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Prevalence and antimicrobial resistance of *Escherichia coli* and *Salmonella* spp. in animal feed in Colombia

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ABSTRACT

Objective. To determine the prevalence and antimicrobial resistance of *Escherichia coli* and *Salmonella* spp. in animal feed samples collected between 2018 and 2021 in Colombia.

Methods. This was a laboratory-based cross-sectional study using routine data from the program for inspection, surveillance, and control of animal feed at the Colombian Agriculture Institute. Samples of animal feed for swine, poultry, canine, feline, leporine, piscine, and equine species were processed for detection of *E. coli* and *Salmonella* spp. using enrichment and selective culture methods. Isolates were tested for antimicrobial susceptibility using an automated microdilution method.

Results. Of 1 748 animal feed samples analyzed, 83 (4.7%) were positive for *E. coli* and 66 (3.8%) for *Salmonella* spp. The presence of *E. coli* and *Salmonella* spp. was highest in feed for poultry (6.4% and 5.5%) and swine (6.1% and 4.3%). Antimicrobial resistance testing was performed in 27 (33%) *E. coli* isolates and 26 (39%) *Salmonella* isolates. Among *E. coli*, resistance was most frequently observed to ampicillin (44.5%) followed by cefazolin (33.3%), ciprofloxacin (29.6%), ampicillin/sulbactam (26%), and ceftriaxone (11.1%). The highest resistance levels in *Salmonella* spp. isolates were against cefazolin (7.7%) and piperacillin/tazobactam (7.7%).

Conclusions. This is the first study from Colombia reporting on the prevalence and antimicrobial resistance of *E. coli* and *Salmonella* spp. in animal feed samples. Its results establish a baseline over a wide geographical distribution in Colombia. It highlights the need to integrate antimicrobial resistance surveillance in animal feed due to the emergence of resistant bacteria in this important stage of the supply chain.

Keywords Drug resistance, microbial; hazard analysis and critical control points; animal feed; one health; operations research; Colombia.

The safety of animal feed is important not only for animals but also for human health, as transmission of infections from animals to humans is a known phenomenon with consequent health and economic implications (1, 2). Therefore, microbial contamination of animal feed or animal by-products is one of the challenges to One Health, which is defined as the collaborative efforts of multiple disciplines, working locally, nationally, and globally, to attain optimal health for people, animals, and our environment (3).

Animal feed plays a vital role in maintaining the nutrition and well-being of animals. Its demand has increased dramatically as

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livestock production and pet ownership are on the rise. Animal feed safety comprises biological hazards such as the presence of pathogenic bacteria like *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, *Aeromonas*, and *Campylobacter*. The most commonly found organisms in animal feed are *E. coli* and *Salmonella* spp. (4, 5). *E. coli* is part of the gastrointestinal microbiota of humans and animals, and in some cases the bacterium can be pathogenic. *S. enterica* can cause salmonellosis in both humans and animals. Transmission of both is usually through contaminated water or food products or by fecal–oral transmission.

The additional problem associated with these pathogens is that of antimicrobial resistance (AMR) (6). Infections caused by resistant bacteria pose a challenge to antimicrobial treatment, requiring selection of effective alternatives. The resistant pathogens contaminating animal feed could spread through the food chain, from primary production to consumers and to humans in direct contact with colonized and infected animals (7). This potential hazard may contribute to enhance AMR in human pathogens, possibly increasing the cost and duration of treatment and leading to unfavorable treatment outcomes (4). Consequently, microbiological evaluation is essential to ensure animal feed is not a source of *E. coli* and *Salmonella* spp. contamination (5, 8) or a propagator of AMR.

Recent studies have shown the presence of *E. coli* and *Salmonella* spp. in animal feed and have further documented their AMR profiles (9). In a study from Kenya, 58% and 28% of poultry feed samples were contaminated with *E. coli* and *Salmonella* spp., respectively. The highest resistance was against ampicillin, at 62% for *E. coli* isolates and 41% for *Salmonella* spp. (10). Another study from the United States of America on animal feed also reported the presence of both organisms, where the frequency of bacteria resistant to two or more antimicrobials was 39.3% for *E. coli* and 22.9% for *Salmonella* spp. (11). Furthermore, AMR to different groups of antimicrobials, including first-line antimicrobials, has been reported in *Salmonella* spp. from animal feed (6). There is no previously published information about the prevalence of AMR in *E. coli* and *Salmonella* in animal feed samples in Colombia or in Latin America.

The Colombian Agriculture Institute (ICA) is the institution responsible for inspection, surveillance, and risk-based control of companies that produce and import animal feed in Colombia. Samples of animal feed for several species including swine, poultry, canine, feline, leporine, piscine, and equine species are tested. To address the safety of animal feed in Colombia and understand the AMR profiles of contaminating pathogens, this study aimed to determine the number (and proportion) of samples of animal feed contaminated with *E. coli* and *Salmonella* spp. and to describe their AMR characteristics.

MATERIALS AND METHODS

This was a laboratory-based cross-sectional study using routinely collected data from the inspection, surveillance, and control of animal feed program carried out by the National Laboratory of Livestock Supplies (LANIP) within ICA.[1] The samples analyzed were obtained between January 2018 and November 2021 and covered 27 of the 32 Colombian administrative departments (provinces) (12, 13). The other five departments were excluded from the sampling because animal production in these provinces is very low, and they do not have factories for the production of animal feed and self-consumption.

Setting

In Colombia, approximately 800 companies produce and market animal feed, of which 400 distribute products to be consumed at the national level and the other 400 import products into the country. In addition, 65 farms produce their own feed products, and they are considered "self-consumption" companies.

ICA works at the national level to ensure the safety of agricultural production in the Colombian countryside. LANIP within ICA verifies the quality of supplies for livestock and the safety of products of animal origin in their primary production phase, to prevent risks to animal and human health. At LANIP, the control of animal feed is carried out following the national sampling plan elaborated and executed by the Technical Directorate of Security and Livestock Supplies of ICA (13).

Sampling

The study comprised samples of animal feed destined for consumption by swine, poultry, canine, feline, leporine, and equine species. The samples originated from 27 of the 32 provinces of Colombia. The type of animal feed was classified according to its physical appearance as either pellet or flour. Animal feed in flour form originated only from self-consumption companies, as defined above.

Samples were collected under the ICA inspection, surveillance, and control of animal feed program according to the guidelines in the ICA resolution 61252 of 2020 (12, 14). Samples were collected *in situ* by ICA staff under aseptic conditions from complete packaged animal feeds received from production companies, chain stores, agricultural distributors, and small stores. One sample consisted of a total of 500 g drawn from 4–16 subsamples proportionally combined. These subsamples correspond to the number of units selected per batch according to the guidelines. The final sample was packaged in a sterile bag and after labeling, shipped to LANIP for analyses.

Upon arrival at the laboratory, only samples that fulfilled the ICA requirements were included. The requirements included samples with a size of 500 g. The samples were analyzed in the food microbiology laboratory of LANIP in Cundinamarca, Colombia. Samples were stored at room temperature (20–30 °C) until they were analyzed.

Isolation of bacteria

Twenty-five grams of each sample was pre-enriched in 225 mL of buffered peptone water and left incubating for 24 hours at 37 °C for *Salmonella* spp. and *E. coli.* For *Salmonella* isolation, 0.1 mL of pre-enriched buffered peptone water was transferred to Rappaport broth and 1 mL to selenite broth. The Rappaport broth was incubated at 42 °C and selenite broth at 37 °C. Both broths were incubated for 24 hours. Then, 10 µL of Rappaport broth was streaked on XLD agar and 10 µL of selenite broth

[1] The dataset used in this article can be made available on request to the corresponding author.

on Hektoen agar. Presumptive *Salmonella* spp. colonies were identified by their H_2S production (black colonies) in XLD agar and Hektoen agar. This method was based on the ISO 6579:2002 *Salmonella* method (15).

For *E. coli* isolation, samples were processed according to ICONTEC-NTC 5652 (16). One mL was transferred to BRILA broth for *E. coli* and incubated at 42 °C. Then, 10 µL was streaked on EMB agar and MacConkey agar. Typical colonies had a metallic shine on EMB, and typical lactose-positive colonies were pink on MacConkey agar.

Antimicrobial susceptibility testing

Investigation for AMR in *E. coli* and *Salmonella* spp. isolates was performed as an addition to the routine surveillance in animal feed described above. Isolates were tested for antimicrobial susceptibility using the Becton Dickinson Phoenix™ Automated Microbiology System. Dilutions of presumptive *E. coli* or *Salmonella* spp. colonies were made in Phoenix™ ID Broth (Becton Dickinson) at a concentration of 0.45–0.5 on the MacFarland scale according to the manufacturer's instructions. The bacterial suspensions were added to the panel for identification of microorganisms and antimicrobial susceptibility NMIC/ID 406 for Gram-negative bacteria (Becton Dickinson). The panel determined minimal inhibitory concentrations (MICs) by broth microdilution of the following antimicrobials: amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin/tazobactam, tigecycline, and trimethoprim/sulfamethoxazole. The panel was incubated in the Phoenix™ according to manufacturer instructions for 24 hours at 35 °C. Resistance breakpoints were evaluated according to the guidelines of the Clinical and Laboratory Standards Institute (17). For isolates of porcine origin, resistance to the third-generation cephalosporins ceftazidime and ceftriaxone was evaluated with the CLSI guidelines for bacteria of veterinary origin VET01S-Ed.5 (18).

Additionally, biochemical screening of carbapenemase production in *Salmonella* spp. isolates was performed with Carba NP test, which is based on the detection of the hydrolysis of the β-lactam ring of a carbapenem (imipenem) by carbapenemase enzymes produced by the bacteria, changing the color of the medium from red to yellow through decreases in the pH of the medium (19).

Data management and analysis

Metadata were extracted from the electronic database that was used for data collection during the surveillance activities of ICA. Data were summarized as counts and proportions using EpiData analysis software (version 2.2.2.181, EpiData Association, Odense, Denmark).

Ethical considerations

Ethics approval was obtained from the Institutional Review Committee of Secretaría Distrital de Salud in Colombia (SDSCTI2021009), The Union Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (EAG28/21), and the Ethics Review Committee of the Pan American Health Organization (PAHOERC.478.01).

RESULTS

A total of 1 748 animal feed samples were analyzed at LANIP-ICA from January 2018 to November 2021. The number of samples tested across the years was similar, though fewer samples were analyzed in 2020 (n = 230). Of all destined animal species, feed for poultry (n = 565) and swine (n = 610) contributed over 65% of samples (Table 1).

Overall, 83 (4.7%) samples were positive for *E. coli* and 66 (3.8%) for *Salmonella* spp. The prevalence of *E. coli* was highest in 2018 (9%) and lowest in 2019 (1.2%). Similarly, the prevalence of *Salmonella* spp. was highest in 2018 (7.1%) and lowest in 2021 (0.7%). The prevalence of *E. coli* and *Salmonella* spp. was highest in feed for poultry (6.4% and 5.5%, respectively) followed by feed for swine (6.1% and 4.3%, respectively).

Among the two types of feed (flour or pellet), flour from self-consumption companies showed the highest prevalence of *E. coli* (7.8%) and *Salmonella* spp. (5.3%) (Table 1).

Cundinamarca (n = 281), Antioquia (n = 272), Valle del Cauca (n = 260), and Santander (n = 128) were the provinces that contributed the highest number of samples. The presence of *E. coli* in these provinces ranged from 3.9% (Cundinamarca) to 6.9% (Valle del Cauca) while the presence of *Salmonella* ranged from 0.8% (Santander) to 5.5% (Antioquia). The samples from Arauca, Cauca, Cesar, Guainía, and Guaviare provinces did not show *E. coli* or *Salmonella* (Table 2).

Of 83 *E. coli* isolates, 27 (33%) were subjected to antimicrobial susceptibility testing. Resistance to ampicillin (44.5%) was most frequently observed, followed by cefazolin (33.3%), ciprofloxacin (29.6%), and ampicillin/sulbactam (26%) (Table 3). Among *E. coli* isolates, 14 (40.7 %) were susceptible to all antimicrobials tested.

TABLE 1. Baseline characteristics of *E. coli* and *Salmonella* spp. isolated from samples of animal feed in Colombia, January 2018 to November 2021

Characteristic	Number of samples	*E. coli*		*Salmonella* spp.	
		n	(%)	*n*	(%)
Total	1 748	83	(4.7)	66	(3.8)
Year					
2018	476	43	(9.0)	34	(7.1)
2019	495	6	(1.2)	14	(2.8)
2020	230	11	(4.8)	14	(6.1)
2021	547	23	(4.2)	4	(0.7)
Destined species					
Canine	152	2	(1.3)	0	(0)
Feline	106	1	(0.9)	2	(1.9)
Swine	610	37	(6.1)	26	(4.3)
Equine	138	3	(2.2)	6	(4.3)
Leporine	114	4	(3.5)	1	(0.9)
Poultry	565	36	(6.4)	31	(5.5)
Piscine	62	0	(0)	0	(0)
Feed type					
Pellet	1 297	48	(3.7)	42	(3.3)
Flour[a]	451	35	(7.8)	24	(5.3)

***Note*:** a. Type of feed for self-consumption, originating from farms that produce and consume their own feed products.
***Source*:** Prepared by the authors based on the study data.

TABLE 2. Distribution of *E. coli* and *Salmonella* spp. isolates from samples of animal feed in Colombia by province, January 2018 to November 2021

Province	Total feed samples	*E. coli*		*Salmonella* spp.	
	N	*n*	(%)	*n*	(%)
Cundinamarca	281	11	(3.9)	10	(3.6)
Antioquia	272	13	(4.8)	15	(5.5)
Valle del Cauca	260	18	(6.9)	14	(5.4)
Santander	128	7	(5.5)	1	(0.8)
Atlántico	88	3	(3.4)	4	(4.5)
Boyacá	76	4	(5.3)	2	(2.6)
Quindío	76	1	(1.3)	2	(2.6)
Huila	61	3	(4.9)	2	(3.3)
Tolima	54	2	(3.7)	3	(5.6)
Caldas	52	5	(9.6)	2	(3.8)
Meta	52	3	(5.8)	0	(0)
Norte de Santander	50	0	(0)	2	(4)
Risaralda	46	3	(6.5)	1	(2.2)
Nariño	41	4	(9.8)	3	(7.3)
Magdalena	31	2	(6.5)	0	(0)
Cordoba	30	0	(0)	1	(3.3)
Bolívar	20	0	(0)	1	(5)
Sucre	19	1	(5.3)	0	(0)
La Guajira	18	1	(5.6)	0	(0)
Putumayo	16	0	(0)	1	(6.3)
Caquetá	8	1	(2.5)	0	(0)
Casanare	7	1	(14.3)	1	(14.3)
Other provinces[a]	42	0	(0)	0	(0)
Total	**1 748**	**83**	**(4.7)**	**66**	**(3.8)**

***Note*:** a. Other provinces: Arauca, Cauca, Cesar, Guainía, and Guaviare.
***Source*:** Prepared by the authors based on the study data.

TABLE 3. Results of antimicrobial susceptibility testing of *E. coli* isolates (*n* = 27) from samples of animal feed in Colombia, January 2018 to November 2021

Antimicrobial	Susceptible [a]		Resistant [b]	
	n	(%)	*n*	(%)
Ampicillin	15	(55.5)	12	(44.5)
Ampicillin/sulbactam	20	(74.0)	7	(26.0)
Cefazolin	18	(66.7)	9	(33.3)
Cefepime	24	(88.9)	3	(11.1)
Cefoxitin	25	(92.6)	2	(7.4)
Ceftriaxone	24	(88.9)	3	(11.1)
Ciprofloxacin	19	(70.4)	8	(29.6)

***Notes*:**
a. All isolates were susceptible to the antimicrobials amikacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin/tazobactam, tigecycline, and trimethoprim/sulfamethoxazole.
b. No intermediate levels of resistance were detected among *E. coli* isolates.
***Source*:** Prepared by the authors based on the study data.

Of 66 *Salmonella* spp. isolates, 26 (39%) were subjected to antimicrobial susceptibility testing. Resistance to cefazolin (7.7%) and piperacillin/tazobactam (7.7%) was most frequently observed. Unlike *E. coli*, where no isolates showed intermediate resistance to any antimicrobial agent, intermediate levels of resistance were observed among *Salmonella* spp. for imipenem (27%), ciprofloxacin (7.7%), cefepime (7.7%), and meropenem (4.3%) (Table 4). Among *Salmonella* spp. isolates, 11 (42.3%) were susceptible to all antimicrobials tested.

Six of seven *Salmonella* spp. isolates with intermediate susceptibility to imipenem and meropenem yielded negative results for carbapenemase activity using the Carba NP test, indicating that this enzymatic mechanism was not involved. One of these showed indeterminate results. Further molecular-based studies are required to characterize this isolate.

DISCUSSION

This is the first study from Colombia reporting on the prevalence and AMR pattern of *E. coli* and *Salmonella* spp. in animal feed samples. We found that 4.7% samples were positive for *E. coli* and 3.8% for *Salmonella* spp. Among *E. coli* isolates, resistance was observed for beta-lactam drugs and fluoroquinolones. Among *Salmonella* isolates, resistance was observed to some beta-lactam drugs. Intermediate levels of resistance to fourth-generation cephalosporins, carbapenems, and fluoroquinolones were observed.

The prevalence rates of *E. coli* and *Salmonella* spp. reported in our study are lower than those reported by the U.S. Food and Drug Administration (FDA) animal food surveillance program: 12.5% and 12% for *E. coli* and *Salmonella* spp., respectively (11, 20). Also, our estimate seems much lower than estimates reported in other studies, such as 39.3% (*E. coli*) and 22.9% (*Salmonella* spp.) reported in 2012 in processing plants in the United States of America (21), and 58% (*E. coli*) and 28% (*Salmonella* spp.) reported in a study from Kenya (10).

In general, in this study we observed a lower prevalence of *E. coli* and *Salmonella* spp. across the years. Interestingly, fewer samples tested positive for *Salmonella* spp. in 2021 compared to the earlier years, even though there was a decrease in the number of samples tested in 2020 due to the COVID-19 pandemic. These results could have been influenced by the fact that, in addition to the monitoring activities, ICA supports producers with training in implementation of good manufacturing practices (GMP). This could have been complemented by the

TABLE 4. Antimicrobial susceptibility testing of *Salmonella* spp. isolates (*n* = 26) from samples of animal feed in Colombia, January 2018 to November 2021

Antimicrobial	Susceptible [a]		Intermediate [b]		Resistant	
	n	(%)	*n*	(%)	*n*	(%)
Ampicillin	25	(95.7)	0	(0)	1	(4.3)
Ampicillin/sulbactam	25	(95.7)	0	(0)	1	(4.3)
Cefazolin	24	(92.3)	0	(0)	2	(7.7)
Cefepime	24	(92.3)	2	(7.7)	0	(0)
Cefoxitin	25	(95.7)	0	(0)	1	(4.3)
Ceftazidime	25	(95.7)	0	(0)	1	(4.3)
Ceftriaxone	25	(95.7)	0	(0)	1	(4.3)
Ciprofloxacin	24	(92.3)	2	(7.7)	0	(0)
Imipenem	19	(73.0)	7	(27.0)	0	(0)
Meropenem	25	(95.7)	1	(4.3)	0	(0)
Piperacillin/tazobactam	24	(92.3)	0	(0)	2	(7.7)

***Notes*:**
a. All isolates were susceptible to the antimicrobials amikacin, ertapenem, gentamicin, tigecycline, and trimethoprim/sulfamethoxazole.
b. Intermediate levels of resistance were observed for imipenem, ciprofloxacin, cefepime, and meropenem.
***Source*:** Prepared by the authors based on the study data.

issuing of resolutions such as 61252 of 2020, in which the regulations of inspection, surveillance, control, and implementation of GMP were reinforced with the purpose of guaranteeing the safety of animal feed (14). Another factor could be the emerging willingness of Colombian companies to export their products, thus being subject to more stringent quality control. All these factors might have contributed to improved food safety and a lower prevalence of microbial contamination. However, despite the low prevalence of *E. coli* and *Salmonella* spp. in animal feed, this is still above the desired levels, as the Colombian regulation mandates that *E. coli* and *Salmonella* spp. should be absent in any 25 g of sample (22). This regulation mandating the absence of *Salmonella* spp. in a sample is reflected in other guidelines, such as those of the European Food Safety Authority, which emphasize that eliminating *Salmonella* spp. in animal feed before it gets to the farm will contribute to reducing the presence of *Salmonella* spp. in food (23).

The highest number of isolates for both *Salmonella* spp. and *E. coli* was found in the flour type of feed. The production of this type of feed is destined for self-consumption. Generally, its production is not done according to commercial standards and is less well-regulated compared to other types of feed. Production is carried out through mechanical homogenization and high temperatures are not usually used. Additional reasons for the contamination of this type of feed could include failures in the cleaning of production lines, poor rodent control practices, storage on the floor and not in food stowage, and poor packaging and storage conditions (24).

Feeds for swine and poultry constituted the largest proportion of the samples, as these are produced in larger quantities than feeds destined for other species. Further, *E. coli* and *Salmonella* spp. were isolated more commonly in this type of feed. This finding is in accordance with the findings for the flour type of feed, which is mostly destined for these animals.

Nariño was the province with the highest percentage of bacterial isolates of *E. coli* (9.8%) and *Salmonella* spp. (7.3%). The feed production in this province concentrates mostly on food destined for leporine species. We therefore recommend further investigation in the production in this province to identify any specific source of the pathogens during production of feed for leporine species.

We found resistance to ampicillin, ampicillin/sulbactam, cefazolin, and ciprofloxacin in *E. coli.* The highest level of resistance was observed against ampicillin, followed by cefazolin and ciprofloxacin. High levels of resistance to ampicillin have also been reported in a study from Kenya (10). However, levels of resistance in *Salmonella* spp. to other drugs in the Kenyan study differed from ours. For instance, no resistance against ciprofloxacin was observed in that study. Our results also contrast with a previous study from the FDA animal food surveillance program, where the highest levels of resistance were observed for tetracycline (11.2%) and streptomycin (4.6%). In the FDA study, low levels of resistance to most beta-lactam drugs, including ampicillin (2.9%), were observed (11).

The AMR levels for *E. coli* observed in our study seem to be specific to the situation in Colombia, and could be related to the use of antimicrobials such as ampicillin, ciprofloxacin, and trimethoprim/sulfamethoxazole in animal production in the country (25). In addition, some companies in Colombia still use non-therapeutic antimicrobials as prophylaxis in animal feed, and this can contribute to the emergence of AMR against these groups. Such AMR patterns, therefore, may limit the use of these antimicrobials in human and animal therapies (26). The resistance against cefazolin and ciprofloxacin is also a matter of concern, as these antimicrobials are in the group of critically important and highly important antimicrobials for human health (27). This has important implications for public health and may lead to calls for more regulated use of these antimicrobials in animals.

Compared with *E. coli,* lower levels of resistance were found in *Salmonella* spp. isolates. The highest levels of resistance in *Salmonella* spp. were against cefazolin and piperacillin/tazobactam. Additionally, intermediate levels of resistance were observed for ciprofloxacin, the fourth-generation cephalosporin cefepime, and the carbapenem drugs imipenem and meropenem. Excluding intermediate resistance levels, most *Salmonella* spp. isolates were susceptible to all the drugs tested. Similar susceptibility patterns have been reported from Kenya and the United States of America (10, 20) where *Salmonella* from animal feed exhibited low resistance levels to most of the antimicrobials tested. Cefepime, imipenem, and meropenem are critically important antimicrobials for human health, where cefepime is in the group of highest priority (27).

The presence of some strains with reduced susceptibility to carbapenems warranted further investigation. After phenotypic screening testing, the presence of carbapenemases (β-lactamases with the ability to hydrolyze β-lactams including carbapenems) in six of seven isolates was excluded. This means that other mechanisms apart from enzymatic actions are contributing to carbapenem and cephalosporin resistance. For instance, the active expulsion of carbapenems from the periplasmic space (28) and porin mutations may be associated with decreased antimicrobial susceptibility, as have been described for other Gram-negative bacilli (29). Our results indicate that uncommon AMR mechanisms may be circulating in *Salmonella* isolates from animal feed in Colombia. This provides evidence to support the design and implementation of better and more sophisticated AMR surveillance in animal feed, considering the impact of cross-transmission of strains between humans and animals as stated by the One Health approach.

The study had several strengths. An analysis was conducted on a large number of samples originating from the nationwide surveillance system covering most of the provinces of the country, collected over a four-year span. The results of the study serve as a baseline against which future reports may be compared. Baseline information is crucial to understanding the effectiveness of current policies on animal feed manufacturing and safety. In addition, given that the sample collection and the analysis were part of a national surveillance system, this process is ongoing and will allow an analysis of trends in the variables evaluated in the future. This information will enable close monitoring and allow suggestions for corrective action when needed.

The study had some limitations. First, the small number of positive samples obtained, despite the large sample size, precluded further statistical analysis of factors associated with the presence of the pathogens in animal feed samples. Second, due to resource constraints, only 27 (33%) and 26 (39%) strains of *E. coli* and *Salmonella* isolates, respectively, were tested for AMR, reducing the scope of the statistical analysis of the distribution of AMR patterns. Third, assessment of the performance of the surveillance system was outside the scope of this study

because we had no access to the specific data system involved; but it would be useful to look into this in the future.

Conclusion

The presence of *E. coli* and *Salmonella* spp. in this study was lower than expected when compared with previous reports from other countries. Despite that, the presence of resistance to antimicrobials critically important for human and animal health deserves attention. Our results highlight the need to integrate antimicrobial susceptibility analyses into the current surveillance program for close monitoring of resistance patterns of isolates to antimicrobials. Future research on how to integrate the multiple sectors and disciplines using a One Health approach will help us to understand the origin, dissemination, and impact on public health of the microbiological and AMR contamination identified in this study.

Author contributions. RP, MD, AK, AP, and KJ were involved in the conception or design of the work. RP, MD, and JM acquired the data. RP, MD, JM, LRC, VC, AK, KJ, and KM were involved in data analyses and interpretation of results. RP, MD, LRC, VC, YC, AK, and KJ drafted the manuscript. LRC, VC, YC, AK, AP, and KJ critically reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

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Prevalencia y resistencia a los antimicrobianos de *Escherichia coli* y *Salmonella spp.* en los alimentos para animales en Colombia

RESUMEN

Objetivo. Determinar la prevalencia y resistencia a los antimicrobianos de *Escherichia coli* y *Salmonella* spp. en muestras de piensos para animales tomadas entre el 2018 y el 2021 en Colombia.

Métodos. Se trata de un estudio transversal realizado en el laboratorio a partir de los datos regulares del programa de inspección, vigilancia y control de alimentos para animales del Instituto Colombiano Agropecuario. Se procesaron muestras de alimentos utilizados en la cría de cerdos, aves de corral, cánidos, félidos, lepóridos, peces y equinos con el fin de detectar *E. coli* y *Salmonella* spp. por medio de métodos de enriquecimiento y cultivo selectivo. Se analizó la sensibilidad a los antimicrobianos de las cepas aisladas mediante microdilución automatizada.

Resultados. De 1748 muestras de alimentos analizadas, 83 (4,7%) resultaron positivas para *E. coli* y 66 (3,8%) para *Salmonella* spp. La presencia de *E. coli* y *Salmonella* spp. fue mayor en los alimentos para aves de corral (6,4% y 5,5%) y cerdos (6,1% y 4,3%). Se realizaron pruebas de resistencia a los antimicrobianos en 27 (33%) cepas de *E. coli* y 26 (39%) de *Salmonella*. En las cepas de *E. coli*, se observó una mayor resistencia a la ampicilina (44,5%), seguida de la resistencia a la cefazolina (33,3%), la ciprofloxacina (29,6%), la ampicilina/sulbactam (26%) y la ceftriaxona (11,1%). En el caso de las cepas de *Salmonella* spp., los niveles de resistencia más elevados fueron para la cefazolina (7,7%) y piperacilina/tazobactam (7,7%).

Conclusiones. Este es el primer estudio realizado en Colombia en el que se informa sobre la prevalencia y la resistencia a los antimicrobianos de *E. coli* y *Salmonella* spp. en muestras de alimentos para animales. Sus resultados establecen una línea de base para una zona geográfica mucho mayor dentro de Colombia. Se subraya la necesidad de integrar la vigilancia de la resistencia a los antimicrobianos en los alimentos para animales debido a la aparición de bacterias resistentes en esta importante etapa de la cadena de suministro.

Palabras clave Farmacorresistencia microbiana; análisis de peligros y puntos de control críticos; alimentación animal; salud única; investigación operativa; Colombia.

Prevalência e resistência a antimicrobianos de *Escherichia coli* e *Salmonella* spp. em ração animal na Colômbia

RESUMO

Objetivo. Determinar a prevalência e a resistência a antimicrobianos de *Escherichia coli* e *Salmonela* spp. em amostras de ração animal coletadas entre 2018 e 2021 na Colômbia.

Métodos. Estudo transversal de base laboratorial, usando dados de rotina do programa de inspeção, vigilância e controle de ração animal do Instituto Colombiano de Agricultura. Amostras de ração animal para as espécies suína, avícola, canina, felina, leporina, piscina e equina foram processadas para detecção de *E. coli* e *Salmonella* spp., usando métodos de enriquecimento e cultura seletiva. Os isolados foram testados quanto à suscetibilidade a antimicrobianos usando um método automatizado de microdiluição.

Resultados. Das 1.748 amostras de ração animal analisadas, 83 (4,7%) foram positivas para *E. coli* e 66 (3,8%) para *Salmonella* spp. A presença de *E. coli* e *Salmonella* spp. foi maior em rações para aves (6,4% e 5,5%) e suínos (6,1% e 4,3%). O teste de resistência a antimicrobianos foi realizado em 27 (33%) isolados de *E. coli* e 26 (39%) isolados de *Salmonella*. Em *E. coli*, a resistência observada com maior frequência foi à ampicilina (44,5%), seguida da cefazolina (33,3%), ciprofloxacino (29,6%), ampicilina/sulbactam (26%) e ceftriaxona (11,1%). Os maiores níveis de resistência em isolados de *Salmonella* spp. foram contra cefazolina (7,7%) e piperacilina/tazobactam (7,7%).

Conclusões. Este é o primeiro estudo da Colômbia a notificar a prevalência e resistência a antimicrobianos de *E. coli* e *Salmonella* spp. em amostras de ração animal. Os resultados estabelecem uma linha de base com ampla distribuição geográfica na Colômbia. Destaca-se a necessidade de integrar a vigilância da resistência a antimicrobianos na ração animal, devido ao surgimento de bactérias resistentes nesta importante etapa da cadeia de abastecimento.

Palavras-chave Resistência microbiana a medicamentos; análise de perigos e pontos críticos de controle; ração animal; saúde única; pesquisa operacional; Colômbia.