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Multidrug-resistant *Salmonella* spp. in fecal samples of pigs with suspected salmonellosis in Antioquia, Colombia, 2019–2021

Juana L. Vidal[1], Viviana Clavijo[2], Luis R. Castellanos[3], Jeyashree Kathiresan[4], Ajay M.V. Kumar[5], Kedar Mehta[6], and Jenny J. Chaparro-Gutiérrez[1]

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ABSTRACT

Objectives. To determine the proportion of *Salmonella enterica* in fecal samples of live pigs with suspected salmonellosis analyzed at the diagnostic unit of the University of Antioquia, Colombia between 2019 and 2021, and examine the serotypes and antimicrobial resistance patterns.

Methods. This was a laboratory-based cross-sectional study of routine data on fecal samples received from pig farms in all nine subregions of Antioquia state, Colombia. *Salmonella* spp. detection at the university is done using enrichment, selective culture, and polymerase chain reaction. Serotypes were identified using the Kauffmann–White scheme and isolates were tested for antimicrobial susceptibility using broth microdilution.

Results. Of 653 samples tested, 149 (23%) were positive for *S. enterica.* Nine serotypes were identified. The most common were *Salmonella* Typhimurium (56%) and its monophasic variant (35%). Resistance to ampicillin (70%) was most frequently observed, followed by ciprofloxacin (55%), and sulfamethoxazole–trimethoprim (52%). No isolates were resistant to amikacin and gentamicin. Multidrug resistance (resistance to ≥ 3 classes of antibiotics) was observed in 61 (44%) isolates. Multidrug resistance was highest in *S.* Typhimurium (57%) compared with the other serotypes. Serotype was associated with multidrug resistance ($p = 0.01$), but age of the pig and sub-region were not.

Conclusions. The proportion of *Salmonella* spp. and the associated high levels of multidrug resistance are of concern and may indicate irrational use of antimicrobials and poor management practices in pig production systems in the region. Strengthened surveillance is needed to monitor and improve farm management practices and the use of antimicrobials in farms in Colombia.

Keywords *Salmonella enterica*; serogroup; drug resistance, multiple; swine; Colombia.

Salmonellosis is a gastrointestinal infection caused by *Salmonella enterica*, a gram-negative bacterium. In humans, this infection is generally contracted through the consumption of contaminated food of animal origin (primarily eggs, pork meat, and chicken meat), although other foods, such as manure-contaminated vegetables and fruits, have also been linked to transmission (1, 2). In the European Union, pork is one of the main sources of human salmonellosis after eggs and egg

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[1] Faculty of Agrarian Sciences, University of Antioquia, Medellin, Colombia. ✉ Juana L. Vidal, juana.vidal@udea.edu.co

[2] Ciencia y tecnologia de Fagos – Sciphage, Bogota, Colombia.

[3] Quadram Institute Bioscience, Norwich, United Kingdom of Great Britain and Northern Ireland.

[4] Indian Council of Medical Research, National Institute of Epidemiology, Chennai, India.

[5] International Union Against Tuberculosis and Lung Disease, Paris, France.

[6] GMERS Medical College, Gotri, Vadodara, Gujarat, India.

products (1, 2). *S. enterica* also causes disease in pigs in a variety of clinical forms. Thus, salmonella infection has substantial economic, public health, and animal health consequences (3). Transmission can occur through pig-to-pig contact, because of infected animals arriving from other farms, or feed contamination from the feces of other carrier animals (4).

S. enterica is widely distributed in domestic and wild animals and over 2 600 serotypes have been identified (5). The *S. enterica* serotypes that mainly cause clinical disease in pigs are *S.* Choleraesuis and *S.* Typhimurium. *S.* Typhimurium is the most frequent serotype detected in pigs. It is sometimes associated with diarrhea in young pigs and is also a common source of food poisoning in humans (6, 7).

Salmonellosis in pigs is treated using antimicrobials including amoxicillin–clavulanic acid, ampicillin, ceftiofur, ciprofloxacin, chloramphenicol, florfenicol, gentamicin, sulfamethoxazole–trimethoprim, tetracycline, and macrolide antibiotics such as tilmicosin – many of these antimicrobials are important for human therapy (8). Antimicrobials are used in animals not only for treatment, but also for controlling the spread of infection (metaphylaxis), preventing infection (prophylaxis), improving feed efficiency, and promoting growth (9).

Although strict regulations exist about the use of antimicrobials in many countries, the enforcement is often weak which has resulted in indiscriminate use of antimicrobials. This overuse has contributed to the emergence of multidrug-resistant (MDR) strains of bacteria (10–12). Antimicrobial-resistant *Salmonella* strains have been found in chicken, pig, and other foods all around the world (13). When humans are infected with resistant *Salmonell*a strains, treatment can be difficult, increasing the likelihood of treatment failure, and even death (14, 15). Hence, it is vital to understand the antibiotic resistance patterns of *Salmonella* spp. infections in pigs.

Limited evidence exists about the prevalence and antibiotic resistance patterns of *Salmonella* spp. in pigs in Colombia (4, 5,16). Previous studies have provided baseline data on antimicrobial resistance patterns of *Salmonella* spp. in pigs in the country (5, 7, 8). However, data from fecal samples in live animals with suspected salmonellosis are limited (4). Furthermore, no studies have investigated the factors associated with MDR. In addition, with the exception of one study in four regions of Colombia (17), most of the previously published studies (8, 10–12) were conducted in specific municipalities, with small sample sizes and limited generalizability (18). Therefore, the aim of the present study was to: (i) determine the proportion of *S. enterica* in fecal samples of pigs with suspected salmonellosis which were analyzed at the veterinary diagnostic laboratory of the University of Antioquia, Colombia, between 2019 and 2021; and (ii) describe the serotypes and antibiotic resistance patterns.

METHODS

Study design, sample and period

This was a laboratory-based cross-sectional study based on the analysis of routine data from fecal samples of pigs with suspected salmonellosis which were received at the diagnostic unit of the Faculty of Agrarian Sciences at the University of Antioquia, Colombia from January 1, 2019 to June 30, 2021. Pigs with gastrointestinal symptoms such as diarrhea and other asymptomatic pigs in contact with them were considered animals with suspected salmonellosis.

Setting

Antioquia is one of the 32 departments (states) in Colombia with a population of 5.8 million; it is divided into nine sub-regions. Antioquia accounts for about one third of the pig population in the country (19, 20). This study was conducted in the diagnostic unit of the University of Antioquia which regularly receives fecal samples from all the sub-regions of the state.

Sample collection

Fecal samples of pigs with suspected salmonellosis are brought to the laboratory by the clients (farmers, veterinarians, or relevant associations). These samples could be from an individual animal or a pool of animals. Upon arrival at the laboratory, clients fill out a request form. Samples that have been adequately maintained with cold chain requirements (2–8 °C.) and are of a sufficient quantity (minimum of 25 g of feces) are investigated further following the sample acceptance standards of ISO/IEC 17025, 2017 (21).

Isolation, identification and serotyping

The basic laboratory procedure for the diagnosis of *Salmonella* spp. has five steps: pre-enrichment; selective enrichment; isolation in selective media; differential biochemical tests; and determination of the serotype (22). First, 10–25 g of the sample are transferred to 225 mL of buffered peptone water, shaken for 2 minutes, and incubated at 35 °C for 18–24 hours. For selective enrichment, the previously incubated sample together with 0.1 mL of the buffered peptone water are inoculated into 10 mL of Rappaport-Vassiliadis broth and incubated at 42 °C for 18–24 hours. After this incubation, the broth cultures are plated on Hektoen agar and xylose lysine deoxycholate agar and then incubated at 35 °C for 18–24 hours. Blue or blue-green colonies (with or without a black center) on Hektoen agar and clear to pinkish-red colonies (with or without a black center) on xylose lysine deoxycholate agar are considered presumptive of *Salmonella* spp. Colonies with typical *Salmonella* spp. characteristics on triple sugar agar and lysine iron agar are put through polymerase chain reaction (PCR) amplification of the *invA* gene (23) and further biochemical characterization is done using the urease, indole, citrate, and motility tests (24). Serotypes are identified using the Kauffmann–White scheme for classification of somatic (O) and flagellar (H) antigen type (22).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MIC) of *Salmonella* isolates are evaluated using a Phoenix automated microbiological system (BD) (25) using the NMIC/ID-406 panel for gram-negative bacteria. This panel includes the following 16 agents: amikacin, ampicillin, ampicillin–sulbactam, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin–tazobactam, sulfamethoxazole–trimethoprim, and tigecycline. The MIC results, both of the antibiotics used for the antibiogram and those used for confirmation of extended-spectrum beta-lactamases (ESBL), are interpreted by the Phoenix expert system.

The MIC for the *Salmonella* isolates tested were interpreted as resistant, intermediate, or susceptible based on the guidelines of Clinical and Laboratory Standards Institute (26). *Escherichia coli* ATCC 25922 and *S.* Enteritidis ATCC 13076 were used as quality control bacteria for each assay. Strains with MIC breakpoint results for ciprofloxacin that included ≤ 0.125 µg/mL were tested by 5 µg ciprofloxacin diffusion disk to confirm susceptibility results. This is needed because the ciprofloxacin breakpoint specific for *Salmonella* spp. in the guidelines of the Clinical and Laboratory Standards Institute is sensitive at ≤ 0.06 µg/mL and resistant at ≥ 1 µg/mL (26), and the BD Phoenix™ NMIC/ID 406 panel has a ciprofloxacin dilution range of 0.125–2 µg/mL (25). Because of this, results ≤ 0.125 µg/mL include values from sensitive (≤ 0.06 µg/mL) to intermediate (0.125 µg/mL).

The categorization of the antimicrobials followed the World Health Organization's (WHO's) list of critically important antimicrobials for human medicine (27). Antimicrobials were classified as critically important in human medicine, highly important, and important.

Data management and statistical analysis

The variables included in the analysis were: age of the pigs; sub-region; serotype; and antibiotic susceptibility results (susceptible, intermediate, and resistant) for selected antibiotics. It was not possible to record if samples came from an individual animal or a pool of animals because this information was not provided by the clients. Data were extracted from the electronic laboratory database at the Faculty of Agrarian Sciences of the University of Antioquia and were analyzed using EpiData analysis software, version 2.2.2.187 (EpiData Association, Odense, Denmark). The proportion of *S. enterica* in the samples was calculated by dividing the number of samples positive for *S. enterica* by the total number of fecal samples processed. For the *Salmonella*-positive isolates, the number and proportion resistant to individual antimicrobials were calculated. Isolates with resistance to three or more classes of antibiotics were classified as MDR, as defined by a joint group of the European Centre for Disease Prevention and Control and the United States Centers for Disease Control and Prevention (28). Associations between variables (such as age, sub-region, and serotype) and MDR were assessed using the chi-squared test. Prevalence ratios and 95% confidence intervals were calculated. A p-value of < 0.05 was considered statistically significant.

Ethics

Permission to access the laboratory data was obtained from the head of the Diagnostic Unit, University of Antioquia, respecting confidentiality policies of owner and farm data. Ethics approvals were obtained from the Institutional Committee on the Care and Use of Experimental Animals at the University of Antioquia (UdeA. 141/03-08-2021), the Ethics Advisory Group at the International Union against Tuberculosis and Lung Disease, Paris, France (EAG number: 23-21/02-09-2021), and the ethics committee of the Pan American Health Organization (PAHOERC.0383.01/ 05-08-2021). We obtained informed consent from the caretakers of the animals who submitted the specimens for their use for research purposes.

RESULTS

Proportion of *S. enterica*

In all, 653 samples were tested during the study period, and of these 149 (22.8%) were positive for *S. enterica* (Figure 1). The proportion of *Salmonella* isolates varied from 0.0% (Occidente) to 40.7% (Oriente) (Table 1).

Serotyping

We identified nine *S. enterica* serotypes: Typhimurium (83 samples), Typhimurium monophasic variant (S.1,4,12:i:–) (52), Enteritidis (7), Virchow (2), Bovismorbificans (1), Edinburg (1), Heidelberg (1), Infantis (1), and Manhattan (1). The most common serotype was *S.* Typhimurium (56%), followed by its monophasic variant (35%) (Figure 1).

Antimicrobial resistance of *S. enterica* serotypes

Of the 149 *Salmonella* isolates, 139 (93.3%) were tested for antimicrobial susceptibility (Figure 1): for the other 10 samples, it was not possible to obtain new growth of the strains from the first culture for antimicrobial susceptibility testing. Resistance to ampicillin (70% of isolates) was most frequently observed, followed by ciprofloxacin (55%), sulfamethoxazole–trimethoprim (52%), cefazolin (39%), ampicillin–sulbactam (35%), ceftazidime (29%), and cefepime and ceftriaxone (28% each). Resistance to piperacillin–tazobactam was also detected in 9% of isolates (Table 2).

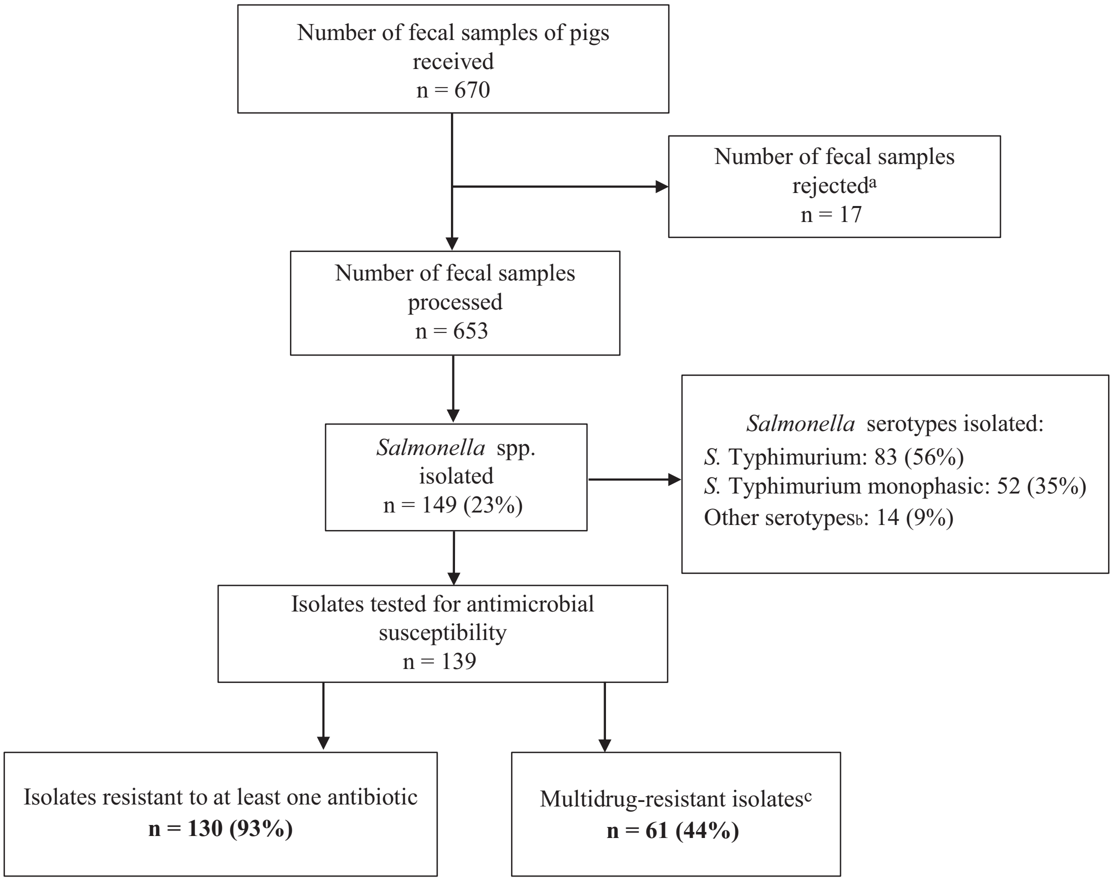
A higher proportion of *S.* Typhimurium and *S.* Typhimurium monophasic variant showed resistance to antibiotics of all classes than the other serotypes (Table 3). Strains with MIC break-point results for ciprofloxacin that included ≤ 0.125 µg/mL were tested by 5 µg ciprofloxacin diffusion disk to confirm sensitivity. Of the 17 strains evaluated, five were confirmed as sensitive with a zone diameter ≥ 31 mm. Of the 139 isolates, 40 (29%) were positive for the production of ESBLs. Additionally,

TABLE 1. *Salmonella* spp. isolated from fecal samples of pigs with suspected salmonellosis in Antioquia, Colombia, by sub-region, January 1, 2019 to June 30, 2021

Sub-region	Total sample	*Salmonella* spp.
	n (%)[a]	n (%)[b]
Norte	363 (55.6)	77 (21.2)
Valle de Aburrá	92 (14.1)	19 (20.7)
Suroeste	83 (12.7)	23 (27.7)
Oriente	54 (8.3)	22 (40.7)
Nordeste	53 (8.1)	7 (13.2)
Occidente	6 (0.9)	0 (0.0)
Urabá	1 (0.2)	1 (100)
Magdalena Medio	1 (0.2)	0 (0.0)
Bajo Cauca	0 (0.0)	0 (0.0)

[a] Column percentage, $n = 653$.
[b] Row percentage.
Source: Prepared by authors from study results.

FIGURE 1. Samples tested, samples positive for *Salmonella enterica*, serotypes identified, and antimicrobial resistance patterns of fecal samples of pigs with suspected salmonellosis received at the University of Antioquia, Colombia from January 1, 2019 to June 30, 2021



[a] The samples rejected did not meet the criteria for further investigation, such as storage temperature (2–8 °C) and quantity (minimum of 25 g of feces), established by the laboratory under the ISO/IEC 17025, 2017 standard (21).
[b] Other serotypes were: S. Enteritidis, S. Bovismorbificans, S. Edinburg, S. Heidelberg, S. Infantis, S. Manhattan, and S. Virchow.
[c] Isolates with resistance to three or more classes of antimicrobials were classified as multidrug resistant.
Source: Prepared by authors from study results.

in one of the isolates, the presence of a positive ESBL test with resistance to meropenem, imipenem, and ertapenem was observed.

No isolates were resistant to amikacin and gentamicin, and only small proportions were resistant to cefoxitin, ertapenem, imipenem, meropenem, and tigecycline (Table 2).

Factors associated with MDR

Overall, MDR was observed in 61 (44%) isolates. The only variable associated with MDR was the serotype (p = 0.01). The serotype with the highest levels of MDR was *S.* Typhimurium (57%) (Table 4). The most common resistance profile in *S.* Typhimurium (20, 45%) was ampicillin–ampicillin/sulbactam–ceftriaxone–ciprofloxacin–sulfamethoxazole/trimethoprim. Age of the pig and sub-region were not associated with MDR.

DISCUSSION

This is the first study from Colombia to assess the proportion and resistance patterns in *S. enterica* isolated from fecal samples of live pigs with suspected salmonellosis. There were three key findings. First, about one in four samples was positive for *S. enterica*. with variations in proportion across the sub-regions of Antioquia Second, the most common serotypes were *S.* Typhimurium and its monophasic variant. Third, about 44% of the isolates showed MDR, with *S.* Typhimurium exhibiting significantly higher levels of MDR than the other serotypes.

The prevalence of *S. enterica* in our study (23%) was higher than that of a previous study in Colombia which reported 8% prevalence in fecal samples. The difference in the results can be explained by the fact that the previous study used fecal samples of healthy pigs (11). Other studies in Colombia have reported a prevalence of *Salmonella* spp. of 3% in pig carcasses, 13% in environmental samples from slaughterhouses, and 28% in samples of pig mesenteric ganglia (8).

Our estimates are similar to those of the European Food Safety Authority in 2008 for Greece, Luxemburg, Portugal, and Spain (29), which reported the prevalence to be between 25% and 30%. A lower prevalence was found in other countries such as Austria, Estonia, Finland, Norway, Poland, and Slovakia where the prevalence was about 5%. These variations might be related to stringent biosecurity standards and better farming practices

TABLE 2. Antimicrobial susceptibility of *Salmonella* spp. serotypes isolated from pigs with suspected salmonellosis in Antioquia, Colombia, by antibiotic, January 1, 2019 to June 30, 2021

Antibiotic	*Salmonella* spp.[a]		
	Susceptible	Intermediate	Resistant
	n (%)	n (%)	n (%)
Critically important[b]			
Amikacin (*n* = 139)	139 (100)	0 (0)	0 (0)
Ertapenem (*n* = 139)	137 (99)	1 (1)	1 (1)
Imipenem (*n* = 137)	135 (98)	1 (1)	1 (1)
Meropenem (*n* = 137)	135 (98)	0 (0)	2 (1)
Gentamicin (*n* = 139)	113 (81)	26 (19)	0 (0)
Piperacillin–tazobactam (*n* = 139)	111 (80)	14 (10)	12 (9)
Ceftriaxone (*n* = 139)	100 (72)	0 (0)	39 (28)
Ceftazidime (*n* = 139)	99 (71)	0 (0)	40 (29)
Cefepime (*n* = 135)	93 (69)	3 (2)	39 (29)
Tigecycline (*n* = 137)	78 (57)	58 (42)	1 (1)
Ampicillin–sulbactam (*n* = 139)	43 (31)	47 (34)	49 (35)
Ampicillin (*n* = 139)	38 (27)	4 (3)	97 (70)
Ciprofloxacin (*n* = 139)	5 (4)	58 (42)	76 (55)
Highly important[b]			
Cefoxitin (*n* = 139)	137 (99)	1 (1)	1 (1)
Sulfamethoxazole–trimethoprim (*n* = 137)	65 (47)	0 (0)	72 (52)
Cefazolin (*n* = 139)	41 (29)	44 (32)	54 (39)

[a] *Salmonella* serotypes: *S.* Typhimurium, *S.* Typhimurium monophasic, *S.* Enteritidis, *S.* Bovismorbificans, *S.* Edinburg, *S.* Heidelberg, *S.* Infantis, *S.* Manhattan, and *S.* Virchow.
[b] As defined by the World Health Organization (27).
Source: Prepared by authors from study results.

TABLE 3. Antimicrobial resistance of *Salmonella* spp. isolated from fecal samples of pigs with suspected salmonellosis in Antioquia, Colombia, by antibiotic, January 1, 2019 to June 30, 2021

Antibiotics to which resistance was documented	*S.* Typhimurium (*n* = 83)	*S.* Typhimurium monophasic (*n* = 52)	Other *Salmonella* serotypes[a] (*n* = 14)
	n (%)	n (%)	n (%)
Critically important[b]			
Ampicillin	56 (67)	36 (69)	4 (29)
Ciprofloxacin	41 (49)	30 (58)	5 (36)
Ampicillin–sulbactam	39 (47)	10 (19)	0 (0)
Ceftazidime	29 (35)	11 (21)	0 (0)
Ceftriaxone	28 (34)	11 (21)	0 (0)
Cefepime	28 (34)	11 (21)	0 (0)
Piperacillin–tazobactam	11 (13)	1 (2)	0 (0)
Meropenem	2 (2)	0 (0)	0 (0)
Ertapenem	1 (1)	0 (0)	0 (0)
Imipenem	1 (1)	0 (0)	0 (0)
Tigecycline	0 (0)	1 (2)	0 (0)
Gentamicin	0 (0)	0 (0)	0 (0)
Amikacin	0 (0)	0 (0)	0 (0)
Highly important[b]			
Sulfamethoxazole–trimethoprim	55 (66)	16 (31)	1 (7)
Cefazolin	38 (46)	14 (27)	2 (14)
Cefoxitin	1 (1)	0 (0)	0 (0)

[a] Other *Salmonella* serotypes: *S.* Enteritidis, *S.* Bovismorbificans, *S.* Edinburg, *S.* Heidelberg, *S.* Infantis, *S.* Manhattan, and *S.* Virchow.
[b] As defined by the World Health Organization (27).
Source: Prepared by authors from study results.

TABLE 4. Factors associated with multidrug resistance in *Salmonella* spp. isolated from fecal samples of pigs with suspected salmonellosis received at the University of Antioquia, Colombia from January 1, 2019 to June 30, 2021

Factors	Total	Multidrug resistant, n (%)	Prevalence ratio (95% CI)	*p*-value[a]
Total	139	61 (43.9)	NA	NA
Age of pig, in days				
≤ 100	64	30 (46.9)	1.17 (0.39–3.54)	0.76
101–200	51	20 (39.2)	0.98 (0.32–3.02)	0.97
>200	5	2 (40)	Ref	
Not recorded	19	9 (NA)	NA	
Serotype				
S. Typhimurium	77	44 (57.1)	1.68 (1.09–2.59)	0.01
S. Typhimurium monophasic	50	17 (34)	Ref	
Other	12	0 (0)	NA	
Sub-region				
Nordeste	6	3 (50)	1.67 (0.58–4.73)	0.33
Norte	75	37 (49.3)	1.64 (0.81–3.33)	0.16
Oriente	20	8 (40)	1.33 (0.56–3.14)	0.51
Suroeste	20	6 (30)	Ref	
Valle de Aburra	18	7 (38.9)	1.29 (0.53–3.14)	0.56

CI, confidence interval; Ref, reference category; NA, not applicable.
[a] Chi-squared test.
Source: Prepared by authors from study results.

in these countries (29). Of note, differences in the results of the prevalence of *Salmonella* spp. could be due to other factors such as the site of sample collection (farms versus slaughterhouses) and the type of samples (fecal versus non-fecal) (30).

The sub-region with the highest prevalence of *Salmonella* spp. in Antioquia was Oriente (40%). This finding might be related to suboptimal farming practices and environmental problems with the management of solid waste reported in this region (12, 31). The variation in the prevalence across sub-regions could be attributed to the production system and management practices of farms. Sub-regions with lower proportions of *Salmonella* spp., such as Nordeste (13%), Valle de Aburra (21%), and Norte (21%), have intensive pig production systems and better implementation of good farming practices, such as farm sanitation, hygienic feeding practices, herd management, and health management (20, 31).

S. Typhimurium and its monophasic variant were the predominant serotypes and accounted for about 90% of all isolates. This finding is similar to the findings in other countries of the European Union, Brazil, China, Japan, and the United States (4, 32, 33). Although studies from other parts of the world also report Derby as another common serotype (34), we did not find this serotype in our study, nor did we detect *S.* Choleraesuis.

Our research showed that *Salmonella* spp. exhibited high levels of resistance to the most commonly prescribed antibiotics for the treatment of swine salmonellosis, such as ampicillin (70%), ciprofloxacin (55%), and sulfamethoxazole–trimethoprim (52%) (8). Our values were higher than those of a recent Colombian study (17), but similar those reported in China (33). Chloramphenicol, ciprofloxacin, sulfamethoxazole–trimethoprim, and ceftriaxone are the first choices for treating salmonellosis in humans and are designated by WHO as critically and highly important for human medicine (27). Brazil, Korea, and Spain have observed similar resistance patterns with percentages of 80%, 94%, and 70%, respectively (32, 34). Of concern in our study was the high prevalence of MDR (44%) – much higher than a previous study in Colombia which reported MDR of 12% (11). MDR was significantly higher in *S.* Typhimurium than the other serotypes, similar to reports from Brazil and China (32, 33). These trends could be a consequence of unregulated antimicrobial use in pig production (11), but further investigation on the levels of antimicrobial use in pig farming is needed to evaluate this hypothesis. *Salmonella* spp. with MDR patterns is a risk to public health as these serotypes can be transmitted to people by different routes such as: indirect transmission through waste management; direct animal–human transmission; or indirect human–food transmission (35). Such MDR patterns thus complicate therapeutic management of *Salmonella* spp. infection in humans.

The finding that one of the isolates positive for ESBL was resistant to meropenem, imipenem, and ertapenem could mean the presence of an ESBL with porin closure or a carbapenemase. This finding requires further investigation.

A strength of our study was that we used fecal samples from pigs with suspected salmonellosis, rather than healthy pigs as in previous Colombian studies, which adds to the limited evidence on this topic. In addition, all tests were performed in an ISO 17025 and quality-assured laboratory, and we followed the guidelines of the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing and interpretation. As such, we aimed to ensure that all our laboratory results were valid and reliable. Another strength was that we had a larger sample than previous studies in Colombia (8, 10, 11) and we included samples from all nine Antioquia sub-regions. As a result, we believe the findings are representative of *Salmonella* spp. infection in pigs in Antioquia.

Our study also had some limitations. The laboratory database did not provide information on several important variables including animal symptoms (whether symptomatic or asymptomatic contacts), type of sample (individual or pooled sample), farm management procedures, and antibiotics used. These variables would have allowed for a more comprehensive and in-depth analysis, and might have provided insights into the reasons for the high prevalence of *Salmonella* spp. and MDR in Antioquia. In addition, we did not assess tetracycline resistance. Tetracycline resistance has been reported to be high in *Salmonella* spp. isolated from pigs and its absence from our analysis may have underestimated overall MDR levels (33).

Despite these limitations, our findings have some important implications. First, the recording system in the laboratory of the University of Antioquia needs to be strengthened to capture all the key variables discussed before. Second, barring evidence from studies such as ours, no surveillance system is in place for *Salmonella* spp. in pigs in Colombia. Given the importance of the growing pig industry, the high consumption of pork by the people of Colombia, and the potential public health implications, we recommend that a surveillance system be instituted with sentinel sites (at farms and slaughterhouses) established across the country. Such a measure will help to capture nationally representative data and monitor the trends in prevalence and resistance levels of *Salmonella* spp. Until such a surveillance system becomes a reality, we need more nationally representative studies to monitor the prevalence of *Salmonella* spp., MDR levels and the practices in the pig production chain (farms, slaughterhouses, storage places, distribution points, and retail outlets) in Colombia. Although fragmented, such information would help to identify the sources of *Salmonella* spp. at each production stage. It would also help to guide interventions to reduce the prevalence of salmonellosis in pigs and the risk to human consumers. Finally, current methods to detect resistance are phenotypic. For the detection of resistance genes, we recommend using molecular tools such as whole genome sequencing and PCR. This information will aid our understanding of the mechanisms underlying antimicrobial resistance in MDR isolates.

The high levels of MDR could be a consequence of indiscriminate use of antibiotics in pig farms. This possibility calls for strategies to understand and regulate antimicrobial use in animal production in Colombia. These measures could include: (i) education of pig farmers and other workers involved in the pig production chain about good husbandry practices, sanitation, disinfection, and the rational use of antibiotics; and (ii) education of veterinarians to be prudent in prescribing antibiotics. According to the guidelines of WHO's global action plan on antimicrobial resistance, a One Health approach with a coordinated response of all sectors (human, animal, and environment) is required (35).

In conclusion, we found a high proportion of *Salmonella* spp. and high levels of MDR in fecal samples of live pigs with suspected salmonellosis in Antioquia, Colombia. These findings deserve urgent attention and action from both the animal health and public health sectors. Our recommendation to develop a national surveillance system to monitor the prevalence of *Salmonella* spp. and antimicrobial resistance levels would enable identification of the sources of *Salmonella* spp. at the different stages of pig production and provide evidence to improve the regulation of antimicrobial use in the animal sector.

Author contributions. JLV, JJC, AMVK, VC, LRC, and JK conceived and designed the study. JLV and KM collected, analyzed or interpreted the data. AMVK, VC, LRC, JK, and JJC drafted or critically revised the report for important intellectual content. All authors approved the final version of the paper.

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Salmonella spp. multirresistentes en muestras fecales de cerdos con presunta salmonelosis en Antioquia, Colombia, 2019-2021

RESUMEN

Objetivos. Determinar la proporción de *Salmonella enterica* en muestras fecales de cerdos vivos con presunta salmonelosis analizadas en la unidad de diagnóstico de la Universidad de Antioquia (Colombia) entre el 2019 y el 2021, así como examinar los serotipos y los patrones de resistencia a los antimicrobianos.

Métodos. Se trata de un estudio transversal de laboratorio sobre datos ordinarios de muestras fecales provenientes de granjas porcinas de las nueve subregiones del departamento de Antioquia (Colombia). La detección de *Salmonella* spp. en la universidad se realiza mediante el enriquecimiento, el cultivo selectivo y la reacción en cadena de la polimerasa. Se identificaron los serotipos con el esquema de Kauffmann-White y se examinaron las cepas aisladas para determinar la susceptibilidad antimicrobiana mediante microdilución en caldo.

Resultados. De las 653 muestras analizadas, 149 (23%) dieron un resultado positivo para *S. enterica.* Se identificaron nueve serotipos. Los más comunes fueron *Salmonella typhimurium* (56%) y su variante monofásica (35%). La resistencia a la ampicilina fue la observada con mayor frecuencia (70%), seguida de la resistencia al ciprofloxacino (55%) y al sulfametoxazol-trimetoprima (52%). Ninguna cepa aislada fue resistente a la amikacina y la gentamicina. Se observó resistencia a múltiples fármacos (resistencia a tres o más clases de antibióticos) en 61 cepas (44%). La resistencia a múltiples fármacos fue más elevada en el caso de *S. typhimurium* (57%) en comparación con los otros serotipos. Se asoció el serotipo con la resistencia a múltiples fármacos ($p = 0,01$), a diferencia de la edad del cerdo y la subregión.

Conclusiones. La proporción de *Salmonella* spp. y los elevados niveles asociados de resistencia a múltiples fármacos son preocupantes y pueden ser un indicativo de uso irracional de antimicrobianos y malas prácticas de gestión en los sistemas de producción porcina de la región. Es necesario reforzar la vigilancia para dar seguimiento y mejorar las prácticas de gestión agropecuaria y el uso de antimicrobianos en las granjas en Colombia.

Palabras clave *Salmonella enterica*; serogrupo; resistencia a múltiples medicamentos; porcinos; Colombia.

Salmonella spp. multirresistente em amostras fecais de suínos com suspeita de salmonelose em Antioquia, Colômbia, 2019–2021

RESUMO

Objetivos. Determinar a proporção de *Salmonella enterica* em amostras de fezes de suínos vivos com suspeita de salmonelose analisadas na unidade de diagnóstico da Universidade de Antioquia, Colômbia, entre 2019 e 2021, e examinar seus sorotipos e padrões de resistência a antimicrobianos.

Métodos. Estudo transversal, de base laboratorial, utilizando dados de rotina de amostras de fezes recebidas de suinocultores em todas as nove sub-regiões do estado de Antioquia, Colômbia. A detecção de *Salmonella* spp. na Universidade é feita por enriquecimento, cultura seletiva e reação em cadeia da polimerase. Os sorotipos foram identificados usando o esquema de Kauffmann-White, e os isolados foram testados quanto à suscetibilidade aos antimicrobianos pelo método de microdiluição em caldo.

Resultados. Das 653 amostras testadas, 149 (23%) foram positivas para *S. enterica.* Foram identificados nove sorotipos. Os mais comuns foram *Salmonella* Typhimurium (56%) e sua variante monofásica (35%). A resistência à ampicilina (70%) foi observada com maior frequência, seguida pela resistência ao ciprofloxacino (55%) e ao sulfametoxazol/trimetoprima (52%). Nenhum isolado apresentou resistência à amicacina ou gentamicina. Multirresistência (resistência a ≥ 3 classes de antibióticos) foi observada em 61 isolados (44%). A multirresistência foi mais comum em *S.* Typhimurium (57%), em comparação aos outros sorotipos. Foi constatada associação da multirresistência com sorotipos ($p = 0{,}01$), mas não com idade do suíno ou sub-região.

Conclusões. A proporção de *Salmonella* spp. e os níveis elevados associados de multirresistência a antimicrobianos aqui constatados são preocupantes, e podem indicar uso irracional de antimicrobianos e práticas inadequadas de manejo nos sistemas de suinocultura da região. É preciso fortalecer a vigilância para monitorar e melhorar as práticas de manejo agrícola e o uso de antimicrobianos em fazendas na Colômbia.

Palavras-chave *Salmonella enterica*; sorogrupo; resistência a múltiplos medicamentos; suínos; Colômbia.