

One Health approach: exploring associated factors for antimicrobial resistance in isolates from companion animals in Medellín, Colombia

Recibido: 12 de julio de 2024 • Aprobado: 30 de agosto de 2024

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How to cite: Yepes-Orozco S y Henao-Villegas S. One Health approach: exploring associated factors for antimicrobial resistance in isolates from companion animals in Medellín, Colombia. *Revista Sanmartiniana de Ciencias de la Salud*. 2024; Vol 1, pág. 46 a 57. ISSN 3028-6689

Conflict of interest statement: Santiago Henao declares no conflict of interest while Sara Yepes had a relationship of employment with the Clínica Veterinaria El Poblado during the time the study was carried out.

Funding statement: The authors received no financial support for the research or publication of this article.

Data availability statement: The database generated and used in the study is available upon reasonable request to the corresponding author.

Ethics approval statement: The study has the endorsement of the Institutional Human Research Ethics Committee of the CES University in its minute 227 of 2023, and the Institutional Committee for Animal Care CICUA minute 58 of 2023. Consent for data management and protection, as well as a confidentiality agreement, were signed with the institution. Participation of guardians was voluntary and was subject to signing written informed consent. To protect the personal information of the participants, the information underwent a reversible data dissociation process.

Abstract

Introduction: Antimicrobial resistance in companion animals is a neglected threat that can compromise public health. Despite this, there is little information and interest in its regard. **Objective:** To establish the factors associated with antimicrobial resistance and multidrug resistance in canine and feline patients from a veterinary clinic in Medellín. **Methods:** A cross-sectional study was performed with 41 canines and 6 felines with positive bacteriological cultures. Data about clinical, zoographic, zootechnic and pet-human interactions factors were collected through surveys to animals' guardians and clinical records. A descriptive and association analysis between those factors, antimicrobial resistance and multidrug resistance was performed using bivariate statistics followed by a binomial logistic regression model with multidrug resistance as the outcome. **Results:** Sixty-nine bacteria were isolated from 57 samples. Out of these, 89.70% were resistant and 48.60% showed multidrug resistance. Clinical, zootechnical, and pet-human interaction factors are mostly associated with multidrug resistance rather than resistance to at least one antibiotic. Cohabitation with health personnel and supplement consumption stood out as variables associated with multidrug resistance. **Conclusions:** This study explores a pathway for antimicrobial resistance research highlighting its occurrence in companion animals and its risk to

public health. It identifies factors associated with resistance and proposes further research to determine a possible interspecies transfer and whether these factors exert selective pressure on the animal microbiome. Additionally, it emphasizes the need to explore this phenomenon from the understanding of the dynamics of dual health and the affective bond between humans and animals.

Keywords: Antimicrobial Drug Resistance, Bacterial Multidrug Resistance, Cats, Companion Animal, dogs, Human-Animal Interaction.

Introduction

Antimicrobial resistance (AMR) is an adaptive phenomenon in which the drugs used for treating infections lose effectiveness, thus representing a major burden for global health systems, the world's economy, food security, and sustainable development^{1,2}.

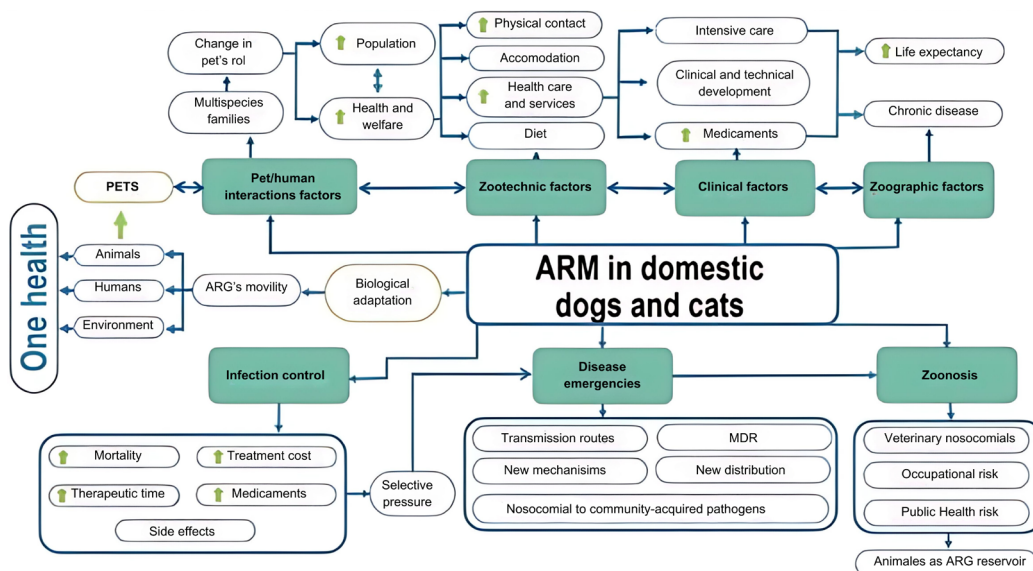
Understanding AMR drivers requires ecological comprehension of antibiotic resistance genes (ARGs) and their circulation within a genetic set called the resistome, where genetic transmission between human, animal, and environmental interfaces is involved³. Because of this, AMR is by no means human-exclusive, and according to its nature, a One Health approach and transdisciplinary research are crucial for combating this global challenge⁴.

Regarding AMR related to animals, there is awareness about the consequences of antibiotic misuse in food-producing animals and its role in the transmission of microorganisms leading to AMR's growth^{5,6}. However, there is limited research contemplating ARGs transfer between

humans and companion animals such as domestic dogs and cats, which not only have a closer bond and direct physical contact with humans, but there is also plenty of evidence supporting the existence of potentially zoonotic bacteria with drug resistance, multidrug resistance (MDR) and extensively drug resistance (XDR) in these species^{7,8}. These animals may act either as reservoirs of ARGs or as populations in which genetic lineages with the potential AMR can emerge, but currently the mechanisms and pathways through which this might occur are poorly understood^{9,10}.

Along with the explained above, in the last decades, there has been a switch in pet-ownership dynamics marked by new conceptions about household animals with an impact over ownership patterns that causes the increase of urban animal population, change of zootechnic management, and transformation of social and physical interactions towards them. This leads to providing and advocating for improved medical care and greater attention to the wellbeing of companion animals^{10,11}.

Figure 1. Scheme of AMR in companion animals, One Health approach considering zoographic, clinical, zootechnical, and pet-human interactions factors. **Source:** own work.



Some behaviors and practices in pet ownership, have been proposed as possible drivers for AMR, especially affective behaviors involving physical contact with animals, such as kissing, grooming, sleeping in the same bed, or direct hand feeding^{10,12}, together with management-related factors like the type of diet offered^{11,13}, and clinical factors such as hospitalization and veterinary intensive care⁹. This article explores the possible association between AMR in companions animals with clinical, zoographic, zootechnic and pet-human interactions factors in their management form o perspective of One Health and understanding of ownership dynamics and the anima-human bond.

The theoretical framework for this study is presented below, classifying the factors proposed to be associated with the increasing hazard of AMR in pets, as well as their main consequences (Figure 1).

Methods

Population and data collection

Data was collected from canine and feline patients of the selected Veterinary Clinic who had at least one sample taken for bacteriological culture with antibiogram between May and October 2023. For each patient, sample collection was carried out according to the clinic protocol and decided independently from the study. Animals with unknown history such as those with less than one month with the guardian, those whose veterinary care was not handled by the clinic personnel, and those whose guardian declined participation in the study were excluded.

Owners were asked to fill out a short survey about clinical, zoographic, zootechnic, and pet-human interaction factors, and the obtained information was verified through medical records. Antibacterial susceptibility was recorded from laboratory reports and was measured by the disk diffusion method according to CLSI. For each isolate, a minimum of five drugs were tested from 13 antimicrobial groups selected by clinical or laboratory criteria, in some cases, an additional antibiogram was required, thus increasing the maximum number of drugs tested to 17 antimicrobials.

Variables

Resistance was defined as reported resistance to at least one antibiotic, while MDR was defined as reported resistance to at least one antibiotic from 3 different antibiotic groups. Intrin-

sically resistance was not included for either classification. Association with resistance and MDR was evaluated for 50 variables.

Statistical analyses

A first analysis reports the isolated bacteria, their resistance levels and affected organ system. Then, clinical, zoographic, zootechnic and pet-human interactions variables were characterized. Both descriptive analyses used absolute and cumulative frequency for qualitative variables, and measures of central tendency and dispersion for quantitative variables according to their distribution.

The association between clinical, zoographic, zootechnic and pet-human interactions variables and drug resistance or MDR was initially evaluated with Chi-squared test or Fisher's exact test, along with their respective prevalence rates (PR).

Afterwards, a regression model for MDR was developed: explanatory variables included those that met the Hosmer Lemeshow criterion ($p < 0.25$), and those that, due to their biological plausibility or findings in previous studies, could explain the outcome. Only two variables are presented in the final model considering the size of the population, and for these adjusted Odds Ratios (ORa) are presented.

The statistical assumptions of independence and absence of collinearity were reviewed considering their theoretical independence and through the Variance Inflation Factor. Normality was not tested because all the included variables were categorical.

Results

From 94 isolates, 60.63% ($n=57$) were positive and 21.05% ($n=12$) reported multiple bacterial growths. A total of 68 bacteria were isolated from seven genera; however, two isolates were non-fermenting Gram-negative bacilli, for which no precise biochemical identification was obtained. The most frequently isolated bacteria were *Staphylococcus pseudointermedius* (41.18%; $n=28$) and *Escherichia coli* (22% $n=15$), followed by *Pseudomonas aeruginosa* and *Enterococcus* spp. (8.82% $n=6$).

Additionally, 63.24% ($n = 43$) of the bacteria were isolated from the skin and its appendages, 25% ($n=17$) were urinary infections, 5.88 % ($n=4$) were isolated from the reproductive system, 2.94%

(n=2) from the digestive system, and 1.47% (n=1) from the respiratory tract. The percentage of positive cultures was higher in canine than feline samples, with 68.12% in contrast with 40%.

Of all isolates, 89.70% (n=61/68) were bacteria resistant to at least one antibiotic, and 48.60% (n=34) were MDR. For all agents, resistance and MDR were 75% and 32.14%. respectively.

Characterization of animals with positive isolates

The characterization of the animals with positive bacteriological cultures was carried out with the data provided by 47 owners who accepted participation in the study and signed the informed consent, representing 82.45% of the animals with positive cultures. (Table 1).

Table 1. Zoographic and zootechnic characterization of participant pets whose owners filled the survey.

Variable	Canines (n=41)		Felines (n=6)		Total (n=47)	
	n	%	n	%	n	%
Sex						
Female	26	63.41	1	16.67	27	57.45
Male	15	36.59	5	83.33	20	42.55
Age group						
Puppy (<12 months old)	3	7.32	0	0	3	6.38
Adult (1 - 6 years old)	13	31.71	3	50	16	34.04
Senior (7 or more years old)	25	60.98	3	50	28	59.57
Reproductive status						
Entire	15	36.59	0	0	15	31.91
Neutered	26	63.41	6	100	32	68.09
Preventive protocols						
Vaccination	10	24.39	3	50	13	27.66
Internal deworming	13	31.71	4	66.67	24	51.06
External deworming	20	48.78	4	66.67	17	36.17
Cohabitee with:						
Human/animal health personnel	13	31.71	1	16.67	14	29.79
Elderly (+60 years old)	16	39.02	3	50	19	40.43
Children under five years old	1	2.44	0	0	1	2.13
People with chronic diseases	14	34.15	5	83.33	19	40.43
Human use of antibiotics (last month)	5	12.20	0	0	5	10.64
Difficulty of oral treatment at home						
Very easy	11	26.83	0	0	11	23.40
Easy	12	29.27	0	0	12	25.53
Normal	8	19.51	2	33.33	10	21.28
Difficult	8	19.51	1	16.67	9	19.15
Impossible	2	4.88	3	50	5	10.64
Affective behaviors						
Sleeping in people's beds	29	70.73	6	100	35	74.47
Waking up people with kisses	17	41.46	0	0	17	36.17

Licking or nibbling people	23	56.10	4	66.67	27	57.45
Feeding with bare hands	17	41.46	1	16.67	18	38.30
Sharing food utensils with human	4	9.76	2	33.33	6	12.77

Owners who completed the survey had an average age of 37.6 ± 11.8 years. A wide variety of professions was found, mostly within finances, but nearly 25% (n=12) of owners had professions related to human or animal health, including anesthesiology, bacteriology, nursing, medical students, veterinary medicine, dentistry, and oral health technology.

Owners also reported a high level of education; 75% had achieved at least a college degree (n=35), and more than 20% had technical education. The remaining two owners with lower education were young adults pursuing degrees in medical and engineering fields. Despite this, prior knowledge about AMR was only 40.43% (n=19).

Zoographical and zootechnical

Data from 41 canines (71.92%) and 6 felines (10.52%) was collected. All cats and 6 canines were mixed breeds, so the frequency of pure-bred canines was 85.37% showing a considerable breed variation. The most common dog breed was the French Bulldog, followed by the Shih Tzu and the American Pit Bull Terrier. Other breeds included English Bulldog, Fox Terrier, Australian Shepherd, Yorkshire Terrier, Akita, American Bully, Beagle, Border Collie, Boxer, Doberman, Labrador Retriever, German Shepherd, Pinscher, German Shorthaired Pointer, Pomeranian, Pug and Shetland Collie.

All cats and most dogs consumed commercial dry food; 27.66% of canines have a mixed diet (commercial dry food plus homemade food or raw diet). No pet was fed an exclusively raw diet. Among the patients who reported consumption of raw animal protein as a complement to the usual diet, owners reported the offering of ossobuco, chicken, and chicken legs.

Regarding the living environment, only ten animals (21.28%), all of them dogs, lived in mixed or rural environments, and nine of them had mainly urban residences with short occasional stays in recreational farms where contact with production species occurred in 12.2% of canines (n=5), including interactions with horses, cattle, and poultry. Also, contact with other pets in the household was common, particularly with other canines, since almost 60% (n=28) of the partici-

pating animals lived with at least one dog and 20 animals (42.56%) lived with at least one cat.

Among the animals' activities, it stands out that only one cat had access to the exterior of its house and no feline had been in daycare or traveled to other places in Colombia or abroad. In contrast, three dogs (7,32%) had stayed in canine daycare, seven dogs traveled nationally and two internationals in the last year.

Clinical factors

Almost 60% of animals had 28% acute diseases, eleven (23,4%) were hospitalized at least 24 hours pre-sampling and four (8.51%) had been hospitalized a month pre-sampling. No cats had previous surgeries the year before the sampling, but four dogs (9.76%) had surgery a month before sampling.

Concomitant diseases were more common in felines, but about 30% of the animals presented concomitant diseases, such as cardiopathy, chronic nephropathy, dermatopathy, endocrinopathy, severe periodontal disease, hemotropic infections, neoplastic conditions with pancytopenia, joint disease, syncope in the diagnostic process, toxoplasmosis, and ventriculomegaly.

Previous antibiotic use (a month before sampling) reached thirty percent in both species, and one female canine was self-medicated at home with oral amoxicillin/clavulanic acid by her owner, who is a nurse. In this regard, self-medication was a phenomenon only presented in four canines (9.76% of dogs), and although no other owner administered antibiotics, drugs given without prescription included topical clotrimazole, oral cannabis, and commercial supplements for the urinary tract.

Prescribed oral supplementation was given to 27.66% of animals (n=13), where 5 patients (10.64%) received supplements for the musculoskeletal system based on chondroitin, 3 Patients (6.38%) received supplements based on Omegas, while other vitamin, cannabinoid and nutraceutical supplements were each given to one animal.

Only one dog presented neutropenia: a female canine with a neoplastic condition cursing with

pancytopenia. This same patient was the only one with a decreased body condition.

Pet-human interaction factors

On average, the households of the animals participating in the study consisted of 2.89 ± 1.34 persons, and feline ownership was more common in single-parent households or childless couples. Approximately, 30% of the canines (n=13) belonged to childless couples, and close to 20% (n=8) couples with children, which were mostly made up of elderly parents with adult children. In fact, 40% of the animals (n=19) lived with adults over 60 years old, and only one ani-

mal lives with children under 5 years old.

Bivariate analysis

Resistance: none of the variables studied had a statistically significant association with resistance in companion animals ($p < 0.05$). However, considering a p-value of up to 0.25 we found that the prevalence of resistance increased by 21% in animals that consumed supplements/vitamins and decreased by 20% in animals with increased body condition scores compared to those with normal body condition scores. Other variables are presented in Table 2.

Table 2. Bivariate association analyses between resistance and clinical, zoographic, zootechnic and pet-human interaction factors with antimicrobial resistance in pets.

Variable	Resistance				P value	PR (CI 05%)
	Yes		No			
	n	%	n	%		
Sex						
Female	22	53.66	5	83.33	0.22	0.85 (0.69 – 1.05)
Male	19	46.34	1	46.34		1
Body condition score (from 1 to 5)						
Under ideal (Score 1 – 2)	1	2.44	0	0	0.19	-
Ideal (Score 3)	24	58.54	6	100		1
Over ideal (Score 4 – 5)	16	39.02	0	0		0.8 (0.66 – 0.95)
Other animals in the household						
Yes	28	68.29	2	33.33	0.17	1.22 (0.92 – 1,62)
No	13	31.71	4	66.67		1
Walker						
Yes	4	9.76	2	33.33	0.16	0.73 (0.41 – 1.31)
No	37	90.24	4	66.67		1
International traveling						
Yes	1	2.44	1	16.67	0.24	0.56 (0.14 – 2.26)
No	40	97.56	5	83.33		1
Supplement/vitamin consumption						
Yes	13	31.71	0	0	0.16	1.21 (1.04 – 1.42)
No	28	68.29	6	100		1

MDR: Animals who wake people up by kissing had a significant increase of 91% in the prevalence of MDR, in contrast, cohabitation with the elderly reduced this outcome by 54%. At the same time, having contact with production animals and consuming supplements/vitamins increased by 50% and 74%, respectively the pre-

valence of MDR with a p-value at the border of statistical significance. Also, hospitalization one month prior to sample collection and culture taken from infected surgical wounds double the prevalence with a non-significant value (p-value under 0.25 but greater than 0,05) (Table 3)

Table 3. Bivariate association analyses between multidrug resistance and clinical, zoographic, zoo-technic and pet-human interaction factors with antimicrobial resistance in pets.

Multidrug resistance						
Variable	Yes		No		P Value	PR (CI 05%)
	n	%	n	%		
Sex						
Female	12	48	15	68,18	0.16	0.68 (0.4-1.16)
Male	13	52	7	31,82		1
Guardian's professions (animal/human health)						
Yes	9	36	3	13,64	0.10	1.64 (1.01 – 2.67)
No	16	64	19	86,36		1
Contact with production animals						
Yes	5	20	0	0	0.05	2.10 (1.53 – 2.88)
No	20	80	22	100		1
International traveling						
Yes	0	0	2	9,09	0.21	0
No	25	100	20	90,91		
Hospitalization a month prior sample						
Yes	4	16	0	0	0.11	2.05 (1.51 – 2.78)
No	21	84	22	100		-
Supplement/vitamin consumption						
Yes	10	40	3	13,64	0.05	1.74 (1.08 – 2.82)
No	15	60	19	86,36		1
Surgical wound						
Yes	3	12	0	0	0.23	2.00 (1.49 – 2.69)
No	22	88	22	100		1
Owners educational stage						
Secondary education	2	8	0	0	0.18	1
Technical Education	7	28	3	13,64		0.7 (0.46 – 1.05)
College degree	16	64	19	86,36		0.45 (0.31 – 0.65)
Cohabitee with health personnel						
Yes	10	40	4	18,18	0.12	1.57 (0.95 – 2.59)
No	15	60	18	81,82		1
Cohabitee with elderly						
Yes	6	24	13	59,09	0.01*	0.46 (0.22 – 0.94)
No	19	76	9	40,91		1
Owners' previous knowledge about AMR						
Yes	13	52	6	27,27	0.13	1.60 (0.94 – 2.70)
No	12	48	16	72,73		1

Waking up people with kisses						
Yes	13	52	4	18,18	0.03*	1.91 (1.15 – 3.19)
No	12	48	18	81,82		1

Multivariate analysis

According to this model, animals that consume supplements have a 6.2 times greater risk

of presenting MDR, while animals that live with health personnel, whether they are the guardian or not, have a 4.65 times greater risk of this outcome (Table 4).

Table 4. Multivariate model explaining associated factors to multidrug resistance in companion animals.

	ORa	SE	Z	P Value	IC (95%)
Supplement/vitamin consumption	6.28	4.98	2.31	0.02	1.32 - 29.79
Cohabitee with health personnel	4.65	3.46	2.07	0.03	1.08 - 20.00
Constant	0.45	0.20	-.174	0.08	0.18 - 1.10
Log likelihood = -28.000386					
AIC= 62					

Discussion

Large-scale studies on AMR prevalence in pets are scarce even in the international literature. The available studies often focus on reporting resistance to specific antibiotics aiming to set epidemiological guidelines for when empirical treatments are required, rather than characterizing AMR and its driver in domestic species¹⁴.

In 2020, MDR in Medellín was reported as 18.7% in canines and 22% in felines⁷. In contrast, this study obtained a sensibility to all antimicrobials evaluated to be around 10% and MDR was close to 50%, results more comparable with one study performed in Tennessee, where MDR was 42,1% of isolated of canine *Staphylococcus*¹⁵, and one study in Portugal, were isolates from a veterinary hospital show a sensibility of 9,2% and an MDR of 60% in dogs and cats¹⁶. The resemblance to the European study can be attributed to their population characteristics, given that they also analyzed samples from canine and feline patients undergoing clinical infections. It does not change that, although the results coincide with international studies, a higher prevalence than reported in local studies was found.

Around 60% of pets were older than seven years old, 30% registered concomitant pathologies, mostly degenerative diseases, and most animals resided exclusively in urban areas. This, as well as the animal characterization present

in the descriptive analysis, agrees with the approach of Wieler et al¹⁰, depicting how changes in animal ownership affect zoographic, zootechnical, and clinical aspects of pet ownership, and how some populations groups like older or immunocompromised dogs can be more susceptible to develop ARM¹⁰. It must be contemplated if the increasing standard of living, health, and well-being in animals have influenced a phenomenon in companion animals alike the demographic and epidemiological transition that occurred in human populations after the industrial revolution, and if this were to be the case, how can it affect ARM in humans, animals, and the environment.

In terms of human risks, all identified isolations have zoonotic potential and may constitute a source for infection and sharing of ARGs among animals and humans. This risk is elevated by the site of infection and the ease of direct contact with the skin or products of the genitourinary and digestive system for the management of urine, saliva, and excreta¹⁷⁻¹⁹, and there is evidence of coincidences in bacterial strains and ARG profiles between pets and owners from the same households, suggesting transmission in home^{18,20}. In this regard, the most common isolated bacteria was *S. pseudointermedius*, with 42.18% of the isolations, mostly collected from the skin, and it has also been reported as the most common bacteria in local studies with isolation frequencies of 43,73% in canine skin sam-

ples²¹.

Some authors who share this approach highlight the risks of environment sharing with pets, and behaviors such as spending time on furniture or even sleeping in the same bed^{10,12,22}. And even when the role of ARGs transfer between companion animals and humans remains unknown, both as a reservoir of genes that AMR, or as a population in which genetic lineages with ARM can be developed^{9,10}, this risk should not be neglected and should be explored instead.

Some pet-human interaction factors were associated with MDR, and previous studies described how the strength of the human-pet bond translates into behaviors and decisions that have the potential to influence the transmission of AMR between humans and animals, and how owners acknowledge the risks but sometimes choose to overlook them in favor the attachment and affection demonstration^{12,23}. Such behaviors were frequent in this study, making clear how the closeness with companion animals has increased and more spaces are shared in the households.

Despite the criticism and ethical considerations that the above can generate, it is a real way of conceiving and living ownership for most people. In this case, proposed effective interventions must be based on knowledge of how the bond between people and animals works and properly analyzing its benefits and hazards while understanding that being subjected to the same environment and lifestyle carries different risks, whose effects on health are shared between species. For example, one of the most significant pet-human interaction factors in the study was cohabitation with health personnel, and when such a person is the owner, the probability of MDR is 4.64 times greater. This relationship may support the possibility of transfer of ARGs between humans and animals, but no studies were found that delve into this relationship.

Meanwhile, consumption of supplements or vitamins was associated with MDR in bivariate and multivariate analysis. No studies were found that explain this; however, Erin et al. reported that the administration of herbal products a month before a fecal sample increased the risk of resistance to at least two antimicrobials in canines (OR=3.37; p 0.029) the authors did not find previous reports of this association so due to the characteristics of the study, it was considered a probable proxy association, or a result of a type I error in the research. In this Canadian study,

glucosamine was the most frequently administered product¹¹ (11), and in the present research, this association occurred yet again and, among the supplements given, the majority are indicated for the musculoskeletal system that contains glucosamine within their composition, so it is a factor that should be explored in depth in future studies.

At the same time, other factors had to be explored, Huttner et al.⁵, for example, called attention to globalization and the movement of people and animals and its impact on AMR, this matches the prevalence ratio in animals with resistance and MDR found with history of international traveling. Also, the possible circulation of ARGs in animal health facilities supports the need to control nosocomial risks in veterinary medicine that affect animal health personnel and patients in these institutions, a risk that is often neglected^{10,24}. Therefore, beyond dismissing medical progress in veterinary, this growth must be combined with the adoption of measures to mitigate the risk of AMR, antimicrobial stewardship, and measures against nosocomial pathogens.

Additionally, no association was found with diet or feeding raw food, but the number of animals with such diet in the study was low. In contrast, isolations of antimicrobial-resistant Enterobacteriaceae related to animal feeding have been reported along with other microbiological hazards for animal diets s containing raw meat^{25,26}; Erin et al¹¹, found higher prevalence of resistant Salmonella spp and E. coli in household dogs fed with homemade food and raw food, while Yildiz M and Demirbilek found association between raw feeding and Salmonella spp carriage¹³. The association between AMR and the type of diet supplied to the animal must continue to be studied along with providing the owners with education on handling pet food, for this, the human-animal bond can be exploited because it is reported that people were more concerned about pets becoming ill from pet food than they were for humans becoming ill for the same reasons²⁷, and also Ma et al, reported that pet owners had better food safety behaviors: hand washing, kitchen cleaning, and food thermometer usage behaviors and most awareness of foodborne pathogens²⁸.

In conclusion, it zoographic and zootechnical factors had no significant association with resistance to at least one antimicrobial but tended to increase its prevalence in animals with some of those factors. Clinical and interaction with human factors were associated with or increa-

sed the prevalence of MDR. This supports the possibility of genetic transfer for AMR between animals and people and marks a path for further research in this field.

The statistical analysis was limited by the population size; however, a moderate non-significant association was found (p-value below 0.25) between resistance with mainly zoographic and zootechnical factors, and between MDR and clinical and pet-human interaction factors. In consequence, they could be driver factors AMR should be further studied. Another important limitation of this was the lack of databases or clinical records with the interest variables, this was controlled by having a prospective data collection from primary and secondary sources at sample size detriment. Additionally, the use of the disk diffusion method for culture and determination of antibacterial susceptibility limited the number of antimicrobials tested for each sample, and antimicrobial groups were selected by clinical criteria and could vary for each isolate.

This study opens the doors to a fundamental field for understanding the dynamics of AMR, not only with the aim of generating evidence in favor of companion animals but also public health in general. That is why this study is significant in terms of generating a conceptual and methodological contribution to develop a new line of transdisciplinary study between public health and veterinary medicine.

Acknowledgements

This manuscript is the result of a thesis submitted by Sara Yepes Orozco to the Universidad CES, Medellín, Colombia, to fulfill the requirements for a master's in public health degree. Therefore, the authors acknowledge this institution for the guide offered and wish to thank the veterinary hospital where this study was performed, Clínica Veterinaria El Poblado, for its support and for providing relevant data from medical records and the information about the animal antimicrobial susceptibility test. Always under the due consent from animal's owners.

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