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Frequency, Resistance Patterns, and Serotypes of *Salmonella* Identified in Samples from Pigs of Colombia Collected from 2022 to 2023

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Abstract: The objective of this study was to determine the frequency of *Salmonella* in pig samples analyzed at the Veterinary Diagnostic Unit of the Faculty of Agricultural Sciences of the University of Antioquia, Colombia, between 2022 and 2023. Out of 5820 serum samples analyzed using indirect enzyme-linked immunosorbent assay, 63.76% were positive. Additionally, *Salmonella* was isolated and identified in a separate group of 848 samples (feces, tissues, etc.) with a positivity rate of 23.47%. Eight serotypes were identified, with the most common being *Salmonella enterica* subsp. *enterica* serotype Typhimurium (49.2%), followed by its monophasic variant I 4,[5],12:i:- (23%), and serotype Choleraesuis (18%). Antimicrobial susceptibility profiles of 105 isolates were evaluated using the Kirby–Bauer method, which demonstrated higher resistance (100%) to ampicillin, tiamulin, penicillin, tylosin, and erythromycin (these were the least tested), followed by florfenicol (44/54), doxycycline (4/5), spectinomycin (18/25), amoxicillin (32/46), chloramphenicol (2/3), tetracycline (2/3), and enrofloxacin (34/64), with lower resistance observed for fosfomycin (2/38) and ceftiofur (5/35). Multi-drug resistance was observed in 59% (62/105) of the isolates. The high proportion of *Salmonella* and the levels of resistance to various drugs raise significant concerns, indicating potential deficiencies in responsible antimicrobial use and management practices on pig farms in the region.

Keywords: *Salmonella*; serotype; antimicrobial resistance; pigs; Colombia

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1. Introduction

Salmonella belongs to the family *Enterobacteriaceae*, where more than 2659 serotypes have been reported, affecting a wide range of animals, including humans [1]. The genus *Salmonella* spp. consists of two species, *enterica* and *bongori* [2]. The species *S. enterica* is classified into six subspecies, with subspecies I *enterica* being the one associated with over 99% of *Salmonella*-caused diseases in warm-blooded animals, including gastroenteritis and enteric fever [3].

Salmonella enterica subsp. *enterica* includes *S.* Typhimurium and *S.* Choleraesuis, which are the most important serotypes in pig production worldwide [4]. In pigs, *Salmonella* strains cause septicemia, enterocolitis, or subclinical infections; the enterocolitis form associated with *S.* Typhimurium has low mortality and high morbidity [5]. On the other

hand, *S*. Choleraesuis in pigs causes generalized sepsis, with rare signs of diarrhea [2]. *S*. Typhimurium is one of the most common food-borne pathogens in humans and is not host-specific, causing a self-limiting gastroenteritis syndrome [6]. The monophasic variant of *S.* Typhimurium, with the antigenic formula I 4,[5],12:i:-, has become one of the most common multi-resistant serotypes responsible for infections in animals and humans [7].

Salmonellosis in animals and humans is typically characterized by a self-limiting gastroenteritis syndrome; however, more severe invasive *Salmonella* infections, such as bacteremia and/or other extraintestinal infections, can occur [8]; antimicrobial use is indicated for treatment in such cases. In pigs, these may include amoxicillin-clavulanic acid, ampicillin, ceftiofur, ciprofloxacin, chloramphenicol, florfenicol, gentamicin, sulfamethoxazole-trimethoprim, tetracycline, and macrolide antibiotics such as tilmicosin [9]; many of these are crucial for human therapy [10,11]. Food can serve as a vehicle for antibiotic-resistant bacteria, which is why it has such a significant impact on public health [12]. Improper antibiotic use can contribute to the generation of resistance that can be transferred to other pathogenic bacteria in both animals and humans [13]. Therefore, information on and the monitoring and control of antibiotic use are of paramount importance.

Understanding the behavior of this pathogenic agent in pigs in each country is important, as it is the cause of the disease known as salmonellosis and it is implicated in the development of antimicrobial resistances [14]. Other countries have managed to understand the importance and evolution of salmonellosis, as well as its dynamics within their respective territories. In the European Union's One Health 2023 annual report on zoonoses, salmonellosis was the second most frequently reported zoonotic disease in 2023, with 77,486 cases, an increase from the 65,208 cases recorded in 2022 [15]. Meanwhile, in the United States, over a 10-year period between 2011 and 2021, the CDC's National Outbreak Reporting System reported 2403 outbreaks of *Salmonella*, resulting in 52,605 illnesses, of which 35.16% were associated with pork [16].

This study aims to reveal the diagnostic frequencies of *Salmonella* in pigs in certain departments (states) of Colombia using the two most frequently employed diagnostic methods. The purpose is to offer critical information to pig producers and consumers on the agent's health state, serotypes, and isolated antibiotic resistances, promoting the awareness and rapid implementation of appropriate biosecurity measures and responsible antibiotic management on farms across the country. This is critical for promoting public health and food safety, avoiding the spread of foodborne infections, and lowering the danger of antibiotic resistance in Colombia.

2. Materials and Methods

2.1. Samples Included in This Study

This cross-sectional study utilized clinical samples referred for isolation and identification, primarily feces and tissues from pigs suspected of having salmonellosis. For enzyme-linked immunosorbent assay (ELISA) tests, serum samples were submitted both for swine production monitoring and suspected disease cases, although serology is predominantly used for surveillance. The exact number of sick animals is unknown, and it is unclear how many samples were collected specifically for monitoring purposes.

The selection of antibiotics for testing, the decision to perform antibiograms, and the choice to conduct serotyping were entirely client-driven. The data analyzed in this study were obtained from the database of the Diagnostic Unit of the Faculty of Agricultural Sciences at the University of Antioquia. As this unit provides diagnostic services to the public, the analyses performed were based on client requests and needs rather than a standardized research protocol. Consequently, variability in testing reflects real-world diagnostic practices rather than methodological bias. Furthermore, client-driven antibiotic

selection, as well as the proportions of serotyping and antibiogram requests, were included in the analysis, as they provide valuable insights into veterinary diagnostic decision-making and antimicrobial resistance monitoring trends.

The information was sourced from the database of the Diagnostic Unit of the Faculty of Agricultural Sciences at the University of Antioquia, covering the years 2022 and 2023. Only complete records containing information on the origin of the samples were included. The total number of samples with complete records was 5820 serum samples tested with ELISA. Additionally, 848 different samples (feces and tissues (most of them intestine)) were submitted to culture for the isolation and identification of *Salmonella*. The serum samples were used for the ELISA test, while the tissue and fecal samples were used for microbiological culture. However, these samples did not always come from the same animals. Although all samples were from pigs, the individuals selected for ELISA testing were not necessarily the same as those selected for microbiological culture. The Ethics Committee for Animal Experimentation (CEEA) of the University of Antioquia, with Act No. 159 dated 18 June 2024, granted approval for the implementation of the project. The clients signed a consent form allowing the laboratory to use the information obtained or created during the tests for academic, scientific, historical, and statistical purposes, or audit processes, adopting all pertinent security measures to ensure the confidentiality of the clients' and farms' personal data.

2.2. Serological Assay for Detection of Antibodies Against Salmonella

The procedure for determining serum antibodies to *Salmonella* by indirect ELISA was carried out using the commercial kit ELISA (IDEXX Swine *Salmonella* Ab Test). The assay was performed following the manufacturer's recommendations, and the results were read using the Epoch ELISA reader (BioTek™, Winooski, VT, USA) with software IDEXX (xChekPlus 4.0.1.65). The optical density (OD10%) was measured at 650 nm. Polyclonal antisera from IDEXX were used, specifically targeting *Salmonella* antigens. The plates are coated with lipopolysaccharides (LPS) from serogroups B, C1, and D, and all reagents used are manufactured by IDEXX.

2.3. Isolation of Salmonella

Regarding the diagnosis by isolation of *Salmonella*, it began with the proper reception of the sample, usually brought by veterinarians and clients of pig farms. The samples, primarily pig feces, had to arrive with proper cold chain management and in sufficient quantity. After being logged in with the provided data, they underwent a 5-step laboratory process, which included: pre-enrichment of the sample followed by selective enrichment; isolation on selective media; differential biochemical tests; and serotype determination [17,18].

The process for *Salmonella* determination involved several steps: first, pre-enrichment, where 10–25 g of the sample is transferred to 225 mL of buffered peptone water, shaken for 2 min, and incubated at 37 °C for a period of 18 to 24 h. For selective enrichment, the pre-incubated sample is inoculated into Rappaport–Vassiliadis broth and incubated at 42 °C for 18 to 24 h. Subsequently, cultures from the broth are plated onto Hektoen agar and xylose lysine deoxycholate agar plates, and then incubated at 36 °C for 18 to 24 h. Blue or bluish-green colonies on Hektoen agar and pink to light red colonies on xylose lysine deoxycholate agar, both with or without a black center, are considered presumptive for *Salmonella* [17]. Once typical colonies are identified, biochemical and serological characterization is conducted for confirmation. Serotyping was performed using the Kauffmann–White scheme; this technique classifies bacteria based on their surface antigens, particularly the O (somatic) and H (flagellar) antigens [19].

2.4. Antibiotic Susceptibility Testing

Susceptibility testing was performed using the Kirby–Bauer disk diffusion method [20]. The zones of growth inhibition were interpreted according to the guidelines of the Clinical and Laboratory Standard Institute [21]. Nineteen antibiotics were used, and each antibiogram varied depending on the specific requirements of the person submitting the sample. As a result, not all isolates were tested for the same antibiotics. Antimicrobial susceptibility profiles were determined for 105 *Salmonella* isolates.

The antibiotics used were ampicillin (10 µg), tetracycline (30 µg), tilmicosin (15 µg), tiamulin (30 µg), penicillin (10 IU), doxycycline (30 µg), tylosin (30 µg), fosfomycin (50 µg), amoxicillin (10 µg), enrofloxacin (5 µg), erythromycin (15 µg), ciprofloxacin (5 µg), spectinomycin (25 µg), chloramphenicol (30 µg), florfenicol (30 µg), trimethoprim-sulfamethoxazole (25 µg), neomycin (30 µg), ceftiofur (30 µg), and gentamicin (10 µg). All antibiotic disks used in this study were manufactured by Oxoid.

2.5. Data Analysis

For statistical analysis, the data were entered into Microsoft Excel 2010®, which was used to calculate the frequencies for each case. The maps were created using Geographic Information System software (QGIS 3.16.15), with the frequency data added to it.

3. Results

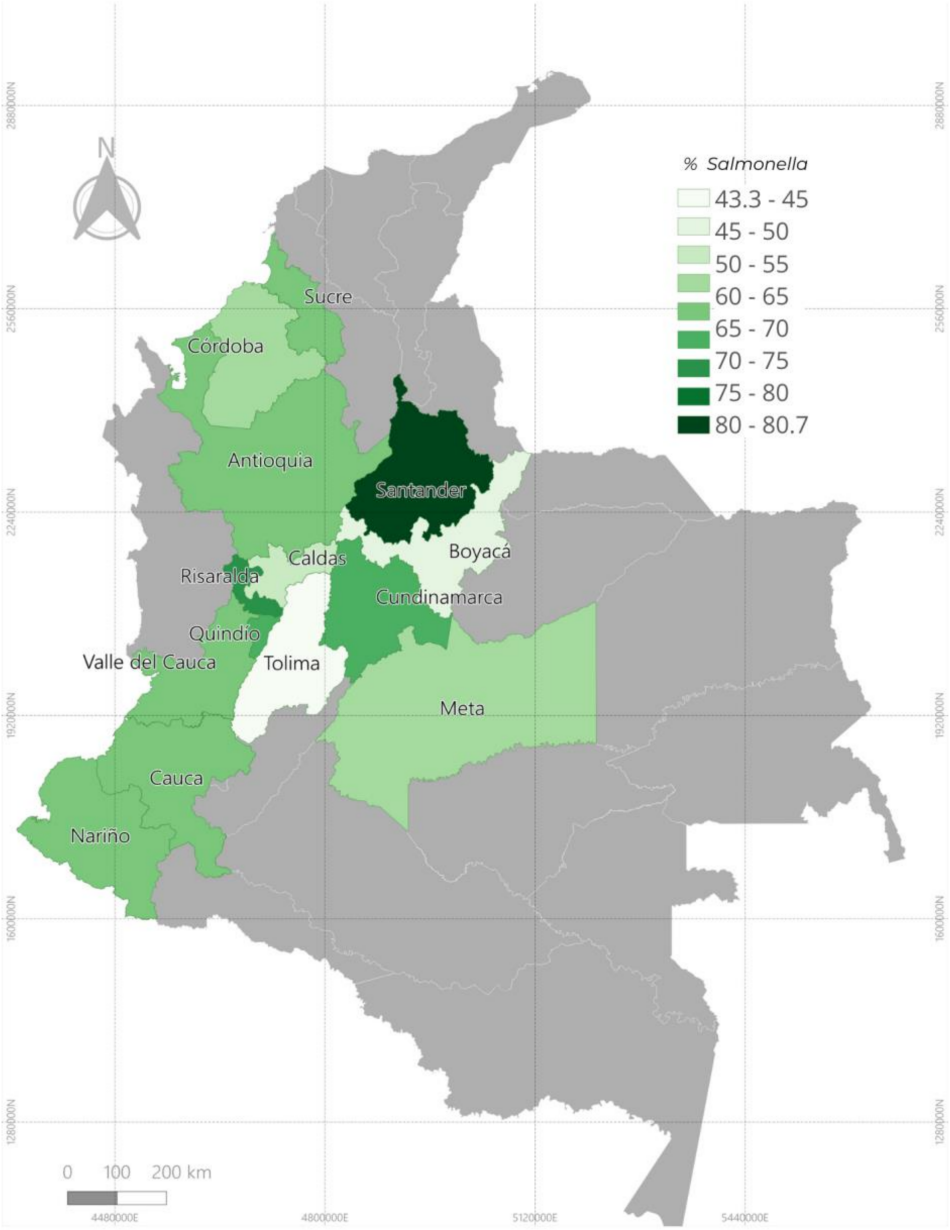
3.1. Detection of Antibodies Against Salmonella in Samples from Colombian Pigs

During the study period, analysis was conducted using the indirect ELISA technique on a total of 5820 porcine serum samples. Of these, 3711 (63.76%) tested positive for *Salmonella*. The received and analyzed samples varied in quantity across different departments (states) of Colombia. Antioquia, Valle del Cauca, and Cundinamarca stood out for the number of samples submitted for analysis, as shown in Table 1 and Figure 1.

Table 1. *Salmonella* positivity by ELISA per Colombian department (state) (2022–2023).

Department (State)	Total Samples Analyzed n (%)		*Salmonella* n (%) *	
Antioquia	3.793	(65.17%)	2.413	(63.62%)
Boyacá	71	(1.22%)	33	(46.48%)
Caldas	171	(2.94%)	89	(52.05%)
Cauca	18	(0.31%)	11	(61.11%)
Córdoba	29	(0.50%)	17	(58.62%)
Cundinamarca	537	(9.23%)	360	(67.04%)
Meta	74	(1.27%)	42	(56.76%)
Nariño	89	(1.53%)	54	(60.67%)
Quindío	100	(1.72%)	67	(67%)
Risaralda	161	(2.77%)	117	(72.67%)
Santander	119	(2.04%)	96	(80.67%)
Sucre	20	(0.34%)	13	(65%)
Tolima	30	(0.52%)	13	(43.33%)
Valle del Cauca	608	(10.45%)	386	(63.49%)
Total samples	5820		3711	(63.76%)

* (%) The proportion of samples positive for *Salmonella* by indirect ELISA per department (state).



Created with QGIS 3.16.15 software

Figure 1. *Salmonella* positivity by ELISA per Colombian department (state) (2022–2023): *Salmonella* positivity varied across Colombian departments. Santander recorded the highest positivity rate at 80.67%, followed by Risaralda at 72.67%. In contrast, Tolima and Boyacá had the lowest rates of 43.33% and 46.48%, respectively.

3.2. Isolation and Identification of Salmonella in Samples from Colombian Pigs

A total of 848 different samples (feces, tissues, etc.) were examined, out of which *Salmonella* was isolated in 199 of them, representing a positive rate of 23.47%. In this context, the departments (states) of Antioquia, Valle del Cauca, and Cundinamarca stood out for the quantity of samples submitted for analysis using this method. The departments where the agent was least isolated were Atlántico, Córdoba, and Tolima with 0 isolations, as shown in Table 2 and Figure 2.

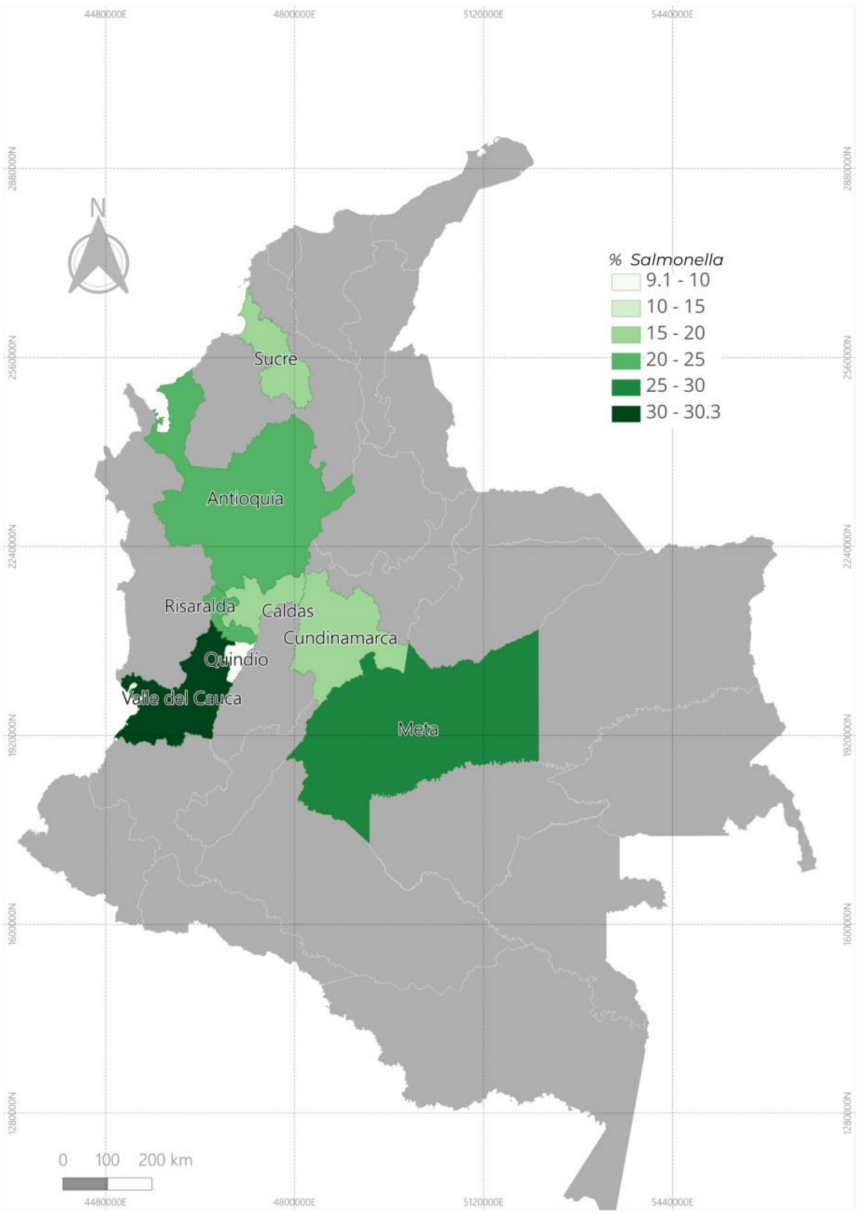


Figure 2. Percentage of *Salmonella* isolation per department (state) of Colombia (2022–2023). In the Valle del Cauca department, the agent was mostly isolated in 30.28% of cases, followed by Meta with 27.27%. The departments where the agent was least isolated were Atlántico, Córdoba, and Tolima with 0 isolations.

Table 2. Samples for diagnosis and percentage of *Salmonella* isolation per Colombian department (state) (2022–2023).

Department (State)	Total Samples Analyzed n (%)		*Salmonella* n (%) *	
Antioquia	566	(66.7%)	133	(23.5%)
Atlántico	1	(0.1%)	0	(0%)
Caldas	5	(0.6%)	1	(20%)
Córdoba	1	(0.1%)	0	(0%)
Cundinamarca	100	(11.8%)	19	(19%)
Meta	11	(1.3%)	3	(27.27%)

Table 2. *Cont.*

Department (State)	Total Samples Analyzed n (%)		*Salmonella* n (%) *	
Quindío	11	(1.3%)	1	(9.09%)
Risaralda	33	(3.9%)	8	(24.24%)
Sucre	5	(0.6%)	1	(20%)
Tolima	6	(0.7%)	0	(0%)
Valle del Cauca	109	(12.9%)	33	(30.28%)
Total samples	848		199	(23.47%)

* (%) Proportion of *Salmonella* isolation per department (state).

The most frequently submitted sample type for *Salmonella* isolation and identification was feces, but where the agent was most isolated was from tissues at 29.70%, followed by feces at 22.67%, as shown in Table 3.

Table 3. Type of samples sent and percentage of *Salmonella* isolation (2022–2023).

Type	Total Samples Analyzed n (%)		*Salmonella* n (%)	
Feces	600	(70.75%)	136	(22.67%)
Tissues	202	(23.82%)	60	(29.70%)
Multiple *	29	(3.42%)	1	(3.45%)
Unknown	17	(2%)	2	(11.76%)
Total samples	848		199	(23.47%)

* Water for animal consumption (n = 12), surface swabs (n = 1), bodily fluid (n = 4), bile (n = 2), fish meal (n = 1), and feed (n = 9).

The selection of antibiotics varied across samples, as it was determined according to the client's specific preferences, making it impossible to use the same set of antibiotics for all samples in the study. Among the antibiograms performed, resistance was observed to ampicillin (3/3), tiamulin (1/1), penicillin (2/2), tylosin (2/2), and erythromycin (1/1), followed by florfenicol (44/54), doxycycline (4/5), spectinomycin (18/25), amoxicillin (32/46), chloramphenicol (2/3), tetracycline (2/3), and enrofloxacin (34/64), with lower resistance observed for fosfomycin (2/38) and ceftiofur (5/35), as shown in Table 4.

Table 4. Antimicrobial susceptibility of *Salmonella* from pig samples received at the veterinary diagnostic unit UdeA (2022–2023).

Antibiotic	Sensitive n (%)		Intermediate n (%)		Resistant n (%)	
Ampicillin (n = 3)					3	(100%)
Tetracycline (n = 3)	1	(33.3%)			2	(66.7%)
Tilmicosin (n = 2)	1	(50%)			1	(50%)
Tiamulin (n = 1)					1	(100%)
Penicillin (n = 2)					2	(100%)
Doxycycline (n = 5)	1	(20%)			4	(80%)
Tylosin (n = 2)					2	(100%)
Fosfomycin (n = 38)	36	(94.7%)			2	(5.3%)
Amoxicillin (n = 46)	13	(28.3%)	1	(2.2%)	32	(69.6%)
Enrofloxacin (n = 64)	17	(26.6%)	13	(20.3%)	34	(53.1%)

Table 4. *Cont.*

Antibiotic	Sensitive n (%)		Intermediate n (%)		Resistant n (%)	
Erythromycin (n = 1)					1	(100%)
Ciprofloxacin (n = 68)	35	(51.5%)	10	(14.7%)	23	(33.8%)
Spectinomycin (n = 25)	6	(24%)	1	(4%)	18	(72%)
Chloramphenicol (n = 3)	1	(33.3%)			2	(66.7%)
Florfenicol (n = 54)	8	(14.8%)	2	(3.7%)	44	(81.5%)
Trimethoprim-sulfamethoxazole (n = 58)	32	(55.2%)			26	(44.8%)
Neomycin (n = 51)	16	(31.4%)	12	(23.5%)	23	(45.1%)
Ceftiofur (n = 35)	29	(82.9%)	1	(2.9%)	5	(14.3%)
Gentamicin (n = 26)	14	(53.8%)			12	(46.2%)
487 antibiotic susceptibility discs	210		40		237	

The 105 antibiograms were tested with 19 antibiotics and 487 sensidiscs. From this analysis, it was found that 210 sensidiscs (equivalent to 43.12%) showed sensitivity, while 40 sensidiscs (8.21%) manifested intermediate sensitivity and 237 sensidiscs (48.67%) demonstrated resistance. It is worth noting that the most frequently evaluated antibiotic was ciprofloxacin, followed by enrofloxacin.

Out of the 199 samples where the agent was isolated, antibiogram was performed on 105 strains of them, with 62 strains showing multi-drug resistance (MDR), meaning 59% of the samples were resistant to three or more antibiotic classes. The serotype that showed the most MDR was *S*. Typhimurium, followed by its monophasic variant.

Out of the 199 isolated *Salmonella* samples, only 61 were serotyped due to the client's specific preferences and testing priorities. The selection of samples for serotyping was based on the client's request, rather than a standardized approach across all isolates, revealing the presence of eight different serotypes of *Salmonella enterica* subspecies *enterica*. Among the serotypes found, the most predominant was Typhimurium, representing 49.2% of the serotypes; following in frequency was its monophasic variant, I 4,[5],12:i:-, at 23%, and then the Choleraesuis serotype, which accounted for 18% of the serotypes identified, as shown in Table 5.

Table 5. Most frequently identified serotypes in *Salmonella* from pig samples received at the veterinary diagnostic unit UdeA (2022–2023).

Serotype	n	(%)
Typhimurium	30	(49.2%)
I 4,[5],12:i:-	14	(23%)
Choleraesuis	11	(18%)
Derby	2	(3.3%)
Enteritidis	1	(1.6%)
Heidelberg	1	(1.6%)
Virchow	1	(1.6%)
Manhattan	1	(1.6%)
Total	61	

Among the 61 serotyped samples, a division was made based on the frequency of each serotype's occurrence in different departments (states), as shown in Table 6. In the case of Antioquia (n = 33), seven serotypes were identified, among which the most prevalent were Typhimurium found in 55% of cases in the department, followed by monophasic variant I 4,[5],12:i:- found in 27%, and Choleraesuis in 6%. The other serotypes Derby, Enteritidis, Heidelberg, and Manhattan were found in low proportions in the department, each at 3%. Regarding Valle del Cauca (n = 17), the serotypes Typhimurium were found in 47% of cases, I 4,[5],12:i:- in 12%, and Choleraesuis in 41% of cases for this department. These percentages of serotype isolation by department may be influenced by the number of samples sent to the diagnostic unit from each department.

Table 6. Most frequently identified serotypes in *Salmonella* per department (state) of Colombia (2022–2023).

Serotype	Antioquia n (%)	Valle n (%)	Cundinamarca n (%)	Meta n (%)	Risaralda n (%)	Quindío n (%)	Caldas n (%)
Typhimurium	18 (55%)	8 (47%)	2 (50%)		1 (25%)	1 (100%)	
I 4,[5],12:i:-	9 (27%)	2 (12%)	1 (25%)	1 (100%)	1 (25%)		
Choleraesuis	2 (6%)	7 (41%)	1 (25%)				1 (100%)
Derby	1 (3%)				1 (25%)		
Enteritidis	1 (3%)						
Heidelberg	1 (3%)						
Virchow					1 (25%)		
Manhattan	1 (3%)						
Total (n = 61)	33	17	4	1	4	1	1

Of the 105 strain samples subjected to antibiogram, 45 underwent serotyping (61 underwent serotyping; however, only 45 of these also had antibiograms performed). In this analysis, 19 different antibiotics were evaluated using 156 antibiotic susceptibility discs; Table 7 shows the analyzed serotypes where it is evidenced that *Salmonella enterica* subsp. *enterica* Typhimurium exhibited the highest antibiotic resistance, reaching a percentage of 33.97%. This was followed by the monophasic variant I 4,[5],12:i:- with 9.62%, and serotype Choleraesuis with 7.05%. These proportions were calculated considering the number of samples that showed resistance to antibiotics for each serotype, within the total of 156 sensidiscs used. Serotype Typhimurium is the one that most commonly exhibits multi-drug resistance (MDR), which is also associated with its higher isolation. The serotypes Typhimurium, monophasic variant I 4,[5],12:i:-, and Choleraesuis share resistance to several clinically important antibiotics, as shown Figure 3.

Table 7. Frequencies of antimicrobial sensitivities and resistances per identified serotype (2022–2023).

	Typhimurium (n = 95)			I 4,[5],12:i:- (n = 35)			Enteritidis (n = 7)			Choleraesuis (n = 13)		Manhattan (n = 6)	
Antibiotic susceptibility disc	S	I	R	S	I	R	S	I	R	S	R	S	R
Ampicillin (n = 2)						2							
Doxycycline (n = 2)						2							
Fosfomycin (n = 12)	7			3			1				1		
Amoxicillin (n = 15)	1		9			2					2	1	
Enrofloxacin (n = 22)	5		9	3	1	1					2	1	
Erythromycin (n = 1)			1										
Ciprofloxacin (n = 21)	5	2	5	4	1		1				2	1	
Spectinomycin (n = 7)	2		4								1		
Chloramphenicol (n = 2)	1		1										

Table 7. *Cont.*

	Typhimurium (n = 95)			I 4,[5],12:i:- (n = 35)			Enteritidis (n = 7)			Choleraesuis (n = 13)		Manhattan (n = 6)	
Florfenicol (n = 15)			10	1		3			1				
Trimethoprim-sulfamethoxazole (n = 18)	4		8	2					1	2		1	
Neomycin (n = 17)	3	4	3	1	1	1		1			2	1	
Ceftiofur (n = 13)	6		1	3		2	1						
Gentamicin (n = 9)	2		2			2	1				1	1	
Total (n = 156)	36	6	53	17	3	15	4	1	2	2	11	6	0

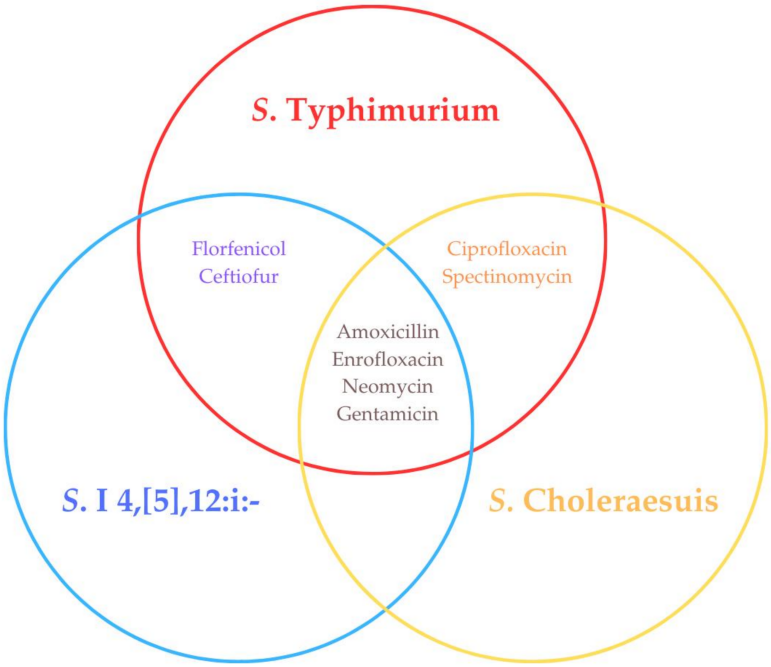


Figure 3. Most frequently identified serotypes and their antibiotic resistances 2022–2023. Typhimurium and monophasic variant I 4,[5],12:i:- exhibit resistance to florfenicol and ceftiofur. Additionally, Typhimurium 1,4,[5],12:i:1,2 and Choleraesuis share resistance to ciprofloxacin and spectinomycin. The three main serotypes also demonstrate combined resistance to amoxicillin, enrofloxacin, neomycin, and gentamicin.

4. Discussion

In the context of Colombian livestock production, the scant diagnosis and reporting of the agent results in a lack of reports addressing the dynamics of this disease in the country and its status in terms of antimicrobial resistance. This study is the only one detailing the frequency of occurrence of *Salmonella* in porcine samples in Colombia using two different laboratory techniques: ELISA and microbiological isolation and identification. It also provides information on resistance and serotypes across various departments (states) of Colombia.

This study compiled information revealing that 63.76% of the samples analyzed tested positive for *Salmonella* antibodies using indirect ELISA technique, indicating that approximately 6 out of every 10 samples tested positive. Among the departments, Santander (80.67%), Risaralda (72.67%), and Cundinamarca (67.04%) recorded the highest positivity levels, while Tolima (43.33%) and Boyacá (46.48%) exhibited the lowest levels. These results are higher than those reported by other studies, like in Tolima, where the positivity was 36.09% in 2014 [22], and, in Cundinamarca, it was 40% in 2019 [23]. In 2005, a study published by the ICA (Colombian Agricultural Institute), which included the departments of Antioquia, Atlántico, Bolívar, Boyacá, Cauca, Caldas, Cundinamarca, Huila, Meta, Quindío,

Risaralda, Santander, Tolima, and Valle, reported a positivity of 57.8% [24], which is lower than the findings in this study. It is also important to highlight that indirect serological analysis has its limitations, such as the inability to identify antibodies generated by the disease and the potential for cross-reactivity with antibodies due to immunization [22].

For the isolation of *Salmonella* through traditional microbiology, a proportion of 23.47% of samples were obtained. This indicates that approximately 2 out of every 10 samples were positive for the agent. It is important to highlight that this prevalence is in line with previous reports of *Salmonella* prevalence in pigs between 2020 and 2021 for Antioquia, Colombia, which showed a prevalence of 22.8% [10]. In the Valle del Cauca department, the agent was mostly isolated in 30.28% of cases, followed by Meta with 27.27%. The departments where the agent was least isolated were Atlántico, Córdoba, and Tolima with 0 isolations, noting that they are among the departments that sent the fewest samples for analysis.

Antioquia had the highest number of samples received and evaluated in this study. This is partly owing to the existence of the veterinary diagnostic unit in the department's capital, which makes it easier to send and process samples. This is followed by the Valle del Cauca and Cundinamarca departments, which have a large concentration of pig population; according to the ICA, in 2023, 64.3% of Colombia's pig population was concentrated in six departments: Antioquia, Valle del Cauca, Cundinamarca, Meta, Córdoba, and Sucre [25]. The high concentration of pig production in these areas may explain the abundant submissions of samples for analysis, given the importance of maintaining pig health in those regions.

The most frequent sample type for *Salmonella* detection was feces, accounting for 70.75% of cases, which was expected given the agent's tropism for intestinal epithelial cells [26] and, hence, its greater preference for collection by professionals, in addition to the ease of obtaining them compared to tissue samples. For this study, a higher percentage of *Salmonella* isolation was observed from tissues at 29.70%, followed by feces at 22.67%. This may be due to the high percentage of agent isolation in systemic infections in the evaluated animals, which may require specialized attention and further investigation to determine the source of infection and prevent its spread [9].

When it comes to the use of antibiotics in the treatment of salmonellosis in pigs, it is a common practice to control the disease [8]. However, the effectiveness of these treatments can be compromised by bacterial resistance, as demonstrated by the results obtained in this study. According to the data, a significant percentage of samples showed resistance to the tested antibiotics. The multi-drug-resistant (MDR) *Salmonella* strains, as presented in this study, account for 59% of the isolates, which is concerning as it may lead to more difficult treatment and, thus, an increase in mortality rates from infections caused by this agent. A limitation of this study was that not all *Salmonella* isolates were tested with the same entire panel of antimicrobials as this study evaluated isolates from multiple sources. Epidemiological studies indicate that MDR *Salmonella* strains are more virulent than susceptible strains, as reflected by greater severity and prolonged illness in patients infected by MDR strains [26]. In Colombia, other studies have reported a multi-drug resistance (MDR) rate of 44% among isolates between the years 2019 and 2021 [10].

The isolated samples that presented resistance to certain antibiotics, such as ampicillin, tiamulin, penicillin, tylosin, and erythromycin, showed 100% resistance (these antibiotics were the least tested). This suggests a lack of effectiveness of these drugs in treating salmonellosis in pigs and raises serious concerns about the selection of resistant strains in the swine environment. Additionally, other important antibiotics for the treatment of this disease, such as florfenicol (44/54), doxycycline (4/5) spectinomycin (18/25), amoxicillin (32/46), chloramphenicol (2/3), and tetracycline (2/3), also showed high levels of resistance, further limiting the available therapeutic options. However, caution must be exercised

when interpreting these results, as some antibiotics were tested on a very limited number of samples due to client preferences. Since the data were sourced from a public diagnostic service institution, the selection of antibiotics tested was not standardized but, rather, determined by the specific needs of each client. Ciprofloxacin and enrofloxacin were the most frequently evaluated antibiotics in this study due to their common use by professionals in Colombia. However, significant resistance to these drugs was also observed, with 53.1% (34/64) resistance in the case of enrofloxacin and 33.8% (23/68) for ciprofloxacin. This resistance to fluoroquinolones is particularly concerning, as these antibiotics are considered critically important for human health by the World Health Organization (WHO), highlighting the potential impact on public health [11].

The isolated samples showed resistance to very few antibiotics below 30%, such as fosfomycin (5.3%) and ceftiofur (14.3%), which were the only two that fell below this threshold. Surprisingly, these two antibiotics constitute only 10% of the list of the 19 antibiotics tested. This suggests that a significant number of antibiotics used in the treatment of salmonellosis in pigs are facing high levels of bacterial resistance, posing significant challenges for the effective treatment of the disease.

As for the correlation between departments and the isolated *Salmonella* serotypes, it is not possible to analyze this due to the variability of samples from each location. More data on the *Salmonella* serotypes isolated in each location are needed to obtain a more comprehensive understanding of the behavior of each serotype in the different departments of Colombia. Currently, the serotyping of this agent is not a common practice among clients, which limits the ability to conduct detailed analyses on the distribution of serotypes and their prevalence in different regions of the country. It is important to encourage greater participation in serotyping to enrich our understanding of the epidemiology of *Salmonella* in Colombia.

The most frequently isolated serotype was *Salmonella enterica* subsp. *enterica* serotype Typhimurium, accounting for 49.2% of isolates, followed by its monophasic variant I 4,[5],12:i:- at 23% and serotype Choleraesuis at 18% isolation. This aligns with the report on animal isolates from USDA National Veterinary Services Laboratories in 2016, which indicated that the main clinical isolates in pigs were monophasic variant I 4,[5],12:i:-, followed by Typhimurium [4]. This is consistent with previous studies in Antioquia, Colombia, where a prevalence of Typhimurium (56%) and its monophasic variant (35%) was found [10].

The serotype Typhimurium (n = 53) exhibited the highest antibiotic resistance, reaching a percentage of 33.97%. This was followed by the monophasic variant Typhimurium I 4,[5],12:i:- (n = 15) with 9.62% resistance. These proportions were calculated considering the number of samples that showed resistance to antibiotics for each serotype, within the total of 156 tests conducted. The resistance behavior of these serotypes is reinforced by previous studies in Colombia [10]. These serotypes have been associated with pork products and the transmission of antimicrobial resistance to humans [8].

It is important to understand the dynamics of *Salmonella* and its resistance in the national context, as well as the urgent need for management strategies and the prudent use of antibiotics in pig production to address this steadily increasing problem. Furthermore, the importance of collecting more data on isolated serotypes and their association with antimicrobial resistance is emphasized to enhance our understanding of *Salmonella* epidemiology in the country. The relevance of these findings for public health is highlighted, along with the need for effective preventive and therapeutic actions to combat this threat to both animal and human health.

Given the high pathogenicity of *Salmonella* and the significant antimicrobial resistance observed in this study, it is crucial to explore new strategies for combating antimicrobial

resistance in pathogenic bacteria. The implementation of innovative therapeutic strategies [27,28] could contribute to mitigating the spread of resistant *Salmonella* strains and improving treatment options in both veterinary and human medicine.

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