

Full Length Research Paper

Detection of multidrug-resistant *Salmonella* Enterica strains in chickens (*Gallus gallus domesticus*) from markets in Abidjan

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Salmonella is a genus of Gram-negative bacilli, causing illnesses in humans, such as typhoid fever, which remain major public health concerns. Based on biochemistry and genomic data, *Salmonella* is divided into many serovars. In West Africa, *Salmonella* infection in humans and in food matrices is reported each year. Recently, the emergence of antibiotic-resistant strains of *Salmonella* represents a high risk for public health. In Côte d'Ivoire, poultry activities and poor hygiene conditions contribute to the emergence of *Salmonella* infection. The main objective of this study is to detect *Salmonella* species strains in chicken from local markets in the city of Abidjan. Four sampling sites in the Abidjan district were selected in the local markets of Yopougon (Siporex and Bagnon), Songon, Adjamé, and Abobo. Several chicken intestines (*Gallus gallus domesticus*) were collected in batches of in each market. The pre-treatment of isolation was performed with classic methods for *Salmonella-Shigella* (SS). Biochemical characterization and antibiotic susceptibility testing were conducted for all isolated strains. The target of the invasive gene (*invA*) of *Salmonella* was used for molecular confirmation and DNA sequencing. Among the 22 *Salmonella* spp. isolates studied, 68% (15/22) of the poultry strains underwent biochemical testing (oxidase, catalase, Gram stain, and motility), with five strains testing positive. Antibiotic susceptibility testing revealed strong resistance to ampicillin and pefloxacin, and weak resistance to gentamicin and clavulanic acid. Furthermore, 59% (13/22) of the isolates were positive by Polymerase chain reaction (PCR) targeting the *invA* gene. Phylogenetic analysis showed a high similarity to the reference strain *Salmonella* Enterica serovar Paratyphi. These findings demonstrate the circulation of multidrug-resistant *Salmonella* strains in poultry in Côte d'Ivoire and highlight the need for continuous surveillance to enhance food safety.

Key words: *Salmonella*, *invA* gene, multidrug-resistant, Abidjan, chicken.

INTRODUCTION

Foodborne illnesses represent a serious public health problem. Often referred to as food poisoning, they can be defined as diseases resulting from the consumption of food contaminated by viruses, parasites, fungi, or bacteria (Garcia and Haddad, 2023). Each year worldwide,

approximately 93.8 million cases of gastroenteritis are caused by species of the genus *Salmonella*, of which 80.3 million cases are foodborne. In Africa, about 70% of recorded foodborne disease cases manifest as diarrheal illnesses, with *Salmonella* being the primary cause. Non-

typhoidal *Salmonella*, often linked to the consumption of contaminated eggs and poultry, is responsible for the highest number of deaths. It alone causes about 32,000 deaths annually in West Africa, accounting for more than half of global deaths from the disease (World Health Organization [WHO], 2015). *Salmonella* is a Gram-negative, non-spore-forming bacillus with peritrichous motility (except *Salmonella* Gallinarum, which is non-motile) and is facultatively anaerobic (Koffi et al., 2014). These bacteria are intestinal pathogens that inhabit the intestines of humans and animals, which serve as their primary reservoirs. Following fecal contamination, they can survive in the environment for several months (Koffi, 2015). Several classes of antimicrobials, including aminoglycosides, tetracyclines, β -lactams, and quinolones, are used to treat infections.

Previous studies have shown that invasive non-typhoidal *Salmonella* infections are common and often multidrug-resistant in sub-Saharan Africa, particularly in Côte d'Ivoire, where high resistance rates to important antibiotics such as doxycycline (94%) and sulfonamides (86%) have been reported (Assoumy et al., 2021). *Salmonella* spp. are increasingly emerging in poultry production, compromising treatment effectiveness (Punchihewage-Don et al., 2024; Bénao et al., 2023). The growing presence of *Salmonella* in poultry farms reduces antibiotic efficacy and poses a major public health risk. However, few studies have characterized *Salmonella* strains circulating in broiler chicken markets, which limits the development of effective control strategies. Therefore, this study aims to isolate and identify *Salmonella* strains present in broiler chickens, perform biochemical tests and antibiograms to determine their resistance profiles, and extract their DNA to confirm their genetic identity using Polymerase chain reaction (PCR) and amplicon sequencing.

MATERIALS AND METHODS

Sampling sites

The sampling lasted two months, in the period from July to August 2024. The samples were collected in Abidjan City, particularly in five sites with local markets for chicken. These markets were: site 1 (Yopougon Siporex), site 2 (Yopougon Bagnon), site 3 (Adjamé Gouro market), site 4 (Abobo Gare), and site 5 (Songon Gravier). The markets offer abattage of chicken and direct delivery to the population (Figure 1).

Biological materials

The biological material for the study consists of several intestinal

tubes collected from freshly slaughtered broilers of chicken intestine (*Gallus gallus domesticus*). Three different batches of chicken intestines were collected from each sampling site. Whole sections of intestine are collected with sterile scissors and stored at 4°C in freezer bags. Two control strains, *Salmonella* Enterica (biocollection of the Pasteur Institute Cote d'Ivoire), were used as controls for validation of the tests.

Isolation and identification of *Salmonella*

Bacteriological analysis of samples for *Salmonella* was carried out in three stages, following protocols of ISO 6579-1:2017 with sample pre-treatment, selective enrichment, and selective isolation (International Organization for Standardization [ISO], 2017). A sample of chicken intestine, around 15 g per 10 cm, was cut and added to 20 mL of sterile Buffered Peptone Water and incubated at 37°C for 18 to 24h (Koffi, 2015). 100 μ L of the first culture was transferred to 10 mL of Rappaport-Vassiliadis broth (RVS broth) modified with soybean peptones and incubated at 41.5°C for 24h (Koffi, 2015). The last step is to inoculate the selective enrichment broth (RVS) using a sterile loop on the *Salmonella*-*Shigella* (SS) and Hektoen selective agars and incubate at 37°C, 24 h. Colonies were purified using Luria Bertani (LB) agar and stored at -20°C in LB broth with 30% glycerol for further analysis (Ammouri and Reik, 2019).

Biochemical identification

After inoculation, twenty-two presumptive strains of *Salmonella*, identified on the basis of their characteristic colonies, were obtained. Fifteen strains were selected and purified on nutrient agar (37°C for 24) for biochemical identification. The main tests performed are Gram staining, mobility, catalase, oxidase, H₂S production, glucose fermentation, and others enzymes production (Nikiema et al., 2021).

Antibiogram tests

The strains confirmed to belong to the genus *Salmonella* on the basis of biochemical tests were then used to perform the antibiogram. One colony of fresh bacterial culture from nutrient agar is resuspended again in 1 mL of physiological water, and the solution is mixed for a homogeneous suspension. MH agar plates were inoculated using a sterile swab to spread the suspension over the entire surface of the agar. Antibiotic discs (Biorad) are placed with a sterile disc dispenser on the medium by spacing them about 2 cm apart from each other and the edges of the dish. The plates were incubated at 37°C, 24 h. The analysis of areas of inhibition is measured in millimeters. Strains isolates are described as resistant, intermediate, and susceptible to the various antibiotics according to the recommendations of the CA-SFM protocol (Jean-Winoc et al., 2023; Kaouthar and Ikram, 2024). Plates were diluted in 100 μ L water and added to the lysis buffer. After washing steps and elution, 60 μ L were stored at -20°C. Quantification of DNA extracts was assayed using the Nanodrop One C (Thermo Scientific). An aliquot of 1 μ L of each DNA extract was used to measure absorbance at 260 nm (Adioumani, 2023). Amplification of the invA gene was then performed as a biomarker for the detection of the

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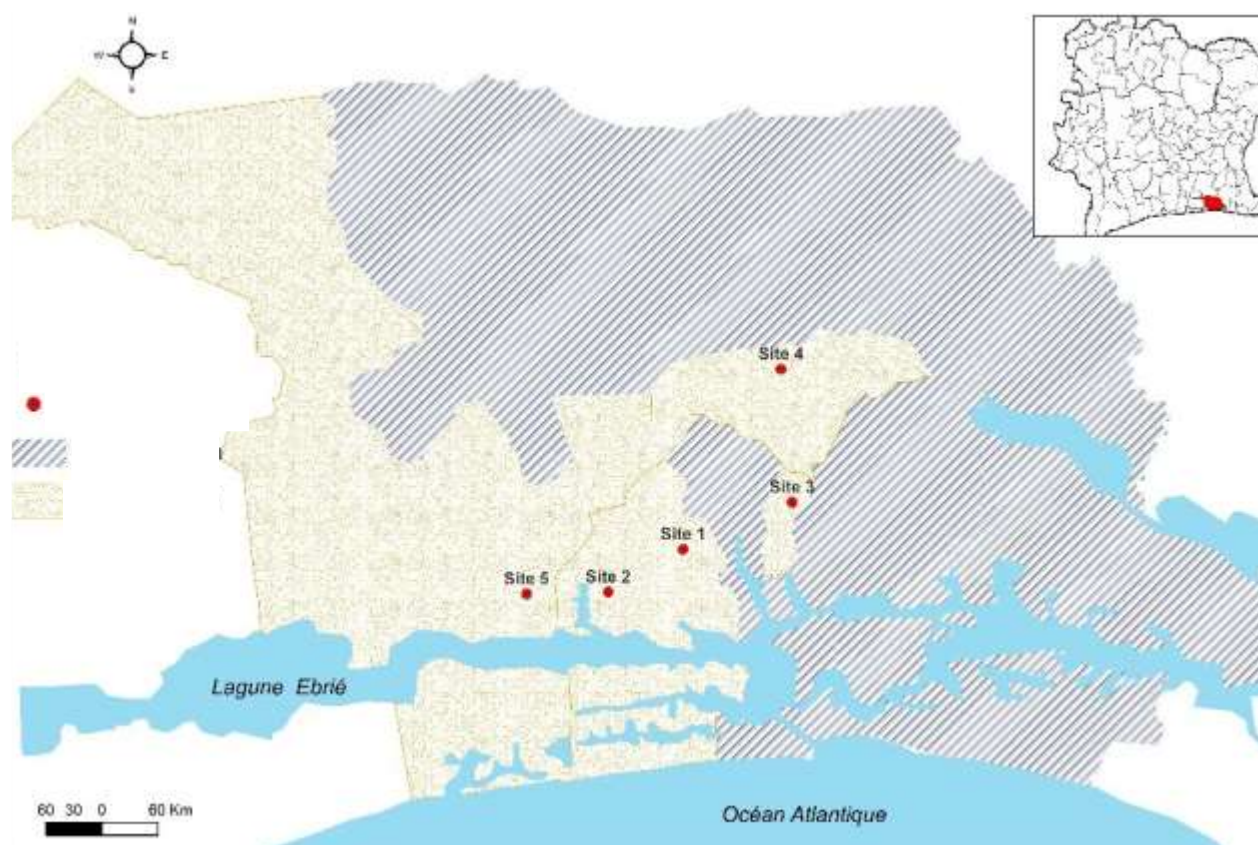


Figure 1. Geographical location of sampling sites. Site 1: Yopougon Siporex; Site 2: Yopougon Bagnon; Site 3: Adjamé Gouro Market; Site 4: Abobo gare; Site 5: Songon gravier.

genus *Salmonella*. The reaction mixture of 25 μ L, consisted of 3 μ L of DNA, 0.5 μ L primer of each primer (10 μ M), 4 μ L $MgCl_2$ (25 mM), 5 μ L 5X Reaction Buffer, 2 μ L dNTPs (25 mM), 0.3 μ L GoTaq G2 Flexi DNA polymerase (5 U/ μ L) (Promega, Wisconsin, USA and 4.7 μ L of molecular water. PCR conditions for the *invA* gene were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, hybridization at 56°C for 30 s, extension at 72°C for 2 min, and a final extension period at 72°C for 10 min. *Salmonella* isolates were confirmed by PCR targeting the *Salmonella*-specific *invA* virulence gene with primer *invA1* (5'-ACA GTG CTC GTT TAC GAC CTG AAT-3') and *invA2* (5'-AGA CGA CTG GTA CTG ATC GAT AAT-3') (Ainyakou-Sanga et al., 2025). After amplification of the DNA extracts in the thermal cycler, 10 μ L of amplicons were deposited on 1.5% agarose gel.

Sequencing of amplicons

DNA purification from amplicons was carried out using the Purification Charge Switch Pro PCR Clean-Up kit (Invitrogen, USA). It was carried out in three steps: DNA binding, washing the column, and DNA elution. The genes studied were sequenced using the *invA1* and *invA2* primers. The Big Dye terminator sequencing kit (Applied Biosystems, USA) was used for the reaction mixture (Furutani et al., 2022). The nucleotide sequences were deposited in the GenBank database. The identity of each strain was determined by comparing the gene sequences studied with the sequences previously published in GenBank, using the BlastN program for the bacterial and fungal strains isolated.

RESULTS

Biocollection of *Salmonella* strains

A total of 22 bacterial strains have positive growth for *Salmonella* on Hektoen and SS agars. 27.27% strains were isolated from site 1, 18.18% strains from site 2 and site 3, 13.6% strains from site 4, and 22.72% strains from site 5. On Shigella-Salmonella agar, *Salmonella* colonies were colourless with black centers, while on Hektoen, they were green or blue-green with black centers (Figure 2A, B, C).

Phenotyping of isolated strains

Biochemical analysis showed that all isolated strains exhibited a negative oxidase, positive catalase (Table 1). Confirmation of these results using the reduced Le Minor showed that 5 strains have specific characteristics of *Salmonella* strains. These strains followings Sa-002, Sa-005, Sa-010, Sa-012, and Sa-015 (Table 2). These characteristics were Gram stain -, bacilli with peritrichous ciliates, catalase +, Oxidase-, Nitrate reductase +, Glucose +, lactose -, ONPG -, LDC +, H_2S +, Simmons citrate +, Indole -, Urea-, TDA-, and Mannitol + (Table 2).

Table 1. Biochemical identification of strains.

Strain	Mobility	Site
Sa-001	MP	S2
Sa-002	MP	S1
Sa-003	MP	S5
Sa-004	MP	S5
Sa-005	MP	S1
Sa-006	IM & M	S2
Sa-007	MP	S3
Sa-008	MP	S3
Sa-009	MP	S5
Sa-010	MP	S2
Sa-011	MP	S4
Sa-012	MP	S2
Sa-013	MP	S1
Sa-014	MP	S1
Sa-015	MP	S1
CS1	MP	ND
CS2	MP	ND

MP: Peritriche mobility; IM: Immobility; M: Mobility; CS1: Control strain 1 *Salmonella* Typhi; CS2: Control strain 2, *Salmonella typhi*, -: negative, +: positive, ND: Not defined. All the strains studied were oxidase-negative, catalase-positive,

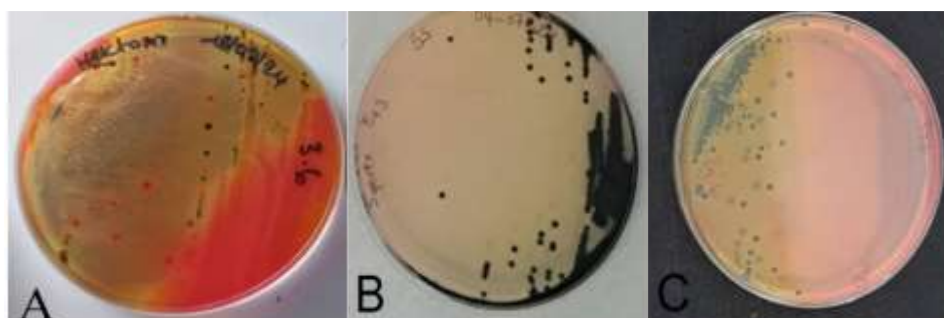


Figure 2. Bacteriological identification of *Salmonella* spp. on selective agar. A: Characteristic *Salmonella* colonies on Hektoen agar, showing blue-green colonies with black centers. B, C: Characteristic *Salmonella* colonies on *Salmonella-Shigella* agar, presence of colorless colonies with a black center.

Antibiotic resistance of strains

To establish their resistance profile, an antibiogram was carried out on five *Salmonella* strains, whose identities were confirmed by biochemical tests. Of the 5 *Salmonella* strains, 4 were resistant to one or more antibiotics tested in this study. The highest percentages of antimicrobial resistance were found for ampicillin (80%), Pefloxacin (80%), ticarcillin clavulanic acid (40%), and Cefepime (40%). The results show that two strains are multi-resistant, as they are resistant to at least two of the eight antibiotics tested, each belonging to a different

family. These families were beta-lactams, aminoglycosides, fluoroquinolones, and phosphonics (Table 3).

Molecular confirmation

Salmonella-specific PCR, using the *invA* gene, revealed the presence of the gene in 13 strains from 22 *Salmonella* strains isolated, with positivity rate of 70.83%. These strains showed an amplified DNA fragment of 284 bp and confirm *Salmonella* strain. The positive control

Table 2. Leminor reduced rack results.

Strain	Test result	Conclusion
Sa-002	GLU+, LACT -, H ₂ S+, CIT+, MOB+	<i>Salmonella</i> spp.
Sa-005	GLU+, LACT -, H ₂ S+, CIT+, MOB+	<i>Salmonella</i> spp.
Sa-010	GLU+, LACT -, H ₂ S+, CIT+, MOB+	<i>Salmonella</i> spp.
Sa-012	GLU+, LACT -, H ₂ S+, CIT+, MOB+	<i>Salmonella</i> spp.
Sa-015	GLU+, LACT -, H ₂ S+, CIT+, MOB+	<i>Salmonella</i> spp.

CIT: Use of citrate; H₂S: H₂S production; GLU: D-glucose; LACT: Lactic Acid; MOB: Mobility.

Table 3. Antibiotic resistance profile of *Salmonella*.

Antibiotic used	Resistance, % (number of strains)	Antibiotic class
TCC	40 (2/5)	β-lactams
FEP	40 (2/5)	β-lactams (Cephalosporins)
CTX	0 (0/5)	β-lactams (Cephalosporins)
GMN	20 (1/5)	Aminoglycoside
AMP	80 (4/5)	β-lactams (penicillins)
IPM	0 (0/5)	β-lactams (Carbapenems)
FOS	0 (0/5)	Phosphonic acids
PEF	80 (4/5)	Fluoroquinolones

TCC: Ticarcillin clavulanic acid; FEP: Cefepime; CTX: Cefotaxime; GMN: Gentamine; AMP: ampicillin; IPM: Imipenem; FOS: Fosfomycin; PEF: Pefloxacin.

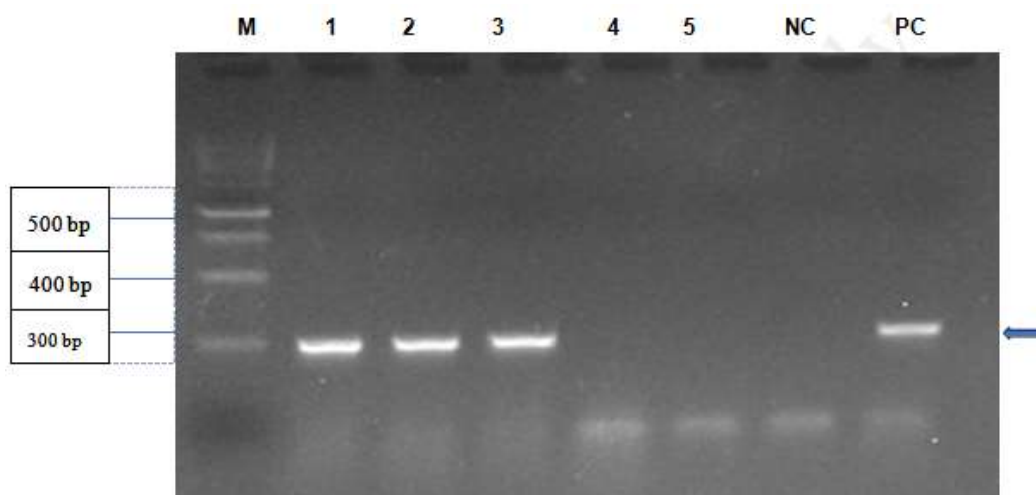


Figure 3. DNA Amplification of the *invA* gene for *Salmonella* strains. M: Molecular weight marker 1 kb (Promega, Germany); 1- 5: (Sa-2025-001; Sa-002; Sa-003; Sa-005; Sa-006); PC: Positive control sample (*Salmonella* Typhi); NC: Negative control (H₂O).

strains were positive in *invA* gene amplification (Figure 3). 33% strains were isolated from site 1; 25% strains s from sites 3 and 5. Confirmed strains were from site 2 and site 4 by 16 and 8%, respectively (Table 4).

Sequencing aligning of isolated strains of *Salmonella*

Thirteen strains were submitted to sequencing; only eleven sequences could be cleaned and retained for the

Table 4. PCR confirmation of *Salmonella* strains.

PCR result	Numbers of strain	Strain
Positive (+)	13	Sa-001; Sa-002; Sa-003; Sa-004; Sa-005; Sa-006; Sa-007; Sa-008; Sa-009; Sa-010; Sa-012; Sa-013; Sa-015
Negative (-)	9	Sa-011; Sa-014; Sa-016; Sa-017; Sa-018; Sa-019; Sa-020; Sa-021; Sa-022

construction of the phylogenetic tree. The phylogenetic analysis of the *invA* gene sequences of different *Salmonella* Enterica strains has identified two main clades. The first clade groups of local isolates (Sa-001, Sa-002, Sa-013) and shows a strong similarity with the reference strain *Salmonella* Paratyphi B (OP599924.1), indicating a possible common origin. The second clade includes well- characterized strains such as *Salmonella* Typhimurium, *Salmonella* Enteridis, *Salmonella* Kentucky, and *Salmonella* Muenchen, with high bootstrap values (90-95%). These results highlight isolated strains that appear to be closer to *Salmonella* Paratyphi B than to other serovars (Figure 4).

DISCUSSION

Salmonella is one of the main pathogens responsible for foodborne illness in humans. Transmission of this bacterium occurs mainly through the consumption of contaminated foods such as eggs, raw milk, cheese, and meat, particularly poultry (Institut Pasteur, 2024). The present study was carried out to isolate strains of multidrug-resistant *Salmonella* from chicken. Twenty-four samples were isolated for *Salmonella* based on morphological characteristics observed on Hektoen agar. The isolated strains were distributed as follows: The site with the most isolated strains is site 1. Indeed, this sampling site is located at the Siporex market. Pollution is quite pronounced at this site due to the perishable goods that remain there for several days and the runoff water present. Site 1 is followed by site 5, which is primarily dedicated to the sale and slaughter of chickens, and we can have a transfer of germs during the handling of contaminated chickens to healthy chickens (Coulibaly-Kalpy et al., 2017). These results are in line with those reported by Silué (2005) and Koffi (2015), who describe *Salmonella* strains as. The results obtained enabled us to reliably identify these four strains as belonging to the *Salmonella* genus, meeting the biochemical criteria accepted in the literature.

Having characterized the isolates by biochemical tests, it was necessary to determine their susceptibility to the antibiotics generally used, which was achieved by carrying out an antibiogram. This is an important step in

the characterization of isolated strains, especially in the context of increasing multi-resistance. Several isolated *Salmonella* strains were subjected to antibiotic susceptibility testing to determine their resistance to different families of antibiotics. The tested strains all showed multi-resistance. Indeed, the use of antibiotics in chicken farming promotes the emergence of numerous resistances. Several studies have shown that chicken intestines are important reservoirs for transmissible resistance genes because they carry determinants of resistance to many classes of antimicrobial agents (Sáenz et al., 2014).

The results obtained revealed resistance to several commonly used antibiotics such as ampicillin, pefloxacin, ticarcillin, clavulanic acid, and gentamycin, but also sensitivity to Imipenem, Fosfomycin, and Cefepime. These results are similar to those reported by Brown and Alhassan (2014) in Ghana, whose strains isolated from chicken were resistant to Ampicillin, Erythromycin, and Tetracycline. Moreover, the ampicillin resistance observed in this study is similar to that reported by Wahome et al. (2014) in Kenya and Compaoré (2006) in Burkina Faso. Several authors also reported an alarming increase in multidrug-resistant *Salmonella* strains, which could be linked to the frequent use of antibiotics in environment (Nacer et al., 2021). Furthermore, antibiogram results reported by El Allaoui et al. (2017) showed that 93.5% (58/62) of strains were resistant to at least one antibiotic. In comparison, multi-resistant strains (significant resistance to several antibiotic classes) make up 80.6% of the total number of strains. The uncontrolled use of antibiotics in poultry production has intensified the emergence of multi-resistant bacteria, according to Nowroozi et al. (2004). This pattern of multidrug resistance is of particular concern, as it limits the available treatment for these infections. The reduced carrier of Le Minor showed that out of 15 strains, 5 strains exhibited the biochemical characteristics of *Salmonella* strains.

The use of PCR proved to be an effective method for identifying virulence genes, in particular *invA*, recognized as a marker specific to the *Salmonella* genus. However, the PCR confirmed *Salmonella* strains. Thirteen strains were positive in the PCR as well as in sequencing. Similar results were reported by Nowroozi et al. (2004) in

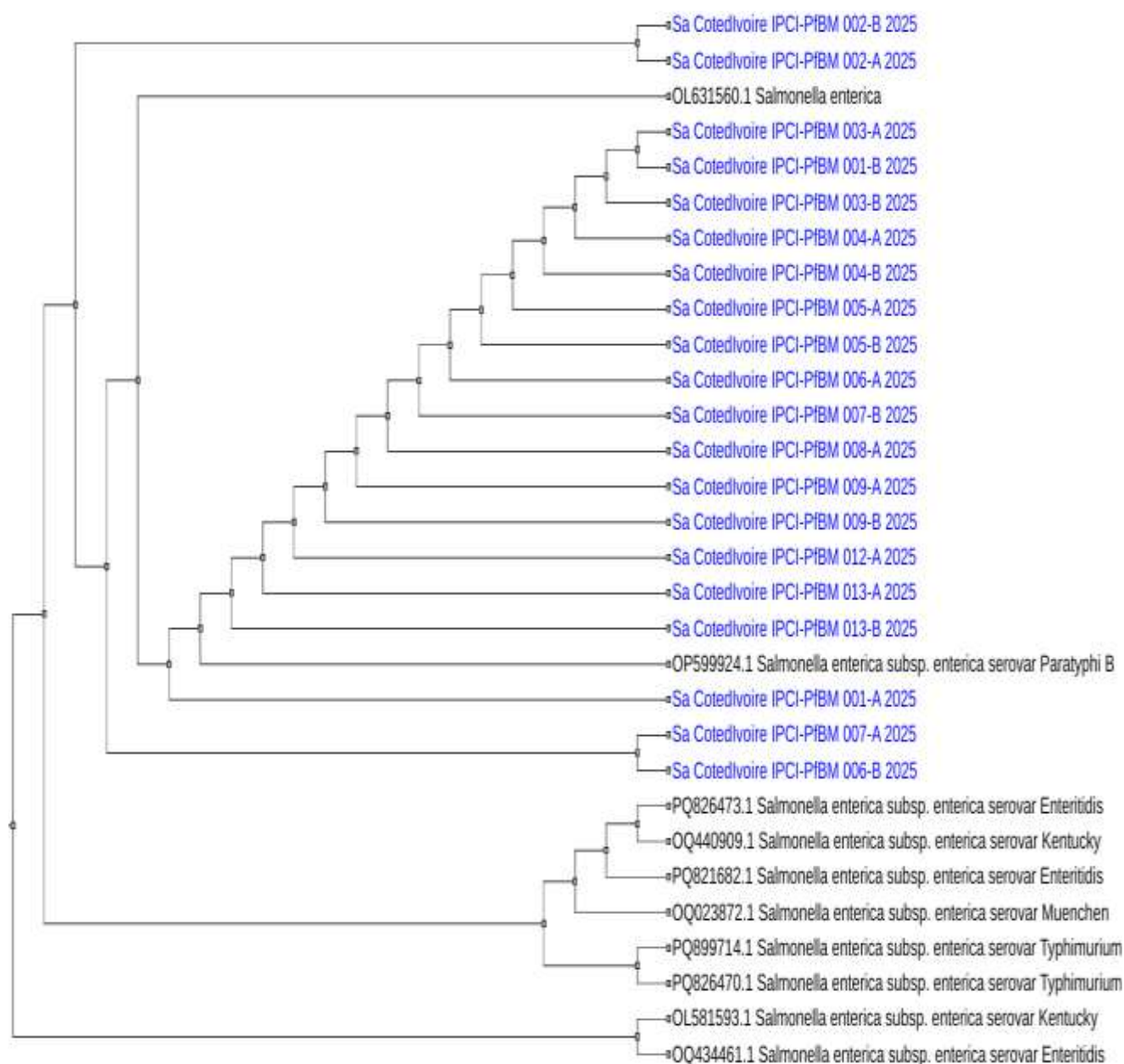


Figure 4. Phylogenetic tree of *Salmonella* strains isolated from chicken intestines.

Iraq and Akinyemi et al. (2021) in Nigeria, who observed prevalence of 25% and 3.9% respectively, of *Salmonella* strains carrying the *invA* gene. Buehler et al. (2019) also confirmed that the *invA* gene is a key virulence gene of *Salmonella* and is used as a target for *Salmonella* detection. In recent years, advances in technology complementing PCR and genome sequencing have transformed the study of infectious diseases (Philippe, 2018). The phylogenetic analysis obtained has enabled us to identify isolated strains with similarities to *Salmonella* Enterica serovars *paratyphi* B.

The results are reinforced by several studies that have identified *Salmonella* using the *invA* gene, which has been confirmed by sequencing. For example, M Kangara et al. (2020) identified *Salmonella* Typhimurium in village chickens using PCR targeting the *invA* and *spvC* genes, revealing 100% homology with similar strains in the databases. In addition, El-Sebay et al. (2017) examined the efficacy of various methods for detecting *Salmonella* in food samples, comparing results from traditional cultures and molecular techniques. Studies based on whole-genome sequencing show great

genetic diversity and highlight the limitations of classical serotyping (Nagpala et al., 2025), suggesting the value of more extensive genomic approaches. However, the limited sample size and market-based sampling may restrict the representativeness and generalizability of results.

Conclusion

At the end of this study, which aimed to isolate multi-resistant *Salmonella* strains from chickens, twenty-four colonies with similar characteristics were identified; five of these were confirmed by biochemical tests to belong to the *Salmonella* genus. Antibiotic susceptibility testing of the isolated strains revealed resistance to several classes of antibiotics, notably fluoroquinolones. Molecular identification using PCR targeting the *invA* gene identified strains, whose sequencing using the Sanger method demonstrated strong similarity to *Salmonella* Enterica serovar Paratyphi B. These results confirm the effective circulation of multidrug-resistant strains of *Salmonella* in chicken in Côte d'Ivoire, precisely in Abidjan, posing significant challenges in terms of food safety and public health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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