of relative insecurity when it came to poultry diseases.

3.1.2. Farm B

- This farm is modern, comprising two buildings approximately 1 km from the homes and nearly 600 m from the river and the main road.
- The buildings are equipped with a battery system with an average size of 2,837 laying hens ranging in age from 5 to 9 months, at a density of 16 animals per square meter.
- The litter is made of perforated metal with pores through which the droppings pass before being collected by a conveyor belt system and then drained into a waste accumulator.
- Most of the feed was produced on site or supplied by local poultry feed companies. The farms were equipped with a borehole and metal waterers and feeders.
- Maintenance of the premises and egg collection were carried out by four employees, including one woman.
- A veterinarian monitored the animals' health. During the sampling, no clinical signs of pathology were detected in the birds.

3.2. Types of Samples

Fresh chicken droppings and cloacal swabs were used as biological material.

Table 1 Number of samples per biological material and per farm

Samples	Farm A	Farm B	Total
Cloacal swabs	15	0	15
Collection of droppings	19	32	51
Total	34	32	66

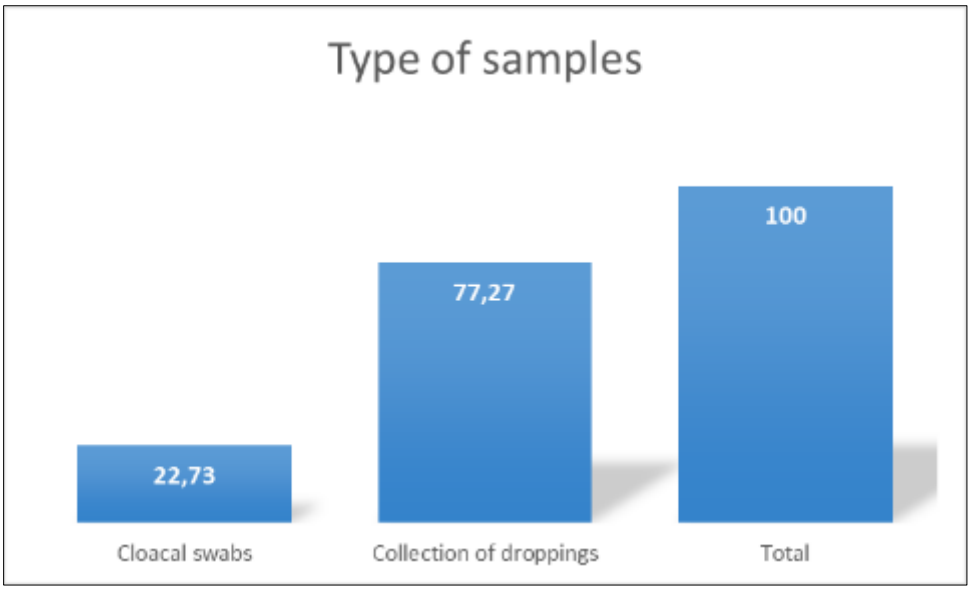

Figure 1 Different types of samples collected in the farms

3.3. Prevalence and types of bacteria found on the two farms

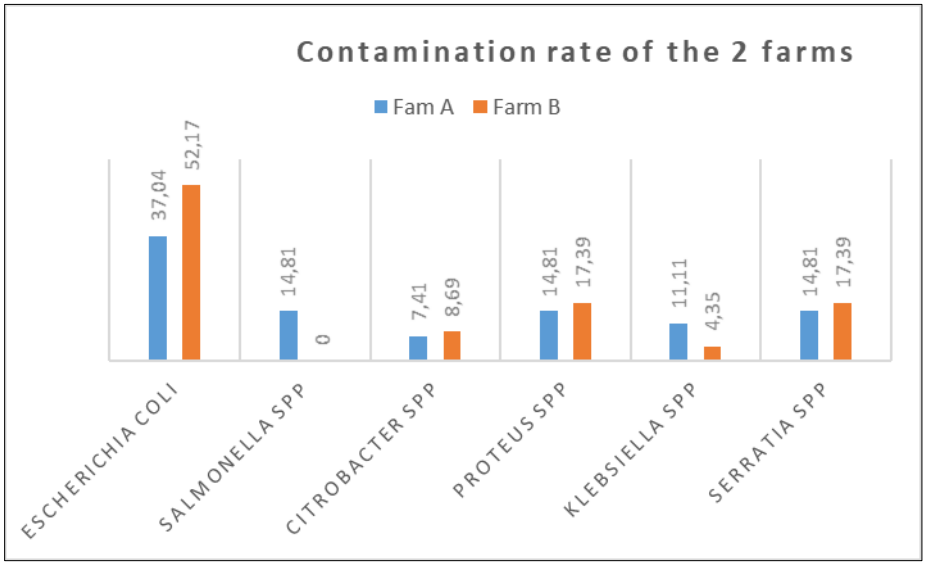

Figure 2 Prevalence of different bacterial germs on the two farms A and B

A total of 27 bacterial strains were isolated on poultry farm A, with a distribution of 14.81% for *Salmonella spp*. During this study, other strains of enterobacteria were also isolated: E. coli (37%), *Proteus spp*. (14.81%), *Serratia spp*. (14.81%), *Klebsiella spp.* (11.11%), and *Citrobacter spp*. (7.41%).

On Farm B, the results showed the absence of *Salmonella spp*. in the analyzed samples. However, other enterobacteria were isolated, including *Escherichia coli*, which was the predominant germ (52.17%), followed by *Proteus spp*. and *Serratia spp*. with equal prevalence (17.39%).

The results of the analyses of the 66 samples taken from the two farms studied, only 4 of these samples were positive for *Salmonella spp*., i.e. an isolation rate of 6.06%. On the other hand, the analysis of all the samples taken from the two farms showed a predominance of E. coli strains on each of the farms, but also an overall predominance on both farms. However, the prevalence of *Escherichia coli* in farm B (52.17%) was significantly higher than that of farm A (47.04%).

In contrast, the results in this figure demonstrate a predominance of *Escherichia coli* on farm B. The isolation rate of germs in all samples was 40.90%.

3.4. Resistance of bacterial strains to antibiotics

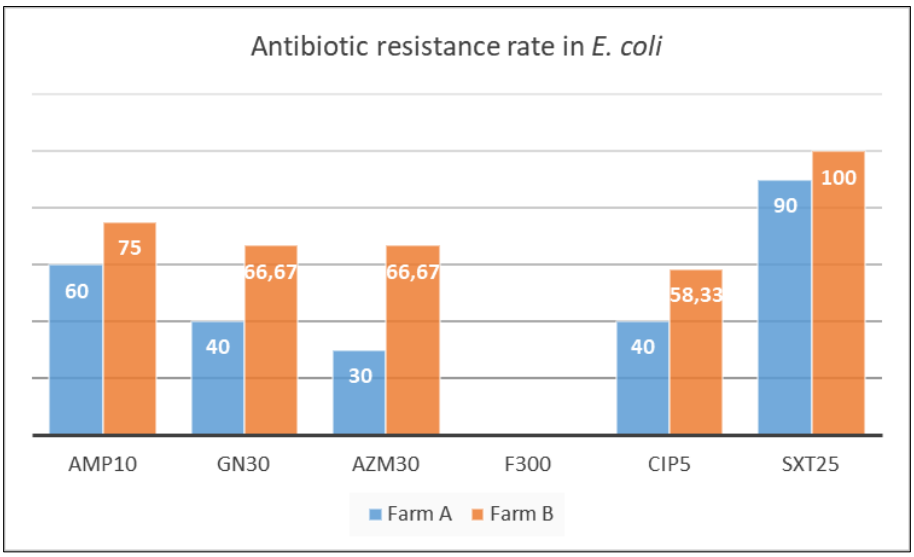

Figure 3 Antibiotic resistance rate in *Escherichia coli* strains isolated from farms

3.4.1. Antibiograms of Escherichia coli strains were performed using six types of antibiotic disks.

The results of the antibiograms of *Escherichia coli* strains showed a very high prevalence of resistance to the Trimethoprim/sulfamethoxazole combination in farms A and B, with 90% in farm B and 100% in farm A, respectively. However, the resistance rate of these *Escherichia coli* strains to ampicillin was 60% in farm B and 75% in farm A.

3.4.2. In contrast, all Escherichia coli germs isolated from both farms were sensitive to nitrofurantoin (100%).

The sensitivity rates of *Escherichia coli* strains to Azithromycin and Ciprofloxacin were different for the two farms. Indeed, for Azitomycin, the reported sensitivity was 70% for farm A and 33.33% for farm B, while for Ciprofloxacin, the sensitivity of Escherichia coli strains was 60% in farm A and 41.67% in farm B.

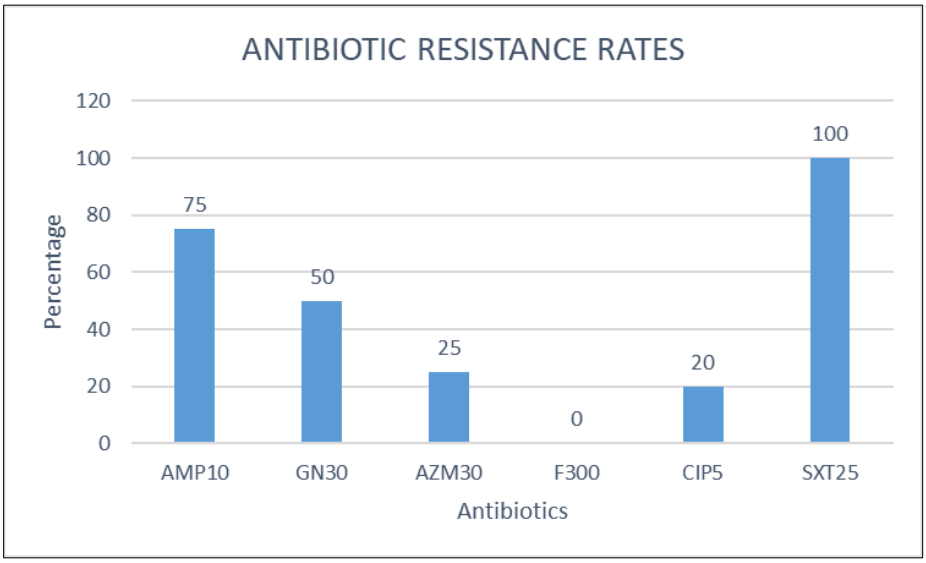

Figure 4 Frequency of antibiotic resistance in *Salmonella spp*. strains

Figure 4 shows that all *Salmonella spp.* strains (100%) were resistant to the trimethoprim/sulfamethoxazole combination. Similarly, the resistance rates of these strains were 75% to Ampicillin and 50% to gentamicin. However, the susceptibility of these strains was 100% to nitrofurantoin, 80% to ciprofloxacin, 75% to azithromycin, and 50% to gentamicin.

4. Discussion

4.1. Characteristics of Poultry Production Systems

The survey results revealed that more than 60% of farm employees were educated. This contrasts with the results reported in Senegal by Nahimana et al. in 2019 [14].

The surveys also show that one in two farms resembles a semi-modern type of operation without a well-defined health plan.

The results of the survey by these authors showed that half of the breeding sites did not have a well-defined health plan, and 85.71% did not have essential isolation measures to reduce health risks. Contrary to the results of the present study, in 37.5% of farms, the laying hens were in an area enclosed by wire mesh with access to a chicken coop made of durable materials and received care and food [15].

4.2. Prevalence of germs isolated in poultry farms

Among all the isolated strains, *Escherichia coli* remains the dominant species for both poultry farms, with an isolation frequency of 33.33% (22/66). This result is lower than that obtained by A. Boering et al. (2018) who reported an *Escherichia coli* isolation frequency of 55.23% [15]. Furthermore, this species is the best indicator of fecal contamination in the search for pathogenic enterobacteria such as *Salmonella spp.* [16].

In the present study, *Salmonella spp*., which is considered the most pathogenic species among the isolated germs, shows an isolation frequency of 6.06%. This rate is higher than that reported by Elgroud R et al., in 2009 (1.66%) during a study carried out in the Wilaya of Constantine in Algeria [17].

Other less pathogenic germs isolated during the analyses include: Proteus spp. and Serratia spp. (12.12%) each, *Citrobacter spp* and *Klebsiella spp.* (6%). These results are significantly higher than those reported in 2023 by Boutaiba [18] which were 2.53% for *Serratia spp.*, 3.49% for *Citrobacter spp*. and 3.17% for *Klebsiella spp.*, However, the results of the present study were lower than those mentioned by Boutaiba in 2023 where *Proteus spp* had a rate of 16.82%.

4.3. Antibiotic Susceptibility of Isolated *Salmonella Spp.* Strains

Salmonella spp. strains showed a high resistance rate (100%) to the Trimethoprim/Sulfamethoxazole combination, and ampicillin (75%). On the other hands, all *Salmonella spp* isolates were susceptible to nitrofurantoine. These results are similar to those published by Abba et al. in 2017 in a study conducted on chicken meat in Chad, which reported resistance to at least one antibiotic [19].

Escherichia coli strains showed high-rate resistance to Trimethoprim/Sulfamethoxazole combination on farms B and A at respective proportions of 90 and 100% whereas these resistance rates to Ampicillin was 60% on Farm and 75% on Farm A. The present result is close to that found in Algeria in 2024 by Aberkane [20].

5. Conclusion

This study characterized the two poultry farms, A and B, in the prefecture of Kindia and assessed the susceptibility of *Salmonella spp*. and *Escherichia coli* isolated from these farms. It clearly appears that, in some areas, the state of infrastructure and the sanitary, environmental, and hygienic conditions of the farms remain factors that favor the circulation of these agents in the sites visited. These results revealed trends in the prevalence and resistance of the isolated strains to certain antibiotics. Furthermore, they emphasize the importance of increased surveillance of antibiotic resistance in *Salmonella* strains from poultry. This should therefore encourage improved management practices in poultry farms in Kindia Prefecture. It would also be interesting to broaden the scope of this work on a larger scale with a more representative sample that would take into account all the poultry farms in the Prefecture of Kindia.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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