PAN AMERICAN JOURNAL OF PUBLIC HEALTH 100 YEARS

Original research

Colistin resistance in *Escherichia coli* and *Klebsiella pneumoniae* in humans and backyard animals in Ecuador

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Suggested citation Bastidas-Caldes C, Guerrero-Freire S, Ortuño-Gutiérrez N, Sunyoto T, Gomes-Dias CA, Ramírez MS, et al. Colistin resistance in *Escherichia coli* and *Klebsiella pneumoniae* in humans and backyard animals in Ecuador. 2023;47:e48. https://doi.org/10.26633/RPSP.2023.48

ABSTRACT

Objective. Colistin is an antibiotic of last resort for treating serious Gram-negative bacterial infections. However, the misuse of colistin, especially as an animal growth promoter, has contributed to increasing antimicrobial resistance, mediated mainly through plasmid transfer of the *mcr-1* gene. This study assessed the prevalence of phenotypic and molecular colistin resistance in *Escherichia coli and Klebsiella pneumoniae* in Ecuador in healthy humans and their chickens and pigs.

Methods. Fecal samples were collected from humans and their chickens and pigs in two rural coastal and Amazon regions between April and August 2020. Gram-negative bacteria were isolated and identified using conventional techniques. Phenotypic resistance was determined using the broth microdilution technique, and the *mcr-1* gene was detected using conventional polymerase chain reaction.

Results. A total of 438 fecal samples were obtained from 137 humans, 147 pigs and 154 chickens. The prevalence of *E. coli* isolates was 86.3% (378/438) and *K. pneumoniae*, 37.4% (164/438). Overall, the *mcr-1* gene was found in 90% (340/378) of *E. coli* isolates, with higher prevalences found in isolates from coastal regions (96.5%, 191/198), humans (95.6%, 111/116) and chickens (91.8%, 123/134); for *K. pneumoniae*, the gene was found in 19.5% (32/164) of isolates, with equal distribution between regions and hosts. Only four isolates, two *E. coli* and two *K. pneumoniae,* showed phenotypic resistance: *mcr-1* was present in both *E. coli* strains but absent in the *K. pneumoniae* strains.

Conclusions. Despite a low prevalence of phenotypic resistance to colistin, the high prevalence of the *mcr-1* gene in *E. coli* is of concern. Ecuador's ban on using colistin in animal husbandry must be enforced, and continual monitoring of the situation should be implemented.

Keywords Colistin; *Escherichia coli*; *Klebsiella pneumoniae*; humans; animals; drug resistance; genes, MDR; operational research; Ecuador.

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Antimicrobial resistance is recognized as one of the most serious global threats to human health. The emergence of multidrug resistance – defined as an organism showing resistance to three or more classes of antibiotics – in *Escherichia coli* and *Klebsiella pneumoniae* is particularly concerning (1). Both are Gram-negative bacteria that cause serious infections and have multiple resistance mechanisms. The commonest examples of these are extended-spectrum β-lactamases, carbapenemases (2) and for colistin, the presence of the *mcr-1* gene.

A recent study has highlighted the growing threat of resistance to colistin mediated through *mcr* genes, with documented examples of resistance in animals, humans, food and the environment (3). Colistin is an antibiotic considered one of the medicines of last resort against multidrug-resistant Gram-negative bacterial infections (4). Colistin is commonly used in animal husbandry for treatment and as a growth promoter in food supplements, either in normal or excessively high doses, thus increasing the selection for colistin resistance in animals (5). In addition, the effectiveness of antibiotics, including colistin, is being threatened by their excessive use in veterinary medicine. Indeed, in some countries, the use of colistin has been 600 times higher in animals than in humans (6).

In 2016, Liu et al. identified in China for the first time a plasmid-mediated colistin- resistance gene, a mobile element that contains the *mcr-1* gene (7). This gene encodes the expression of phosphoethanolamine transferase, affecting lipid A, which confers antibiotic resistance in Enterobacterales (7). Colistin-resistant Enterobacterales, particularly *E. coli* and *K. pneumoniae*, carrying the *mcr-1* gene have since been reported worldwide, including in the Americas (in Argentina, Bolivia [Plurinational State of], Brazil, Colombia, Peru and the United States) (8). Studies in Latin America have suggested that this spread might be associated with the horizontal transfer of colistin-resistance genes (9). Since the discovery of the *mcr-1* gene and its presence in Enterobacterales plasmids, worldwide surveillance of colistin resistance has been strengthened (5).

In 2016, Ortega-Paredes et al. reported the first clinical isolate of colistin-resistant *E.coli* harboring the *mcr-1* gene in Ecuador in an adolescent with appendicitis (10). Since then, studies of animals on rural farms where there is extensive use of colistin as a growth promoter have shown widespread distribution of colistin-resistant *E.coli*, with a high proportion of isolates containing *mcr-1* genes (11–13). With the growing importance of the One Health concept, documenting the prevalence of colistin-resistance genes in both humans and animals in Ecuador is critical to establish a baseline of how far the *mcr* genes have dispersed (14). This information will help to reinforce national surveillance and guide policies for the rational use of colistin. Such a study will also provide evidence about the possible cross-transfer of *mcr-1* genes between different host species. Therefore, this study aimed to assess the prevalence of phenotypic colistin resistance and the presence of *mcr-1* in *E. coli and K. pneumoniae* from humans and their backyard animals (chickens and pigs) living in selected rural communities in Ecuador between April and August 2020.

MATERIALS AND METHODS

Study design and sampling

This was a cross-sectional study using primary data and conducted in rural areas of Ecuador.

Ecuador is a country that straddles the equator on South America's west coast. It has a diverse landscape that includes the Amazon jungle, Andean highlands, a coastal region and the wildlife-rich Galapagos Islands. Rural hamlets in the coastal region (province of Santo Domingo de los Tsáchilas) and the Amazon region (province of Pastaza) were included in the study. Each hamlet had a population of around 700 inhabitants in about 140 households, with a mean household size of 5 persons. There were approximately 15 hamlets and farmhouses in each of the study regions. Most inhabitants earn their livelihood from agriculture and farming activities, with every family usually having chickens, pigs, ducks and some cattle in their backyard. These hamlets were selected because there was a preliminary report disclosing information about resistance to colistin and cefotaxime in *E. coli* in backyard animals in these regions (12).

Human fecal samples were collected and placed in semisolid Cary–Blair transport medium for coliforms (Eiken Chemical, Tokyo, Japan). For samples from backyard animals, cloacal and rectal swabs were taken from, respectively, chickens and pigs, and these were placed in transport medium similar to that used for humans. These specimens were all kept at 4 °C until transportation. Laboratory testing was conducted by the microbiology laboratory at the Universidad de las Américas.

Phenotypic identification of isolates

All samples from humans and animals were screened by inoculating onto selective CHROMagar COL-APSE chromogenic medium (Paris, France), and they were incubated at 37 °C for 18 hours. Plates were considered positive when at least one typical (coliform) colony had formed (15). Subsequently, the typical colonies were inoculated onto BD DIFCO MacConkey Agar medium (Becton Dickinson, Franklin Lakes, New Jersey, USA). Suspected colonies were separated and further confirmed as *E. coli* or *K. pneumoniae* through biochemical testing that included inoculation onto Simmons citrate medium, and the triple sugar iron, urea, sulfur indole motility and the methyl red/Voges–Proskauer tests (all media and solutions were from Becton Dickinson); isolates were preserved for future experiments. All isolates were preserved in brain heart infusion broth with 10% glycerol; those used in the study were frozen at –20 °C and those preserved for future research were frozen at –80 °C. ATCC (Manassas, Virginia, USA) strains 25922 for *E. coli* and 700603 for *K. pneumoniae* were used for quality control.

Phenotypic resistance to colistin

The isolates of *E. coli* and *K. pneumoniae* were incubated in brain heart infusion broth for 18 hours at 37 °C for enrichment. Subsequently, colonies were isolated on nutrient agar and cultivated at 37 °C for 18 hours. These colonies in Muller Hinton broth medium were compared with the 0.5 McFarland standard. Briefly, the broth microdilution test was carried out in line with the recommendations of the Clinical and Laboratory Standards Institute (CLSI standard M100, 2022), in which bacteria diluted to the 0.5 McFarland standard are cultured together with the test antibiotic. Two cut-off points were used: ≤ 2 µg/mL to indicate intermediate resistance and ≥ 4 µg/mL to indicate colistin resistance (CLSI standard M100, 2022). All isolates were then cultured in brain heart infusion broth for cryopreservation for

future experiments. The plates were processed in a reader at the recommended wavelength (260 nm). Each isolate was inoculated and subsequently measured in duplicate.

Bacterial DNA extraction

DNA was extracted following the Chelex 100 and proteinase K methods previously described (16). Briefly, all confirmed isolates of *E. coli* and *K. pneumoniae* were resuspended in 200 µL of Chelex 10% (Sigma-Aldrich, St. Louis, Missouri, USA) and 5 µL of proteinase K (Invitrogen, Waltham, Massachusetts, USA). Samples were incubated at 56 °C for 60 min. Following this, the samples were vortexed and centrifuged at 8000 g for 2 min. They were then incubated at 96 °C for 20 min. Finally, samples were centrifuged at 8000 g for 5 min and the supernatant was transferred to a clean tube and stored at –20 °C.

Molecular detection of *mcr*

One-step polymerase chain reaction (PCR) was performed for the *mcr-1* gene using the forward primer 5'-GCTACTGATCACCACGCTGT-3' and the reverse primer 5'-AGCTGAACATACACGGCACA-3', giving a product size of 698 bp. The QIAGEN PCR kit (QIAGEN, Hilden, Germany) was used. The reaction mix for the PCR contained an amplification mixture of 10 µL, with a concentration of 1 µM of multiplex master mix, 0.2 µM of the mixture of primer pairs for the *mcr-1* gene, and 1 µM of Q solution, following the manufacturer's instructions for multiplex PCR kits (17, 18).

PCR was performed in a Mastercycler thermocycler (Eppendorf, Hamburg, Germany) using gradient operation, with the initial denaturation step at 95 °C for 15 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 90 s, extension at 72 °C for 90 s and a final extension at 72 °C for 10 min. PCR products were analyzed using SyBr Safe DNA Gel Stain (Invitrogen) in 2% (w/v) agarose gel in 1X tris–borate–EDTA (ethylenediaminetetraacetic acid) buffer, and electrophoresis was performed at 100 V for 40 min in an Enduro Gel XL Electrophoresis System (Labnet International, Edison, New Jersey, USA). The positive control DNA for the *mcr-1* gene was kindly donated by the Osaka Institute of Public Health, Japan. Finally, to confirm the variant of the *mcr* gene, the 3130 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) was used for Sanger sequencing on positive amplification products from PCR. The sequences obtained were aligned with others already described and characterized in the GenBank database using the Geneious 8.0 (Biomatters, Auckland, New Zealand) and MEGA X (Molecular Evolutionary Genetics Analysis, https://www.megasoftware.net/docs) bioinformatics programs. A summary of study procedures is presented in Figure 1.

Sample size calculation

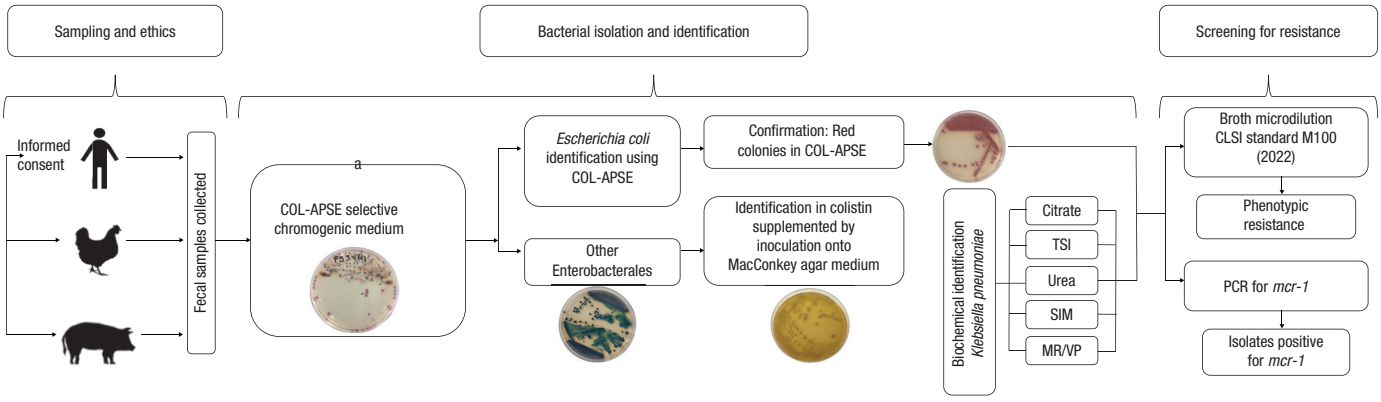
The sample size was calculated based on the formula $N = (Z1- \alpha/2)2p(1 - p)/d2$, where $Z1 - \alpha/2$ is the value of normal deviation at a 95% confidence level, p is the expected frequency of colistin resistance in about 50% of farms, according to the reports by Yamamoto et al. (12), with 15% relative precision (d), a design effect of 2 for cluster sampling and a nonresponse rate of 10%.

Cluster random sampling was adopted to select the host from which samples were collected. A total of 20 clusters were selected from the available hamlets. Then 20 households were selected from each cluster by simple random sampling. From each household that agreed to participate, one adult human or one animal was recruited into the study. This process was followed until the desired total sample size of 400 was achieved.

Data entry and statistical analysis

Data were extracted from the study forms and laboratory records, double entered into a Microsoft Excel 2010 spreadsheet (Microsoft, Redmond, Washington, USA) and analyzed using EpiData v. 2.2.3.187 (EpiData, Odense, Denmark). The prevalence of bacterial isolates and their genotypic and phenotypic resistance to colistin were calculated and reported with a 95% confidence interval (CI). Phenotypic resistance was defined as bacterial growth occurring in the broth microdilution test at

FIGURE 1. Flow chart of the study's methodology[a]



CLSI: Clinical and Laboratory Standards Institute (standard M100, 2022); *mcr*: mobile colistin resistance gene; MR/VP: methyl red/Voges–Proskauer; PCR: polymerase chain reaction; TSI: triple sugar iron; SIM: sulfur indole motility.

[a] Phenotypic resistance breakpoints followed CLSI standard M100 for 2022. Using microbroth dilution, bacteria are considered to have intermediate resistance to colistin if growth occurs at ≤ 2 µg/µL of the antibiotic and considered resistant to colistin if growth occurs at ≥ 4 µg/µL.

Source: Figure prepared by the authors for this study.

≥ 4 µg/mL of colistin and genotypic resistance as the presence of *mcr-1*. The prevalence of genotypic and phenotypic resistance in relation to geographical location and different hosts was compared using the χ^2 test. A P value < 0.05 was considered statistically significant.

Ethics

Approval was obtained from the Ethics Committee for the Investigation on Human Beings at Universidad San Francisco de Quito (approval code 2018-110E), in accordance with the Declaration of Helsinki. Ethics approval was also obtained from the Union Ethics Advisory Group (EAG approval number 19/21) of the International Union Against Tuberculosis and Lung Disease, Paris, France. All participants included in the study signed approved informed consent forms.

RESULTS

Prevalence of *E. coli* and *K. pneumoniae*

A total of 438 individual fecal samples were obtained from rural hamlets of the coastal and the Amazon regions of Ecuador. These included 226 samples from Santo Domingo province in the coastal region (72 from humans, 78 from pigs, 76 from chickens) and 212 samples from Pastaza province in the Amazon region (65 from humans, 69 from pigs, 78 from chickens).

The overall prevalence of *E. coli* isolates was 86.3% (378/438; 95% CI: 82.1 to 90.5) and the overall prevalence of *K. pneumoniae* was 37.4% (164/438; 95% CI: 32.0 to 42.0]. The prevalences of these isolates, stratified by province and by host, are shown in Table 1 and Table 2, respectively. For *E. coli* isolates, the prevalence was similar in Santo Domingo and in Pastaza among chickens, pigs and humans and ranged between about 85% and 88%. For *K. pneumoniae* isolates, however, there was a statistically significant higher prevalence in Santo Domingo (42.9%) compared with Pastaza (31.6%) ($P < 0.05$) and a significantly higher prevalence in humans (72.3%) compared with chickens (27.9%) and with pigs (15.0%) ($P < 0.01$).

Prevalence of *mcr-1* in *E. coli* and *K. pneumoniae*

The overall prevalence of *mcr-1* in *E. coli* isolates was 90.0% (340/378; 95% CI: 86.1 to 93.8) and the overall prevalence in *K. pneumoniae* isolates was 19.5% (32/164; 95% CI:12.1 to 27.9). The prevalences of *mcr-1* in *E. coli* and *K. pneumoniae*, stratified by province and by host, are also shown in Table 1 and Table 2, respectively. For *E. coli* isolates, there was a statistically significant higher prevalence in isolates from Santo Domingo (96.5%, 191/198) compared with Pastaza (82.7%, 149/180) ($P < 0.001$) and a statistically significant higher prevalence in humans (95.6%, 111/116) and chickens (91.8%, 123/134) compared with pigs (82.8%, 106/128) ($P < 0.0.5$). For *K. pneumoniae* isolates, the prevalence of *mcr-1* was similar in Santo Domingo and Pastaza, at about 19%, and similar between humans, chickens and pigs, at about 16–21%. The GenBank accession number MW527090, corresponding to the phosphoethanolamine–lipid A transferase *mcr-1* gene, was obtained from isolate LR37 from a healthy human in Santo Domingo province.

TABLE 1. Prevalence of *Escherichia coli* isolates and the *mcr-1* gene in fecal samples from humans, pigs and chickens in two rural provinces of Ecuador, 2020 (*N* = 438)

Variables	No. of samples	Prevalence of confirmed isolates		Prevalence of *mcr-1* gene	
		n	% (95% CI)	*n*	% (95% CI)
Province					
Pastaza	212	180	84.9 (78.0 to 91.7)	149	82.7 (74.7 to 90)
Santo Domingo	226	198	87.6 (81.5 to 93.6)	191	96.5 (93.1 to 99)
Host					
Human	137	116	84.7 (76.1 to 93.3)	111	95.6 (91.2 to 100)
Chicken	154	134	87.0 (79.5 to 94.5)	123	91.8 (85.7 to 98)
Pig	147	128	87.1 (79.3 to 94.6)	106	82.8 (73.6 to 92)

CI: confidence interval.
***Source*:** Table prepared by the authors with data from their study.

Prevalence of phenotypic resistance to colistin

Two (0.5%) of the 378 *E. coli* isolates showed statistically significant phenotypic resistance to colistin (Table 3). Two (1.2%) of the 164 *K. pneumoniae* isolates showed statistically significant phenotypic resistance to colistin, with these two isolates being identified in pigs (Table 4).

Correlation between colistin-resistant phenotypes and *mcr-1*

Correlations between phenotypic resistance and the presence of *mcr-1* for *E. coli* and *K. pneumoniae* are shown in Table 3 and Table 4, respectively. For *E. coli*, *mcr-1* was found in all isolates from humans, pigs and chickens showing phenotypic intermediate resistance (≤ 2 µg/mL) to colistin. The *mcr-1* gene was also found in the two *E. coli* isolates showing resistance (≥ 4 µg/mL) to colistin (Table 3). For *K. pneumoniae*, *mcr-1* was found in isolates from humans, pigs and chickens with intermediate resistance to colistin. The *mcr-1* gene was not found in the two *K. pneumoniae* isolates showing resistance to colistin (Table 4).

DISCUSSION

To our knowledge, this is the first study in Ecuador, and one of the first in Latin America, that has assessed the prevalence of fecal carriage of colistin-resistance among *E. coli* and *K. pneumoniae* isolates from healthy humans and their backyard animals living in rural areas of two different regions. We found a surprisingly low prevalence of phenotypic resistance to colistin (< 1%), but a high prevalence of molecular resistance in *E. coli*, with *mcr-1* present in 90% of fecal samples with *E. coli*.

The high prevalence of *E. coli* in all types of hosts was consistent with previous studies, confirming that this bacterium is one of the most common colonizers of the gastrointestinal tract in both animals and humans (14). The overall prevalence of *K. pneumoniae* in the three types of hosts was lower, at 37%, although humans had a higher prevalence of the organism compared with chickens and pigs. *K. pneumoniae* naturally

TABLE 2. Prevalence of *Klebsiella pneumoniae* isolates and the *mcr-1* gene in fecal samples from humans, pigs and chickens in two rural provinces of Ecuador, 2020 (*N* = 438)

Variables	No. of samples	Prevalence of confirmed isolates		Prevalence of *mcr-1* gene	
		n	% (95% CI)	*n*	% (95% CI)
Province					
Pastaza	212	67	31.6 (16.9 to 46.2)	13	19.4 (0 to 74.6)
Santo Domingo	226	97	42.9 (29.9 to 55.8)	19	19.6 (0 to 43.1)
Host					
Human	137	99	72.3 (60.3 to 83.6)	21	21.2 (0 to 43.9)
Chicken	154	43	27.9 (10.3 to 45.6)	7	16.3 (0 to 51.6)
Pig	147	22	15.0 (0 to 34.6)	4	18.2 (0 to 67.5)

CI: confidence interval.
Source: Table prepared by the authors with data from their study.

TABLE 3. Association between phenotypic intermediate resistance and resistance to colistin and presence of the *mcr-1* gene in *Escherichia coli* isolates from humans and backyard animals in Ecuador, 2020 (*N* = 378)[a]

Host	Results of broth microdilution	*mcr-1* gene		
		No. (%) negative	No. (%) positive	Total
Human	I	5 (4.3)	111 (95.7)	116
	R	0	0	
Pig	I	22 (17.2)	104 (81.3)	128
	R	0	2* (1.6)	
Chicken	I	11 (8.2)	123 (91.7)	134
	R	0	0	

I: intermediate resistance to colistin is determined when growth occurs at ≤ 2 µg/µL of the antibiotic; R: bacteria are considered resistant to colistin if growth occurs at ≥ 4 µg/µL; *: statistically significant difference at $P < 0.05$ using the χ^2 test.
[a] The cut-off points for determining resistance were from the Clinical and Laboratory Standards Institute standard M100, 2022.
Source: Table prepared by the authors with data from their study.

colonizes the respiratory and gastrointestinal tracts of humans, and the bacterium is one of the main etiological pathogens of infections with clinical relevance (19). The low prevalence of *K. pneumoniae* in chickens and pigs in our study has been found elsewhere (20) and might be due to their diet, competition in the environment or genetic virulence factors responsible for the colonization of enteric systems in animals (21, 22).

There was a surprisingly low prevalence of phenotypic resistance to colistin in our study. Our results concur with a recent review that found a global prevalence of colistin resistance of 1.6%, with incidence rates rising during the past 5 years on all continents, largely as a result of inappropriate use of the antibiotic in animal husbandry (23). We used standard laboratory methodology (broth microdilution) for determining the minimum inhibitory concentration values for colistin, so we believe our results are accurate. Of note, however, when laboratory susceptibility testing of our samples was conducted in 2020 and 2021, the CLSI break point of ≤ 2 µg/mL of colistin was regarded as indicating susceptibility. The 2022 version of the CLSI standard indicates that bacterial growth at that concentration should be considered as indicating intermediate resistance, which is how we have presented the data.

A high prevalence of *mcr-1* (90%) was found in the *E. coli* isolates, and this aligns with the rapid worldwide spread of this gene since it was first reported in 2016 in China. There are several variants of the *mcr* gene, but *mcr-1* appears to be the predominant variant and has spread at a rate that is 95% faster than the other 9 variants (*mcr-2* to *mcr-10*) (3, 8). The *mcr-1* gene is certainly the most common in Latin America and the Caribbean, and the country with the highest prevalence is Brazil, with nearly 45% of *E. coli* isolates testing positive for the gene (24).

We found a higher prevalence of *mcr-1* in *E. coli* isolates from the coastal region compared with the Amazon region, and in humans and chickens compared with pigs. Other studies in Ecuador have also found differing prevalence rates, depending on the source. For example, a low prevalence was found in dogs, at 25% (11), and irrigation water, at 18% (13), whereas in chickens rates of more than 95% were found (12, 25). We do not have a clear explanation for why there are differences, but diet, animal husbandry practices and the environment might play a part.

In *K. pneumoniae* isolates, we found a lower prevalence of *mcr-1*, at less than 20%, with little difference between geographical regions and type of host. However, this prevalence in our study was higher than those found elsewhere: in Taiwan, 5.5% of chickens and 0.4% of pigs were reported to harbor *mcr-1* (26, 27), while in China 11% of chickens and 7% of pigs with resistant *K. pneumoniae* isolates harbored the gene (28).

There was no correlation between phenotypic resistance and the presence of *mcr-1*. This finding has been previously described by others (29, 30), and it may be explained by the multifactorial aspects of antimicrobial resistance, such as (i) the diversity of plasmid types carrying *mcr-1* (31), (ii) strong or weak plasmid promotors influencing the expression of the genes (32), and (iii) the fusion plasmid phenomenon that increases the transfer capabilities of *mcr* genes between hosts (9). Only four bacterial isolates were resistant, and all were found in pigs. The two resistant *E. coli* isolates were positive for *mcr-1*, indicating plasmid-induced resistance, while the two resistant *K. pneumoniae* isolates were negative for *mcr-1*, presumably indicating resistance due to chromosomal mutations. The high prevalence *mcr-1* in *E. coli* with intermediate resistance, however, is cause for concern, as this might lead to further full-blown resistance if the misuse of colistin continues.

Strengths and limitations

The strengths of this study were the large sample size, which exceeded our estimated sample size; a robust system for collecting specimens; sound laboratory techniques that followed international guidelines; and conducting and reporting the study in line with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (33). A further strength was the implementation of the study in two different regions and among rural settlements, together with the inclusion of humans and two types of backyard animals.

However, there were some limitations. It was difficult to collect samples consistently during the study period due to the COVID-19 pandemic, and we had several refusals to participate from households in the settlements. Genetic and epidemiological studies are needed to compare these findings

TABLE 4. Association between phenotypic intermediate resistance and resistance to colistin and presence of the *mcr-1* gene in *Klebsiella pneumoniae* isolates from humans and backyard animals in Ecuador, 2020 (*N* = 164)[a]

Host	Results of broth microdilution	*mcr-1* gene		
		No. (%) negative	No. (%) positive	Total
Human	I	78 (78.8)	21 (21.2)	99
	R	0	0	
Pig	I	16 (72.7)	4 (18.2)	22
	R	2* (9.1)	0	
Chicken	I	36 (83.7)	7 (16.3)	43
	R	0	0	

I: intermediate resistance to colistin is determined when growth occurs at ≤ 2 µg/µL of the antibiotic; R: bacteria are considered resistant to colistin if growth occurs at ≥ 4 µg/µL; *: statistically significant difference at $P < 0.05$ using the χ^2 test.
[a] The cut-off points for determining resistance were from the Clinical and Laboratory Standards Institute standard M100, 2022.
Source: Table prepared by the authors with data from their study.

and determine whether there are other strains in the country. Furthermore, studies on the expression of the *mcr-1* gene are necessary to understand better the relationship between molecular and phenotypic resistance because this is dynamic and multifactorial for colistin. The clonal distribution between humans, chickens and pigs, and the presence of the *mcr-1* gene in plasmids were not determined, and these are areas to pursue in further research. Finally, we did not collect any information about colistin use in animals in the settlements that we studied.

Conclusions

The prevalence of colistin resistance in bacterial isolates of *E. coli* and *K. pneumoniae* was evaluated from fecal samples from healthy humans and their backyard animals living in rural Ecuador.

The prevalence of molecular resistance to colistin (identified by the presence of *mcr-1*) was high, especially in *E. coli* isolates (90%). However, the low prevalence of phenotypic resistance associated with these strains was striking (< 1%). In *K. pneumoniae* isolates, phenotypic resistance occurred independently of the presence of *mcr-1* (1.2%), with the chromosomal mechanism of colistin resistance probably being the main factor involved. These findings raise important concerns about the potential spread of drug-resistant pathogens in the community, which will add to the overall burden of antimicrobial resistance unless firm action is taken.

To counter this, we make two important recommendations. First, our study methodology should serve as a basis for ongoing surveillance of colistin resistance in three species of hosts, or carriers, and this should be replicated in sentinel sites all over the country. Second, there is an urgent need to regulate the use of colistin in animal husbandry and to avoid its overuse in human clinical cases for which there is no clear indication. In January 2020, Ecuador banned the veterinary use of colistin, and it has since been shown that the sales of colistin as a growth promoter have decreased (34). An encouraging report from China showed that banning colistin as an animal growth promoter led to substantial decreases in colistin-resistant *E. coli* in pigs, chickens and humans (35). Therefore, regulatory policy combined with continual colistin monitoring can work and will help prevent the spread of colistin resistance in Ecuador and elsewhere.

Authors' contributions. All authors conceived the original idea, planned the experiments, collected and analyzed the data, contributed data or analysis tools, interpreted the results, and wrote and reviewed the paper. All authors reviewed and approved the final version of the paper.

Acknowledgements. This research study was developed through the Structured Operational Research and Training Initiative (SORT IT), a global partnership coordinated by the Special Programme for Research and Training in Tropical Diseases (TDR), the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank; it is hosted at the World Health Organization. The specific SORT IT program that led to this study included an implementation partnership between TDR and the Pan American Health Organization, the WHO country offices of Colombia and Ecuador; the Ministry of Health and Social Protection, Colombia; the Food and Agriculture Organization, Sierra Leone; Sustainable Health Systems, Freetown, Sierra Leone; the Tuberculosis Research and Prevention Center, nongovernmental organization, Armenia; the International Union Against Tuberculosis and Lung Disease, France and India offices (South-East Asia); Institute of Tropical Medicine, Antwerp, Belgium; Damien Foundation, Belgium; Indian Council of Medical Research–National Institute of Epidemiology; Jawaharlal Institute of Postgraduate Medical Education and Research; GMERS Medical College, Gotri, India; India Medical College Baroda; Sri Manakula Vinayagar Medical College, India; Public Health Ontario, Canada; Universidade Federal de Ciências de Saúde de Porto Alegre, Brazil; Universidade de Brasilia, Brazil; Universidad de Concepción, Chile; Universidad de los Andes, Colombia; Universidad Pontificia Bolivariana, Colombia; Universidad Pedagógica y Tecnológica de Colombia; Universidad Central del Ecuador; California State University, Fullerton, California, USA; and Universidad Autónoma de Yucatán, México.

Conflicts of interest. None declared.

Funding. This study was supported by funding from the Universidad de las Américas, Quito, Ecuador (grant number VET.MCA.19.03). The SORT IT antimicrobial resistance program is funded by the National Institute for Health and Care Research, Department of Health and Social Care, United Kingdom, and supported by implementing partners. All open access and ethics-related costs were covered by TDR. Funding was also provided by Consejería de Economía e Infraestructuras, La Junta de Extremadura (grant number IB18047).

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Manuscript submitted 26 October 2022. Revised version accepted for publication on 29 October 2022.

Resistencia a la colistina de las bacterias *Escherichia coli y Klebsiella pneumoniae* en humanos y animales de granja en Ecuador

RESUMEN

Objetivo. La colistina es un antibiótico de último recurso para tratar infecciones graves por bacterias gramnegativas. Sin embargo, su uso indebido, especialmente para estimular el crecimiento animal, ha contribuido con el aumento de la resistencia a los antimicrobianos, mediada principalmente por la transferencia de plásmidos del gen *mcr-1*. En este estudio se evaluó la prevalencia de la resistencia fenotípica y molecular a la colistina de las bacterias *Escherichia coli* y *Klebsiella pneumoniae* en humanos sanos, sus pollos y cerdos en Ecuador.
Métodos. Se recolectaron muestras fecales de humanos, así como de sus pollos y cerdos, en dos zonas rurales de la región costera y la región amazónica entre abril y agosto del 2020. Se aislaron las bacterias gramnegativas y se identificaron empleando técnicas convencionales. Se determinó la resistencia fenotípica mediante la técnica de microdilución en caldo y se detectó el gen *mcr-1* con la técnica convencional de reacción en cadena de la polimerasa.
Resultados. Se obtuvo un total de 438 muestras fecales de 137 humanos, 147 cerdos y 154 pollos. La prevalencia de *E. coli* en las cepas aisladas fue del 86,3% (378/438) y la de *K. pneumoniae*, del 37,4% (164/438). En general, se detectó el gen *mcr-1* en el 90% (340/378) de las cepas aisladas de *E. coli* y la mayor prevalencia encontrada fue en cepas aisladas de la región costera (96,5%, 191/198), humanos (95,6%, 111/116) y pollos (91,8%, 123/134); en el caso de *K. pneumoniae*, el gen se encontró en el 19,5% (32/164) de las cepas, con una distribución equitativa entre regiones y hospedadores. Únicamente cuatro cepas aisladas, dos de *E. coli* y dos de *K. pneumoniae*, mostraron resistencia fenotípica: el gen *mcr-1* estaba presente en ambas cepas de *E. coli* y ausente en las cepas de *K. pneumoniae*.
Conclusiones. Si bien hubo una baja prevalencia de resistencia fenotípica a la colistina, la alta prevalencia del gen *mcr-1* en *E. coli* es preocupante. Es necesario hacer cumplir la prohibición del uso de colistina en la cría de animales en Ecuador, así como realizar un seguimiento continuo de la situación.

Palabras clave Colistina; *Escherichia coli*; *Klebsiella pneumoniae*; humanos; animales; resistencia a medicamentos; genes MDR; investigación operativa; Ecuador.

Resistência à colistina em *Escherichia coli e Klebsiella pneumoniae* em humanos e animais de quintal no Equador

RESUMO

Objetivo. A colistina é um antibiótico de último recurso para o tratamento de infecções graves por bactérias Gram-negativas. Entretanto, o uso indevido da colistina, principalmente como promotor de crescimento animal, tem contribuído para o aumento da resistência a antimicrobianos, principalmente por transferência horizontal do gene *mcr-1* mediada por plasmídeos. Este estudo avaliou a prevalência de resistência fenotípica e molecular à colistina em *Escherichia coli* e *Klebsiella pneumoniae* no Equador em humanos hígidos e em galinhas e porcos por eles criados.
Métodos. Entre abril e agosto de 2020, foram coletadas amostras de fezes de habitantes de duas regiões litorâneas e amazônicas do Equador e de galinhas e porcos por eles criados. Bactérias Gram-negativas foram isoladas e identificadas por meio de técnicas convencionais. A resistência fenotípica foi determinada pela técnica de microdiluição em caldo, e o gene *mcr-1* foi detectado por reação em cadeia da polimerase convencional.
Resultados. Foram obtidas 438 amostras fecais de 137 humanos, 147 suínos e 154 galinhas. A prevalência de isolados de *E. coli* foi de 86,3% (378/438), e de *K. pneumoniae*, 37,4% (164/438). Em geral, o gene *mcr-1* foi encontrado em 90% (340/378) dos isolados de *E. coli*, com maiores prevalências encontradas em isolados de regiões litorâneas (96,5%, 191/198), humanos (95,6%, 111/116) e galinhas (91,8%, 123/134); para *K. pneumoniae*, o gene foi encontrado em 19,5% (32/164) dos isolados, com igual distribuição entre regiões e hospedeiros. Somente quatro isolados, dois de *E. coli* e dois *de K. pneumoniae,* demonstraram resistência fenotípica: o gene *mcr-1* estava presente em ambas as cepas de *E. coli*, mas ausente nas de *K. pneumoniae.*
Conclusões. Apesar da baixa prevalência de resistência fenotípica à colistina, a alta prevalência do gene *mcr-1* em *E. coli* é preocupante. É preciso fiscalizar a proibição ao uso agropecuário de colistina no Equador e implementar o monitoramento contínuo da situação.

Palavras-chave Colistina; *Escherichia coli*; *Klebsiella pneumoniae*; humanos; animais; resistência a medicamentos; genes MDR; Equador.