

Original Article

Prevalence of Antimicrobial Resistance in *Escherichia coli* Isolated From Guinea Pigs in Andean PeruRodolfo Gamarra-Ramírez¹ , María Díaz-Pereyra¹ , Norma Gamarra-Ramírez² , Luis Vargas-Rocha^{1*}

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ABSTRACT

Background: Guinea pig breeding has gained increasing economic and nutritional importance in various regions worldwide. However, guinea pig farming exposes livestock to multiple infectious diseases that require treatment with different antibiotics—leading to resistance if not properly managed.

Objectives: This study aimed to determine the prevalence of bacterial resistance in *Escherichia coli* isolated from guinea pigs raised in Cajamarca City, Peru, against five commonly used antimicrobials in 2022.

Methods: Rectal swabs were collected from 105 clinically healthy guinea pigs with no history of antibiotic treatment. The identification of *E. coli* was confirmed through IMViC biochemical tests, and antimicrobial susceptibility was assessed using the disk diffusion method.

Results: All samples showed resistance to at least one antibacterial. According to the The European Food Safety Authority (EFSA)-European Centre for Disease Prevention and Control (ECDC) classification, the prevalence of neomycin resistance was “extremely high (>70%),” of sulfamethoxazole/trimethoprim and chloramphenicol “low (>1-10%)” and of tetracycline and enrofloxacin “very low (>0.1-1%).” Additionally, 93 cultures (88.57%) were resistant to only one antibacterial, eight (7.62%) to two antibacterials, and only two (1.90%) to three antibacterials.

Conclusion: A high prevalence of neomycin resistance was observed in *E. coli* isolated from guinea pigs. Antibacterials with low resistance prevalences are an alternative for use in these rodents. However, continuous monitoring of antimicrobial susceptibility profiles and specific implementation and sustainable strategies are necessary to preserve the efficacy of antibacterials with high sensitivity rates.

Keywords: Antibiotic therapy, *Cavia porcellus*, Enterobacteria, Resistance, Susceptibility

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Introduction

Guinea pig farming has gained significant importance due to its provision of a regular source of high-quality animal protein for consumption (Ngoula et al., 2017; Sánchez-Macías et al., 2018). Additionally, these rodents (*Cavia porcellus*) are prolific animals, capable of growing and reproducing on a variable diet and they adapt to a wide range of climates, which has enabled their distribution all over the world (Lammers et al., 2009; Sánchez-Macías et al., 2018). However, these animals are susceptible to multiple diseases of different etiologies, including viral, fungal, parasitic, metabolic, neoplastic, and bacterial infections.

Bacterial diseases have gained more relevance due to their frequency and impact, manifesting as lymphadenitis, pododermatitis, pneumonia, otitis, enteritis, mastitis, and the like (Anderson, 1987). For the treatment of these infections, various antibiotics, such as erythromycin and rifampicin (Fraser et al., 1978), have been used over the decades as fluoroquinolones for respiratory diseases (Pechère & Gootz, 1998) and combinations of fluoroquinolones and metronidazole for oral diseases (Minarikova et al., 2016).

However, resistance and reduced efficacy have also been reported in some studies. Even decades ago, penicillin, chloramphenicol, tetracycline, and gentamicin showed no significant effects (Fraser et al., 1978). In a later study, the efficacy of potassium benzylpenicillin, cephalothin, amoxicillin-clavulanate, doxycycline, gentamicin, trimethoprim-sulfamethoxazole, clindamycin, and metronidazole has decreased (Minarikova et al., 2016). In Peru, it has been reported that beta-hemolytic *Streptococcus* sp. isolated from cervical abscesses in guinea pigs was resistant to enrofloxacin and trimethoprim-sulfamethoxazole (Angulo-Tisoc et al., 2021). Additionally, resistance of *Trueperella pyogenes* to cephalexin, tetracycline, trimethoprim-sulfamethoxazole, and penicillin was reported in a study conducted in Cajamarca (Vargas-Rocha et al., 2023).

The bacterium *Escherichia coli* is a gram-negative enterobacterium that has been found in various diseases in guinea pigs. This bacterium has been shown to cause emaciation, depression and death in weaned pups (Hurlet et al., 1995). *E. coli* infections in guinea pigs are facilitated by poor nutrition, overcrowding, environmental stress, and the administration of oral antibiotics (Harkness et al., 2002). Its presence has also been reported in cases of mastitis and cystitis (Kinkler et al., 1976). Furthermore, the *E. coli* O124 K72 strain has been identified to have adverse effects on intestinal barrier function, capable of disrupting the integrity of structural proteins and potentially playing a role in colon carcinogenesis (Ren et

al., 2017). Additionally, resistance of *E. coli* to cephalosporins has been reported (Tenover, 2006).

Cajamarca is one of Peru's main guinea pig-producing regions, with a population of more than 2.4 million (SENASA, 2019). However, according to a previous study conducted in a nearby province, between one and five guinea pigs die per week per producer, especially in the lactating class, with the most reported disease being salmonellosis (Ortiz-Oblitas et al., 2021). This figure suggests that guinea pigs suffer from various infectious diseases that producers treat, but the proven efficacy of these treatments is unknown. Therefore, this study aimed to determine the susceptibility and prevalence of bacterial resistance in *E. coli* isolated from guinea pigs in Cajamarca City to commonly used antimicrobials. *E. coli* was chosen as a model and representative bacterium, as it is part of the normal microbiota of guinea pigs. Thus, the effects of antibiotics on this bacterium could be reflective of other bacteria.

Materials and Methods

Place and sample size

The study was conducted in 2022 in Cajamarca City, located in the Cajamarca region in northeastern Peru. Since there was no updated census of the guinea pig population, based on the Equation 1:

$$1. n = \frac{Z^2 \times p \times q}{d^2}$$

The sample size (n) was estimated based on an unknown population, considering a 95% confidence level (Z), a 50% expected proportion (p) and a 10% of precision (d) (Charan et al., 2021).

Based on this, it was determined that 97 samples should be collected; however, to account for the different sectors of Cajamarca, the sample size was increased to 105 samples. During the study, Cajamarca was divided into 15 administrative sectors.

Seven samples were collected from each sector. Visits were made to households and those who raised guinea pigs and voluntarily agreed to participate after being informed about the study were included. If a household did not have guinea pigs or declined to participate, the next house was visited until a suitable case was found. Clinically healthy guinea pigs with no history of antimicrobial treatment were sampled. Additionally, information on breeding conditions, purpose, sex and age of the animals was collected to identify possible associated or risk factors.

Sampling and sample transportation

A single fecal sample was collected from the rectum of each properly restrained guinea pig using sterile swabs. These swabs were placed in test tubes containing 0.1% peptone water. The samples were then transported in an expanded polystyrene box to the Veterinary Microbiology Laboratory at the [National University of Cajamarca](#).

Bacteriological culture and isolation

The primary culture was performed on Petri dishes with MacConkey agar (Merck, Germany) at 37 °C for 18 to 24 hours. Typical *E. coli* colonies were then read and phenotypically identified. A pure culture was obtained from these colonies in 1% peptone broth, followed by biochemical tests using IMViC to confirm the bacteria's identity ([MacWilliams, 2009](#); [MacWilliams, 2012](#); [McDevitt, 2009](#)).

For the indole test, *E. coli* was inoculated into test tubes containing peptone broth and incubated at 37 °C for 24 h. After adding 5 drops of Kovac's reagent (Liofilchem, Italy), a red ring appeared on the surface of the broth, confirming a positive result and the bacterium's ability to produce the enzyme tryptophanase.

In the methyl red test, an aliquot of the *E. coli* culture was inoculated into MR-VP (Merck, Germany) broth and incubated at 37 °C for 24 to 48 h. After adding 2–3 drops of methyl red reagent and gentle mixing, the medium displayed a bright reddish-pink color, characteristic of mixed acid fermentation, indicating a positive result.

For the Voges-Proskauer test, a pure culture was inoculated into MR-VP broth and incubated at 37 °C for 18–24 h. Subsequently, 0.6 mL of α -naphthol and 0.2 mL of 40% KOH were added, with gentle mixing after each addition. The tubes were then incubated for an additional 1–2 hours. No color change was observed in the medium, confirming a negative result characteristic of *E. coli*.

Finally, in the citrate utilization test, *E. coli* was streaked onto the slanted surface of Simmons' Citrate Agar (Merck, Germany) and incubated at 37 °C for 24–48 h. No color change was observed in the medium, indicating that *E. coli* did not utilize citrate as a carbon source, resulting in a negative outcome.

Antibiogram

The disk diffusion test ([Bauer et al., 1966](#)) was performed on Mueller Hinton agar plates (Merck, Ger-

many), incubated at 37 °C for 18 hours, followed by evaluation of inhibition zone (IZs). Commonly used antimicrobials were tested: Tetracycline (30 μ g), neomycin (30 μ g), sulfamethoxazole/trimethoprim (1.25/23.7 μ g), enrofloxacin (30 μ g) and chloramphenicol (30 μ g) (Oxoid™, UK).

These five antimicrobials were selected based on surveys conducted at Cajamarca City's main veterinary product outlets. The most frequently sold products for treatment in guinea pigs were identified, and the chemical composition of these products was reviewed to determine the most common active ingredients.

Interpretation was performed according to the [Clinical and Laboratory Standards Institute \(CLSI\)](#) guidelines. The isolates were classified as susceptible, intermediate, or resistant. Since the [CLSI \(2024\)](#) does not provide standardized inhibition zone diameters for neomycin, the values for amikacin, a human-use aminoglycoside with a similar mechanism of action, were used as a reference.

Resistance prevalence was categorized based on the [European Food Safety Authority- European Centre for Disease Prevention and Control \(2023\)](#) guidelines: <0.1% (rare), >0.1% to 1% (very low), >1% to 10% (low), >10% to 20% (moderate), >20% to 50% (high), >50% to 70% (very high), and >70% (extremely high).

Statistical analysis

Data were processed using basic and analytical statistics. Prevalence rates and their corresponding 95% confidence intervals were calculated. The association between antimicrobial resistance and the variables of interest was initially assessed through bivariate analysis using the chi-square test to identify potential associated factors. Subsequently, univariate logistic regression analysis was conducted to determine possible risk factors. All analyses were performed using SPSS software, version 27, with a significance level set at $P < 0.05$.

The odds ratio (OR) analysis was applied to evaluate the association between *E. coli* resistance to antibiotics and the independent variables: Sex, breeding place, cohabitation with other animals, productive purpose and type of feed. The reference categories selected were "male" for sex, "cage" for breeding place, "no" for cohabit, "consumption" for productive purposes, and "alfalfa" for type of feed. These reference categories were coded as 0, while the comparative categories were coded as 1.

The OR was used to estimate the strength of the association between an exposure factor and an outcome, in this case, the probability of antibiotic resistance. An $OR > 1$ indicates a higher likelihood of resistance in the comparative category relative to the reference, an $OR < 1$ shows a lower probability, and an $OR = 1$ suggests no difference between categories. The 95% confidence intervals (CI) were calculated for each OR. If the value of 1 was included within the CI, the result was considered statistically non-significant, further corroborating by the P. This analysis was restricted to chloramphenicol due to the sufficient number of recorded cases, in contrast to other antimicrobials where low or high resistance prevalence precluded robust statistical analyses.

Results

All the guinea pig samples were raised in household environments and ranged in age from 1 to 24 months (4.65 ± 3.68 months). Some were raised in cages, while others were kept on the ground. Other animal species, such as rabbits, chickens, dogs, and cats, cohabited in the same breeding environment. Most guinea pigs were raised for consumption, with fewer kept as pets. Due to the variability in breeds and types of guinea pigs, these characteristics were not considered in the study.

All *E. coli* cultures exhibited resistance to at least one of the evaluated antimicrobials. Also, 100% of the samples showed resistance to neomycin, while the lowest resistance prevalence was observed for tetracycline and enrofloxacin (Table 1).

The prevalence of resistance to a single antimicrobial (neomycin) was classified as “extremely high ($>70\%$).” In contrast, both tetracycline and enrofloxacin were classified in the “very low ($>0.1\%$ to 1%)” category (Table 2).

Additionally, there were cases where a single *E. coli* culture showed resistance to more than one antimicrobial. However, most cases demonstrated resistance to only one antimicrobial (Figure 1).

The bivariate analysis did not reveal any variables associated with the prevalence of *E. coli* resistance to any of the evaluated antimicrobials (Table 3). Moreover, since all isolates were resistant to neomycin, this antimicrobial could not be further analyzed.

In the univariate regression analysis, four of the five antibiotics did not meet the statistical criteria due to a high number of resistant or susceptible cases. The only antimicrobial that met the statistical criteria was chloramphenicol. However, none of the variables (sex, breeding place, cohabitation with other animals, production purpose and type of diet) were found to be risk factors ($P > 0.05$) (Table 4).

Discussion

All *E. coli* cultures exhibited resistance to at least one of the evaluated antimicrobials. Neomycin showed an extremely high prevalence ($>70\%$), while the lowest prevalence was observed for tetracycline and enrofloxacin, classified as very low ($>0.1\%$ to 1%). Due to extreme values, no factors associated with *E. coli* antimicrobial resistance were identified.

Table 1. Reference measurements of the IZ of the antibacterials used and percentages of sensitive, intermediate and resistant cases in a guinea pig-producing area in Peru

Antibacterial	IZ (mm)*			Count (%)		
	S	I	R	S	I	R
Tetracycline	15 $\geq$	12-14	11 $\leq$	104(99.05)	0	1(0.95)
Neomycin	20 $\geq$	17-19	16 $\leq$	0	0	105(100)
Sulfamethoxazole/ trimethoprim	16 $\geq$	11-15	10 $\leq$	102(97.14)	1(0.95)	2(1.90)
Enrofloxacin	23 $\geq$	17-22	16 $\leq$	101(96.19)	3(2.86)	1(0.95)
Chloramphenicol	18 $\geq$	13-17	12 $\leq$	52(49.52)	43(40.95)	10(9.52)

S: Sensitive; I: Intermediate; R: Resistant.

*Clinical and Laboratory Standards Institute (2024).

Table 2. Prevalence (%) and resistance classification of *E. coli* isolates from guinea pigs (n=105) from a production area in Peru against the evaluated antibacterials

Antibacterial	No.	% Resistant (95% CI)	EFSA-ECDC*
Tetracycline	1	0.95 (0.00-2.81)	Very low
Neomycin	105	100 (0.00-0.00)	Extremely high
Sulfamethoxazole/trimethoprim	2	1.90 (0.00-4.52)	Low
Enrofloxacin	1	0.95 (0.00-2.81)	Very low
Chloramphenicol	10	9.52 (3.91-15.14)	Low

Abbreviations: EFSA: European Food Safety Authority; ECDC: European Centre for Disease Prevention and Control.

*EFSA and ECDC: Extremely high (>70%), low (>-10%), very low (>0.1-1%).

Although there are no specific studies on *E. coli* resistance in guinea pigs, there have been studies documenting antibiotic resistance in other bacteria. In Lima (Peru), intensively raised guinea pigs found that *Salmonella typhimurium* exhibited resistance to several antibiotics, including 60% to erythromycin, 40% to nitrofurantoin, 30% to streptomycin, 25% to penicillin, and 10% to enrofloxacin (Salvatierra et al., 2018). In the same region (Lima), an analysis revealed genotypic resistance to multiple antibiotics in at least 50% of *S. Typhimurium*

isolates, including nalidixic acid, aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and fluoroquinolones (Hurtado et al., 2023). Another research in Azuay (Ecuador) found that 6.25% of guinea pigs carrying *Staphylococcus aureus* were resistant to methicillin (Zambrano-Mila et al., 2019). In Cusco (Peru), resistance to enrofloxacin (1.8%) and trimethoprim-sulfamethoxazole (3.6%) was identified in family-farmed guinea pigs infected with beta-hemolytic *Streptococcus* species (Angulo-Tisoc et al., 2021).

Table 3. Prevalence (%) and bivariate analysis of resistance of *E. coli* isolates from guinea pigs in a guinea pig-producing area in Peru

Variables	Covariables	No.	Tetracycline		Sulf/trim*		Enrofloxacin		Chloramphenicol	
			Cases (%)	P	Cases (%)	P	Cases (%)	P	Cases (%)	P
Sex	Male	47	0	0.366	2(4.26)	0.113	1(2.13)	0.264	5(10.64)	0.762
	Female	58	1(1.72)		0		0		5(8.62)	
Age (m)	≤6	86	1(1.16)	0.637	2(2.33)	0.502	1(1.16)	0.637	9(10.47)	0.485
	>6	19	0		0		0		1(5.26)	
Breeding place	Cage	68	0	0.137	1(1.47)	0.659	1(1.47)	0.173	4(5.88)	0.085
	Floor	37	1(2.70)		1(2.70)		0		6(16.22)	
Cohabit	No	66	1(1.52)	0.440	2(3.03)	0.272	1(1.52)	0.440	6(9.06)	0.884
	Yes	39	0		0		0		4(10.26)	
Productive purpose	Consumption	92	1(1.09)	0.706	2(2.17)	0.591	1(1.09)	0.706	9(9.78)	0.810
	Pet	13	0		0		0		1(7.69)	
Feed	Alfalfa	66	0	0.191	2(3.03)	0.272	1(1.52)	0.440	9(13.64)	0.062
	Alfalfa+other	39	1(2.56)		0		0		1(2.56)	

Significance level $P < 0.05$ (the chi-square test).

*Sulfamethoxazole/trimethoprim.

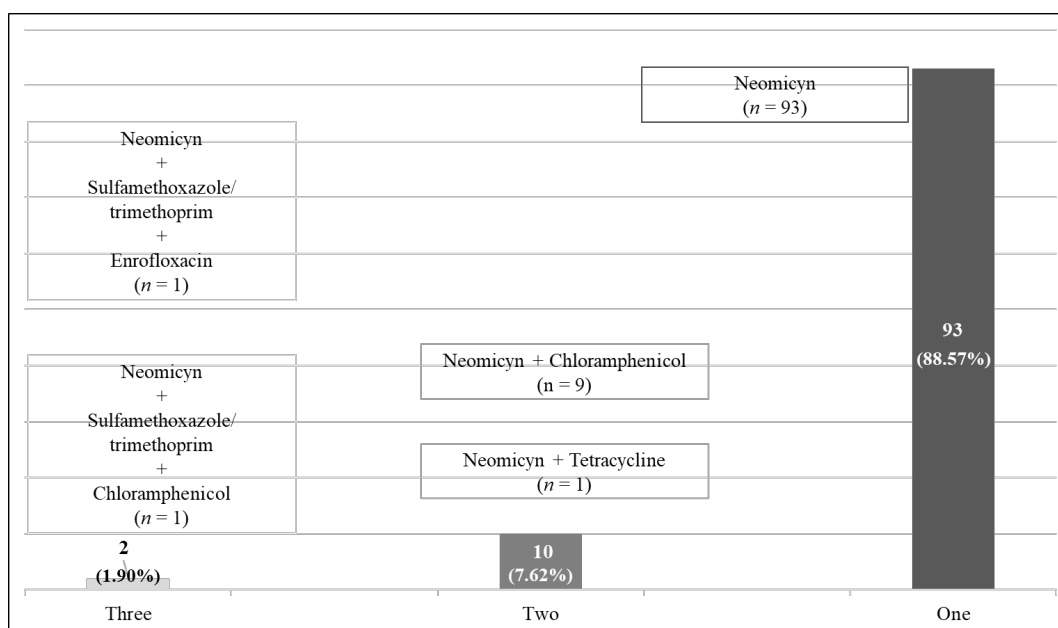


Figure 1. Resistance of *E. coli* isolated from guinea pigs in Cajamarca, according to the number of antibacterials

Although cultures resistant to two or three antimicrobials were identified, these were not as prevalent as resistance to a single antimicrobial. This could be considered favorable in cases of multidrug resistance, as it allows the use of other antimicrobials and potential combinations. According to the [European Food Safety Authority \(EFSA\)](#) and [European Centre for Disease Prevention and Control \(ECDC\)](#), bacteria are considered multidrug-resistant if they are resistant to at least three different classes of antimicrobials, and co-resistance refers to combined resistance to two critically important anti-

crobials ([EFSA & EFSA 2023](#)). Multidrug resistance represents a significant global health threat. The lack of adequate disease treatments leads to animal suffering and welfare issues ([Vaarten, 2012](#)).

Since the guinea pigs in this study had no history of antimicrobial treatments yet showed resistance, horizontal transmission of resistant bacteria from cohabiting mammal species could be considered. Additionally, it has been reported that multidrug-resistant pathogens, including *E. coli*, are spreading at an unprecedented rate

Table 4. Univariate logistic regression analysis of *E. coli* resistance to chloramphenicol in guinea pigs from a guinea pig-producing area in Peru

Variables	Covariable	No.	OR	95% CI	P
Sex	Ref. (male)	47			
	Female	58	1.384	[0.313-6.116]	0.668
Breeding place	Ref. (cage)	68			
	Floor	37	0.59	[0.113-3.084]	0.532
Cohabit	Ref.	66			
	Yes	39	1.771	[0.417-7.526]	0.438
Productive purpose	Ref. (consumption)	92			
	Pet	13	1.012	[0.114-8.958]	0.992
Feed	Ref. (alfalfa)	66			
	Alfalfa+other	39	0.222	[0.026-1.875]	0.167

globally, with *E. coli* having a high capacity to accumulate resistance genes (Cantas et al., 2013; Poirel et al., 2018; Theuretzbacher, 2013). There is also a greater possibility of *E. coli* transmission from mother to offspring, as maternal-offspring interaction is highest post-partum.

Given the expanding presence of guinea pig farming in various parts of the world and the limited information on antimicrobial resistance in this species, special attention should be given. The irrational use of veterinary antimicrobials can lead to the selective pressure of antimicrobial-resistant pathogens, which could endanger animal, public, and environmental health (Hao et al., 2014).

Comprehensive (one health) measures must be considered to prevent the increase of bacterial resistance in livestock. Since the constant use of antimicrobials contributes to developing resistant bacterial strains (Tenover, 2006), antimicrobial management should be addressed through coordinated approaches and interventions. In addition to reducing antibiotic use, efforts should encompass infection control, clinical microbiology, antimicrobial use, resistance surveillance, pharmacovigilance, education, guidelines, and government regulations (Bengtsson & Greko, 2014).

Conclusion

In conclusion, widespread resistance of *E. coli* isolated from guinea pigs was observed in Cajamarca City. The prevalence of resistance reached 100% for neomycin, while the lowest was found for tetracycline and enrofloxacin (0.95%). This scenario underscores the need to implement antimicrobial susceptibility monitoring measures and to develop specific and sustainable strategies to preserve the efficacy of antimicrobials in guinea pig production in Cajamarca.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the National University of Cajamarca, Cajamarca, Peru (Code: 002866). The authors adhered to the ethical guidelines and principles for using animals in research. The owners were informed about the objectives and methodology of the study, and they provided their oral consent for participation. The authors complied with the provisions of the Ley de Protección y Bienestar Animal of the Peruvian State (Law No.: 30407).

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Authors' contributions

Conceptualization and resources: Rodolfo Gamarra-Ramírez and María Díaz-Pereyra; Methodology: Rodolfo Gamarra-Ramírez and Norma Gamarra-Ramírez; investigation: Rodolfo Gamarra-Ramírez, María Díaz-Pereyra, and Norma Gamarra-Ramírez; Project administration: Rodolfo Gamarra-Ramírez; Formal analysis, visualization, and writing: Luis Vargas-Rocha.

Conflict of interest

The authors declared no conflict of interest.

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