

Biochemical Characterization of Salmonella Strains and Evaluation of Hygiene, Disinfection, and Antibiotic Use Practices Inpoultry Farms in Mahajanga, Madagascar

ASTIRFADHUL Ahamadi³, RANDIMBIZAKA Ranjivah Frandley³, RAZAFINDRAKOTO Jean Louis², RABEMANANTSOA Flavien³, ROUKIA Djoudi^{1,3}, RANDRIAMIALLY Jean Dominique^{1,3}, RALISON Farasolo Paule Aimée^{1,3}

¹Faculty of Science, Technology and Environment (FSTE), University of Ambondrona, Mahajanga

²Research Laboratory in Technology, Environment and Health (CHU PEZAGA ANDROVA)

³Doctoral School: Life Engineering and Modeling (EDGVM), University of Mahajanga Contact: +261 340 584 940 – astir3401@gmail.com

Abstract— The sanitary context of urban poultry farming in Mahajanga (Madagascar) remains insufficiently documented despite the growing risks associated with bacterial contamination and the uncontrolled use of antibiotics. This study primarily aimed to characterize *Salmonella* species circulating in poultry farms and to analyze cleaning, disinfection, and medication practices that may favor their emergence. The specific objectives were to: (i) biochemically identify *Salmonella* strains present in different types of poultry samples; (ii) describe the zoosanitary practices applied on farms; and (iii) establish potential links between these practices and the observed contamination. A field survey and microbiological analyses were conducted on 108 samples (meat, offal, feces). Biochemical identification, performed according to ISO 6579:2017 (motility, H₂S, glucose, lactose, citrate, indole, urea, mannitol tests), confirmed the presence of three pathogenic species: *Salmonella typhi*, *S. Paratyphi A*, and *S. arizonae*. In parallel, the evaluation of hygiene practices showed a predominance of cleaning with water and Kline soap (80–85%), with highly variable use of disinfectants (lime, Virunet, Virocide). The study also revealed frequent use of antibiotics such as Tetracolivite® and Ashidox®, often combined with vitamins (Vitaflash, Carnitole, Vinlite®), reflecting medication practices that are sometimes poorly regulated. The results highlight a major public health risk: the coexistence of pathogenic *Salmonella* strains and insufficient biosecurity measures, which may promote the persistence and spread of potentially resistant germs. Standardization of hygiene protocols, regulation of antibiotic use, and strengthened microbiological surveillance are recommended to reduce public health risks.

Keywords— *Salmonella*, poultry farming, biosecurity, antibiotics, hygiene, Madagascar.

I. INTRODUCTION

The poultry sector occupies a strategic position in Madagascar's food security, particularly in urban areas where demand for chicken meat is steadily increasing (FAO, 2022). In Mahajanga, chicken meat now represents a significant share of total animal protein consumption due to its accessibility, nutritional value, and rapid preparation (Rakotondrazaka et al., 2021). However, this positive trend is accompanied by major biosecurity challenges. As highlighted by several recent studies in Sub-Saharan Africa, the predominance of traditional farming systems with limited infrastructure increases sanitary vulnerabilities and facilitates the circulation of microbial contaminants (Hoffmann et al., 2020; Minga et al., 2023). At the same time, the uncontrolled use of antibiotics—widely documented in family and semi-intensive farms in the region—promotes the emergence of resistant bacteria, a phenomenon now recognized as a global threat by the WHO (WHO, 2021; O'Neill, 2020). Among concerning pathogens, *Salmonella* remains one of the leading causes of foodborne illness and a sensitive indicator of hygiene levels along the poultry production chain (EFSA, 2022). In this context, the central problem lies in the lack of up-to-date data on the microbiological quality of broiler chicken meat produced and

marketed in Mahajanga, as well as on zoosanitary practices that may influence the emergence of bacterial contamination. A better understanding of these factors is essential to guide prevention efforts and strengthen local biosecurity strategies (Randrianantoandro et al., 2020). The guiding hypothesis of this study is that farming practices and the unregulated use of antibiotics in poultry farms in Mahajanga significantly increase the risk of microbial contamination, particularly by *Salmonella*. To test this hypothesis, the study addressed three fundamental research questions: (1) What levels of hygiene and disinfection are actually applied in poultry farms? (2) Which antibiotics and vitamins are used, and under what modalities? (3) Does the marketed chicken meat show contamination by pathogenic bacteria, particularly *Salmonella* strains? These considerations led to the selection of the study theme stated above. The main objective was to evaluate the microbiological quality of broiler chicken meat marketed in Mahajanga. The specific objectives were: (i) to characterize hygiene and disinfection practices in poultry farms; (ii) to inventory antibiotics and vitamins used and document their administration; and (iii) to detect and identify pathogenic bacteria in poultry samples, with particular focus on *Salmonella* strains.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study area

The study was conducted in several broiler farms located in different urban sectors of Mahajanga (Ambohimandamina, Belobaka, Antanimalandy, Ambodrona, Amborovy, and Mahajanga City, among others), northwestern Madagascar (15°43'S; 46°19'E). The region has a dry tropical climate with a short rainy season (December–March), favorable to predominantly semi-intensive poultry farming.

2.1.2 Biological material

- Broiler chicken meat samples (pectoral muscle) and offal;
- Samples collected from five identified sectors of the Mahajanga region;
- Sterile 50 g samples collected for each category.

2.1.3 Chemical materials and reagents

- Sterile physiological saline (0.85% NaCl);
- Non-selective enrichment medium: buffered peptone water;
- Selective enrichment medium: Rappaport–Vassiliadis broth (Kauffmann formulation);
- Isolation media: SS agar, XLD agar;
- API 20E biochemical gallery;
- Kovac's reagent;
- Reagents for complementary biochemical tests (citrate, urea, glucose, lactose, mannitol).

2.1.4 Survey material

- Semi-structured questionnaires for farmers, sellers, and veterinary agents;
- Zoosanitary data collection forms (hygiene, antibiotics, vitamins).

2.1.5 Equipment and instruments

- Gloves, sterile bags, disposable spatulas and forceps;
- Portable refrigerator for sample transport;
- Incubators (37 °C and 42 °C);
- Laminar flow hood;
- Homogenizer shaker;
- Optical microscope.

2.2 Methods

2.2.1 Collection of zoosanitary data

Semi-structured surveys were conducted among farmers, sellers, and veterinary agents to assess sanitary practices. Data focused on:

- Cleaning methods (water only; water + Kline soap);
- Disinfectants used (Virunet, Virocide, white lime, bleach, or combinations);
- Use of antibiotics and vitamins (Tetracolivit, Ashidox, Vitaflash, Total Amino, Carnitole, Vinlite).

2.2.2 Sample collection

Meat and offal samples were collected from six selected poultry farms (E1 to E6):

- E1, E2, and E3: broiler offal samples;
- E4, E5, and E6: broiler meat samples.

For each sampling type:

- Three 50 g samples were collected;

- Using gloves and sterile bags;
- Transported in a portable refrigerator at <10 °C to the laboratory.

2.2.3 Microbiological analyses

Analyses were conducted according to ISO 6579:2017 for detection of *Salmonella* spp.

Protocol steps:

- *Pre-enrichment*: 25 g of homogenized sample in 100 mL buffered peptone water; incubation 24 h at 37 °C.
- *Selective enrichment*: transfer to 10 mL Rappaport–Vassiliadis broth; incubation 18–24 h at 42 °C.
- *Isolation*: inoculation on SS and XLD media; incubation 24 h at 37 °C; selection of suspected colonies (H₂S⁺, black centers, red halo on XLD).
- *Biochemical identification*: API 20E gallery; complementary tests (motility, glucose/lactose/mannitol fermentation, H₂S production, urease, indole with Kovac's reagent, citrate utilization).

Biochemical profiles were compared with reference profiles of *S. Typhi*, *S. Paratyphi A*, and *S. arizonae*.

2.2.4 Data analysis

- Frequencies of zoosanitary practices were calculated from survey data;
- Biochemical profiles were compared with API 20E identification matrices;
- Results were validated based on ISO 6579:2017 interpretation thresholds.

III. RESULTS

3.1. Sanitary practices in broiler chicken farms

Analysis of the survey data reveals that hygiene practices in poultry farms in Mahajanga remain largely basic. The majority of farmers (80–85%) clean their poultry houses using a mixture of water and soap, while a minority (14–16%) rely solely on water, without the use of detergents. The use of disinfectants is heterogeneous, with a predominance of products such as Virunet, Virocide, white lime, and Ciprofloxx, applied with varying frequencies and methods. This variability reflects the absence of standardized protocols and limited control over sanitary practices. At the same time, the administration of antibiotics and vitamin supplements is widespread. Tetracolivit is the most commonly used antibiotic, frequently combined with various vitamins, with the aim of supporting growth and preventing infections.

To illustrate the distribution of cleaning methods across the different sectors studied, Figure 1 shows the proportion of poultry houses using water alone versus soapy water.

Figure 2: Distribution of disinfectants used in poultry houses according to the products and combinations applied.

3.2. Use of antibiotics and vitamins

Analysis of treatment practices and nutritional supplementation in the sectors of Ambohimandamina, Ambodrona, Belobaka, and Amborovy shows that Tetracolivit is the predominant antibiotic, used in the majority of poultry house categories (up to 28.5% depending on the group), whereas Ashidox is used more sporadically. Some groups

received no antibiotics, reflecting either economic constraints or specific preventive choices.

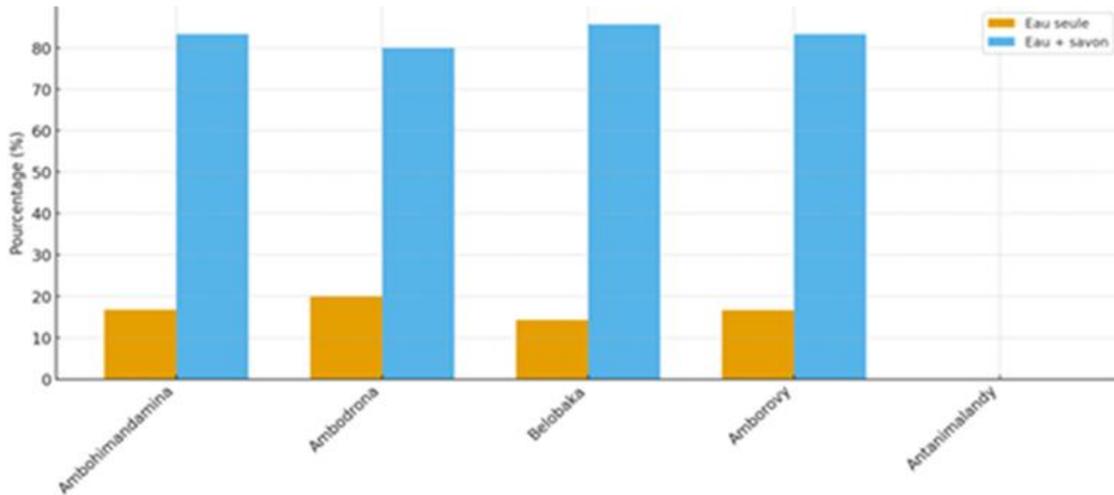


Figure 1: Distribution of poultry house cleaning methods by sector (water only vs. water + soap).

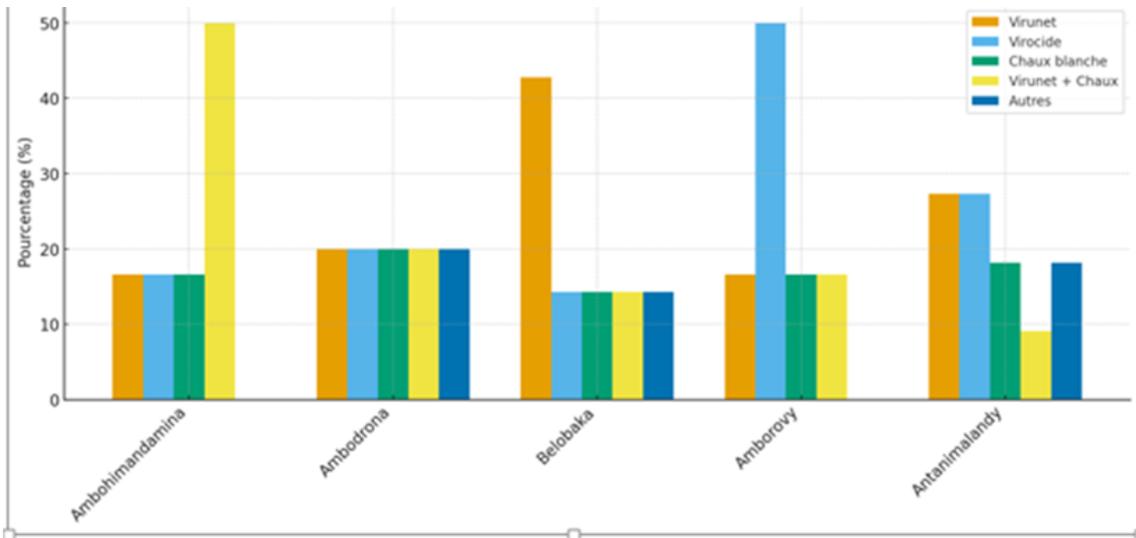


Figure 2: Distribution of the use of disinfectants applied in poultry houses (Virunet, Virocide, white lime, Ciproflox, and combinations).

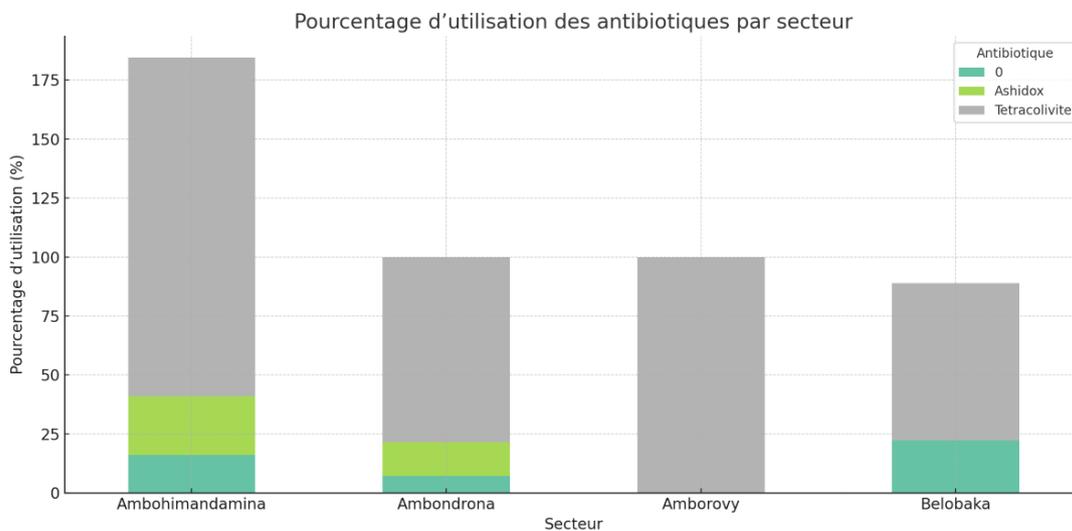


Diagram 1: Distribution of antibiotic use by sector (Tetracolivit, Ashidox, no antibiotics).

Diagram 1: Distribution of antibiotics used by sector, highlighting the predominant use of Tetracolivit, the occasional use of Ashidox, and the absence of antibiotics in certain groups. In addition, vitamins such as Vitaflash, Amine Totale, Carnitole, and Vinlite are administered, often in combination, to support poultry growth and health. The diversity of vitamin

supplementation strategies reflects the variable availability of products and the individual experience of farmers. Diagram 2: Distribution of vitamin combinations by sector, highlighting the heterogeneity of vitamin supplementation practices.

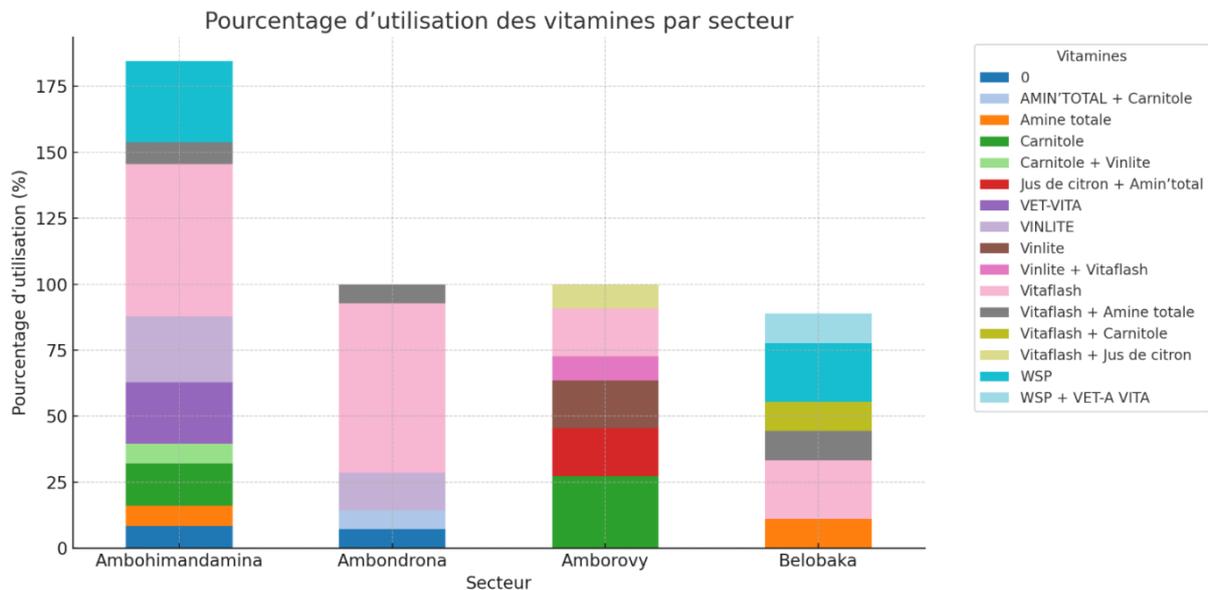


Diagram 2: Distribution of vitamin combinations by sector.

Overall, the relatively standardized use of Tetracolivit reflects an intention to prevent common bacterial infections. In contrast, the variability in vitamin combinations may influence treatment effectiveness and the overall health of the animals. These observations highlight the need to regulate the use of antibiotics and vitamins through standardized protocols in order to limit the risk of bacterial resistance while optimizing poultry performance and welfare.

3.3. Results of microbiological analyses

To assess the presence of pathogenic bacteria in poultry houses in Mahajanga, samples of muscle tissue, organs (offal), and feces were analyzed on selective media (SS and XLD). Initial observation revealed the presence of suspicious H₂S⁺ colonies in several samples, suggesting potential fecal contamination. These colonies were subsequently subjected to in-depth biochemical identification to determine their correspondence with pathogenic *Salmonella* species.

Table 1 presents the initial reading and determination of the biochemical characteristics of the suspicious colonies, compared with reference profiles of *Salmonella typhi*, *S. Paratyphi A*, and *S. arizonae*. This step allows the characterization of preliminary biochemical traits and the assessment of potential contamination risk.

Preliminary results show variable biochemical profiles with no complete match to pathogenic *Salmonella* strains. This suggests an environmental risk rather than true product contamination.

TABLE 1: Initial reading and determination of *Salmonella* isolates from broiler chicken offal samples compared with the reference table obtained in the Ambohimandamina sector, Mahajanga.

Bacterial species	<i>S. typhi</i>	<i>S. Paratyphi A</i>	<i>S. arizonae</i>	E1	E2	E3
Motility (gas)	+	+	+	-	+	+
Glucose	+	-	+	+	+	-
Lactose	-	-	±	+	-	-
H ₂ S ⁺	+	+	-	+	-	+
Urease	-	-	-	±	±	±
Indole	-	-	-	+	+	-
Mannitol	+	+	+	-	+	-
Citrate	+	-	-	+	+	+
LDC (Lysine Decarboxylation)	+	-	+	+	+	+

Note: The symbols + and - indicate the presence and absence of the characteristic, respectively. ± indicates a variable reaction. E1, E2, E3: Suspected broiler chicken offal samples.

To further investigate this observation, a first series of biochemical confirmation tests was performed on the same samples to verify the possible presence of *Salmonella* spp.

Table 2 summarizes the results of this first confirmation series, indicating the biochemical characteristics of the suspicious colonies. This step confirmed the absence of *Salmonella* spp., while highlighting the persistence of H₂S⁺ colonies, suggesting possible environmental contamination.

TABLE 2: Confirmation of *Salmonella* isolates (first series) from suspected samples compared with the reference table obtained in the Belobaka sector, Mahajanga.

Bacterial species	<i>S. typhi</i>	<i>S. Paratyphi A</i>	<i>S. arizonae</i>	E 1	E 2	E 4	E 5
Motility (gas)	+	+	+	-	+	+	-
Glucose	+	-	+	+	-	+	+
Lactose	-	-	±	+	-	-	+
H ₂ S ⁺	+	+	-	+	-	+	+
Urease	-	-	-	±	±	±	±
Indole	-	-	-	-	+	-	-
Mannitol	+	+	+	+	+	-	+
Citrate	+	-	-	-	-	-	+
LDC (Lysine Decarboxylation)	+	-	+	+	+	+	+

Finally, a final analysis was carried out following an in-depth comparison with reference biochemical profiles.

E1, E2: Suspected broiler chicken offal samples
E4, E5: Suspected broiler chicken meat samples

Table 3 presents the final determination of *Salmonella* isolates in all samples. These analyses confirm the absence of *Salmonella* spp. in meat, offal, and fecal samples. Nevertheless, the initial detection of H₂S⁺ colonies indicates residual fecal contamination, highlighting that the sanitary practices implemented, although effective to some extent, remain insufficient to completely eliminate microbiological risks in the studied poultry farms.

TABLE 3: Final determination of *Salmonella* isolates according to sample type compared with the reference table obtained at the Mahabibo market, Mahajanga.

Bacterial species	<i>S. typhi</i>	<i>S. Paratyphi A</i>	<i>S. arizonae</i>	E 1	E 2	E 3	E 4	E 5	E 6
Motility (gas)	+	+	+	+	+	+	+	+	+
Glucose	+	-	+	+	-	-	+	-	-
Lactose	-	-	±	-	-	-	-	-	-
H ₂ S ⁺	+	+	-	+	+	+	+	+	+
Urease	-	-	-	±	±	±	±	±	±
Indole	-	-	-	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Citrate	+	-	-	+	-	-	+	-	-
LDC (Lysine Decarboxylation)	+	-	+	-	-	+	+	+	-

LDC: Lysine Decarboxylation

E4, E5, and E6: Suspected broiler chicken meat samples

E1, E2, and E3: Suspected broiler chicken offal samples

IV. DISCUSSION

4.1. Insufficiency and variability of sanitary practices

The results show that poultry house cleaning relies mainly on the use of water or soapy water, an approach that is insufficient to effectively reduce the microbial load. Previous studies have demonstrated that these methods, although accessible, do not eliminate persistent pathogens in poultry environments (Harrigan, 1998; Manyi-Loh et al., 2016).

The heterogeneity of disinfectants observed across farms reflects the absence of standardized protocols and limits the consistency of biosecurity measures. This variability

constitutes a structural vulnerability factor for the local poultry sector.

4.2. Unregulated use of antibiotics and risk of resistance

The intensive and often unsupervised use of antibiotics, particularly Tetracolivite®, reflects a preventive rather than therapeutic approach. Such uncontrolled use exerts selective pressure that promotes the emergence of resistant bacteria, a phenomenon widely reported in livestock systems in Africa and Southeast Asia (Phu et al., 2019; Tufa et al., 2022). Even in the absence of identified pathogens in the samples, this context of high antimicrobial pressure represents a latent risk to public health and compromises the future effectiveness of treatments.

4.3. Potential contamination and public health implications

The confirmed absence of *Salmonella* spp. through biochemical testing is an encouraging result. However, the initial presence of suspicious H₂S⁺ colonies suggests residual fecal contamination. This type of indicator flora reflects deficiencies in environmental hygiene and a persistent risk of introducing enteric pathogens into the production chain, as observed in other African poultry contexts (Van Immerseel et al., 2004; WHO, 2023). These findings highlight the need to improve cleaning and disinfection conditions to prevent the future emergence of contamination.

4.4. Food safety challenges and operational recommendations

The results highlight a structural fragility of sanitary practices which, despite the absence of *Salmonella*, exposes consumers to a potential risk of foodborne illness. Irregular disinfection procedures and the extensive, uncontrolled use of antibiotics threaten both food safety and the sanitary sustainability of poultry farming systems. Similar observations have been reported in other high-density poultry contexts, where insufficient biosecurity combined with indiscriminate antibiotic use promotes the emergence of resistant strains (Modi et al., 2014; Batie, 2018).

Strengthening farmer awareness, ensuring regular veterinary supervision, and establishing standardized biosecurity protocols are essential levers for sustainably improving hygiene in poultry farms in Mahajanga and reducing contamination risks throughout the poultry value chain.

V. CONCLUSION

This study assessed sanitary practices and the microbiological quality of broiler chicken farms in Mahajanga, Madagascar. The results reveal that cleaning and disinfection practices are heterogeneous and often insufficient, while the unregulated use of antibiotics, particularly Tetracolivite® combined with vitamins, is widespread. Although microbiological analyses confirmed the absence of *Salmonella* spp. in meat, offal, and fecal samples, the detection of suspicious H₂S⁺ colonies indicates a residual risk of fecal contamination, underscoring that current hygiene measures remain incomplete. These findings highlight the urgent need to:

- Strengthen biosecurity through standardized cleaning and disinfection protocols;
- Regulate antibiotic use to limit the development of antimicrobial resistance;

- Raise farmer awareness and ensure regular veterinary monitoring;
- Improve microbiological surveillance of poultry.

In conclusion, the poultry sector in Mahajanga has significant potential to meet the growing demand for animal protein. However, its sustainability and consumer health safety depend on strict management of farming practices and antimicrobial use. These results provide essential data to guide foodborne disease prevention strategies and the development of biosecurity policies adapted to urban poultry farming systems in Madagascar.

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