



FACULTAD DE CIENCIAS DE LA SALUD

**“FRECUENCIA DE LA RESISTENCIA DE *Escherichia coli* A
ANTIMICROBIANOS Y SUS FACTORES DE RIESGO EN PERROS DE
DOS CLINICAS VETERINARIAS DE LIMA, PERÚ”**

Tesis para optar el grado académico de:

**MAESTRO EN EPIDEMIOLOGÍA CLÍNICA Y
BIOESTADÍSTICA**

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Para optar por el Grado de **Maestro en Epidemiología Clínica y Bioestadística**

Con la asesoría de: **Dra. María Jesús Pons Casellas**.

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INFORME DE REVISIÓN DE ORIGINALIDAD

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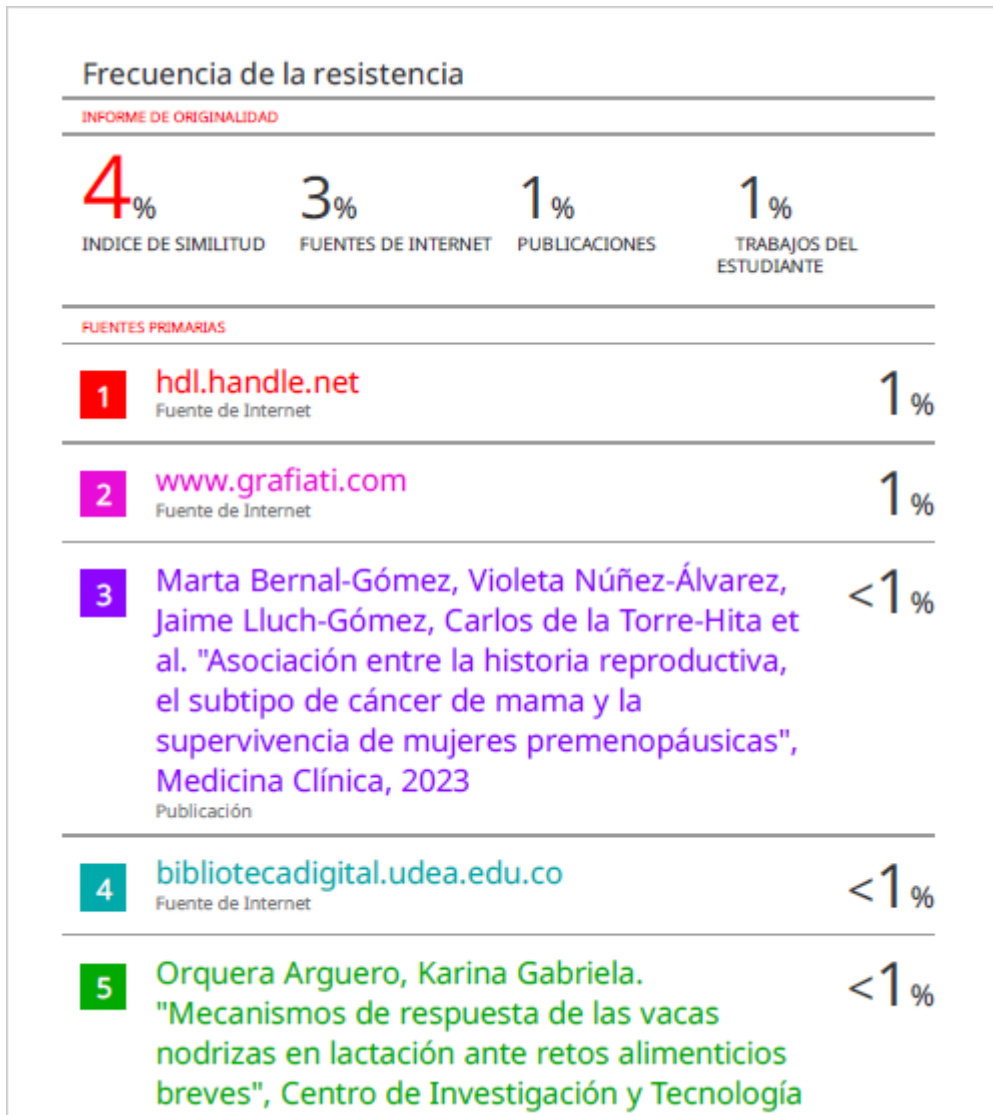
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RESUMEN

Antecedentes

Los sistemas de vigilancia de la resistencia a los antimicrobianos (RAM) en animales de compañía son escasos, especialmente en los países de ingresos medios-bajos. En Perú, la información sobre RAM en perros es limitada. Por ello, el objetivo del presente estudio fue establecer los niveles de RAM y sus factores asociados en perros clínicamente sanos atendidos en dos clínicas veterinarias de distritos socioeconómicos diferentes de Lima (Perú).

Métodos

Se incluyeron noventa perros en el estudio y se analizaron sus muestras fecales para detectar la presencia de *Escherichia coli*. Además, se evaluó la resistencia antimicrobiana de las muestras positivas de *E. coli* mediante el método de difusión en disco y se usó microdilución para determinar la susceptibilidad a colistina. Se caracterizó molecularmente los aislados que dieron positivo para β -lactamasas de espectro extendido (BLEE) y resistentes a la colistina. Se evaluaron las relaciones clonales de los aislados mediante electroforesis en gel de campo pulsado. Por último, se analizaron estadísticamente los factores asociados a la resistencia a los antimicrobianos.

Resultados

Se detectaron 35 (38,9%) aislados de *E. coli* a partir de 90 muestras de hisopados rectales de perros. Se detectaron niveles elevados de resistencia a la ampicilina (57,1%), ácido nalidíxico (54,3%), tetraciclina (48,6%) y azitromicina (25,7%). Los niveles de resistencia a las cefalosporinas fueron $\geq 20\%$ y 14,3% para la colistina. Se detectaron aislados productores de BLEE en 12 (34,2%) aislados; específicamente, 6 *bla*_{CTX-M-55} (50,0%), dos (16,6%) *bla*_{CTX-M-15}, y dos (16,6%) *bla*_{CTX-M-8-like}. Se observaron sustituciones de codones de aminoácidos en el gen *mgrB* (V8A) y mutaciones en el promotor de *mgrB* (a12g, g98t y c89t) relacionados con la resistencia a colistina en *E. coli* resistentes. Se observó que los aislados resistentes a la colistina mostraban una filogenia diversa, no mostrando una relación clonal. Además, se observaron asociaciones entre la edad del perro, <6 años ($p = 0,027$) y la dieta cruda ($p = 0,054$) con la presencia de resistencia a más familias de antibióticos.

Conclusión

Se determinó que los perros clínicamente sanos eran portadores frecuentes de *E. coli* multirresistente, incluidos aislados resistentes a antimicrobianos utilizados como último recurso como la colistina. La estrecha relación entre el propietario y su mascota realzan la importancia de conocer la presencia de bacterias resistentes en esta especie, pudiendo representar un problema de salud pública que podría comprometer el futuro tratamiento de infecciones en clínicas humanas y veterinarias.

Palabras clave: Resistencia a los antibióticos, animales de compañía, Perú, beta-lactamasas de espectro extendido, colistina, factores de riesgo.

ABSTRACT

Background

No established antimicrobial resistance (AMR) surveillance system exists in companion animals, especially in low-middle-income countries. In Peru, information about AMR in dogs is limited. Thus, dogs attended at two veterinary clinics in Lima (Peru) were studied to analyze the AMR status of *Escherichia coli* and its risk factors in dogs.

Methods

We included ninety dogs in the study and tested their fecal samples for *E. coli* presence. Additionally, we assessed the *E. coli* positive sample antimicrobial susceptibility by disk diffusion and used microdilution to determine colistin susceptibility. Also, we characterized molecularly all the isolates that tested positive for extended-spectrum β -lactamases (ESBL) and colistin-resistant. We assessed the isolates' clonal relationships by XbaI-pulsed-field gel electrophoresis. Finally, analysis of factors associated with antimicrobial resistance was performed.

Results

Thirty-five *E. coli* strains were isolated from 90 rectal swab samples from dogs. We detected high levels of resistance to ampicillin (57.1%), nalidixic acid (54.3%), tetracycline (48.6%), and azithromycin (25.7%). Cephalosporin resistance levels were $\geq 20\%$ and 14.3% for colistin. We detected ESBL-producing strains in 12 (34.2%) isolates; of these, six *bla*CTX-M-55 (50.0%), two (16.6%) *bla*CTX-M-15, and two (16.6%) *bla*CTX-M-8-like genes. We observed amino acid codon substitutions in the *mgrB* gene (V8A) and mutations in the *mgrB* promoter (a12g, g98t, and c89t) in colistin-resistant *E. coli*. We observed colistin-resistant isolates showed diverse phylogeny, being clonally unrelated. Furthermore, associations between dog age, <6 years ($p = 0.027$) and raw diet ($p = 0.054$) with resistance to more antibiotic families.

Conclusion

We found that dogs were frequent carriers of multidrug-resistant *E. coli*, including isolates resistant to last-resort antimicrobials. Thereby dogs could play a potential zoonotic role in antimicrobial resistance. This fact may represent a public health problem due to close contact between dogs and humans, which could compromise future infection treatment in human and veterinary clinics.

Keywords: Antibiotic resistance, companion animals, Peru, ESBL, colistin, risk factors

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RESEARCH ARTICLE

Antimicrobial resistance and associated factors in *Escherichia coli* isolated from Peruvian dogs: A focus on extended-spectrum β -lactamases and colistin

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Abstract

Background and Aim: Established antimicrobial resistance (AMR) surveillance in companion animals is lacking, particularly in low-middle-income countries. The aim of this study was to analyze AMR and its risk factors in *Escherichia coli* isolated from dogs at two veterinary centers in Lima (Peru).

Materials and Methods: Ninety dogs were included in the study. Antimicrobial susceptibility was established by disk diffusion, whereas microdilution was used to determine colistin susceptibility. Extended-spectrum β -lactamases (ESBL) and the mechanisms of colistin resistance were determined by polymerase chain reaction. Clonal relationships of colistin-

resistant isolates were assessed by *Xba*I-pulsed-field gel electrophoresis.

Results: Thirty-five *E. coli* strains were isolated. High levels of resistance to ampicillin (57.1%), nalidixic acid (54.3%), tetracycline (48.6%), and azithromycin (25.7%) were detected. Cephalosporin resistance levels were $\geq 20\%$ and 14.3%, respectively, for colistin. Twelve (34.2%) isolates were ESBL producers; of these, six *bla*CTX-M-55 (50.0%), 2 (16.6%) *bla*CTX-M-15, and 2 (16.6%) *bla*CTX-M-8-like genes were found. The five colistin-resistant isolates were clonally unrelated, with four of them presenting amino acid codon substitutions in the *mgrB* gene (V8A) or mutations in the *mgrB* promoter (a12g, g98t, and c89t). Furthermore, dog age, <6 years ($p = 0.027$) and raw diet ($p = 0.054$) were associated with resistance to a greater number of antibiotic families.

Conclusion: The dogs studied were carriers of multidrug-resistant *E. coli*, including last-resort antimicrobials, representing a public health problem due to close contact between dogs and humans.

Keywords: antibiotic resistance, colistin, dogs, extended-spectrum β -lactamases, Peru, risk factor.

<H1>Introduction

Antimicrobial resistance (AMR) is a significant public health threat worldwide, highlighting the health crisis linked to the worldwide increase in infections caused by pathogens resistant to

last-line antibiotics [1]. In this sense, companion animals are often exposed to antibiotics because of the treatment of infections or prophylaxis [2] or through the consumption of food products [3], increasing selective pressure and leading to the development of multidrug resistance in their commensal bacteria [4]. Thus, pets can be reservoirs and hosts for resistant bacteria, raising concerns about the risks to both human and animal health [5].

Escherichia coli is a common member of the intestinal microbiota of humans and companion animals and is involved in many intestinal and extraintestinal infections [6, 7]. Because of its ubiquity and high facility for acquiring AMR genes, it is a good marker of antibiotic resistance [6–8]. In recent years, the emergence and rapid dissemination of *E. coli* producers of extended-spectrum β -lactamases (ESBL) [9, 10], carbapenemases [11, 12], and even the harboring of genes of resistance to last-resort antibiotics such as colistin [13] have become a paramount concern for animal health.

While a great variety of ESBLs have been described in the literature, the most widely distributed genes conferring resistance to broad-spectrum cephalosporins are the *bla*CTX-M genes. Among these, *bla*CTX-M-15 is the most common ESBL in most regions of the world, whereas others, such as *bla*CTX-M-27 and *bla*CTX-M-55, are particularly relevant in specific areas [14]. Furthermore, regarding carbapenemases, reports of *bla*NDM, *bla*KPC, and *bla*OXA-48 have increased among enterobacterial in recent years, with this increase also

being reported in veterinary microorganisms [15, 16]. Finally, regarding colistin resistance mechanisms, plasmid-mediated colistin resistance *mcr* genes and mutations in chromosomal genes have been frequently reported [17–19].

In small animals, antibiotic resistance and its associated factors have important implications for veterinary and public health [20]. However, scientific literature on this critical problem is scarce. In Peru, studies on the frequency of antibiotic-resistant microorganisms and their risk factors in companion animals are limited [8]. Thus, this study evaluated AMR levels and risk factors in dogs from veterinary clinics in Lima, Peru.

<H1>Materials and Methods

<H2>Ethical approval

This study was approved by the Institutional Ethics Committee in Research with Animals and Biodiversity and the Ethical Committee of the Universidad Científica del Sur (452-2020-POS99).

<H2>Study period and location

A cross-sectional study was conducted from January to July 2021 on clinically healthy dogs (i.e. Dogs attending for bathing or vaccination services) at two veterinary clinics in districts with different socioeconomic characteristics (Surco and Villa El Salvador) in Lima, Peru. Surco is one of the largest high-income residential districts of Lima (population ~400,000), whereas

Villa El Salvador is one of the largest low-income residential districts of Lima (population ~2,400,000). All dogs that had consumed antibiotics within the 3 months before sampling were excluded from the study. In addition, all the owners of the canine companion animals provided written informed consent for sampling their animals.

<H2>Sampling

Convenience sampling was performed. Rectal swab samples were collected from the dogs after obtaining the owner's consent. The samples were placed in Cary Blair preservation and maintained at 4–8°C during transportation to the Universidad Científica del Sur laboratories according to standardized protocols [5].

<H2>Epidemiologic information

The owners provided epidemiological information on the canine companion animals. The canine data collected included sex, age (classified into two groups: <6 years and ≥6 years), diet (raw meat diet/prepared dry diet/mixed/home diet), owner's history of antimicrobial use, and contact with other companion animals (yes/no).

<H2>*E. coli* isolation

Samples were homogenized and seeded on MacConkey plates (Oxoid, Basingstoke, UK) or in McConkey agar containing disks of ceftazidime (30 µg) at one end of the plate and incubated at 37°C for 24 h. Then, isolated colonies compatible with *E. coli* growing within the ceftazidime-

treated halos or without antibiotic pressure were selected and confirmed by polymerase chain reaction (PCR) amplifying the *E. coli*-specific *uidA* gene [21].

<H2>Antimicrobial susceptibility

Antimicrobial susceptibility to ampicillin (10 µg), amoxicillin plus clavulanic acid (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), aztreonam (30 µg), trimethoprim-sulfamethoxazole (25 µg), nitrofurantoin (300 µg), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), levofloxacin (5 µg), norfloxacin (10 µg), and meropenem (10 µg) was determined by the disk (Oxoid) diffusion method according to the clinical laboratory standard institute guidelines (CLSI) [22]. For azithromycin, a halo of ≤ 12 mm was considered to classify the isolates as resistant [23]. *E. coli* isolates were classified as multi-drug resistant (MDR) according to the classification of Magiorakos *et al.* [24] when resistant to at least three or more antibiotic families To establish the presence of ESBLs, disks of amoxicillin plus clavulanic acid, cefotaxime, and ceftazidime were disposed as reported previously [22]. In addition, colistin susceptibility was determined using the microdilution technique following the recommendations of CLSI [22] and the European Committee on Antimicrobial Susceptibility Testing [23]. When more than one *E. coli* was isolated from the same dog, only one isolate was included in the study, except when the AMR patterns differed

<H2>Antibiotic resistance genes

In isolates with an ESBL-positive phenotype, the presence of *bla*CTX-M (including *bla*CTX-M1, *bla*CTX-M2, *bla*CTX-M8, and *bla*CTX-M9) and *bla*SHV genes was determined by PCR [25]. The amplified products were recovered and purified using the EZNA Gel Extraction Kit (Omega Bio Tek, Norcross, GA) and sequenced (Macrogen, Seoul, South Korea) to determine the specific gene. Meanwhile, the genes involved in carbapenem resistance were amplified by PCR: *bla*VIM, *bla*IMP, *bla*KPC, *bla*NDM, and *bla*OXA-48 [26]. In the case of colistin-resistant isolates, the presence of transferable *mcr*-1 to *mcr*-5 genes was analyzed by PCR [27]. In addition, the presence of point mutations in chromosomal genes, such as *mgrB*, was assessed by PCR [28] and subsequent sequencing (Macrogen, Seoul, South Korea).

<H2>Phylogenetic and clonal analyses

The phylogenetic groups of the colistin-resistant isolates were established according to the Clermont extended scheme [29]. Clonal relationships among the colistin-resistant isolates were determined by pulsed-field gel electrophoresis (PFGE) of the total DNA digested with 40 U of *Xba*I restriction enzyme, as previously described [30]. DNA fragments were resolved in 1% agarose gels using the CHEF-DRIII system (Bio-Rad Laboratories Inc, Hercules, USA). The PFGE conditions used were at 6 V cm⁻² at 14°C and with a pulse time ranging from 1 s to 30 s for 19 h. Normalization and processing of the PFGE images and construction of the dendrogram were performed using GelJ v2.0 software (<https://sourceforge.net/projects/gelj/>)

[31]. A UPGMA tree was constructed using Dice similarity indices, complete linkage, 1% optimization, and position % position tolerance. Isolates were considered clonally related with a Dice similarity index $\geq 80\%$ [32].

<H2>Statistical analysis

A descriptive analysis was performed to summarize the qualitative variables according to their absolute and relative frequencies and the quantitative variables according to their mean and standard deviation. During this analysis, intermediate-resistant phenotypes were considered to be resistant. In addition, AMR-associated factors were assessed by modeling the number of resistant antimicrobial families using a Poisson regression model. ESBL and multidrug resistance were also evaluated separately as secondary outcomes of interest. Finally, we estimated the prevalence ratio (PR) as the magnitude of the association of interest. During these analyses, diet categories included dry, raw meat, mixed, and homemade; however, for the regression analysis, we converted this variable to a dichotomous variable (raw vs. no raw diet). All statistical analyses were performed using the STATA MP v14.0 statistical package (Stata Corp LP, College Station, Texas), and each estimate was reported with their respective 95% confidence intervals (CI).

<H1>Results

<H2>Study population

Fecal samples from 90 dogs attending two veterinary clinics from different districts of Lima (Peru) were analyzed: 46 from Villa El Salvador (51.1%) and 44 from Surco (48.9%) (Table-1). Most samples were collected from female dogs (73.3%), those aged 6 years or older (51.1%), and dogs that lived with other pets (87.8%). Most dog diets were based on prepared dry food (52.2%), followed by raw meat (25.6%), mixed food (18.9%), and home food (3.3%). Regarding the owner's history of antimicrobial use, 44.4% of the animal owners had consumed antibiotics within the last month.

<H2>***E. coli* isolation and identification**

Of the 90 fecal analyzed samples, 35 (38.9%) were *E. coli*-positive (of these, eight strains were isolated with the presence of CAZ and 27 from the medium without antibiotic disk). The characteristics of the dogs are shown in Table-1. When comparing the characteristics of the animals with *E. coli*-positive versus *E. coli*-negative fecal samples, there was a significantly frequent history of owners who had consumed antibiotics in the last month among the samples positive for *E. coli* (65.7% vs. 30.9%, $p = 0.001$) (Table-1).

<H2>**Antibiotic susceptibility**

The isolates showed high levels of resistance to antibiotics and were susceptible only to nitrofurantoin. The highest levels of resistance were to ampicillin (57.1%), nalidixic acid (54.3%), tetracycline (48.6%), levofloxacin (34.3%), and norfloxacin (31.4%). Resistance

levels to cephalosporins were >20%, and the ESBL phenotype was detected in 12 isolates (34.3%). Meanwhile, colistin resistance reached 14.3%, with all five colistin-resistant isolates presenting a minimum inhibitory concentration (MIC) of 4 µg/mL (Figure-1). Twenty (57.1%) isolates were classified as MDR.

Regarding antibiotic resistance patterns, 3 (8.57%) isolates showed resistance to seven antimicrobial agents of different groups, 5 (14.28%) isolates showed resistance to six antimicrobial agents of different groups, and 3 (8.57%) isolates showed resistance to four and three antimicrobials, respectively. In addition, 1 (2.86%) isolate presented resistance to 2 antimicrobial agent groups and 5 (14.28%) isolates to one antimicrobial group. Note that 4 (11.4%) isolates did not present resistance to any antimicrobial agent (Supplementary Table-S1).

<H2>ESBL characterization

Twelve ESBL-producing *E. coli* isolates were detected (13.3% of the tested samples and 34.3% of the *E. coli*-positive samples). The *bla*CTX-M-1G gene was amplified in 8 (66.7%) patients, whereas no *bla*SHV was detected. The variant *bla*CTX-M-55 was detected in six isolates, and *bla*CTX-M-15 was identified in the remaining two isolates. In addition, in two isolates *bla*CTX-M-8G was amplified, and three isolates were positive for universal *bla*CTX-M, but no variant was determined. Finally, none of the ESBL-encoding genes sought were

amplified in the two remaining isolates.

<H2>Carbapenemase characterization

All isolates presented an intermediate phenotype to carbapenems. No positive results were detected among the carbapenemase genes assessed.

<H2>Colistin characterization

Five isolates were colistin-resistant, and mutations in the *mgrB* gene were found in four isolates. Three of these isolates presented alterations in the promoter region, including two possessing the base changes a-12→g and g-98→t and the remaining isolate showing the change c-89→t. Meanwhile, one isolate presented an amino acid codon substitution V8A (Figure-2). In addition, no *mcr* genes were detected.

<H2>Phylogeny and clonal relationship between colistin-resistant isolates

The five colistin-resistant *E. coli* isolates were clonally unrelated. Three isolates belonged to group B1, and the other two belonged to groups D and B2, respectively (Figure-2).

<H2>Factors associated with AMR

Regression analysis showed that animals aged 6 years presented *E. coli* isolates with resistance to a lower number of antimicrobial families (PR = 0.41; 95% CI: 0.19–0.90; p = 0.027). Although marginally significant (p = 0.054), dogs consuming raw food presented *E. coli* resistant to a higher number of antimicrobial families. The study did not have sufficient power

to perform a multivariate Poisson regression model; likewise, no factor associated with ESBL or multidrug resistance was identified (Table-2).

<H1>Discussion

The present results demonstrate that companion animals, such as dogs, have antimicrobial-resistant *E. coli* that may be transmitted between animals and humans due to direct or indirect contact. Significantly high levels of antibiotic resistance were observed in canine *E. coli* isolates, with over half of the isolates being resistant to ampicillin and over half resistant to nalidixic acid and tetracycline. Moreover, approximately 40% of the isolates were cephalosporine-resistant and levofloxacin. Resistance levels to these antibiotics are important because these drugs are mainly used in human and veterinary medicine. Furthermore, this study highlights the presence of *E. coli* resistant to carbapenems and polymyxins, which is of concern because these antibiotic families are considered last resort antimicrobial treatment in human infections. In addition, we observed an association between dog age and the type of diet and the presence of *E. coli* resistance to a greater number of antibiotic families.

Previous studies characterizing resistance levels and mechanisms in *E. coli* recovered from companion animals in Peru are scarce [8]. However, studies conducted in Cajamarca (Northern Peru) [33] showed resistance levels of 61% for streptomycin and 51% for ampicillin, the antibiotics most commonly used in daily clinical practice in the region. The levels of

antibiotic resistance in the present study were similar to those reported from Minas Gerais, a populous state in Brazil [3], although they did not report colistin resistance.

Our results are consistent with the increase in ESBL-producing *E. coli* reported in dogs worldwide, mainly related to the high use of β -lactams in clinical practice in veterinary medicine [34, 35]. The percentage of samples possessing ESBL-producing *E. coli* was 13.3%, similar to that in other countries in the region, such as Ecuador [36]. In the comparison among isolates, we found 34.3% of ESBL producers, which is a lower percentage than that reported in Thailand [10].

Regarding CTX-M variants, various ESBLs were found in the study, with *bla*CTX-M-55 being the most frequent. In addition, CTX-M-15 and CTX-M-8G were found. It is of note that CTX-M-55 has been previously reported in Peru in studies including samples of diverse origins (healthy human and clinical samples, marketed food, drinking water, and animals including wild mammals, poultry, livestock, and companion animals) [37–39]. Moreover, in ESBL studies in this region, CTX-M-55 has been the gene most frequently reported [3]. It should also be noted that CTX-M-15, the dominant variant worldwide [40], was not very frequently identified in this study. Similarly, other studies in the area have described the same finding, suggesting that CTX-M-15 is not the predominant ESBL in Peru [37, 41]. CTX-M-8G, a rare ESBL group, has been previously detected in dog feces in Northern Peru [38]. It is worth mentioning that

some of the ESBL-positive strains did not amplify CTX-M, suggesting that other mechanisms may be involved, such as TEM or other less frequent ESBLs, a scenario that has already been reported in other cases in the country [25].

Colistin is used as a last resort for human clinical use against multiresistant bacteria, and it has not been marketed for veterinary use in small animals in the country since 2019 [42].

However, the results of our study showed that 14.3% of *E. coli* isolates were resistant to colistin, even in *E. coli* isolated from animals considered to be clinically healthy. In the previous studies, *mcr-1* carriage was associated with colistin resistance in *E. coli* from dogs and their owners [35]; however, resistance to colistin in the present study was probably related to chromosomal mutations, which were reported as a major mechanism in other Enterobacteria such as *Klebsiella pneumoniae* [43]. In this sense, three of five colistin-resistant isolates possessed mutations in the promoter region of *mgrB*, and another isolate possessed the amino acid substitution V8A. While no data about the effect of mutations in the promoter region of *mgrB* have been found in the literature, alterations in the promoter region of genes have the potential to affect (increasing, decreasing, or avoiding) the final expression levels of subsequently encoded peptides [44]. However, while the role of these alterations in promoter regions in final colistin MIC levels cannot be ruled out, they are outside the PhoP box and do not affect the 10 regions or transcription start sites. Nevertheless, further studies are necessary

to elucidate the effects of these alterations [45]. Meanwhile, the amino acid substitution V8A has been described in both colistin-resistant and colistin-susceptible isolates, suggesting a polymorphism or little effect on final colistin MIC levels [46]. Furthermore, PFGE and phylogenetic analysis demonstrated that these strains were clonally unrelated. Thus, exposure to colistin, which has been used in the avian sector for many years as a possible source of exposure through the food chain, can be considered [47].

When exploring the factors associated with AMR, we observed that diet appears to be a critical factor in resistance to certain families of antimicrobials. Although a dry diet based on different types of proteins, carbohydrates, grains, and other components that undergo dehydration was reported to be consumed by most of the dogs in our study, we found an association between consumption of a raw diet and resistance to a higher number of antimicrobial families. Raw food as the basis of a dog diet has gained interest recently because of studies or cases of cardiac pathologies related to different types of preservatives or ingredients used in dry foods [48]. However, the consumption of raw meat has been associated with a high risk of antibiotic-resistant *E. coli* and other food-borne bacteria, such as *Salmonella* [49]. It has also been associated with MDR *E. coli* in dogs with a raw meat-based diet in Brazil [3], emphasizing the importance of surveillance and control of this type of food, the consumption of which has increased in recent years. Moreover, this study found younger age to be a risk factor for AMR,

despite age being related to longer exposure time to antimicrobials [50]. This fact could be related to the use of antimicrobials for treating diarrhea associated with dietary changes in puppies.

Close contact between pets, their owners, and the environment has led to the sharing of bacteria, including resistant bacteria [51]. Thus, the spread of bacteria from humans to animals and vice versa poses a risk for treating non-human infections and may compromise the treatment of infections in veterinary medicine. Therefore, the high consumption of antimicrobials by animal owners is noteworthy in our study, with more than 40% of the owners having consumed antibiotics in the previous month.

One of the limitations of this study was the lack of data related to the clinical history of the dogs from which the samples were collected, thereby precluding the analysis of the association between antibiotic resistance and underlying pathologies. Another limitation was that while the study power was sufficient to recognize the strongest associated factors, it was not sufficient to adjust a multivariate regression model.

<H1>Conclusion

Our study demonstrates that dogs can be an important source of antibiotic resistance genes, including ESBLs and those involved in resistance to antibiotics of last resort, such as colistin.

This is likely related to the high veterinary and dietary use of antibiotics, despite the prohibition

of their use. The results of this study provide a better understanding of the presence of antibiotic-resistant bacteria in companion animals. They also highlight the need for strategies to prevent the spread of resistant strains in small animal clinics and domestic settings.

<H1>Data Availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

<H1>Authors' Contributions

MJP, JR, and MV: Conceptualization. MV, RO, KE, FG, NV, ML, and BRB: Experimental work in the laboratory. MV and AMQ: Statistical analysis. MJP and JR: Funding Acquisition. MV, MJP, JR and YS: Writing – Original Draft. All authors have read, – reviewed, and approved the final version of manuscript.

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<H1>Competing Interests

The authors declare that they have no competing interests.

<H1>Publisher's Note

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Tables

Table-1: Epidemiological characteristics of the sampled dogs (total) and of those *Escherichia coli* positive.

Characteristics	<i>Escherichia coli</i>		Total (n = 90)
	Negative (n = 55)	Positive (n = 35)	
Districts			
Villa El Salvador district	28 (50.9)	18 (51.4)	46 (51.1)

Surco district	27 (49.1)	17 (48.6)	44 (48.8)
Age			
Age ≥6 years	30 (54.6)	16 (45.7)	46 (51.1)
Age <6 years	25 (45.5)	19 (54.3)	44 (48.8)
Sex			
Female	41 (74.6)	25 (71.4.)	66 (73.3)
Male	14 (25.4)	10 (28.6)	24 (26.7)
Diet			
Dry	29 (52.7)	18 (51.4)	47 (52.2)
Raw meat	16 (29.1)	7 (20.0)	23 (25.6)
Mixed	7 (12.7)	10 (28.6)	17 (18.9)
Home	3 (5.5)	0 (0.0)	3 (3.3)
Lives together with other pets	49 (89.1)	30 (85.7)	79 (87.8)
The owner consumed antibiotics in the last month	17 (30.9)*	23 (65.7)*	40 (44.4)
*p = 0.001			

Table-2: Factors associated with antimicrobial family resistance.

Associated factors	PR (95% CI)	p-value
Age		
<6 years	Ref	
≥6 years	0.41 (0.19–0.90)	0.027*
Raw diet		
No	Ref.	
Yes	1.78 (0.99–3.20)	0.054
Owner consumes antibiotics		
No	Ref.	
Yes	0.64 (0.35–1.19)	0.159
District		
Surco	Ref.	
Villa El Salvador	0.67 (0.36–1.25)	0.212
Sex		
Female	Ref.	

Male	0.86 (0.43–1.72)	0.678
Coexists with other animals		
No	Ref.	
Yes	0.96 (0.49–1.89)	0.912
PR=Prevalence ratio, *p < 0.05		

Figure Legends

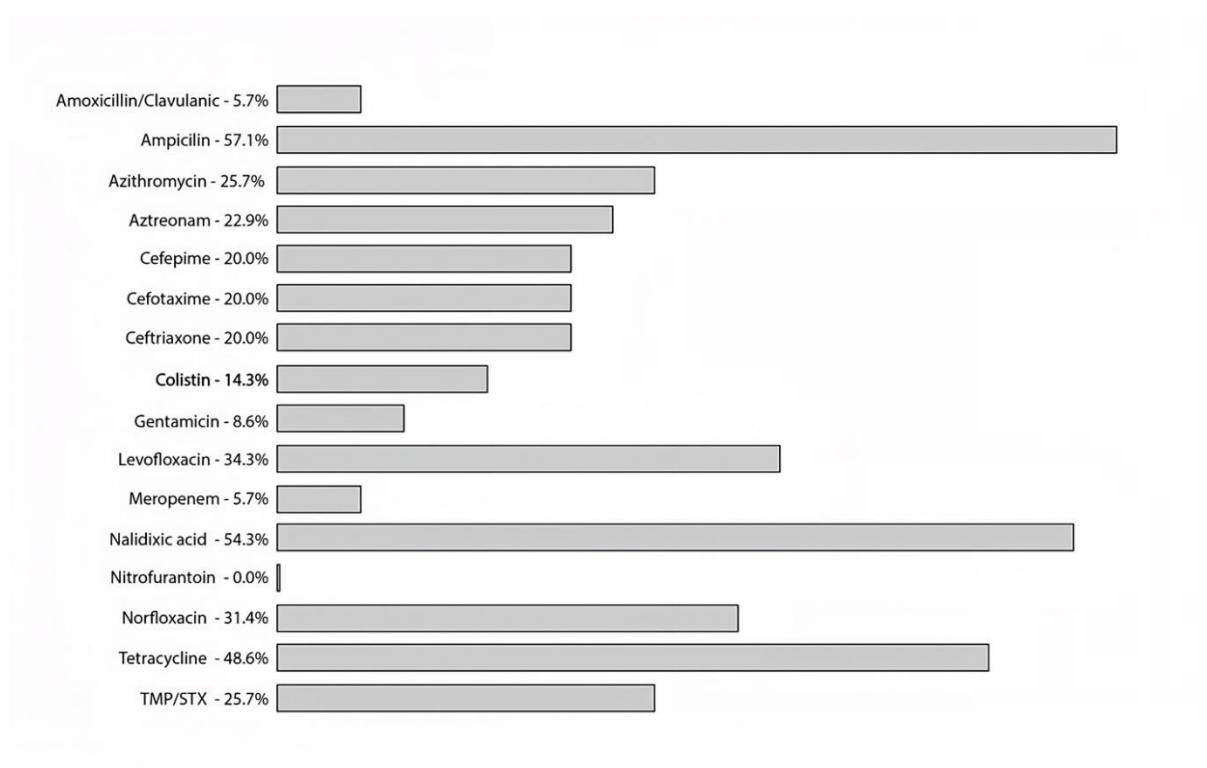


Figure-1: Percentage of resistance to different antibiotics in the 35 *Escherichia coli* isolates analyzed.

(TMP/SXT=Trimethoprim-sulfamethoxazole).

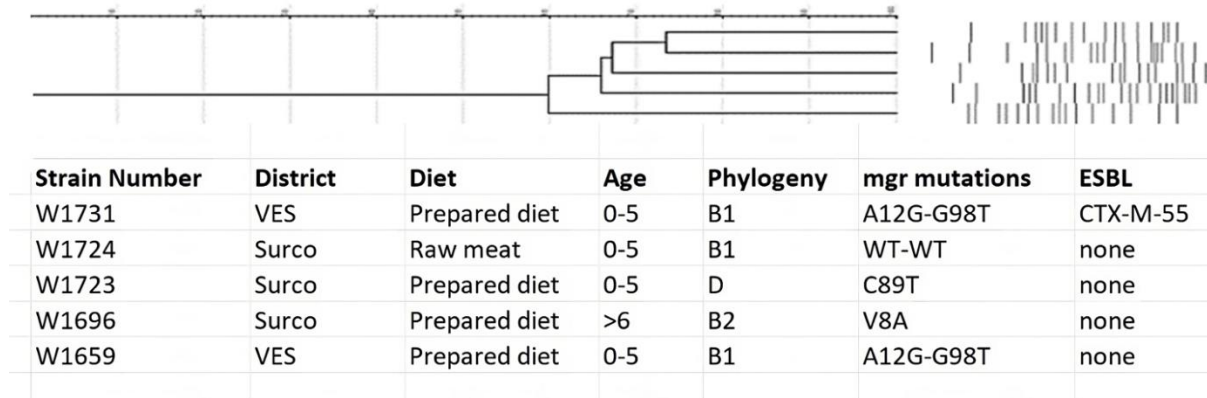


Figure-2: Clonal relationship and phylogeny of colistin-resistant isolates.

Supplementary Table

Table-S1: Antibiotic resistance patterns observed in the <i>Escherichia coli</i> isolates from fecal samples of dogs.		
No. Antibiotics	Antibiotic pattern	No. Isolates
1	AMP	4
	AZM	2
	Q	2

	CFS	1
	COL	1
2	TET, AZM	1
3	Q, AMP, AZM	2
	COL, TET, AZM	1
	TET, AMP, AZM	1
4	TET, Q, AMP, AZM	2
	COL, SXT, AMP, AZM	1
	CFS, ATM, TET, Q	1
6	AMC, CFS, ATM, TET, AMP, AZM	2
	CFS, ATM, SXT, TET, GEN, Q	2
	CFS, ATM, SXT, TET, Q, AMP	2
	CFS, ATM, SXT, TET, Q, AZM	2
	COL, CFS, ATM, SXT, Q, AMP	1
7	AMC, CFS, ATM, TET, Q, AMP, AZM	1
	CFS, ATM, SXT, TET, Q, AMP, AZM	1
	CFS, ATM, SXT, TET, GEN, Q, AMP	1
AMP=Ampicillin, AZM=Azithromycin, Q=Quinolones, CFS=Cephalosporines, COL=Colistin, TET=Tetracycline, SXT=Sulfamethoxazole/trimethoprim, ATM=Aztreonam, GEN=Gentamicin		

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Antimicrobial resistance and associated factors in *Escherichia coli* isolated among Peruvian dogs: focus on extended-spectrum β -lactamases and colistin

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ABSTRACT

Background and objective: Established antimicrobial resistance (AMR) surveillance in companion animals is lacking, especially in low-middle-income countries. The aim of this study was to analyze AMR and its risk factors, in *Escherichia coli* isolated in dogs attended at two veterinary centers in Lima (Peru).

Material and methods: Ninety dogs were included in the study. Antimicrobial susceptibility was established by disk diffusion while microdilution was used to determine colistin susceptibility. Extended-spectrum β -lactamases (ESBL) and the mechanisms of colistin-resistance were determined by polymerase chain reaction. Clonal relationships of colistin-resistant isolates were assessed by *Xba*I-pulsed-field gel electrophoresis in .

Results: Thirty-five *E. coli* strains were isolated. High levels of resistance to ampicillin (57.1%), nalidixic acid (54.3%), tetracycline (48.6%), and azithromycin (25.7%) were detected. Cephalosporin resistance levels were $\geq 20\%$ and 14.3% for colistin. Twelve (34.2%) isolates were ESBL-producers; of these, six *bla*_{CTX-M-55} (50.0%), two (16.6%) *bla*_{CTX-M-15}, and two (16.6%) *bla*_{CTX-M-8-like} genes were found. The five colistin-resistant isolates were clonally unrelated, with four of them presenting amino acid codon substitutions in the *mgrB* gene (V8A) or mutations in the *mgrB* promoter (a12g, g98t, and c89t). Furthermore, dog age, <6 years ($p = 0.027$) and raw diet ($p = 0.054$) were associated with resistance to a greater number of antibiotic families.

Conclusion: The dogs studied were carriers of multidrug-resistant *E. coli*, including last-resort antimicrobials, representing a public health problem due to close contact between dogs and humans.

Keywords Antibiotic resistance, dogs, Peru, ESBL, colistin, risk factor

Introduction

Antimicrobial resistance (AMR) is a significant public health threat worldwide, highlighting the health crisis linked to the worldwide increase in infections caused by pathogens resistant to last-line antibiotics [1]. In this sense, companion animals are often exposed to antibiotics due to the treatment of infections or prophylaxis [2] or through the consumption of food products [3], increasing selective pressure and leading to the development of multidrug resistance in their commensal bacteria [4]. Thus, pets can be reservoirs and hosts for resistant bacteria, raising concerns about the risks to both human and animal health [5].

Escherichia coli is a common member of the intestinal microbiota of humans and companion animals and is involved in many intestinal and extra-intestinal infections [6,7]. Due to its ubiquity and high facility for acquiring antimicrobial resistance genes, it has been considered a good marker of antibiotic resistance [6,7,8]. In the last years, the emergence and rapid dissemination of *E. coli* producers of extended-spectrum β -lactamases (ESBL) [9,10], carbapenemases [11,12], and even the harboring of genes of resistance to last-resort antibiotics such as colistin [13], are considered of paramount concern of animal health.

While a great variety of ESBLs are described in the literature, the most widely distributed genes conferring resistance to broad-spectrum cephalosporins are the *bla*_{CTX-M} genes. Among these, *bla*_{CTX-M-15} has been considered the most common ESBL in most regions of the world, while others, such as *bla*_{CTX-M-27} and *bla*_{CTX-M-55}, are especially relevant in specific areas [14]. Furthermore, regarding carbapenemases, reports of *bla*_{NDM}, *bla*_{KPC}, and *bla*_{OXA-48} have increased among enterobacteria in recent years, with this increase also being reported in veterinary microorganisms [15,16]. Finally, regarding colistin resistance mechanisms, plasmid-mediated colistin resistance *mcr* genes and mutations in chromosomal genes are frequently reported [17-19].

In small animals, antibiotic resistance and its associated factors have important implications for veterinary and public health [20]. However, the scientific literature on this critical problem is scarce. In Peru, studies on the frequency of antibiotic-resistant microorganisms and their risk factors in companion animals are limited [8]. Thus, this study aimed to evaluate antimicrobial resistance levels and the risk factors in dogs from veterinary clinics in Lima, Peru.

Material and methods

Study

A cross-sectional study was carried out in clinically healthy dogs (i.e.: dogs attending for bathing or vaccination services), at two veterinary clinics in districts with different socioeconomic characteristics (Surco and Villa El Salvador) in Lima, Peru, from January to July 2021. Surco is one of the largest high-income residential districts of Lima (population ~400,000), while Villa El Salvador is one of the largest low-income residential districts of Lima (population ~2,400,000). All the dogs that had consumed antibiotics within the three months before sampling were excluded from the study. In addition, all the owners of the canine companion animals gave written informed consent for sampling their animals.

Samples

Convenience sampling was performed. Rectal swab samples were collected from the dogs after obtaining the owner's consent. The samples were placed in Cary Blair preservation and maintained at 4-8 °C during transportation to the Universidad Científica del Sur laboratories according to standardized protocols [5].

Epidemiologic information

The owners provided epidemiological information about the canine companion animals. The

canine data collected included sex, age (classified into two groups: < 6 years and ≥ 6 years), diet (raw meat diet/ prepared dry diet/ mixed/ home diet), owner's history of antimicrobial use, and contact with other companion animals (yes/no).

***Escherichia coli* isolation**

Samples were homogenized and seeded on MacConkey plates (Oxoid, Basingstoke, UK), or in McConkey agar containing disks of ceftazidime (30 μg) at one end of the plate, and incubated at 37°C for 24 hours. Then, isolated colonies compatible with *E. coli* growing within the halos for ceftazidime or without antibiotic pressure were selected and confirmed by polymerase chain reaction (PCR) amplifying the *E. coli*-specific *uidA* gene [21].

Antimicrobial susceptibility

Antimicrobial susceptibility to ampicillin (10 μg), amoxicillin plus clavulanic acid (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cefepime (30 μg), aztreonam (30 μg), trimethoprim-sulfamethoxazole (TMP/SXT) (25 μg), nitrofurantoin (300 μg), tetracycline (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), levofloxacin (5 μg), norfloxacin (10 μg), and meropenem (10 μg) was determined by the disk (Oxoid), diffusion method according to the Clinical Laboratory Standard Institute guidelines (CLSI) [22]. For azithromycin, a halo of ≤ 12 mm was considered to classify the isolates as resistant [23]. *E. coli* isolates were classified as multi-drug resistant (MDR) according to the classification of Magiorakos *et al.* when resistant to at least three or more antibiotic families [24]. To establish the presence of ESBLs, the disks of amoxicillin plus clavulanic acid, cefotaxime, and ceftazidime were disposed as reported previously [22]. In addition, colistin susceptibility was determined using the microdilution technique following the recommendations of the CLSI [22] and the European Committee on Antimicrobial Susceptibility Testing [23]. When more than one *E. coli* was isolated from the same dog, only one isolate was included in the study, excepting when the antimicrobial

resistance patterns differed

Antibiotic resistance genes

In isolates with an ESBL-positive phenotype, the presence of *bla*_{CTX-M} (including *bla*_{CTX-M1}, *bla*_{CTX-M2}, *bla*_{CTX-M8}, *bla*_{CTX-M9}) and *bla*_{SHV} genes was determined by PCR [25]. The amplified products were recovered and purified using the EZNA Gel Extraction Kit (Omega Bio Tek, Norcross, GA) and sequenced (Macrogen, Seoul, South Korea) to determine the specific gene. Meanwhile the genes involved in carbapenem resistance were amplified by PCR: *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48} [26]. In the case of colistin-resistant isolates, the presence of transferable *mcr-1* to *mcr-5* genes was analyzed by PCR [27]. In addition, the presence of point mutations in chromosomal genes, such as *mgrB*, was assessed by PCR [28] and subsequent sequencing (Macrogen, Seoul, South Korea).

Phylogeny and clonal analysis

The phylogenetic groups of the colistin-resistant isolates were established according to the Clermont extended scheme [29]. Clonal relationships among the colistin-resistant isolates were determined by pulsed-field gel electrophoresis (PFGE) of the total DNA digested with 40U of *Xba*I restriction enzyme as previously described [30]. DNA fragments were resolved in 1% agarose gels using the CHEF-DRIII system (Bio-Rad Laboratories Inc, Hercules, USA). The PFGE conditions used were at 6 V cm⁻² at 14°C and with a pulse time ranging from 1 s to 30 s during 19 h. Normalization and processing of PFGE images and the construction of the dendrogram were performed using the GelJ v2.0 software (<https://sourceforge.net/projects/gelj/>) [31]. A UPGMA tree was constructed using Dice similarity indices, complete linkage, optimization 1%, and position tolerance 1.3 %. Isolates were considered to be clonally related with a Dice similarity index ≥ 80 % [32].

Statistical analysis

A descriptive analysis was performed summarizing the qualitative variables according to their absolute and relative frequency and the quantitative variables according to their mean and standard deviation. During this analysis, intermediate-resistant phenotypes were considered as resistant. In addition, AMR-associated factors were assessed by modeling the number of resistant antimicrobial families using a Poisson regression model. ESBL and multidrug resistance were also evaluated separately as secondary outcomes of interest. Finally, we estimated the prevalence ratio (PR) as the magnitude of the association of interest. During these analyses, diet categories included dry, raw meat, mixed, and home-made; however, for the regression analysis we converted this variable to a dichotomous variable (raw vs. no raw diet) . All the statistical analyses were performed using the STATA MP v14.0 statistical package (Stata Corp LP, College Station, Texas), and each of the estimates was reported with their respective 95% confidence intervals (CI).

Results

Study population

Fecal samples from 90 dogs attending two veterinary clinics from different districts of Lima (Peru) were analyzed: 46 from Villa El Salvador (51.1%) and 44 from Surco (48.9%) (Table 1). Most samples were collected from female dogs (73.3%), six years or older (51.1%), and dogs that lived with other pets (87.8%). Most dog diets were based on prepared dry food (52.2%), followed by raw meat (25.6%), mixed food (18.9%), and home food (3.3%). Regarding the owner's history of antimicrobial use, 44.4% of the animal owners had consumed antibiotics within the last month.

Escherichia coli isolation and identification

Of the 90 fecal analyzed samples, 35 (38.9%) were *E. coli*-positive (of these, 8 strains were isolated with the presence of CAZ and 27 from the medium without antibiotic disk). The

characteristics of the dogs are shown in Table 1. When comparing the characteristics of the animals with *E. coli* positive vs. negative fecal samples, there was a significantly frequent history of owners who had consumed antibiotics in the last month among the samples positive for *E. coli* (65.7% vs. 30.9%, $p = 0.001$) (Table 1).

Antibiotic susceptibility

The isolates showed high levels of resistance to antibiotics, being susceptible only to nitrofurantoin. The highest levels of resistance were to ampicillin (57.1%), nalidixic acid (54.3%), tetracycline (48.6%), levofloxacin (34.3%), and norfloxacin (31.4%). The resistance levels to cephalosporins were greater than 20%, and the ESBL phenotype was detected in 12 isolates (34.3%). Meanwhile, colistin resistance reached levels of 14.3%, with all five colistin-resistant isolates presenting a minimum inhibitory concentration (MIC) of 4 µg/ml (Figure 1). Twenty (57.1%) of the isolates were classified as MDR.

Regarding antibiotic resistance patterns, 3 (8.57%) isolates showed resistance to 7 antimicrobial agents of different groups, 5 (14.28%) isolates to 6 antimicrobial agents of different groups, and 3 (8.57%) isolates showed resistance to 4 and 3 antimicrobials, respectively. Additionally, 1 (2.86%) isolate presented resistance to 2 antimicrobial agent groups and 5 (14.28%) isolates to 1 antimicrobial group. It should be noted that 4 (11.4%) isolates did not present resistance to any antimicrobial (Supplemental material S1).

ESBL characterization

Twelve ESBL-producing *E. coli* isolates were detected (13.3% of the tested samples and 34.3% of the *E. coli*-positive samples). The *bla*_{CTX-M-1G} gene was amplified in eight (66.7%) while no *bla*_{SHV} was detected. The variant *bla*_{CTX-M-55} was detected in six isolates and *bla*_{CTX-M-15} was identified in the remaining two isolates. Additionally, in two isolates *bla*_{CTX-M-8G} was amplified, and three isolates were positive for universal *bla*_{CTX-M} but no variant was determined.

Finally, none of the ESBL-encoding genes sought were amplified in the two remaining isolates.

Carbapenemase characterization

All the isolates presented an intermediate phenotype to carbapenems. No positive results were detected among the carbapenemase genes assessed.

Colistin characterization

Five isolates were colistin-resistant, and mutations in the *mgrB* gene were found in four. Three of these isolates presented alterations in the promoter region, including two possessing the base changes a-12→g and g-98→t and the remaining isolate showing the change c-89→t. Meanwhile, one isolate presented the amino acid codon substitution V8A (Figure 2). Additionally, no *mcr* genes were detected.

Phylogeny and the clonal relationship between colistin-resistant isolates.

The five colistin-resistant *E. coli* isolates were clonally unrelated. Three isolates belonged to group B1, and the other two belonged to groups D and B2, respectively (Figure 2).

Factors associated with antimicrobial resistance.

Regression analysis showed that animals six years or older presented *E. coli* isolates with resistance to a lower number of antimicrobial families (PR = 0.41; 95% CI: 0.19 – 0.90; $p = 0.027$). While marginally significant ($p = 0.054$), dogs consuming raw food presented *E. coli* resistant to a higher number of antimicrobial families. The study did not have enough power to perform a multivariable Poisson regression model; likewise, no factor associated with ESBL or multidrug resistance was identified (Table 2).

Discussion

The present results demonstrate that companion animals, such as dogs, have antimicrobial-resistant *E. coli* that might be transmitted between animals and humans due to direct or indirect contact. Significantly high levels of antibiotic resistance were observed in canine *E. coli* isolates, with over half of the isolates being resistant to ampicillin and over half resistant to nalidixic acid and tetracycline. Moreover, around 40% of the isolates were resistant to cephalosporines and levofloxacin. Resistance levels to these antibiotics are important because these drugs are used mainly in human and veterinary medicine. Furthermore, this study highlights the presence of *E. coli* resistant to carbapenems and polymyxins, which is of concern since these antibiotic families are considered for last resort antimicrobial treatment in human infections. In addition, we observed an association between dog age and the type of diet and the presence of *E. coli* resistance to a greater number of antibiotic families.

Previous studies characterizing resistance levels and mechanisms in *E. coli* recovered from companion animals in Peru are scarce [8]. However, studies carried out in Cajamarca (Northern Peru) [33], showed resistance levels of 61% for streptomycin and 51% for ampicillin, the antibiotics most commonly used in daily clinical practice in the region. The levels of antibiotic resistance in the present study were similar to reports from Minas Gerais, a populous state in Brazil, [3], although they did not report colistin resistance.

Our results are consistent with the increase of ESBL-producing *E. coli* reported in dogs worldwide, mainly related to the high use of β -lactams in clinical practice in veterinary medicine [34,35]. The percentage of samples possessing ESBL-producing *E. coli* was 13.3%, similar to other countries in the region, such as Ecuador [36]. In the comparison among isolates, we found 34.3% of ESBL producers, which is a lower percentage compared to reports in Thailand [10].

Regarding CTX-M variants, a variety of ESBL was found in the study, with *bla*_{CTX-M-55} being the

most frequent. Also, CTX-M-15 and CTX-M-8G were found. It is of note that CTX-M-55 has been previously reported in Peru in studies including samples of diverse origins (healthy human and clinical samples, marketed food, drinking water, and animals including wild mammals, poultry, livestock, and companion animals) [37-39]. Moreover, in ESBL studies in the region, CTX-M-55 has been the gene most frequently reported [3]. It should also be noted that CTX-M-15, the dominant variant worldwide [40], was not very frequently identified in the present study. Similarly, other studies in the area have described the same finding, suggesting that CTX-M-15 is not the predominant ESBL in Peru [37, 41]. Interestingly, CTX-M-8G, a rare ESBL group, has been previously detected in dog feces in northern Peru [38]. It is worth mentioning that some of the ESBL-positive strains did not amplify CTX-M, suggesting that other mechanisms may be involved, such as TEM, or other less frequent ESBLs, being a scenario that has already been reported in other cases in the country [25].

Colistin is used as a last resort for human clinical use against multiresistant bacteria, and it has not been marketed for veterinary use in small animals in the country since 2019 [42]. However, the results of our study show a surprising 14.3% of *E. coli* isolates resistant to colistin, even in the *E. coli* isolated from animals considered to be clinically healthy. In previous studies, *mcr-1* carriage was associated with colistin resistance in *E. coli* from dogs and their owners [35]; however, resistance to colistin in the present study was probably related to chromosomal mutations, which were reported as a major mechanism in other Enterobacterias such as *Klebsiella pneumoniae* [43]. In this sense, 3 out of 5 colistin-resistant isolates possessed mutations in the promoter region of *mgrB* and another isolate possessed the amino acid substitution V8A. While no data about the effect of mutations in the promoter region of *mgrB* have been found in the literature, alterations in the promoter region of genes has the potential to affect (increasing, decreasing, or avoiding) final expression levels of subsequent encoded peptides [44]. However, while the role of these alterations in promoter regions in final

colistin MIC levels cannot be ruled out, they are outside the PhoP box and do not affect -10 region or transcription start sites. Nevertheless, further studies are necessary to elucidate the effects of these alterations [45]. Meanwhile, the amino acid substitution V8A has been described in both colistin-resistant and colistin-susceptible isolates, suggesting a polymorphism or a little effect on final colistin MIC levels [46]. Furthermore, the PFGE and phylogeny analysis demonstrated that these strains were clonally unrelated. Thus, exposure to colistin, which was used in the avian sector for many years as a possible source of exposure through the food chain, can be considered [47].

When exploring the factors associated with antimicrobial resistance, we observed that diet appears to be a critical factor in resistance to certain families of antimicrobials. Although a dry diet based on different types of proteins, carbohydrates, grains, and other components that undergo dehydration was reported to be consumed by most of the dogs in our study, we found an association between consumption of a raw diet and resistance to a higher number of antimicrobial families. Raw food as the basis of a dog diet has gained interest in recent years due to studies or cases of cardiac pathologies related to different types of preservatives or ingredients used in dry foods [48]. However, the consumption of raw meat has been associated with a high risk of antibiotic-resistant *E. coli* and other food-borne bacteria, such as *Salmonella* [49]. It has also been associated with MDR *E. coli* in dogs with a raw meat-based diet in Brazil [3], emphasizing the importance of surveillance and control of this type of food, the consumption of which has increased in recent years. Moreover, this study found younger age to be a risk factor for antimicrobial resistance, despite age being related to longer exposure time to antimicrobials [50]. This fact could be related to use of the antimicrobials in the treatment of diarrhea associated with dietary changes in puppies.

The close contact between pets and their owners and the environment has led to the sharing

of bacteria, including resistant bacteria [51]. Thus, the spread of bacteria from humans to animals and vice versa poses a risk for treating not only human infections but it may also compromise the treatment of infections in veterinary medicine. Therefore, the high consumption of antimicrobials by animal owners is noteworthy in our study, with more than 40% of the owners having consumed antibiotics in the previous month.

One of the limitations of this study was the lack of data related to the clinical history of the dogs from which the samples were collected, thereby precluding the analysis of associations of antibiotic resistance with underlying pathologies. Another limitation was that while the study power was sufficient to recognize the strongest associated factors, it was not enough to adjust a multivariable regression model.

To sum up, our study demonstrates that dogs can be an important source of antibiotic resistance genes, including ESBLs and those involved in resistance to antibiotics of last resort, such as colistin. This is likely related to high veterinary and dietary use of antibiotics, despite the prohibition of their use. The results of this study provides a better understanding of the presence of antibiotic-resistant bacteria in companion animals. They also highlight the need for strategies to prevent the spread of resistant strains in small animal clinics and the domestic setting.

Author Contributions

MJP, JR, MV- Conceptualization. MV, RO, KE, FG, NV, ML, BRB Experimental work in the laboratory MV, AMQ Statistical analysis MJP, JR- Funding Acquisition. MV, MJP, JR, YS Writing – Original Draft. All authors are writing – Review and approved the final version of manuscript.

Ethical issues

This study was approved by the Institutional Ethics Committee in Research with Animals and Biodiversity and the Ethical Committee of the Universidad Científica del Sur (452-2020-POS99).

Disclosure Statement

The authors report there are no competing interests.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Table 1: Epidemiological characteristics of the sampled dogs (total) and of those *Escherichia coli* positive.

Characteristics	<i>Escherichia coli</i>		Total (n = 90)
	Negative (n = 55)	Positive (n = 35)	
Districts			
Villa El Salvador district	28 (50.9)	18 (51.4)	46 (51.1)
Surco district	27 (49.1)	17 (48.6)	44 (48.8)
Age			
Age ≥6 years	30 (54.6)	16 (45.7)	46 (51.1)
Age <6 years	25 (45.5)	19 (54.3)	44 (48.8)
Sex			
Female	41 (74.6)	25 (71.4)	66 (73.3)
Male	14 (25.4)	10 (28.6)	24 (26.7)
Diet			
Dry	29 (52.7)	18 (51.4)	47 (52.2)
Raw meat	16 (29.1)	7 (20.0)	23 (25.6)
Mixed	7 (12.7)	10 (28.6)	17 (18.9)
Home	3 (5.5)	0 (0.0)	3 (3.3)
Lives together with other pets	49 (89.1)	30 (85.7)	79 (87.8)
The owner consumed antibiotics in the last month	17 (30.9)*	23 (65.7)*	40 (44.4)

* p -value = 0.001

Table 2: Factors associated with antimicrobial family resistance.

Associated factors	PR (95% CI)	p-valor
Age		
< 6 years	Ref.	
≥ 6 years	0.41 (0.19 - 0.90)	0.027*
Raw Diet		
No	Ref.	
Yes	1.78 (0.99 - 3.20)	0.054
Owner consumes antibiotics		
No	Ref.	
Yes	0.64 (0.35 - 1.19)	0.159
District		
Surco	Ref.	
Villa El Salvador	0.67 (0.36 - 1.25)	0.212
Sex		
Female	Ref.	
Male	0.86 (0.43 - 1.72)	0.678
Coexists with other animals		
No	Ref.	
Yes	0.96 (0.49 - 1.89)	0.912

PR: Prevalence Ratio, *p-value <0,05

Figure legends:

Figure 1: Percentage of resistance to different antibiotics in the 35 *Escherichia coli* isolates analyzed. (TMP/SXT: trimethoprim-sulfamethoxazole).

Figure 2. Clonal relationship and phylogeny of colistin-resistant isolates.

Supplemental material:

Table S1: Antibiotic resistance patterns observed in the *Escherichia coli* isolates from fecal samples of dogs.

No. ANTIBIOTICS	ANTIBIOTIC PATTERN	No. ISOLATES
	AMP	4
	AZM	2
	Q	2
	CFS	1
1	COL	1
2	TET, AZM	1

	Q, AMP, AZM	2
	COL, TET, AZM	1
3	TET, AMP, AZM	1
	TET, Q, AMP, AZM	2
	COL, SXT, AMP, AZM	1
4	CFS, ATM, TET, Q	1
	AMC, CFS, ATM, TET, AMP, AZM	2
	CFS, ATM, SXT, TET, GEN, Q	2
	CFS, ATM, SXT, TET, Q, AMP	2
	CFS, ATM, SXT, TET, Q, AZM	2
6	COL, CFS, ATM, SXT, Q, AMP	1
	AMC, CFS, ATM, TET, Q, AMP, AZM	1
	CFS, ATM, SXT, TET, Q, AMP, AZM	1
7	CFS, ATM, SXT, TET, GEN, Q, AMP	1

AMP: ampicillin, AZM: azithromycin, Q: quinolones, CFS: cephalosporines, COL: colistin, TET: tetracycline, SXT: sulfamethoxazole/trimethoprim, ATM: aztreonam, GEN: gentamicin

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Introducción

La resistencia a los antimicrobianos (RAM) es una importante amenaza para la salud pública en todo el mundo, destacando la crisis sanitaria vinculada al aumento mundial de infecciones causadas por patógenos resistentes a los antibióticos de última línea (Antimicrobial Resistance, 2022). En este sentido, los animales de compañía a menudo están expuestos a antibióticos debido al tratamiento de infecciones, profilaxis (Guardabassi *et al.*, 2004) o a través del consumo de productos alimenticios (Ramos *et al.*, 2022), hecho que aumenta la presión selectiva y conduce al desarrollo de resistencia a múltiples fármacos en sus bacterias comensales (Wu *et al.*, 2019). De esta manera los animales de compañía pueden ser reservorios y hospedadores de bacterias resistentes, lo que causa preocupación por los riesgos tanto para la salud humana como animal (Carvalho *et al.*, 2021).

Escherichia coli es un miembro común de la microbiota intestinal de humanos y animales de compañía y está implicada en muchas infecciones intestinales y extraintestinales (Bourne *et al.*, 2019; Jang *et al.*, 2017). Debido a su ubicuidad y alta facilidad para adquirir genes de resistencia a los antimicrobianos, se considera un buen marcador de exposición a la resistencia a los antibióticos (Bourne *et al.*, 2019; Castillo *et al.*, 2022; Jang *et al.*, 2017). En los últimos años, la aparición y rápida diseminación de *E. coli* productores de β -lactamasas de espectro extendido (ESBL) (Garcias *et al.*, 2021; Nittayasut *et al.*, 2021), carbapenemasas (Bandyopadhyay *et al.*, 2021; Cole *et al.*, 2020), además de cepas portadoras genes de resistencia a antibióticos de último recurso como la colistina (Sato *et al.*, 2022), se consideran una preocupación primordial para la salud animal.

Aunque en la literatura se describe una gran variedad de BLEEs, los genes más ampliamente distribuidos que confieren resistencia a las cefalosporinas de amplio espectro son los genes *bla*_{CTX-M}. Entre ellos, *bla*_{CTX-M-15} se ha considerado la BLEE más común en la mayoría de las regiones del mundo, mientras que otros, como *bla*_{CTX-M-27} y *bla*_{CTX-M-55}, son especialmente relevantes en áreas específicas (Bevan *et al.*, 2017). En cuanto a las carbapenemasas, *bla*_{NDM}, *bla*_{KPC}, y *bla*_{OXA-48} se ha reportado un aumentado entre las enterobacterias en los últimos años, también en veterinaria (Das S., 2023; Formenti *et al.*, 2021). Por último, en lo que respecta a los mecanismos de resistencia a la colistina, no sólo se informa con frecuencia de genes *mcr* de resistencia a la colistina mediada por plásmidos, sino también de mutaciones en genes cromosómicos (Naha *et al.*, 2022; Conceicao-Neto *et al.*, 2022; Ortega-Paredes *et al.*, 2019).

La resistencia a los antibióticos en animales pequeños y sus factores asociados tienen importantes implicaciones para la salud animal y pública (Lappin *et al.*, 2017). Sin embargo, la literatura científica sobre este problema crítico es escasa. En el Perú, los estudios sobre la frecuencia de microorganismos resistentes a antibióticos y sus factores de riesgo en animales de compañía son limitados (Castillo *et al.*, 2022). Por lo tanto, el objetivo de este estudio fue evaluar los niveles de resistencia antimicrobiana y los factores de riesgo en perros de clínicas veterinarias de Lima, Perú.

Materiales y Métodos

Estudio

Se realizó un estudio transversal y se evaluaron perros clínicamente sanos, es decir aquellos que acudían por servicio de baño o vacunas a dos clínicas veterinarias de los distritos de características socioeconómicas diferentes (Surco y Villa El Salvador) en Lima, Perú, de enero a julio de 2021. Surco es uno de los distritos residenciales de altos ingresos más grandes de Lima (población ~400,000),

mientras que Villa El Salvador es uno de los distritos residenciales de bajos ingresos más grandes de Lima (población ~2,400,000) (INEI, 2020). Se excluyeron del estudio todos los perros que habían consumido antibióticos en los tres meses anteriores al muestreo. Además, todos los propietarios de los perros dieron su consentimiento informado por escrito para la toma de muestras de sus animales.

Muestras

Se obtuvieron muestras de hisopados rectales de cada perro que acudía a las consultas o a los servicios de baño de las instalaciones correspondientes tras obtener el consentimiento del propietario. Las muestras se colocaron en medio Cary Blair para su conservación y se mantuvieron a 4-8 °C durante el transporte a los laboratorios de la Universidad Científica del Sur según protocolos estandarizados (Carvalho *et al.*, 2021).

Información epidemiológica

Los propietarios proporcionaron información epidemiológica sobre los animales de compañía. Los datos de los perros recogidos incluyeron sexo (hembra/macho), edad (< 6 años/≥ 6 años), dieta (dieta de carne cruda/ dieta seca preparada/ dieta mixta/ dieta casera), historial de uso de antimicrobianos por parte del propietario y convivencia con otros animales de compañía (sí/no).

Aislamiento de *Escherichia coli*

Las muestras se homogeneizaron y se sembraron en placas MacConkey (Oxoid, Basingstoke, Reino Unido), que contenían discos de ceftazidima (30 µg) en un extremo de la placa, y se incubaron a 37°C durante 24 horas. A continuación, se seleccionaron las colonias aisladas compatibles con *E. coli* que crecían dentro de los halos para ceftazidima o sin presión antibiótica y se confirmaron mediante reacción en cadena de la polimerasa (PCR) amplificando el gen *uidA* específico de *E. coli* (Ji *et al.*, 2010).

Sensibilidad antimicrobiana

Se determinó la susceptibilidad antimicrobiana para ampicilina, amoxicilina más ácido clavulánico, ceftriaxona, cefotaxima, cefepime, aztreonam, trimetoprim-sulfametoxazol (TMP/SXT), nitrofurantoína, tetraciclina, gentamicina, ácido nalidíxico, levofloxacino, norfloxacino y meropenem mediante el método de difusión en disco de acuerdo con las directrices del Clinical Laboratory Standard Institute (CLSI, 2021). Para la azitromicina, se consideró un halo de ≤ 12 mm para clasificar los aislados como resistentes (EUCAST, 2022). Los aislados de *E. coli* se clasificaron como multirresistentes (MDR) según la clasificación de Magiorakos *et al.* (Magiorakos *et al.*, 2012). Para establecer la presencia de BLEEs, se dispusieron los discos de amoxicilina más ácido clavulánico, cefotaxima y ceftazidima como se informó previamente (Palma *et al.*, 2017). Además, se determinó la susceptibilidad a la colistina mediante la técnica de microdilución siguiendo las recomendaciones del CLSI (CLSI, 2021) y del Comité Europeo de Pruebas de Susceptibilidad a los Antimicrobianos (EUCAST, 2022). Se incluyeron las *E. coli* que no presentaban el mismo patrón genético de resistencia procedentes del mismo perro para evitar duplicidades.

Genes de resistencia a los antibióticos

La presencia de los genes *bla*_{CTX-M} y *bla*_{SHV} se determinó mediante PCR (Palma *et al.*, 2017) en aquellos aislados con fenotipo positivo a BLEE. Los productos amplificados se recuperaron y purificaron utilizando el kit de extracción de gel EZNA (Omega Bio Tek, Norcross, GA) y se secuenciaron (Macrogen, Seúl, Corea del Sur) para determinar la variante del gen específico. Mientras tanto, se utilizó una PCR para amplificar los genes implicados en la resistencia a los carbapenemasas: *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{NDM}, y *bla*_{OXA-48} (Pons *et al.*, 2020). En el caso de aislados resistentes a colistina, se analizó mediante PCR la presencia de genes *mcr-1* a *mcr-5* transferibles (Lescat-2018). Por su parte, la presencia de mutaciones puntuales en genes cromosómicos, como el *mgrB*, se evaluó mediante PCR (Huang *et al.*,

2021) y posterior secuenciación (Macrogen, Seúl, Corea del Sur).

Filogenia y análisis clonal

Los grupos filogenéticos de los aislados resistentes a la colistina se establecieron según el esquema ampliado de Clermont (Clermont *et al.*, 2013). La relación clonal entre los aislados resistentes a la colistina se determinó mediante electroforesis en gel de campo pulsado (PFGE) del ADN total digerido con 40U de enzima de restricción *XbaI*, como se describió previamente (Gautom, 1997). Los fragmentos de ADN se corrieron en gel de agarosa al 1 % utilizando el sistema CHEF-DRIII (Bio-Rad Laboratories Inc, Hércules, EEUU.). Las condiciones de PFGE utilizadas fueron a 6 V cm⁻², a 14°C y con tiempo de pulso que osciló entre 1 s y 30 s durante 19 h. La normalización y el procesamiento de las imágenes de PFGE y la construcción del dendrograma se realizó utilizando el software GelJ v2.0 (<https://sourceforge.net/projects/gelj/>) (Heras *et al.*, 2015). Se construyó un árbol UPGMA utilizando índices de similitud Dice, ligamiento completo, optimización 1% y tolerancia de posición 1,3%. Los aislados se consideraron clonalmente relacionados si su índice de similitud Dice era ≥ 80 % (Guiral *et al.*, 2018).

Análisis estadístico

El cálculo de tamaño de muestra se determinó por diferencia de proporciones en cuanto al factor edad en función a la presencia de BLEE en estos grupos, separando los perros jóvenes/adultos de los geriátricos, utilizando la herramienta “power two proportions” de Stata (Aguilar-Gamboa *et al.*, 2016). Obteniendo como resultado 45 muestras por grupo etario (menor a 6 años/mayor a 6 años). Se definió como criterio que solo se tomaría una muestra de perro por propietario que cumpla los criterios de inclusión (clínicamente sano y no haber consumido antibióticos en el mes previo a la toma de muestra). Los propietarios tuvieron de 18 años a más y completaron el formulario de consentimiento para incluir a su perro en el estudio. Para la determinación de factores asociados se les pidió a los propietarios completar un cuestionario durante el proceso.

Se realizó un análisis descriptivo resumiendo las variables cualitativas según su frecuencia absoluta y relativa y las variables cuantitativas según su media y desviación estándar. Durante este análisis, se consideraron resistentes aquellos fenotipos con resistencia intermedia y se describieron por separado la resistencia a BLEE y a múltiples fármacos. Además, se evaluaron los factores asociados a la RAM modelando el número de familias de antimicrobianos resistentes mediante el modelo de regresión de Poisson. Dado que se trata de una variable desenlace de tipo conteo y se usó un modelo de regresión de Poisson con varianzas robustas, se estimó el “prevalence ratio” (razón de prevalencias o RP) como magnitud de la asociación de interés. Para el análisis de regresión se consideraron como potenciales factores asociados edad (dicotomizada como <6 años vs. ≥ 6 años), dieta (dicotomizada como dieta no cruda [dieta seca, mixta y casera] vs. cruda), Distrito (Surco vs. Villa El Salvador), Sexo (hembra vs macho) y convive con otros animales (No vs. Sí). El análisis de regresión multivariado fue planificado utilizando modelos anidados, el método forward y el Criterio de Información de Akaike para selección el modelo con mejor bondad de ajuste. Todos los análisis estadísticos se realizaron utilizando el paquete estadístico STATA MP v14.0 (Stata Corp LP, College Station, Texas) y se reportó cada una de las estimaciones con sus respectivos intervalos de confianza del 95% (IC 95%).

Resultados

Población de estudio

Se analizaron muestras fecales de 90 perros que acudían a dos clínicas veterinarias de diferentes

distritos de Lima : 46 de Villa El Salvador (51,1%) y 44 de Surco (48,9%) (Tabla 1). La mayoría de las muestras se recogieron de perros hembras (73,3%), de seis años o más (51,1%) y que convivían con otras mascotas (87,8%). La mayoría de las dietas de los perros se basaban en comida seca (52,2%), seguida de carne cruda (25,6%), comida mixta (18,9%) y comida casera (3,3%). En cuanto a los antecedentes de uso de antimicrobianos por parte del propietario, el 44,4% de los dueños de animales habían consumido antibióticos el mes anterior a la toma de muestra.

Aislamiento e identificación de *Escherichia coli*

Del total de 90 muestras fecales, 35 (38,9%) fueron positivas para *E. coli* (de éstas, 8 aislados se aislaron con presencia de CAZ y 27 a partir del medio sin disco antibiótico). Las características de los perros se muestran en la Tabla 1. Al comparar las características de los animales con muestras fecales positivas a *E. coli* frente a las negativas, se observó una frecuencia significativa de antecedentes de propietarios que habían consumido antibióticos en el último mes entre las muestras positivas a *E. coli* (65,7% frente a 30,9%, $p = 0,001$) (Tabla 1).

Tabla 1: Características epidemiológicas de los perros muestreados (total) y de los perros portadores de *Escherichia coli* (positivos).

Características	<i>Escherichia coli</i>		Total (n = 90)	
	Negativo (n = 55)	Positivo (n = 35)		
Distrito				
	Villa El Salvador	28 (50,9)	18 (51,4)	46 (51,1)
	Surco	27 (49,1)	17 (48,6)	44 (48,8)
Edad				
	≥6 años	30 (54,6)	16 (45,7)	46 (51,1)
	<6 años	25 (45,5)	19 (54,3)	44 (48,8)
Sexo				
	Hembra	41 (74,6)	25 (71,4)	66 (73,3)
	Macho	14 (25,4)	10 (28,6)	24 (26,7)
Dieta				
	Seca	29 (52,7)	18 (51,4)	47 (52,2)
	Cruda	16 (29,1)	7 (20,0)	23 (25,6)
	Mixta	7 (12,7)	10 (28,6)	17 (18,9)
	Home	3 (5,5)	0 (0,0)	3 (3,3)
Conviven con otro animal	49 (89,1)	30 (85,7)	79 (87,8)	
Propietario consumió antibióticos en el último mes	17 (30,9)*	23 (65,7)*	40 (44,4)	

* p -valor = 0,001

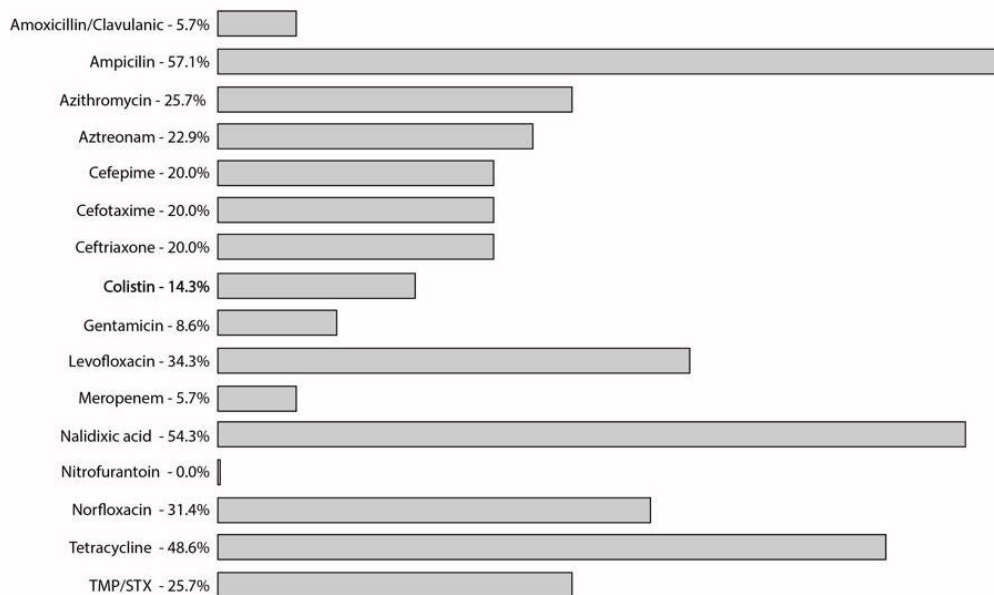
Resistencia a los antibióticos

Los aislados mostraron altos niveles de resistencia a los antibióticos, siendo susceptibles únicamente a la nitrofurantoína. Los mayores niveles de resistencia se observaron frente a ampicilina (57,1%), ácido nalidíxico (54,3%), tetraciclina (48,6%), levofloxacina (34,3%) y norfloxacina (31,4%). Los niveles de resistencia a las cefalosporinas fueron superiores al 20%, y se detectó el fenotipo BLEE en 12

aislados (34,3%). Por su parte, la resistencia a colistina alcanzó niveles del 14,3% de resistencia, presentando una concentración mínima inhibitoria (CMI) de 4 µg/ml (Figura 1). 20 (57,1%) de los aislados se clasificaron como MDR.

En cuanto a los patrones de resistencia a los antibióticos, 3 (8,57%) aislados mostraron resistencia a 7 agentes antimicrobianos de diferentes grupos, 5 (14,28%) aislados a 6 agentes antimicrobianos de diferentes grupos, 3 (8,57%) aislados mostraron resistencia a 4 y 3 antimicrobianos, respectivamente. Además, 1 (2,86%) aislado presentó resistencia a 2 grupos de agentes antimicrobianos y 5 (14,28%) aislados a 1 grupo de antimicrobianos. Cabe destacar que 4 (11,4%) aislados no presentaron resistencia a ningún antimicrobiano (Anexo S1).

Figura 1: Porcentaje de resistencia a diferentes antibióticos en los 35 aislados de *Escherichia coli* analizados.



Caracterización de BLEE

Se detectaron 12 aislados de *E. coli* productores de BLEE (13,3% de las muestras analizadas y el 34,3% de las muestras positivas para *E. coli*). El gen *bla*_{CTX-M-1G} se amplificó en 8 (66,7%), mientras que no se detectó ningún *bla*_{SHV}. Se detectó la variante *bla*_{CTX-M-55} en 6 de ellos y *bla*_{CTX-M-15} en los dos aislados restantes. Además, dos aislados amplificaron *bla*_{CTX-M-8G}, y 3 aislados fueron positivos para *bla*_{CTX-M} universal pero no se determinó ninguna variante. Por último, no se amplificaron genes codificantes de BLEE buscados en los dos aislados restantes.

Caracterización de las carbapenemasas

Todos los aislados presentaron un fenotipo intermedio a los carbapenemasas. No se detectaron resultados positivos de los genes de carbapenemasas evaluados.

Caracterización de la colistina

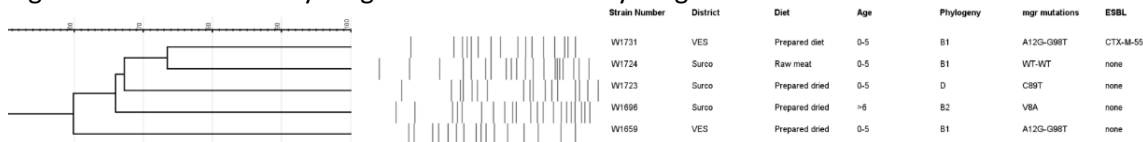
Cinco aislados eran resistentes a la colistina, y en cuatro de ellos se encontraron mutaciones en el gen *mgrB*. Así, tres aislados presentaban alteraciones en la región promotora, incluyendo dos que poseían los cambios de base a-12→g y g-98→t y el restante el cambio c-89→t. Mientras tanto, un aislado mostró la sustitución del codón aminoácido V8A (Figura 2). Además, no se detectaron genes

plamídicos *mcr*.

Filogenia y relación clonal entre aislados resistentes a la colistina.

Los cinco aislados de *E. coli* resistentes a la colistina no tenían relación clonal. Tres aislados pertenecían al grupo B1, y los otros dos a los grupos D y B2, respectivamente (Figura 2).

Figura 2. Relación clonal y filogenia Relación clonal y filogenia de los aislados resistentes a la colistina



Factores asociados al conteo de familias de antimicrobianos resistentes.

En el análisis de regresión observamos que en los aislados de *E. coli* de perros el principal factor asociado al conteo de familias de antimicrobianos era la edad (RP = 0,41; IC 95%: 0,19 - 0,90; p = 0,027). Específicamente, observamos que una edad ≥ 6 años representaba un factor protector y que el riesgo de incremento del conteo de familias de antimicrobianos en una unidad se reducía 0,59 veces comparando aquellos perros con ≥ 6 años versus aquellos con <6 años. Adicionalmente observamos que el consumo de dieta cruda representa un factor asociado marginalmente significativo al conteo de familias de antimicrobianos (RP = 1,78; IC 95%: 0,99 - 3,20; p = 0,054), y que en este caso este sería un factor de riesgo (Tabla 2). Al intentar ajustar un modelo de regresión multivariado observamos que el modelo de mejor bondad de ajuste seguía siendo el modelo bivariado como edad como factor asociado al número de familias de antimicrobianos. Finalmente, se realizó el análisis de regresión de la resistencia a BLEE o a múltiples fármacos, pero no se logró identificar ningún factor asociado estadísticamente significativo.

Tabla 2: Factores asociados a la resistencia de las familias de antimicrobianos.

Factor asociado	RP (IC 95%)	p-valor
Edad		
< 6 años	Ref	
≥ 6 años	0,41 (0,19 – 0,90)	0,027*
Dieta cruda		
Dieta no cruda	Ref.	
Dieta cruda	1,78 (0,99 – 3,20)	0,054
Propietario consume antibióticos		
No	Ref.	
Sí	0,64 (0,35 – 1,19)	0,159
Distrito		
Surco	Ref.	
Villa el Salvador	0,67 (0,36 – 1,25)	0,212
Sexo		
Hembra	Ref.	
Macho	0,86 (0,43 – 1,72)	0,678
Convive con otros animales		
No	Ref.	
Sí	0,96 (0,49 – 1,89)	0,912

VES: Villa El Salvador; RP: Razón de prevalencia, *p-valor <0,05

Discusión

Los resultados mostraron que los perros, tienen *E. coli* resistente a los antimicrobianos que podría transmitirse entre animales y humanos por contacto directo o indirecto. Se observaron niveles significativamente altos de resistencia a los antibióticos en los aislados de *E. coli*, siendo más de la mitad de los aislados resistentes a la ampicilina, ácido nalidíxico y tetraciclina. Además, alrededor del 40% de los aislados eran resistentes a las cefalosporinas y levofloxacina. Todos los niveles de resistencia a estos antibióticos son importantes porque se utilizan principalmente en medicina humana y veterinaria. Además, el estudio destaca la presencia de *E. coli* resistentes a carbapenemasas y polimixinas, lo cual es preocupante porque estas familias de antibióticos se consideran el último recurso de tratamiento antimicrobiano en infecciones humanas. Además, observamos una asociación entre la edad del perro, el tipo de dieta y la presencia de *E. coli* resistentes a un mayor número de familias de antibióticos.

Los estudios previos que caracterizan los niveles y mecanismos de resistencia en *E. coli* aislados de animales de compañía del Perú son escasos (Castillo *et al.*, 2022). Sin embargo, estudios realizados en Cajamarca, al norte del país (Gamarra, 2018), mostraron niveles de resistencia de 61% para estreptomomicina y 51% para ampicilina, los antibióticos más utilizados en la práctica clínica diaria en la región (Gamarra, 2018), siendo valores menores a los reportados previamente por nuestro grupo. Los niveles de resistencia a antibióticos fueron similares a los reportados en Minas de Geras, un populoso estado de Brasil, (Ramos *et al.*, 2022) aunque no reportaron resistencia a colistina.

Nuestros resultados son consistentes con el aumento de *E. coli* productor de BLEE reportado en perros a nivel mundial, principalmente relacionado con el alto uso de β -lactámicos en la práctica clínica de la medicina veterinaria (Collognon P *et al.*, 2009; Lei *et al.*, 2021). El porcentaje de muestras con *E. coli* productoras de BLEE fue de 13,3%, similar a otros países de la región, como Ecuador (Mitman *et al.*, 2022), e inferior al reportado por Chile (Benavides *et al.*, 2021). Comparando dentro de los aislamientos, encontramos un 34,3% de productores de BLEE, menor a otros reportes en Thailandia (Nittayasurt *et al.*, 2021).

En cuanto a las variantes CTX-M, el estudio reportó una variedad de BLEE, siendo *bla*_{CTX-M-55} la más frecuente. También se encontraron *bla*_{CTX-M-15} y *bla*_{CTX-M-8G}. Cabe destacar que *bla*_{CTX-M-55} ha sido reportado previamente en Perú en estudios que incluyeron muestras de diversos orígenes (muestras humanas y clínicas sanas, alimentos comercializados, agua potable y animales incluyendo mamíferos silvestres, aves de corral, ganado y animales de compañía) (Benavides *et al.*, 2022; Medina-Pizzali *et al.*, 2022; Murray *et al.*, 2021); también en estudios de BLEE en la región, ha sido el gen más reportado (Ramos *et al.*, 2022). Cabe destacar que CTX-M-15, la variante dominante en todo el mundo (Castanheira *et al.*, 2021), no fue muy frecuente en el presente estudio. De manera similar, otros estudios en el área han mostrado el mismo hallazgo, sugiriendo que CTX-M-15 no es la variante BLEE predominante en el Perú (Benavides *et al.*, 2022). Curiosamente, CTX-M-8G, un grupo de BLEE poco frecuente, ha sido detectado previamente en heces de perro en el norte de Perú (Medina-Pizzali *et al.*, 2022). Cabe mencionar que algunas de los aislados BLEE-positivas no amplificaron CTX-M, sugiriendo que otros mecanismos pueden estar involucrados como TEM, u otros BLEEs menos frecuentes, y ese escenario ya ha sido reportado en otros casos en el país (Palma *et al.*, 2017).

La colistina se utiliza como antimicrobiano de último recurso para uso clínico humano contra bacterias multirresistentes, y no se comercializa para uso veterinario en animales menores dentro del país desde 2019 (MINAGRI, 2019). Sin embargo, los resultados de nuestro estudio muestran un sorprendente 14,3% de aislados de *E. coli* resistentes a colistina, incluso en aquellos *E. coli* aislados de animales

considerados clínicamente sanos. En estudios anteriores, la presencia de *mcr-1* se asoció con la resistencia a la colistina en *E. coli* de perros y sus propietarios (Lei *et al.*, 2021); sin embargo, la resistencia a la colistina en el presente estudio está probablemente relacionada con mutaciones cromosómicas, que se informó como un mecanismo importante, en otras Enterobacterias como *Klebsiella pneumoniae* (Khoshbayan *et al.*, 2022). En este sentido, 3 de los 5 aislados resistentes a la colistina poseían mutaciones en la región promotora de *mgrB* y otro la sustitución del aminoácido V8A. Aunque no se han encontrado datos en la literatura sobre el efecto de las mutaciones en la región promotora de *mgrB*, las alteraciones en la región promotora de los genes tienen el potencial de afectar (ya sea aumentando, disminuyendo o evitando) los niveles finales de expresión de los péptidos codificados posteriormente (Ince y Hooper, 2003). No obstante, aunque no se puede descartar el papel de estas alteraciones de la región promotora en los niveles finales de MIC de colistina y son necesarios más estudios para dilucidar su efecto, Existen reportes de mutaciones fuera de la región PhoP, del mismo modo que estas no se encuentran en los sitios de inicio de transcripción ni afectan la región -10 (Eguchi *et al.*, 2004). Mientras tanto, la sustitución de aminoácidos V8A se ha descrito tanto en aislados resistentes a la colistina como en aislados susceptibles a la colistina, lo que sugiere un polimorfismo o un efecto bajo en los niveles finales de MIC de colistina (Luo *et al.*, 2017). Además, el análisis de PFGE y filogenia demostró que estas cepas no estaban relacionadas clonalmente. Por lo tanto, se podría considerar como consecuencia de la exposición a la colistina que se utilizó en el sector aviar durante muchos años, como una posible fuente de exposición a través de la cadena alimentaria (Monte *et al.*, 2017).

Al explorar los factores asociados con el número de familias de antimicrobianos resistentes, observamos que la dieta cruda podría comportarse como un factor de riesgo. Esto es una observación importante porque, aunque la mayoría de los perros del estudio consumía una dieta seca basada en distintos tipos de proteínas, carbohidratos, cereales y otros componentes que sufren deshidratación, el consumo de dieta cruda se asoció marginalmente a un incremento del número de familias de antimicrobianos resistentes. La alimentación cruda como base de la dieta canina ha cobrado interés en los últimos años debido a estudios o casos de patologías cardíacas relacionadas con distintos tipos de conservantes o ingredientes utilizados en la alimentación seca (Smith *et al.*, 2021). Sin embargo, el consumo de carne cruda se ha asociado con un alto riesgo de presentar *E. coli* resistente a antibióticos y otras bacterias de transmisión alimentaria como *Salmonella* (Groat *et al.*, 2022), o con *E. coli* MDR en perros con dieta basada en carne cruda en Brasil (Ramos *et al.*, 2022), enfatizando la importancia de la vigilancia y control de este tipo de alimentación, cuyo consumo ha ido en aumento en los últimos años. Además, este estudio informó de la baja edad como factor de riesgo para la resistencia a mayor número de antimicrobianos, aunque previamente se ha descrito que mayor edad está relacionada con un mayor tiempo de exposición a los antimicrobianos (Ekakoro *et al.*, 2022). Este hecho podría estar relacionado con el tratamiento antimicrobiano de la diarrea en cachorros asociado a cambios en la dieta.

El estrecho contacto de las mascotas con sus dueños y con el entorno ha provocado el intercambio de bacterias, incluidas las resistentes (Dazio *et al.*, 2021). Así, esta propagación de bacterias de humanos a animales y viceversa supone un riesgo para el tratamiento de infecciones en la práctica clínica humana y también puede comprometer el tratamiento de infecciones en animales (Ramos *et al.*, 2022). Por ello, en nuestro estudio se destaca el elevado consumo de antimicrobianos por parte de los propietarios de animales, siendo más del 40% los que consumieron antibióticos en el último mes.

Entre las principales limitaciones de este estudio hay que destacar el poder de estudio limitado para poder ajustar un análisis de regresión multivariado. Esto es importante porque esta es una de las principales estrategias para controlar potenciales sesgos de confusión. Finalmente, otra limitación

importante es el diseño transversal del estudio, el mismo que por su naturaleza es prono a sesgos de selección, información y confusión. De ahí que, independientemente de las importantes hipótesis que nuestro estudio permite plantear a partir de sus resultados, es altamente recomendable reproducir el estudio con un mayor poder de estudio para poder verificarlos. Además, considerando también la parte experimental que ocurre en el laboratorio como la falta de crecimiento bacteriano en la totalidad de la población muestreada pudiendo deberse esto a la biología de la muestra o a las variaciones entre cada individuo muestreado.

En resumen, nuestro estudio demuestra la presencia en los perros de bacterias portadoras de genes de resistencia a los antibióticos, incluidos los BLEE y los implicados en la resistencia a los antibióticos de último recurso, como la colistina, probablemente relacionada con el elevado uso veterinario y dietético, a pesar de la prohibición de su uso. Este hallazgo permite comprender mejor las bacterias resistentes a los antibióticos en los animales de compañía. También pone de relieve la necesidad de una estrategia para prevenir la propagación de cepas resistentes en clínicas de animales menores y en el ámbito doméstico.

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Anexo:

Table S1: Antibiotic resistance patterns observed in the *E. coli* isolates from fecal samples of dogs.

N°	ANTIBIOTICS	ANTIBIOTIC PATTERN	N° ISOLATES
		AMP	4
		AZM	2
		Q	2
		CFS	1
1		COL	1
2		TET,AZM	1
		Q, AMP, AZM	2
		COL, TET, AZM	1
3		TET, AMP, AZM	1
		TET, Q, AMP, AZM	2
		COL, SXT, AMP, AZM	1
4		CFS, ATM, TET, Q	1
		AMC, CFS, ATM, TET, AMP, AZM	2
		CFS, ATM, SXT, TET, GEN, Q	2
		CFS, ATM, SXT, TET, Q, AMP	2
		CFS, ATM, SXT, TET, Q, AZM	2
6		COL, CFS, ATM, SXT, Q, AMP	1
		AMC, CFS, ATM, TET, Q, AMP, AZM	1
		CFS, ATM, SXT, TET, Q, AMP, AZM	1
7		CFS, ATM, SXT, TET, GEN, Q, AMP	1

AMP: ampicillin, AZM: azithromycin, Q: quinolones, CFS: cephalosporines, COL: colistin, TET: tetracycline, SXT: Sulfamethoxazole/Trimethoprim, ATM: aztreonam, GEN: gentamicin

Anexos:

- Carta de aceptación de la revista

By E-mail

Ref. No. VW/Accept/71/2024

Date: 26-03-2024

To,

Maria J. Pons
Grupo de Investigación en Dinámicas y Epidemiología de la
Resistencia a Antimicrobianos - "One Health",
Universidad Científica del Sur,
Lima, Peru
E-mail: ma.pons.cas@gmail.com

and

Joaquim Ruiz
Grupo de Investigación en Dinámicas y Epidemiología de la
Resistencia a Antimicrobianos - "One Health",
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E-mail: joruiz.trabajo@gmail.com

Acceptance of article for publication in Veterinary World

Dear Dr.

I am pleased to inform you that your manuscript titled as -

Antimicrobial resistance and associated factors in Escherichia coli isolated from Peruvian dogs: a focus on extended-spectrum β -lactamases and colistin - Margot Ventura, Rosario Oporto, Kathya Espinoza, Fernando Guibert, Antonio M. Quispe, Nidia Vilar, María López, Beatriz Rojo-Bezares, Yolanda Sáenz, Joaquim Ruiz, and Maria J. Pons

is accepted for publication in *Veterinary World*.

We have received the payment for publication (bill no. 321 dated 26-03-2024). So, you will receive the galley proof within 2-4 weeks. You must have to solve the query, if we point out any in galley proof.

After correction of galley proof, your article will be published online at www.veterinaryworld.org in chronological order.

Thanking You.

Yours Sincerely,



Dr. Anjum V. Sherasiya
Editor-in-Chief
Veterinary World



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- Copia de documento de aprobación del comité de ética. Agregar las enmiendas o extensiones si hubieran. (como fotos)

COMITÉ INSTITUCIONAL DE ÉTICA EN INVESTIGACIÓN CON ANIMALES Y BIODIVERSIDAD



Constancia N° 017-CIEI-AB-CIENTÍFICA-2021

El presidente del Comité Institucional de Ética en Investigación con Animales y Biodiversidad de la Universidad Científica del Sur (CIEI-AB-CIENTÍFICA) da constancia que el proyecto de investigación, cuyos datos se registran a continuación, ha sido **aprobado**.

Código de registro: **452-2020-POS99**
 Título: **Frecuencia de la resistencia a antimicrobianos y sus factores de riesgo en animales menores de Lima, Perú**
 Investigador(a): **Cristina Margot Ventura Valencia**

La aprobación del proyecto de investigación implica que el documento presentado a evaluación cumple con los lineamientos de Universidad en materia de investigación y ética, los cuales se sustentan en la normativa internacional vigente. Adicionalmente, en el contexto de la pandemia de enfermedad por COVID-19, le recomendamos cumplir con las normas publicadas por el estado peruano.

En tal sentido, esta aprobación carecerá de valor si es que el proyecto de investigación arriba indicado es modificado de cualquier forma. Toda enmienda, añadido, eliminación o eventualidad (eventos adversos, etc.) posterior a la fecha de aprobación debe ser reportado al CIEI-AB-CIENTÍFICA.

La vigencia de la aprobación de este documento es de dieciocho (18) meses (**hasta el 08 de agosto del 2022**), periodo en el que puede desarrollarse el proyecto. Cualquier trámite para su renovación deberá ser enviado antes del término de la vigencia de este documento, debiendo suspenderse todo desarrollo hasta que la renovación sea aprobada.

Villa El Salvador, 09 de febrero de 2021




Méd. Vet. Gianmarco Paolo Rojas Moreno
 Presidente
 Comité Institucional de Ética en Investigación
 con Animales y Biodiversidad

**COMITÉ INSTITUCIONAL DE ÉTICA EN
INVESTIGACIÓN CON ANIMALES Y
BIODIVERSIDAD**



Constancia

Luego de la revisión de la solicitud, el presidente del Comité Institucional de Ética en Investigación con Animales y Biodiversidad de la Universidad Científica del Sur (CIEI-AB-CIENTÍFICA) hace constar que se ha aprobado de forma expedita la **enmienda/modificación** del proyecto de investigación que se detalla continuación:

Código de registro: **452-2020-POS99**

Título: **Frecuencia de la resistencia a antimicrobianos y sus factores de riesgo de animales menores de Lima, Perú**

Investigador(es): **Cristina Margot Ventura Valencia**

Constancia de aprobación: **017-CIEI-AB-CIENTÍFICA-2021**

La **enmienda/modificación** corresponde a los siguientes:

1. **Proyecto de investigación**, versión recibida en fecha 30 de octubre del 2022. Se detallan los siguientes cambios:
 - a. **Renovación de vigencia de aprobación ética.** Vista la solicitud, se da la ampliación de la vigencia de la aprobación ética por un periodo de seis meses; es decir, desde el 03 de enero de 2023 hasta el 02 de julio del 2023. El proyecto solo podrá ejecutarse entre los periodos detallados tanto en la constancia 017-CIEI-AB-CIENTÍFICA-2021 como en esta enmienda.

Todas las demás características de la investigación descritas en el proyecto original permanecen; por lo que cualquier otra modificación deberá informarse a este Comité, para su evaluación.

Lima, 11 de enero de 2023.




Méd./Vet. Milena Paola Montenegro Veja
Presidenta (e)
Comité Institucional de Ética en Investigación
con Animales y Biodiversidad

**COMITÉ INSTITUCIONAL DE ÉTICA EN
INVESTIGACIÓN CON ANIMALES Y
BIODIVERSIDAD**



Constancia

Luego de la revisión de la solicitud, el presidente del Comité Institucional de Ética en Investigación con Animales y Biodiversidad de la Universidad Científica del Sur (CIEI-AB-CIENTÍFICA) hace constar que se ha aprobado de forma expedita la **enmienda/modificación** del proyecto de investigación que se detalla continuación:

Código de registro: **452-2020-POS99**

Título: **Frecuencia de la resistencia a antimicrobianos y sus factores de riesgo en perros de Lima, Perú.**

Investigador(es): **Cristina Margot Ventura Valencia**

Constancia de aprobación: **017-CIEI-AB-CIENTÍFICA-2021**

Enmienda anterior: **Constancia Ref. 017-CIEI-AB-CIENTÍFICA-2021 (11/01/2023)**

La **enmienda/modificación** corresponde a los siguiente:

1. **Proyecto de investigación**, versión recibida en fecha 15 de setiembre del 2023. Se detallan los siguientes cambios:
 - a. **Toma de conocimiento de cambio en título.** Se toma conocimiento del cambio en el título, realizado a solicitud del comité evaluador de tesis. Así, la nueva versión es **“Frecuencia de la resistencia de *Escherichia coli* a antimicrobianos y sus factores de riesgo en perros de dos clínicas veterinarias de Lima, Perú”**.

Todas las demás características de la investigación descritas en el proyecto original permanecen; por lo que cualquier otra modificación deberá informarse a este Comité, para su evaluación.

Lima, 24 de octubre del 2023.




Méd. Vet. Milena Paola Montenegro Veja
Presidenta (e)
Comité Institucional de Ética en Investigación
con Animales y Biodiversidad

- Copia de resolución directoral de aprobación del proyecto. Agregar las Resoluciones de las enmiendas o extensiones si hubieran. (como fotos)

**RESOLUCIÓN DIRECTORAL ACADÉMICA DE CARRERA No. 159 - DACMH-DAFCS-U. CIENTIFICA-2021**

Lima, 05 de marzo del 2021

VISTO:

El informe de revisión independiente académica y la aprobación de un Comité de Ética del proyecto de tesis titulado: **“FRECUENCIA DE LA RESISTENCIA A ANTIMICROBIANOS Y SUS FACTORES DE RIESGOS EN ANIMALES MENORES DE LIMA, PERÚ”**.

CONSIDERANDO:

Que, de acuerdo al Reglamento General de la Universidad Científica del Sur y el reglamento y Procedimientos de trabajos de Investigación para obtener el **Grado de Maestro**.

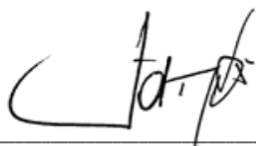
Que, de acuerdo a la normativa vigente de la Universidad Científica del Sur, en uso de las atribuciones conferidas al Director Académico de la Carrera.

SE RESUELVE:

ART. 1° Aprobar, en vías de regularización e inscribir el proyecto de tesis titulado: **“FRECUENCIA DE LA RESISTENCIA A ANTIMICROBIANOS Y SUS FACTORES DE RIESGOS EN ANIMALES MENORES DE LIMA, PERÚ”**, presentado por la alumna **CRISTINA MARGOT VENTURA VALENCIA** con N° de registro: **452-2020-POS99**.

ART. 2° Nombrar a la Docente María Jesús Pons Casellas, como asesor para el desarrollo de la tesis en cuestión.

Regístrese, comuníquese y archívese.



Mg. JORDI GRAU MONGE
Director Académico
Carrera de Medicina Humana



RESOLUCIÓN DIRECTORAL ACADÉMICA DE CARRERA No. 151 - DACMH-DAFCS-U. CIENTIFICA-2023

Lima, 03 de marzo del 2023

VISTO:

El informe de revisión independiente académica y la aprobación de un Comité de Ética del proyecto de investigación titulado: **“FRECUENCIA DE LA RESISTENCIA A ANTIMICROBIANOS Y SUS FACTORES DE RIEGO DE ANIMALES MENORES DE LIMA, PERÚ”**.

CONSIDERANDO:

Que, de acuerdo al Reglamento General de la Universidad Científica del Sur y el reglamento y Procedimientos de trabajos de Investigación para obtener el **Grado de Maestro**.

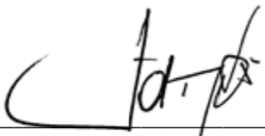
Que, de acuerdo a la normativa vigente de la Universidad Científica del Sur, en uso de las atribuciones conferidas al Director Académico de la Carrera.

SE RESUELVE:

ART. 1° Aprobar, en vías de regularización e inscribir el proyecto de tesis titulado **“FRECUENCIA DE LA RESISTENCIA A ANTIMICROBIANOS Y SUS FACTORES DE RIEGO DE ANIMALES MENORES DE LIMA, PERÚ”**, presentado por la alumna Cristina Margot Ventura Valencia con N° de registro: **452-2020-POS99**.

ART. 2° Nombrar al Docente Maria J. Pons Casellas , como asesor para el desarrollo de la tesis en cuestión.

Regístrese, comuníquese y archívese.


Mg. JORDI GRAU MONGE
Director Académico (e)
Carrera de Medicina Humana



RESOLUCIÓN DIRECTORAL ACADÉMICA DE CARRERA No. 652 - DACMH-DAFCS-U. CIENTIFICA-2023

Lima, 27 de octubre del 2023

VISTO:

El informe de revisión independiente académica y la aprobación de un Comité de Ética del proyecto de tesis titulado: "FRECUENCIA DE LA RESISTENCIA DE ESCHERICHIA COLI A ANTIMICROBIANOS Y SUS FACTORES DE RIESGO EN PERROS DE DOS CLÍNICAS VETERINARIAS DE LIMA, PERÚ".

CONSIDERANDO:

Que, de acuerdo al Reglamento General de la Universidad Científica del Sur y el reglamento y Procedimientos de trabajos de Investigación para obtener el **Grado de Maestro**.

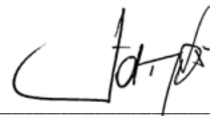
Que, de acuerdo a la normativa vigente de la Universidad Científica del Sur, en uso de las atribuciones conferidas al Director Académico de la Carrera.

SE RESUELVE:

ART. 1° Aprobar e inscribir el proyecto de tesis titulado: "FRECUENCIA DE LA RESISTENCIA DE ESCHERICHIA COLI A ANTIMICROBIANOS Y SUS FACTORES DE RIESGO EN PERROS DE DOS CLÍNICAS VETERINARIAS DE LIMA, PERÚ", presentado por la alumna Cristina Margot Ventura Valencia con N° de registro: **452-2020-POS99**

ART. 2° Nombrar al docente: María Jesus Pons Casellas, como asesor para el desarrollo de la tesis en cuestión.

Regístrese, comuníquese y archívese.



Mg. JORDI GRAU MONGE
DECANO
CARRERA DE MEDICINA HUMANA



c.c. Archivo