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Antimicrobial resistance profiles of non-pathogenic *Escherichia coli* in pig farms in Colombia

Perfiles de resistencia antimicrobiana de Escherichia coli no patógena en granjas porcinas de Colombia

Perfis de resistência antimicrobiana de Escherichia coli não patogênica em granjas de suínos na Colômbia

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Abstract

Background: Antibiotic resistance is a global public health problem. To date, there are limited studies focused on *Escherichia coli* isolated from pig herds in Colombia. **Objective**: This study aimed to evaluate antibiotic resistance in non-pathogenic strains of *E. coli* isolated from pig farms located in Valle del Cauca, Colombia. **Methods:** The hemolytic capacity and the presence of heat-labile (LT) and heat-stable (STa and STb) toxins were evaluated. Subsequently, Antibiotic resistance was assessed in γ-hemolytic strains against 11 commonly used antibiotics. **Results:** A total of six *E. coli* strains were isolated. The strains exhibited a high level of antibiotic resistance. The highest resistance prevalence was observed against amikacin (20%), ceftiofur (20%), fosfomycin (20%), ciprofloxacin (40%), gentamicin (40%), florfenicol (80%), enrofloxacin (80%), norfloxacin (80%), apramycin (100%), ampicillin (100%), and doxycycline (100%). **Conclusions:** Commensal *E. coli* strains from piglets pose a high epidemiological risk as reservoirs and disseminators of resistance genes within the pig production chain. Additionally, these strains may represent a potential risk to the food chain.

Keywords: *Antibiotics; antibiotic resistance; Escherichia coli; hemolytic capacity; pathogens; piglets; public health; resistance genes.*

Resumen

Antecedentes: La resistencia a los antibióticos es un problema de salud pública mundial. A la fecha, existen pocos estudios centrados en *Escherichia coli* aislada de hatos porcinos en Colombia. **Objetivo:** Este estudio tuvo como objetivo evaluar la resistencia a antibióticos de cepas no patógenas de *E. coli* aisladas de granjas porcinas del Valle del Cauca, Colombia. **Métodos:** Se usaron seis cepas de *E. coli* no patógenas aisladas de estudios previos. Se evaluó la capacidad hemolítica de las cepas y la presencia de toxinas termolábiles (LT) y

termoestables (STa y STb) mediante amplificación genética, con tamaños esperados de STa: 163 bp, STb: 368 bp, y LT: 275 bp. Finalmente, se evaluó la resistencia a 11 antibióticos de uso común en cepas γ-hemolíticas. **Resultados:** Las cepas presentaron multirresistencia a antibióticos. La mayor prevalencia de resistencia se observó contra amikacina (20%), ceftiofur (20%), fosfomicina (20%), ciprofloxacina (40%), gentamicina (40%), florfenicol (80%), enrofloxacina (80%), norfloxacina (80%), apramicina (100%), ampicilina (100%) y doxiciclina (100%). **Conclusiones:** Las cepas comensales de *E. coli* aisladas de lechones representan un alto riesgo de diseminación de genes de resistencia en la cadena productiva porcina y pueden ingresar a la cadena alimentaria, contribuyendo a la transferencia de resistencia antimicrobiana a humanos. Estos hallazgos son de gran importancia para la implementación de estrategias de control y prevención de la resistencia antimicrobiana en la industria porcina y la protección de la salud pública.

Palabras clave: *Antibióticos; Escherichia coli; genes de resistencia; lechones; patógenos; resistencia a antibióticos; salud pública; capacidad hemolítica.*

Resumo

Antecedentes:A resistência aos antibióticos é um problema de saúde pública global. Até o momento, existem poucos estudos focados em *Escherichia coli* isolada de rebanhos suínos na Colômbia. **Objetivo:** Este estudo teve como objetivo avaliar a resistência a antibióticos de cepas não patogênicas de *E. coli* isoladas de granjas de suínos. **Métodos:** Foram utilizadas seis cepas de *E. coli* não patogênicas isoladas de estudos anteriores. Avaliou-se a capacidade hemolítica e a presença de toxinas termo-lábeis (LT) e termo-estáveis (STa e STb) por meio de amplificação genética, com tamanhos esperados de STa: 163 bp, STb: 368 bp e LT: 275 bp. Por fim, avaliou-se a resistência a 11 antibióticos de uso comum em cepas γ-hemolíticas. **Resultados:** As cepas apresentaram multirresistência a antibióticos. A maior prevalência de resistência foi observada contra amicacina (20%), ceftiofur (20%), fosfomicina (20%), ciprofloxacina (40%), gentamicina (40%), florfenicol (80%), enrofloxacina (80%), norfloxacina (80%), apramicina (100%), ampicilina (100%) e doxiciclina (100%). **Conclusões:** Cepas comensais de *E. coli* isoladas de leitões representam um alto risco de disseminação de genes de resistência na cadeia produtiva suína e podem ingressar na cadeia alimentar, contribuindo para a transferência de resistência antimicrobiana a humanos. Esses achados são de grande relevância para a implementação de estratégias de controle e prevenção da resistência antimicrobiana na indústria suína e para a proteção da saúde pública.

Palavras-chave: *Antibióticos; Escherichia coli; genes de resistência; leitões; patógenos; resistência aos antibióticos; saúde pública; capacidade hemolítica.*

Introduction

Some strains of *Escherichia coli (E. coli)* possess pathogenic characteristics and can cause life-threatening diseases (Torres-León et al., 2022). The hemolytic capacity of *E. coli* is an important virulence factor, as the ability of the bacterium to lyse red blood cells is associated with the presence of oligomeric toxins capable of forming pores in the cell membrane. In livestock production, *E. coli* is responsible for significant economic losses (Torres-León et al., 2022). In humans, *E. coli* is associated with urinary and skin infections, meningitis, myositis, osteomyelitis, and orchiepididymitis (Vila et al., 2016).

Antibiotics such as aminoglycosides, penicillins, streptomycin, cephalosporins, sulfonamides, tetracyclines, and quinolones are commonly used to treat diseases caused by *E. coli* (Younis et al., 2017). However, many *E. coli* strains have developed resistance to these antibiotics (Roth et al., 2019). Approximately 20–45% of *E. coli* isolates exhibit resistance to first-line antibiotics such as tetracyclines, penicillins, and sulfonamides (Pitout, 2012). Although humans are the main carriers of antibiotic-resistant *E. coli* strains, antibiotic-resistant strains have also been reported in livestock species such as cattle, pigs, and poultry (Alonso et al., 2017). The presence of antibiotic-resistant microorganisms not only impacts animal health and welfare but also poses a serious public health concern, as over 60% of resistant bacteria originate from animals (Mcarthur, 2019).

Antibiotic resistance arises from a combination of natural and artificial factors, such as the high adaptability of microorganisms (Fernández-Rodríguez et al., 2020), their ability to exchange genetic material, the misuse of antibiotics (including self-medication and administration without veterinary supervision), incomplete treatments, and misdiagnoses (Torres-León et al., 2021). Common resistance mechanisms include antibiotic inactivation by enzymes, bacterial modifications, and alterations at the target site (Reygaert, 2018; Van Boeckel et al., 2015).

The remarkable adaptability of bacteria to extrinsic factors facilitates the development of multidrug resistance (Mendoza et al., 2019). Bacteria can acquire and transfer resistance genes through horizontal gene transfer mechanisms, including conjugation, transduction, and transformation. Genetic material is exchanged through plasmids, integrons, and transposons (Quiñones-Pérez, 2017). The transmission of resistance genes is a highly dynamic process driven by the movement of mobile genetic elements (Murray et al., 2022).

In intensive animal production systems, the widespread use of antimicrobials exerts selection pressure on microorganisms, leading to the accumulation and spread of resistance genes. *E. coli* can serve as a reservoir for resistance genes, making it a valuable sentinel organism for monitoring antimicrobial resistance trends in both animal and human populations (Loayza et al., 2020).

Antibiotic-resistant *E. coli* is recognized as one of the most pressing global public health concerns (Jang et al., 2017). The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organisation for Animal Health (OIE) have declared antimicrobial resistance a critical threat to humanity (WHO, 2014). Antibiotic resistance not only limits treatment options for infectious diseases but also increases the risk of pathogen transmission and outbreaks (Mcarthur, 2019; Quiñones-Pérez, 2017). While the incidence of *E. coli*-induced diarrhea has declined in developing countries, this disease continues to disproportionately affect children (Anderson et al., 2019). The objective of this study was to evaluate the antibiotic resistance of non-pathogenic *E. coli* strains isolated from pig farms.

Materials and Methods

Bacterial strains

Non-hemolytic *Escherichia coli* (γ-hemolytic) strains were obtained by rectal swabbing of 77 piglets randomly selected from six intensive swine farms in Valle del Cauca, Colombia. The selection criteria were based on farm records indicating the presence of diarrhea in piglets. Samples were collected in the cities of Yumbo (3° 34' 56'' N, 76° 29' 29'' W) and Palmira (3° 31' 1'' N, 76° 18' 0'' W). Piglets aged 4–40 days, weighing approximately 2–11.2 kg, were chosen, corresponding to the lactation or nursery phase. The methodology followed the protocols described in previous studies by Pabón-Rodríguez et al. (2023). The six bacterial isolates were designated as strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12.

Hemolytic activity

The isolated strains were cultured in triplicate on blood agar plates supplemented with 5% defibrinated sheep red blood cells. The plates were incubated for 24 h at 37°C. Strains classified as γ-hemolytic did not exhibit red blood cell lysis around the colonies.

Molecular identification of strains

γ-Hemolytic *E. coli* strains were confirmed through PCR amplification of the 16S ribosomal RNA gene, using primers 27F and 1492R.

Gene amplification of LT, STa, and STb toxins

The presence of thermolabile (LT) and thermostable (STa and STb) toxin genes in *E. coli* strains was determined by PCR. To reactivate the strains, cryopreserved samples (-60°C) were thawed at room temperature (25°C). Briefly, 1 mL of thawed sample was inoculated into a test tube containing 4 mL of soy broth and incubated for 24 h at 37°C. Following incubation, the cultures were streaked onto MacConkey agar for isolation. *E. coli* strains that were γ-hemolytic and tested negative for the LT, STa, and STb genes were considered as non-pathogenic.

The presence of toxin genes was confirmed by visualizing the amplification bands of the target genes, with expected fragment sizes of

STa: 163 bp, STb: 368 bp, and LT: 275 bp. PCR products were separated on 2% agarose gels via electrophoresis and visualized using photo-documentation equipment.

Antibiotic resistance of E. coli strains

The antimicrobial susceptibility of molecularly identified *E. coli* strains was assessed following the standardized disk diffusion method, measuring inhibition zone diameters (Begum et al., 2016).

Briefly, 50 µL of each bacterial inoculum was evenly spread on Mueller-Hinton agar using a sterile swab. Antibiotic discs were placed on the agar surface, including: ampicillin (10 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), florfenicol (30 µg), fosfomycin (50 µg), doxycycline (30 µg), norfloxacin (10 µg), amikacin (30 µg), ceftiofur (30 µg), and apramycin (15 µg). The Petri dishes were then incubated at 37°C for 24 h.

At the end of the incubation period, standardized photographs were taken of the Petri dishes, and inhibition zones were analyzed using ImageJ software (Valencia-Hernández et al., 2016). Each antibiotic susceptibility test was performed in triplicate.

Results

Hemolytic Activity

The *Escherichia coli* strains did not exhibit a hemolytic halo around their colonies, indicating the absence of hemolysis. This is attributed to the lack of an enzyme system capable of lysing red blood cells (Sánchez-Neira and Angarita-Merchán, 2018). The hemolytic capacity of *E. coli* strains is associated with the expression of four genes in the *hlyCABD* operon (Soares et al., 2022). Hemolysis is a characteristic associated with pathogenicity (Sarowska et al., 2019). However, our results indicate that pathogenic *E. coli* strains can still carry virulence factors despite lacking hemolytic activity.

Molecular Identification of Strains

A fragment of the 16S rRNA gene of *E. coli* strains was amplified via PCR and subsequently sequenced. Figure 1 illustrates the amplification bands of the 16S rRNA gene from strains isolated via rectal swabs from piglets. Sequencing was performed using a reverse primer to generate two sequences per strain, minimizing the risk of false polymer formations.

The bands in lanes 1, 2, 3, 4, 5, and 6 appear at a higher position than the molecular marker lane (Mm), with a size of 1000 bp. Consequently, the observed band size was approximately 1500 bp, which is consistent with the expected size for the 16S rRNA gene in isolates 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12, using molecular markers of 100 bp and 1 kb. Additionally, no contamination was observed in the reactions.

The use of growth promoters, antibiotics, and environmental conditions in animal husbandry influences the composition and abundance of microbial strains. These strains are commonly identified through variation in the 16S rRNA gene sequence (Mantilla and Torres, 2019).

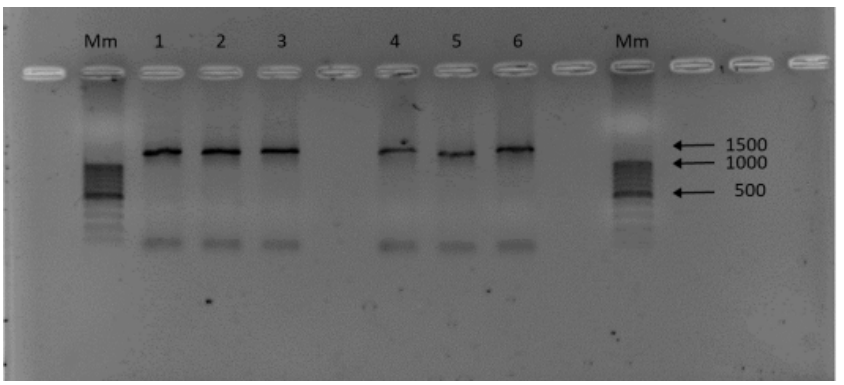

Figure 1. PCR amplification of the 16S rRNA gene from *E. coli* strains isolated from piglets. Lanes (1–6) correspond to strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12. Lane (Mm) contains the 100 bp molecular marker.

Table 1 presents the molecular identification results for strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12, corresponding to partial 16S rRNA gene sequences. The percent identity with reference *E. coli* strains ranged between 94% and 98%, supporting the microbiological culture results.

Table 1. Genus and species identification of PCR fragments from *E. coli* strains isolated via rectal swabs from piglets.

Encoded strain	E-value.	Identity percentage	Molecular identification	Length
1:2	0,0	96,77%	*E. coli* Strain UT03	408pb
1:6	0,0	97,78%	*E. coli* isolate RS16	406pb
2:1	0,0	96,78%	*E. coli* Strain UT03	559pb
2:11	0,0	98,02%	*E. coli* Strain wid10	605 pb
3:2	0,0	94,04%	*E. coli* Strain LR09	688pb
3:12	0,0	98,95%	*E. coli* isolate 68	286pb

Amplification of LT, STa, and STb Genes

The *E. coli* strains (1:2, 1:6, 2:1, 2:11, 3:2, and 3:12) did not exhibit amplification of the LT (thermolabile), STa (thermostable type A), or STb (thermostable type B) toxin genes. Consequently, these strains are considered non-pathogenic.

Enterotoxigenic *E. coli* strains are a major cause of morbidity and mortality in neonatal pigs. Post-weaning diarrhea is associated with two primary virulence factors: adhesins (K88/F4, K99/F5, 987P/F6, F41/F7, and F18) and enterotoxins (LT, STa, and STb).

Antibiotic Resistance of E. coli Strains

Table 2 summarizes the antibiotic susceptibility profiles of *E. coli* strains (1:2, 1:6, 2:1, 2:11, 3:2, and 3:12), classified as resistant, intermediate, or susceptible.

The overall resistance profile of the strains was as follows: amikacin (20%), ceftiofur (20%), fosfomycin (20%), ciprofloxacin (40%), gentamicin (40%), florfenicol (80%), enrofloxacin (80%), norfloxacin (80%), apramycin (100%), ampicillin (100%), and doxycycline (100%).

Thus, the study strains exhibited multidrug resistance. Strain 1:2 demonstrated the highest resistance capacity, being resistant to 10 out of 11 tested antibiotics.

Table 2. Antibiotic resistance of *E. coli* strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12.

Antimicrobial	Inhibition zone diameter (mm)					Reference (mm)
	1:2	1:6	2:1	2:11	3:12	
Ampicillin	0	0	0	0	0	≤ 13 14 - 16 ≥ 17
Ciprofloxacin	11	22	28	23	8	≤ 19 20 - 21 ≥ 22

Gentamicin	0	13	19	0	15	≤ 12 13 - 14 ≥ 15
Florfenicol	0	0	0	16	0	≤ 14 15 - 18 ≥ 19
Fosfomycin	0	26	32	21	25	≤ 12 13 - 15 ≥ 16
doxycycline	0	3	3	4	7	≤ 12 13 - 15 ≥ 16
norfloxacin	19	16	30	19	0	≤ 19 20 – 21 ≥ 22
Amikacin	19	18	21	17	14	≤ 14 15 – 16 ≥ 17
Ceftiofur	9	19	24	18	19	≤ 17 18 – 20 ≥ 21
Apramycin	15	15	14	14	12	≤ 15 16-19 ≥ 20
Enrofloxacin Baytril	15	10	24	14	0	≤ 16 17 – 22 ≥ 23

Discussion

Resistance of *Escherichia coli* to fluoroquinolones (nalidixic acid, cinoxacin, pipemidic acid, enoxacin, ofloxacin, ciprofloxacin, pefloxacin, norfloxacin, lomefloxacin, levofloxacin, sparfloxacin, tosufloxacin, gatifloxacin, trovafloxacin, clinafloxacin, and moxifloxacin) is likely due to the acquisition and modification of plasmids that carry resistance genes (Singh et al., 2022). These results are consistent with those reported by Begum et al. (2016), who observed fluoroquinolone resistance in 84% of *E. coli* strains. This trend has also been reported in studies conducted in China and Japan (Terahara and Nishiura, 2019).

Previously, antibiotic-resistant *E. coli* strains isolated from pigs have been reported in Colombia (Mantilla et al., 2022). The observed resistance percentages were as follows: tetracycline (100%), sulfamethoxazole-trimethoprim (97.5%), ampicillin (95.2%), amoxicillin (83.1%), tylosin (82.1%), and florfenicol (74.6%) (Mantilla et al., 2022). These results align with the findings

of the present study, particularly regarding resistance to ampicillin (100%) and florfenicol (80%). Additionally, the γ-hemolytic *E. coli* strains analyzed in this study were compared to a group of pathogenic β-hemolytic *E. coli* strains isolated from piglets, as reported by Pabón-Rodríguez et al. (2023). Both groups showed 100% resistance to ampicillin, apramycin, and doxycycline, as well as high resistance to enrofloxacin (80%) and florfenicol (80%).

The resistance of non-pathogenic *E. coli* strains to ampicillin and other antibiotics reflects the high prevalence of multidrug-resistant strains in the Colombian swine industry. This phenomenon is not unexpected, given the widespread availability and use of antibiotics in livestock production. The indiscriminate use of these drugs exerts selective pressure on bacterial populations, favoring the emergence and spread of resistant strains (Monger et al., 2021). A major contributing factor is the administration of subtherapeutic antibiotic doses in animal feed and drinking water. This practice creates an environment conducive to the development and dissemination of resistant bacteria (Garcias et al., 2024).

The high prevalence of multidrug resistance in Colombia underscores the urgent need to implement effective antimicrobial control and regulation strategies in the swine industry. This includes restricting the use of antibiotics for disease treatment and as growth promoters (Mantilla et al., 2022). Proper regulation is critical to minimizing the risk of disease outbreaks caused by resistant bacteria and safeguarding public health (Monger et al., 2021; Nowaczek et al., 2021; Daneman et al., 2023; Magiorakos et al., 2012).

Based on the results of this study, stricter monitoring and the adoption of responsible antibiotic management practices in pig farms are imperative to mitigate the risk of outbreaks and the spread of antibiotic-resistant bacteria. Therefore, regulatory enforcement by organizations such as the Colombian Agricultural Institute (ICA) is essential to ensure that antibiotics used in pig production are properly supervised and administered (Arenas and Melo, 2018).

The horizontal transfer of resistance genes among different bacterial populations highlights the need for continuous surveillance. The ability of *E. coli* to acquire and disseminate antimicrobial resistance genes makes it a key indicator for monitoring resistance trends in both animal and human populations (Loayza et al., 2020; O'Neill et al., 2023).

In Colombia, the ICA currently regulates the use of antibiotics in livestock production. However, these regulations must be strengthened and rigorously enforced to protect public health and ensure the long-term sustainability of the swine industry.

Conclusions

This study demonstrated that *Escherichia coli* strains isolated from pig farms exhibit resistance to multiple antibiotics. The strains were molecularly identified as non-hemolytic and lacked thermolabile (LT) and thermostable (STa and STb) toxins. The antibiotic resistance observed in these bacteria poses a risk to both animal and human health.

Therefore, it is crucial to implement stricter controls and regulations to mitigate the spread of antimicrobial resistance. The identification and characterization of these *E. coli* strains contribute to Colombia's national antimicrobial resistance response plan, as established by the Ministry of Health.

Our findings emphasize the need for stricter oversight in antibiotic use in swine production. The indiscriminate use of antibiotics should be reduced to prevent the selection and dissemination of resistant strains.

Declarations

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Conflict of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

Author contribution

Methodology: Daniela Buitrago-Angel, Gloria Casas-Bedoya, Liliana Serna-Cock; Formal

analysis and investigation: Jesús Quintana-Moreno, Omar Pabón-Rodríguez; Writing - original draft preparation: Daniela Buitrago-Angel, Gloria Casas-Bedoya, Liliana Serna-Cock, Omar Pabón-Rodríguez, Cristian Torres-León; Funding acquisition: Liliana Serna-Cock; Writing - review and editing: Cristian Torres-León.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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