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## Prevalence of *Salmonella* and *Escherichia coli* in Pig Farms in Yopougon (Côte d'Ivoire)

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### Abstract

The microbiological contamination of pork is an increasingly important concern as pork consumption rises. This study aims to examine the microbiological contamination by *Salmonella* and *Escherichia* species in a pork production environment. To this end, forty (40) fecal samples from pigs raised in enclosures on four (04) farms in the Yopougon municipality were collected and analyzed. Classical microbiological methods, including enrichment, pre-enrichment, selective media culture, isolation on ordinary agar, and biochemical tests, were employed to identify the specific biochemical characteristics of each bacterium. The overall prevalence of *Escherichia* species was 80% [70–90] across farms and 80% [66.7–100] based on pig status. Furthermore, *Salmonella* species showed a prevalence of 12.5% [10–20] according to the farm and 12.5% [0–20] based on pig status. These findings highlight the necessity of raising awareness among stakeholders in the pig farming sector about environmental hygiene and monitoring pig microbiota to reduce consumers' exposure to pathogenic and endemic bacteria of the *Salmonella* and *Escherichia* genera.

### Introduction

The regular presence of pathogenic microorganisms in food represents a public health concern. Indeed, bacteria ingested by humans are responsible for endemic infections worldwide. According to the World Health Organization (WHO, 2022), bacteria contribute significantly to numerous infections associated with antibiotic resistance, which increases risks to human health. The contamination process by these pathogens often occurs through daily habits, potentially creating a link between a host and an individual. Consequently, food remains a constant source of pathogens for humans. In modern consumer societies, farmed meat plays a key role in meeting the dietary protein needs of populations. Pork is the second most consumed meat globally, following beef (Organisation for Economic Co-operation and Development/Food and Agriculture Organization [OECD/FAO], 2024). This trend has driven an increase in global pork production. In Côte d'Ivoire, for example, the pork sector has shown significant growth, with production rising from 8,583 tons of carcass equivalent (TEC) in 2014 to 11,620 TEC in 2019—an increase of approximately 35%. The total pig population (from modern and traditional farming) grew from 375,669 heads in 2014 to 431,389 heads in 2019, reflecting an average growth rate of 15% (Ministry of Animal Resources and Fisheries [MIRAH], 2022).

The exposure to pathogenic bacteria associated with pork is primarily explained by a contamination chain that begins during the fattening phase in pig farms (Tian et al., 2021). Ultimately, these bacteria exhibit pathogenic potential for humans during the handling and preparation of meat for consumption. In essence, the exposure to pathogenic bacteria starts on the farm and ends on the consumer's table. Within this contamination chain, pork is often contaminated by intestinal contents (Xu et al., 2021). As a result, the most frequently encountered pathogenic bacteria are of fecal origin.

Due to the presence of both a normal microbiota and intestinal pathogenic bacteria in fecal matter, *E. coli* has emerged as a critical health indicator, reflecting the contamination potential of pigs' digestive systems and the associated risks to human health (Kusturov et al., 2017). Thus, the genus *Escherichia* is commonly used to assess the overall risk of contamination by pathogenic microorganisms. Furthermore, since pork is recognized as one of the primary sources of *Salmonella* infections in humans and one of the main reservoirs of *Salmonella* species, risk assessments of microorganisms related to pork have also focused on *Salmonella* (Gopinath et al., 2012). Consequently, *Escherichia coli* and *Salmonella* serve as indicators of the microbiological quality of pork. It is essential to investigate the contamination potential of pig feces in feeding and maintenance environments, particularly on farms, based on the pigs' status (fattening pigs, boars, sows, or piglets). This approach to analyzing bacterial contamination related to pigs has been relatively underexplored in Côte d'Ivoire.

The objective of this study is to determine the prevalence of enterobacteria, specifically of the genera *E. coli* and *Salmonella*, in the feces of pigs raised on farms in Abidjan.

## Materials and Methods

### Materials

This prospective study was conducted from June 1 to September 30, 2023, in pig farms located in the peri-urban area of Yopougon. Sample collection was carried out during this period, and laboratory analyses were performed at the Environmental Chemistry and Microbiology Unit (UCME) of the Pasteur Institute of Côte d'Ivoire. The farms selected for this study included enclosures housing breeding sows, lactating sows, weaned piglets, boars, and fattening pigs. Appropriate materials were chosen to collect fecal samples for evaluating the presence of *Salmonella sp.* and *Escherichia coli* across all available pig groups in these four farms. The Environmental Chemistry and Microbiology Unit, within the Department of Environment and Health, served as the platform for pathogen identification.

### Materials for the Isolation and Identification of Pathogenic Germs

- **Enrichment Media:** Buffered Peptone Water (BPW) and Rappaport-Vassiliadis broth (RV10).
- **Selective Isolation Media:** Eosin Methylene Blue (EMB) agar for detecting *Escherichia coli* and Salmonella-Shigella (SS) agar for *Salmonella sp.*
- **Non-Selective Agar Media:** Ordinary agar was used for general bacterial isolation.

- **Biochemical Identification Media:** Le Minor's reduced portoir allowed selective identification of biochemical or metabolic characteristics specific to *E. coli* and *Salmonella sp.*

Reagents were used to detect enzymatic activities, including cytochrome oxidase, urease, catalase, and indole production.

## Methods

### Fecal Sampling

This study examined the prevalence of pathogens of the *Escherichia* and *Salmonella* genera in pig farms. Modern pig farms with enclosures were selected as appropriate sites for evaluating the presence of these human pathogens. Fecal samples were collected using specialized tools adapted for this purpose. In each farm, a sample was taken from each enclosure. Within an enclosure, freshly excreted feces were collected using sterile spatulas. The sample was taken from the submerged portion of the feces to ensure that the identified pathogens originated from the intestinal tract rather than the environment, such as the enclosure. The fecal samples were placed in sterile tubes labeled with a unique order number, the type of enclosure, and the animal's status. A total of 40 samples were collected, and stored in coolers containing ice packs and transported to the Pasteur Institute laboratory in Adiopodoumé, located in the Yopougon municipality.

### Microbiological Analysis

#### Isolation of Pathogenic Bacteria of the *Escherichia* Genus

The isolation process for pathogenic bacteria of the *Escherichia* genus begins with the enrichment of 5 grams of feces in 5 milliliters of sterile distilled water in a sterile Falcon tube. One milliliter (1 mL) of this mixture is added to 9 mL of Buffered Peptone Water (BPW) and incubated at 37°C in an incubator. After 24 hours of incubation, the sample is cultured on EMB agar, followed by another incubation at 37°C for 24 hours. Colonies suspected to be *Escherichia coli* are identified by their characteristic metallic sheen on purple-colored colonies. These colonies are subcultured on ordinary agar and subjected to biochemical tests to confirm specific characteristics of *E. coli*.

#### Isolation of *Salmonella* Genus

For *Salmonella*, the pre-enrichment is conducted using BPW medium. After 24 hours of incubation, 1 mL of the pre-enriched medium is pipetted with sterile pipettes and added to 9 mL of Rappaport-Vassiliadis broth (RV10). The sample is incubated at 37°C for 4–6 hours. Subsequently, a few drops of the RV10 broth are streaked on SS agar and incubated for 24 hours. After incubation, colonies suspected to be *Salmonella* are subcultured on ordinary agar for biochemical identification specific to *Salmonella sp.*

### Statistical Analysis

Data on samples testing positive or negative for *Escherichia coli* and *Salmonella* through the various steps of the microbiological procedure were recorded in an Excel table. These data were expressed as frequencies. The Chi-square test ( $\chi^2$ ) was applied to identify significant differences in frequencies of *Escherichia coli* and *Salmonella sp.* based on pig status and the specific farm. Statistical analyses were performed using SPSS software, version 23.

## Results

**Table I: Prevalence (%) of *Escherichia coli* by Farm**

<b>Farm</b>	<b><i>Escherichia coli</i> Positive</b>	<b><i>Escherichia coli</i> Negative</b>	<b>Total</b>
Farm 1	n (%) 8 (80)	2 (20)	10 (100)
Farm 2	n (%) 8 (80)	2 (20)	10 (100)
Farm 3	n (%) 7 (70)	3 (30)	10 (100)
Farm 4	n (%) 9 (90)	1 (10)	10 (100)
<b>Total</b>	<b>32 (80)</b>	<b>8 (20)</b>	<b>40 (100)</b>

**Chi-square ( $\chi^2$ ):** 0.946, ns (*not significant*)

The proportion of fecal samples positive for *Escherichia coli* (80%) is higher than the proportion of negative samples (20%). However, the prevalence of *Escherichia coli* in fecal samples from the four farms does not show statistically significant differences ( $p = 0.946$ ) (Table I).

**Table II: Prevalence (%) of *Escherichia coli* by Animal Status**

<b>Animal Status</b>	<b><i>Escherichia coli</i> Positive</b>	<b><i>Escherichia coli</i> Negative</b>	<b>Total</b>
Fattening pig	n (%) 5 (100)	0 (0.0)	5 (100)
Weaned piglet	n (%) 7 (70)	3 (30)	10 (100)
Lactating sow	n (%) 2 (66.7)	1 (33.3)	3 (100)
Breeding sow	n (%) 10 (76.9)	3 (23.1)	13 (100)
Boar	n (%) 8 (88.9)	1 (11.1)	9 (100)
<b>Total</b>	<b>32 (80)</b>	<b>8 (20)</b>	<b>40 (100)</b>

**Chi-square ( $\chi^2$ ):** 0.614, ns (*not significant*)

The prevalence of *Escherichia coli* in pig feces based on animal status shows that the proportion of positive samples is consistently higher than negative samples across all categories: fattening pigs (100% positive), weaned piglets (70% positive), lactating sows (66.7% positive), breeding sows (76.9% positive), and boars (88.9% positive) (Table II).

**Table III: Prevalence (%) of *Salmonella sp.* by Farm**

Farm	<i>Salmonella sp.</i> Positive	<i>Salmonella sp.</i> Negative	Total
Farm 1	n (%) 1 (10)	9 (90)	10 (100)
Farm 2	n (%) 1 (10)	9 (90)	10 (100)
Farm 3	n (%) 1 (10)	9 (90)	10 (100)
Farm 4	n (%) 2 (20)	8 (80)	10 (100)
<b>Total</b>	<b>5 (12.5)</b>	<b>35 (87.5)</b>	<b>40 (100)</b>

**Chi-square ( $\chi^2$ ):** 1.000, ns (*not significant*)

The results for *Salmonella sp.* prevalence differ from those for *Escherichia coli*. The proportion of fecal samples positive for *Salmonella sp.* is lower (12.5%) compared to the negative samples (87.5%). However, these differences are not statistically significant ( $p = 1.000$ ) (Table III).

**Table IV: Prevalence (%) of *Salmonella sp.* by Animal Status**

Animal Status	<i>Salmonella sp.</i> Positive	<i>Salmonella sp.</i> Negative	Total
Fattening pig	n (%) 1 (20.0)	4 (80)	5 (100)
Weaned piglet	n (%) 0 (0.0)	10 (100)	10 (100)
Lactating sow	n (%) 1 (33.3)	2 (66.7)	3 (100)
Breeding sow	n (%) 2 (15.4)	11 (84.6)	13 (100)
Boar	n (%) 1 (10)	8 (90)	9 (100)
<b>Total</b>	<b>5 (12.5)</b>	<b>35 (87.5)</b>	<b>40 (100)</b>

**Chi-square ( $\chi^2$ ):** 0.422, ns (*not significant*)

Similar trends are observed when comparing the proportion of fecal samples positive for *Salmonella sp.* based on animal status. The observed prevalences in this study support the hypothesis of a high presence of enterobacteria, specifically *E. coli* and *Salmonella sp.*, in farmed pigs.

## Discussion

The incidence of pathogenic agents in pigs has been the focus of various studies. Some research suggests that controlling pathogens that could contaminate pork begins during the fattening phase of pigs raised for consumption (Raufu et al., 2021). Others emphasize the slaughter and preparation stages as critical points for contamination control (Yang et al., 2022; Bernad-Roche et al., 2023). The examination of enterobacteria prevalence, particularly *Escherichia coli* and *Salmonella*, in pigs raised on farms revealed a high prevalence regardless of the farm or the animals' roles within it. The observed differences were not statistically significant. However, the findings highlight that the bacterial flora of pigs in farms could act as a reservoir for pathogens, posing potential health risks to consumers and the environment. Pigs exhibited fecal samples

positive for *Escherichia coli* and *Salmonella* across all farms (see Tables I and III). The regular presence of these bacteria is often associated with enclosure hygiene (Smith et al., 2023).

Notably, excrement collection was conducted daily in most enclosures, with feces being deposited near the farms for composting purposes in neighboring crops. This hygiene management could explain the lower proportion of *Salmonella* bacteria compared to *Escherichia coli* in the analyzed samples. In essence, the presence of these bacteria serves as an indicator of hygiene levels in pig farming operations. According to Harrison et al. (2022), pig contamination by *Salmonella* occurs primarily through feces, contact with infected animals or materials, visitors, drinking water, or pests. These results reveal shortcomings in the biosafety management of farms in controlling animal exposure to pathogenic bacteria. This could explain the presence of samples positive for *E. coli* and *Salmonella sp.* in the farms included in this study.

The results also highlighted the prevalence of infectious agents across farm sections based on animal roles (status). In the farms studied, enclosures housing breeding sows had the highest prevalence of *E. coli* compared to boars, weaned piglets, fattening pigs, and lactating sows (see Tables II and IV). These differences suggest that the level of care varies between farm sections, depending on the direct impact an infection could have on the farm's productivity. This observation is supported by previous studies. Le Floc'H et al. (2021) reported that within a single farm, cleaning and disinfection protocols are less frequently applied during the fattening phase compared to the post-weaning and maternity phases. These findings also hold for differences in *Salmonella* prevalence among the farm sections. Hygiene levels were higher in the breeding sow, lactating sow, and fattening pig enclosures compared to the weaned piglet enclosures. Similar trends were noted in other studies. For instance, Ntakiyisumba et al. (2023) found that *Salmonella* prevalence in fattening pigs was one of the most significant factors associated with *Salmonella* contamination in processed pork carcasses.

Pig enclosures are where fecal matter accumulates, making it challenging for this study to isolate the specific health risks *E. coli* poses to pork consumers from a farm environment perspective. This limitation arises from the natural presence of *E. coli* in the intestines of animals (WHO, 2018). However, certain *E. coli* strains can cause diseases in consumers. Future studies should focus on contamination from toxin-producing *Escherichia coli* strains in pigs, such as Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), which includes Edema disease-associated *E. coli* (EDEC) and Enterohemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), and Extraintestinal Pathogenic *E. coli* (ExPEC) (Barros et al., 2023; Remfry et al., 2021). Additionally, research should address the antibiotic resistance of these strains.

## Conclusion

This study examined the prevalence of enterobacteria of the genera *Escherichia coli* and *Salmonella* in pig farms. The results indicate that pig farms face a higher prevalence of *Escherichia coli* compared to *Salmonella*. Moreover, statistical tests revealed no significant variations in prevalence across farms or treatments. However, these findings underscore the importance of implementing biosafety measures on farms to reduce contamination by pathogenic agents such as *E. coli* and *Salmonella* in pork production. They also highlight the need for farms to maintain consistent hygiene standards across all sections of the pig farming operation.

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## Conflicts of Interest

The authors declare no conflicts of interest. They affirm that no funding was received for conducting this study.

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