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Diversity and molecular characterization of the virulence genes of antibiotic-resistant *Salmonella* strains isolated from bovine faeces in Abidjan district (Côte d'Ivoire)

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Abstract

The emergence of potentially pathogenic and antibiotic-resistant *Salmonella* strains in livestock has become a public health concern and is the subject of increased scientific interest.

The overall objective of this study is to determine the diversity and molecularly characterize the virulence of antibiotic-resistant *Salmonella* strains isolated from cattle faeces in the district of Abidjan (Côte d'Ivoire).

Salmonella strains were isolated from various samples of fresh cattle faeces using conventional methodology in accordance with ISO 6579:2002 (E), and the identity of the strains was then confirmed by MALDI-TOF mass spectrometry, followed by the determination of the different serotypes. An antibiotic sensitivity test was used to determine the resistance profiles of the isolated strains. The search for different virulence genes was carried out using the PCR technique with specific primers (*invA,* spvC, *iroN* and *pefA*, etc.).

Thus, out of a total of 420 faecal samples analysed, 84 *Salmonella* strains (20%) were isolated. Serotyping detected 50 different serotypes, with a predominance of *Salmonella* serotype II. The resistance profile of *the Salmonella* strains showed relatively high levels of resistance to tetracycline, minocycline and colistin, with rates ranging from 20.2% to 33.3%. However, low levels of resistance were observed to β-lactams, aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole. PCR testing for virulence genes showed that these strains possess at least one virulence gene. In *Salmonella,* the *invA, spvC* and *iroN* genes were detected in 100%, 3.9% and 53.9% of cases, respectively.

Poor antibiotic use practices in livestock farming contribute to the spread of potentially pathogenic and antibiotic-resistant bacteria among the human population. Hence the need to collect data to develop strategies based on a One Health approach in order to protect public health in Côte d'Ivoire.

Keywords: *Salmonella*; Virulence; Antibiotic Resistance; Cattle; Abidjan; Ivory Coast

1. Introduction

The genus *Salmonella*, a ubiquitous bacterium [1] belonging to the *Enterobacteriaceae* family, is widespread and is a major cause of foodborne illness worldwide, causing approximately 180 million cases of diarrhoea each year [2] [3].

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Salmonella infections represent a major challenge for global public health, affecting both human and animal populations. *Salmonella enterica* is the species responsible for a variety of diseases [4], ranging from common gastroenteritis to potentially fatal typhoid and paratyphoid fever, as well as invasive infections that can lead to serious complications, particularly in at-risk individuals such as children, the elderly and immunocompromised individuals [5] [6] [7] [8].

Domestic and wild animals are the main reservoirs of infection in humans, and contamination occurs through direct contact or consumption of contaminated food or water [9]. Salmonellosis in cattle is widespread throughout the world and is now considered the most important animal zoonosis [10] due to the significant role of cattle in the spread of *Salmonella* and their impact on the contamination of the food chain and the environment [11] [12].

The virulence of *Salmonella* is a complex process governed by a multiplicity and variability of factors involving adhesion to host cells, invasion of host cells, multiplication within these cells and resistance to the body's immune defences [13] [14]. The genes responsible for *Salmonella* virulence, such as *invA, spvC, pefA* and *iroN,* are often grouped into pathogenicity islands [15] [16]. Indeed, cattle can carry *Salmonella* in their intestines without necessarily showing clinical symptoms, thus becoming a silent reservoir. The excretion of the bacterium in faeces contaminates the environment, slaughter surfaces, raw milk and meat, thereby promoting the spread of potentially pathogenic strains [17].

The increase in antibiotic resistance rates in *Salmonella* is a growing threat, particularly in developing countries where the unregulated and indiscriminate use of antibiotics in livestock farming is common [18]. This practice significantly reduces the effectiveness of antibiotic treatments in humans and animals and further increases the spread of multi-resistant strains in the bacterial environment [19]. These resistances, often carried by plasmids that can be transmitted between bacteria, contribute to the rapid spread of resistance genes in various ecosystems [20]. Thus, the detection of resistance carriers associated with virulence carriers provides a better understanding of the pathogenicity of these circulating *Salmonella* strains. In cattle, the presence of antibiotic-resistant *Salmonella* poses a public health risk because these strains can be transmitted to humans through the consumption of contaminated products or through direct contact with these animals.

In Côte d'Ivoire, although research has been conducted on the prevalence, phenotypic and molecular characterization of antibiotic resistance in *Salmonella spp.* strains in cattle farms [21] [22], data on the molecular characterization of virulence and resistance factors in bovine strains remain limited. In the district of Abidjan, where agri-food activity is intense, it is therefore essential to characterize the *Salmonella* strains present in cattle in order to assess the risks to public and animal health and to implement appropriate control measures.

The overall objective of this study is to determine the diversity and molecularly characterize the virulence of antibiotic-resistant *Salmonella* strains isolated from bovine faeces in the Abidjan district (Côte d'Ivoire).

2. Materials and Methods

2.1. Collection of Salmonella strains from bovine faeces

Over a six-month period, between April and September 2016, a study was conducted on the prevalence and characterization of Salmonella strains in cattle faeces in the district of Abidjan (Côte d'Ivoire). This study isolated and identified a total of 84 *Salmonella* spp strains from 420 fresh bovine faecal samples collected during this period. Specifically, sixty (60) faecal samples were collected in each municipality, except in the municipality of Port-Bouët (3 sites) where 180 samples were collected. Two hundred (200) grams of fresh faeces were randomly selected from the various sites. Initial detection of *Salmonella* spp. was carried out using the conventional methodology in accordance with ISO 6579:2002 (E) as described by Yao et al., [20] and Julien et al, [21]. The identity of the strains was then confirmed by MALDI-TOF mass spectrometry (BioMérieux, France), thus ensuring rigorous identification. More specifically, the study identified 5 strains from Abobo, 30 strains from Adjamé, 4 from Yopougon, 4 from Bingerville and, finally, 41 strains from Port-Bouët.

2.2. Serotyping test of isolated strains

The serotypes of the strains isolated from cattle faeces and confirmed by MALDI-TOF were determined by slide agglutination tests with O, H and Vi antisera (Bio Mérieux, France) [20]. The results were read according to the Kauffmann-White scheme (1934).

2.3. Antibiotic susceptibility testing of isolated strains

Antibiotic sensitivity testing was performed on all *Salmonella* strains using the agar disc diffusion method, and the results were interpreted according to the standards of the Antibiogram Committee of the French Society for Microbiology (EUCAST/CA-SFM, 2016). The reference strain E*. coli* ATCC 25922 was used for internal quality control. The following antibiotic discs (Bio-Rad France) were used: ampicillin (10 µg), amoxicillin + clavulanic acid (30 µg), cefalotin (30 µg), cefepime (30 µg), aztreonam (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), imipenem (10 µg), tetracycline (30 µg), minocycline (30 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), nalidixic acid (30 µg), norfloxacin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), colistin (50 µg) and trimethoprim/sulfamethoxazole (25 µg).

2.4. Detection of virulence genes by PCR

Molecular detection of virulence genes was performed by PCR on all *Salmonella* strains exhibiting phenotypic resistance to at least one antibiotic.

Bacterial DNA was extracted from 500 µL of *Salmonella* isolate suspension using nuclease-free water (pure water) and total DNA extraction was performed by heat shock [23] [21]. The various DNA extracts obtained were then used as a template for PCR reactions using specific primers listed in Table 1.

Genomic amplification was performed in a final volume of 50 µl of reaction mixture, containing 5X coloured buffer (Promega, USA), 5X uncoloured buffer (Promega, USA), 25 mM $MgCl_2$(Promega, USA), 10 mM of each dNTP (Biorad, France), 10 µM specific primers (Reverse and Forward), 5U of Taq polymerase (Go taq ® G2 Flexi DNA Polymerase) (Promega, USA), and 5 µL of DNA. Reference strains provided by the National Food Institute (DTU Food) collection were used as positive controls for PCR (Table 2) and a reaction mixture without DNA extract was used as a negative control. The amplification conditions are shown in Table 3.

The PCR products were analysed by agarose gel electrophoresis. The gels, prepared with 10X TAE (Tri-Acetate-EDTA) buffer and 5 µL of EZ-vision® solution (Inqaba biotec, West Africa), had a concentration of 2%. Electrophoresis was conducted at 120 V/cm for 1 hour.

A 100 bp molecular weight marker was used to approximately determine the size of the fragments to be analysed. After migration, the DNA was observed under UV light (λ = 312 nm) using the Gel Doc EZ Imager automated system (BioRad, USA).

Table 1 Specific primer for the detection of resistance genes

Gene	Primer	Sequence (5'-3')	Hybridisation temperature	Fragment size (bp)	References
invA	INVA-1 INVA-2	ACAGTGCTCGTTTACGACCTGAAT AGACGACTGGTACTGATCGATAAT	56	244	[24]
spvC	SPVC-1 SPVC-2	ACTCCTTGCACAACCAAATGCGGA TGTCTTCTGCATTTCGCCACCATCA	56	571	[24]
iroN	iroN-F iroN-R	ACTGGCACGGCTCGCTGTCGCTCTAT CGCTTTACCGCCGTTCTGCCACTGC	66.5	1205	[25]
pefA	pefA-r pefA-f	AGGGAATTCTTCTTGCTTCCATTCCATTATTGCACTGGG TCTGTCGACGGGGGATTATTTGTAAGCCACT	50	157	[25]

Table 2 Reference strains

Species	Usage	References/Origin
E. coli ATCC25922	Controls for culture media and antibiograms	DTU Food (Denmark)
Sal*monella P5002212 DT104*	Positive control for detection of *invA*, *spvC*, *pefA* and *iroN* genes	DTU Food (Denmark)

Table 3 PCR programmes

Amplification step	Temperature condition/duration	
	invA, spvC	*iroN, pefA*
Initial denaturation	94°C/2 min	34°C/2 min
Cyclic denaturation	94°C/30 sec	94°C/30 s
Hybridisation	56°C/30 s	65°C/30 s
Cyclic elongation	72°C/2 min	72°C/1 min
Final elongation	72°C/10 min	72°C/10 min
Number of cycles	35	35

3. Results

3.1. Frequency of isolation and distribution of *Salmonella* serotypes isolated from bovine faeces in the district of Abidjan

After isolation and confirmation by MALDI-TOF mass spectrometry, 84 Salmonella strains were identified in a total of 420 bovine faecal samples collected in the Abidjan district, indicating an overall prevalence of 20% in the bovine faeces sampled. In addition, the geographical distribution of the isolated *Salmonella* strains varied considerably between municipalities. The municipality of Port-Bouët had the highest number of isolates with 41 strains, followed by the municipality of Adjamé with 30 strains. The municipalities of Abobo, Yopougon and Bingerville had the lowest numbers of isolates with 5 strains, 4 strains and 4 strains respectively (**Table 1)**.

In addition, serological analysis revealed a diversity of *Salmonella* serotypes in the bovine faeces sampled (**Table 4**). A total of 50 different serotypes were identified among the 84 *Salmonella* strains isolated from bovine faeces. The most frequently identified serotypes were *Salmonella enterica* subsp. *enterica* serotype II (11 strains, 13.1%), Salmonella *enterica* subsp. *enterica* serotype Agbeni (7 strains, 8.3%) and *Salmonella enterica* subsp. *enterica* serotype *Hohentwiel* (7 strains, 8.3%). Other serotypes were identified with lower frequencies ranging from 1.2% to 3.6%.

Table 4 Distribution of samples and number of *Salmonella* strains isolated in faeces

Salmonella ssp	
Common/ Number of samples	Number of strains isolated
Abobo (n= 60)	5
Adjamé (n= 60)	30
Yopougon (n= 60)	4
Bingerville (n= 60)	4
Port-Bouët (n= 180)	41
Total (N=420)	84

Table 5 *Salmonella* serotypes isolated from cattle faeces

Number	Serotypes	Number	Frequency (%)
1	*Salmonella Virchow*	2	2.4
2	*Salmonella Muguga*	2	2.4
3	*Salmonella IIIa*	1	1.2
4	*Salmonella Agbeni*	7	8.3
5	*Salmonella Enteritidis*	1	1.2%
6	*Salmonella Neumenster*	1	1.2%
7	*Salmonella II*	11	13.1
8	*Salmonella Othmerschen*	1	1.2
9	*Salmonella Brikama*	1	1.2%
10	*Salmonella Durcham*	2	2.4
11	*Salmonella Catanzaro*	1	1.2
12	*Salmonella Parkroyal*	1	1.2%
13	*Salmonella Senftenberg*	2	2.4%
14	*Salmonella Schwarzengrund*	1	1.2
15	*Salmonella Kambole*	1	1.2%
16	*Salmonella Umbadah*	1	1.2%
17	*Salmonella Dessau*	1	1.2%
18	*Salmonella Tudu*	1	1.2
19	*Salmonella Gustavia*	2	2.4
20	*Salmonella Canton*	1	1.2%
21	*Salmonella Muenster*	1	1.2%
22	*Salmonella Salford*	1	1.2%
23	*Salmonella Potsdam*	2	2.4%
24	*Salmonella Fufu*	1	1.2
25	*Salmonella Budapest*	1	1.2%
26	*Salmonella Urbana*	1	1.2%
27	*Salmonella Wichita*	1	1.2%
28	*Salmonella Torhout*	1	1.2%
29	*Salmonella Baguirmi*	2	2.4%
30	*Salmonella Africana*	1	1.2%
31	*Salmonella Kedougou*	1	1.2%
32	*Salmonella Dublin*	3	3.6%
33	*Salmonella Alfort*	1	1.2%
34	*Salmonella Hohentwiel*	7	8.3%
35	*Salmonella Clerkenwell*	2	2.4

36	*Salmonella Kisangani*	1	1.2
37	*Salmonella Bradford*	1	1.2%
38	*Salmonella Shubra*	2	2.4%
39	*Salmonella Chicago*	1	1.2
40	*Salmonella Erfurt*	1	1.2%
41	*Salmonella Scarborough*	1	1.2%
42	*Salmonella Schleissheim*	1	1.2%
43	*Salmonella Langensalza*	2	2.4
44	*Salmonella Preston*	1	1.2
45	*Salmonella Wuiti*	1	1.2%
46	*Salmonella Bron*	1	1.2%
47	*Salmonella Typhi*	1	1.2%
48	*Salmonella Bochum*	1	1.2%
49	*Salmonella Atakpame*	1	1.2%
50	*Salmonella Chomedey*	1	1.2
Total		*84*	100%

3.2. Resistance profile of *Salmonella* strains

Analysis of antibiotic resistance in the 84 *Salmonella spp* strains showed that 26 strains were resistant to at least one antibiotic. The rate of resistance to beta-lactams and quinolones was generally low (1.2% to 6% and 3.6%, respectively). The highest resistance was observed for colistin (33.3%), followed by tetracycline and minocycline (20.2% each). Gentamicin (1.2%), tobramycin (3.6%) and trimethoprim/sulfamethoxazole (6%) showed low levels of resistance, while no resistance was observed with cefoxitin, imipenem, amikacin and chloramphenicol (**Table 6)**.

Table 6 Antibiotic resistance profil

Antibiotic families	**Resistance frequency (%) R(n=84)**
Betalactams	
Ampicillin (AMP)	2.4
Amoxicillin/clavulanic acid (AMC)	1.2
Cefalotin (CEF)	6
Cefuroxine (CXM)	2.4
Cefoxitin (FOX)	0
Ceftriaxone (CRO)	1.2
Ceftazidime (CAZ)	1.2
Cefepime (FEP)	1.2
Aztreonam (ATM)	1.2
Imipenem (IMP)	0
Quinolones	

Nalidixic acid (NAL)	3.6
Ciprofloxacin (CIP)	3.6
Norfloxacin (NOR)	3.6
Cyclines	
Tetracycline (TET)	20.2
Minocycline (MNO)	20.2
Aminoglycosides	
Amikacin (AKN)	0
Tobramycin (TMN)	3.6
Gentamicin (GEN)	1.2
Others	
Colistin (CST)	33.3
Chloramphenicol (CHL)	0
Trimethoprim/Sulfamethoxazole (SXT)	6

3.3. Detection of virulence genes

The search for genes linked to the virulence of *Salmonella* strains was carried out using single-plex PCR (*iroN* and *pefA*) and multiplex PCR (*invA* and *spvC*). The presence of the genes was confirmed by the size of the amplicons obtained (157 bp for *pefA,* 244 bp for *invA,* 571 bp for *spvC* and 1205 bp for *iroN)* (**Figure 1)**. Thus, *no pefA* gene was detected in the strains analysed. However, all 26 resistant strains possessed at least one of the virulence genes sought. The *invA* gene was present in all strains with a frequency of 100%. The spvC gene showed a relatively low frequency (3.8%). The iroN gene was detected with a frequency of 53.8% (**Table 7)**.

Overall, all *Salmonella* serotypes possessed at least one virulence gene. Some serotypes carried only the *invA* gene (12 serotypes), while others carried both the *invA* and *spvC* genes (13 serotypes). Only the ***Schwarzengrund*** serotype had a combination of three (3) virulence genes (*invA + spvC+ iroN) (***Table 8)**.

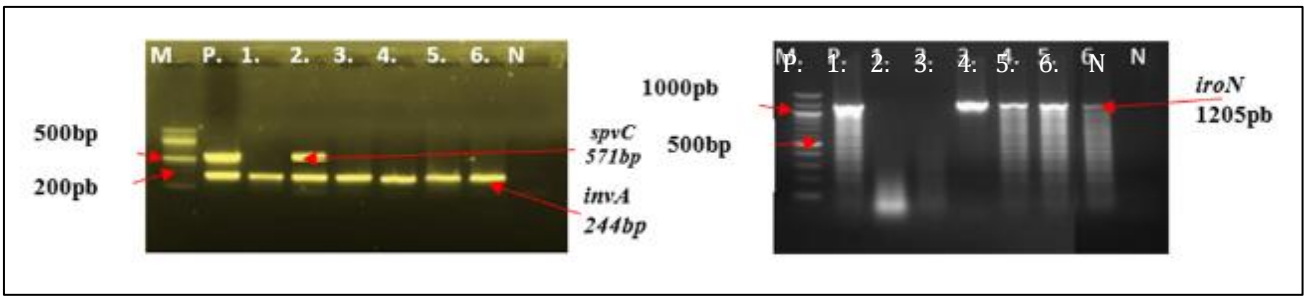

Figure 1 Electrophoretic profiles of virulence genes detected in *Salmonella*

A: detection of the *invA (244bp)* and *spvC* (571bp) genes by multiplex PCR; B: detection of the *iroN* gene (1205) by simplex PCR ; M: molecular weight marker (100 to 2000 bp) ;P: positive control (*Salmonella* DT104 strain);N: negative control (contains ppi water instead of bacterial DNA). Numbers 1 to 6: bacterial strains

Table 7 Detection of virulence genes

Strains	**Number of genes detected**				**Total**
	invA	*spvC*	*iroN*	*pefA*	
Salmonella	26	1	14	0	26
Frequency (%)	100	3.8	53.8	0	100

Table 8 Association between virulence genes and serotypes

Salmonella					
Strains	Genes	Serotypes	Strains	Genes	Serotypes
E32S	invA	Virchow	E93S	invA	Muenster
E38S	invA	IIIa	E108S	invA,iroN	Fufu
E44S	invA,iroN	Enteritidis	E28ADS	invA,iroN	Africana
E46S	invA,iroN	Neumenster	E31ADS/A	invA,iroN	Dublin
E60S	invA,iroN	Brikama	E31ADS/C	invA,iroN	Alfort
E61S	invA	Agbeni	E32ADS/C	invA,iroN	hohentwiel
E64S	invA	II	E35ADS/A	invA,iroN	Kisangani
E67S/B	invA	Senftenberg	E35ADS/C	invA,iroN	Shubra
E68S	invA,spvC,iron	schwarzengrund	E45ADS/A	invA,iroN	hohentwiel
E73S	invA	Umbadah	DCE28AB/C	invA,iroN	Agbeni
E74S	invA	Dessau	DCE29PB/B	invA,iroN	Agbeni
E88S	invA	II	E32BIS	invA	chomedey
E89S	invA	Gustavia	E66S	invA	II

4. Discussion

In this study, *Salmonella* strains were isolated from bovine faeces using the method based on standard NF EN ISO 6579 (ISO-6579, 2002E). The process included pre-enrichment, selective enrichment, selective isolation and identification via MALDI-TOF [21] [22]. The isolation results showed a prevalence of 20% in the sampled cattle faeces. This average prevalence of 20% is higher than that reported in several studies in Ethiopia (2.3%) and Nairobi (2.6%) [26] [27]**.** However, much lower carriage rates have been reported in cattle in Japan and Great Britain, with prevalences of 0.5% and 1.4% respectively [28] [29]. On the other hand, this prevalence is relatively comparable to that reported by **Akoachere *et al.*** [30] in Cameroon, which is 28.7%. A study conducted in Burkina Faso revealed a much higher carriage rate of *Salmonella* (52%) in bovine faeces [31]. The prevalence observed in this study may be related to the geographical location and hygiene conditions on farms. The prevalence observed in this study may be related to the geographical location and hygiene conditions on our farms. Farms and livestock parks are located close to human populations, and excrement and food waste are not regularly disposed of. These conditions can provide sufficient nutrients in moist soils for Salmonella to remain viable [32].

Serotyping of the 84 *Salmonella* strains isolated revealed 50 different serotypes. Previous studies have reported that the serotypes most frequently observed in cattle are *S. Typhimurium, S. Dublin, S. Agona, S. Orion, S. Aintpul, S. Braenderup, S. Muenchen, S. Croft, S. Kentucky, S. Telaviv, S. Montevideo, S. Kpeme, S. Infantis, S. Abadina, S. Cerro, S. Mismarhaenek, S. Enteritidis, S. Guildford, S. Anatum, S. Gozo, S. Mbandaka, S. Senftenberg, S. Newport, S. Give,* and S. *Muenster* [33] [34] [35] [36] [37]**.** Research conducted by [26] on cattle faeces in Ethiopia reported several serotypes,

among which S*. Typhimurium,* S*. Saint-paul,* S*. Kentucky, and* S*. Virchow* were the most common. However, in this study, serotype II was the most dominant. The *Typhi* serotype was observed with a frequency of 1.19%. Although present in low proportions, the presence of S*. Typhi* explains the failure of hygiene measures, but also the presence of livestock in urban areas. Indeed, S*. Typhi* is a strictly human pathogen that causes invasive fever (typhoid fever), whereas most other *Salmonella* serotypes mainly cause gastrointestinal symptoms without systemic invasion [38].

The growing resistance of Salmonella to antibiotics commonly used in veterinary and human medicine is a growing public health concern. This is often attributed to poor management practices in livestock farming, which lead to the emergence of resistant bacteria, as observed in this study. Isolated bovine faecal samples showed a high rate of antibiotic-resistant Salmonella strains, confirming the hypothesis that bovine faeces can serve as reservoirs for resistant bacteria, thereby increasing the likelihood of transmission to humans [39]. *Salmonella* strains showed low resistance to beta-lactams. Resistance to this molecule could be attributed to its misuse in livestock farming. Resistance to beta-lactams, particularly third- and fourth-generation cephalosporins (C3G/C4G), is less common in animal Salmonella strains, suggesting that this bacterium is not a major reservoir of resistance genes [40]. Moreover, cumulative data from *Salmonella* strain surveillance networks in France confirm this very low proportion of C3G/C4G-resistant *Salmonella* strains of animal origin. Several authors have also reported similar resistance rates [41]**.** Variable rates of resistance to third-generation cephalosporins have been reported in China (1.6%), Romania (11.4%) and the United States (16%) [42] [43] [44]**.** All *Salmonella* strains in this study were susceptible to imipenem, probably due to its efficacy, good stability, and high bactericidal activity, making it an antibiotic of choice for the treatment of infections caused by resistant bacteria [45] [46]. *Salmonella* strains remained largely susceptible to quinolones, with average resistance rates of 3.6% observed for nalidixic acid, ciprofloxacin and norfloxacin. Similar quinolone resistance rates have been reported in the Republic of Ireland (2.6% for nalidixic acid) and India (5.7% for ciprofloxacin) [47] [41]. However, higher rates of resistance to nalidixic acid have been reported in Kenya (12%) [27] and Romania (65.1%) [43]. Even higher resistance rates for ciprofloxacin (25.8% to 42.95%) have been reported in China [43] [44]. High resistance rates for nalidixic acid (75%) and ciprofloxacin (75%) have been reported in Nigeria [39]. The emergence of fluoroquinolone resistance is concerning because these antibiotics are commonly used to treat invasive salmonellosis in veterinary medicine. The low rate observed in this study may be due to the fact that fluoroquinolones are not widely used in cattle farming in Côte d'Ivoire. The isolated *Salmonella* strains showed high resistance to cyclines (20.2% tetracycline, 20.2% minocycline). This increase in cycline resistance has also been observed in the United States [48], with a reported resistance rate of 20.9%. Higher resistance rates have been observed in Nigeria [39], Ethiopia [26] and the United States [49]. High resistance rates to tetracycline (62.2%) and minocycline (46.3%) were observed in South Africa [50]. Lower rates of resistance to tetracycline have been reported in other studies [51] [52]. In addition, resistance to colistin was quite high in this study, affecting 33.3% of *Salmonella* strains*.* The rates obtained in this study are worrying because they are much higher than those reported in several studies [53] [54] [55]**.** This high prevalence of colistin resistance may be due to the increased use of colistin in livestock farming.

In addition to the resistance profiles observed, all resistant *Salmonella* strains studied possessed at least one virulence gene. The *invA*, *spvC* and *iroN* genes were detected with frequencies of 100%, 3.8% and 53.8%, respectively. Only the *pefA* gene was not detected. The high detection rate of the *invA* gene obtained in this study was also reported by **Abouzeed *et al.*** [56] in Iceland. The results obtained are similar to those of [57], with 98.8% of *Salmonella* carrying the *invA* gene originating from cattle. Indeed, the *invA* virulence gene is common to all *Salmonella* and is used as a PCR target gene for the detection of *Salmonella* [58]**.** The *invA* gene is essential for the full virulence of *Salmonella* and is important in the invasion of phagocytic epithelial cells and entry into the intestinal mucosa. As for the *spvC* gene, its detection frequency in this study is similar to that reported by **Chuanchuen et *al.*** [57], which is 1.3%. The *spvC* gene is one of the virulence genes that play a role in controlling the immune response and regulating host cell interaction. The *spvC* gene also controls the growth rate of *Salmonella* in the host cell [59]. Thus, the very low prevalence of *spvC* genes observed in our study could be explained by the integration of the virulence plasmid into the chromosome [56]**.** The *pefA* gene, which codes for the fimbriae adhesin necessary for *Salmonella* adaptation to the host, was not detected in this study. However, **Chuanchuen *et al.*** [57] reported a detection frequency of 0.6% in their work. Other studies on *Salmonella* strains from sick birds [60] **and** pigs [61] showed high detection frequencies of 68.2% (15/22) and 92% (23/25) respectively. *The pefA* virulence genes are associated with the plasmid. The *iroN* gene*,* detected in 53.9% of cases, plays an important role in the survival of the bacterium in hostile environments [62]. It enables *Salmonella* to acquire iron and distinguishes *Salmonella* from other bacteria. Iron is an essential element for many bacterial species. Thus, the high rate of the *iroN* gene observed in this study may be linked to the bioavailability of iron [62] [63] in bovine faecal matter.

PCR profiles of *Salmonella* strains revealed genotypic variations in virulence depending on serotype. *Salmonella* serotype *Schwarzengrund*, for example, presented a more diverse range of virulence genes, including the *invA, spvC and iroN* genes*.* This study shows that certain antibiotic-resistant *Salmonella* serotypes are also capable of expressing

multiple virulence factors, which is very important for public health. Concerningly, this study reveals that certain antibiotic-resistant *Salmonella* serotypes are also capable of expressing multiple virulence genes, which represents a major challenge for public health.

5. Conclusion

This study has provided important information on the diversity and virulence factors of antibiotic-resistant *Salmonella* strains circulating in cattle populations in the Abidjan district of Côte d'Ivoire. The resurgence of antibiotic resistance and virulence genes observed in various *Salmonella* serotypes highlights the significant risk to public health, which is likely to increase the severity and spread of *Salmonella* infections in the Abidjan district. For this reason, in-depth studies are needed to investigate the factors that promote the emergence and spread of resistant and virulent *Salmonella* strains, including local farming practices, antibiotic use and environmental contamination. These data are essential for developing One Health strategies to protect public health in Côte d'Ivoire.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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