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

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

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

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Received: *September 26, 2023.* ***Accepted:*** *July 11, 2024*

eISSN: 2256-2958 Rev Colomb Cienc Pecu

To cite this article:

Pabón-Rodríguez OV, Buitrago-Angel D, Quintana-Moreno JD, Casas-Bedoya GA, Serna-Cock Liliana, Torres-León C. Antibiotic resistance profiles of non-pathogenic *Escherichia coli* in pig farms in Colombia. Rev Colomb Cienc Pecu. *Year, Vol, number, and pages pending.* DOI: https://doi.org/10.17533/udea.rccp.v38n2a4

Abstract

Background: Antibiotic resistance is a global public health problem. So far, there are limited studies focused on *Escherichia coli* isolated from pig herds in Colombia. **Objective:** The objective of this work was to evaluate the resistance to antibiotics of non-pathogenic strains of *E. coli* isolated from pig farms. **Methods:** The hemolytic capacity and the presence of thermolabile (LT) and thermostable toxins (STa and STb) were evaluated. Finally, the resistance capacity of the strains to 11 commonly used antibiotics was evaluated in γ - hemolytic strains. **Results:** A total of 6 *E. coli* were isolated. The strains presented a high resistance capacity to antibiotics. The highest prevalence of resistance was against Amikacin (20%), Ceftiofur (20%), Fosfomycin (20%), Ciprofloxacin (40%), Gentamicin (40%), Florfenicol (80%), Enrofloxacin Baytril (80%), Norfloxacin (80%), Apramycin (100%), Ampicillin (100%) and Doxycycline (100%). **Conclusions:** Therefore, commensal strains of *E. coli* from piglets represent a high epidemiological risk to conserving and disseminating resistance genes in the pig production chain. In addition, these strains have a potential risk of presence in the food chain.

Keywords: *Antibiotics; antibiotics resistance; Escherichia coli; hemolytic capacity; pathogen; piglets; public health; resistance genes.*

Resumen

Antecedentes: La resistencia a los antibióticos es un problema de salud pública mundial. Hasta el momento, existen estudios limitados centrados en *Escherichia coli* aislada de hatos porcinos en Colombia. **Objetivo**: El objetivo de este trabajo fue evaluar la resistencia a antibióticos de cepas no patógenas de *E. coli* aisladas de granjas porcinas del Valle del Cauca, Colombia. **Métodos**: Se usaron 6 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 la observación de bandas de amplificación de genes de interés con tamaño esperado de STa: 163 bp, STb: 368 bp, and LT: 275 bp. Finalmente, se evaluó la capacidad de resistencia de

las cepas a 11 antibióticos de uso común en cepas γ - hemolíticas. **Resultados**: Las cepas presentaron multiresistencia a antibióticos. La mayor prevalencia de resistencia fue contra Amikacina (20%), Ceftiofur (20%), Fosfomicina (20%), Ciprofloxacina (40%), Gentamicina (40%), Florfenicol (80%), Enrofloxacina Baytril (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 y transferirse a humanos generando resistencia a antibióticos. Por lo tanto, esta información es de alta relevancia 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; capacidad hemolítica; Escherichia coli; genes de resistencia; lechones; patógeno; resistencia a antibióticos; salud pública.*

Resumo

Antecedentes: A resistência aos antibióticos é um problema de saúde pública global. Até agora, existem estudos limitados focados em *Escherichia coli* isolada de rebanhos suínos na Colômbia. **Objetivo:** O objetivo deste trabalho foi avaliar a resistência a antibióticos de cepas não patogênicas de *E. coli* isoladas de granjas de suínos. **Métodos:** A capacidade hemolítica e a presença de toxinas termolábeis (LT) e termoestáveis (STa e STb) foram avaliadas. Finalmente, a capacidade de resistência das cepas a 11 antibióticos comumente usados foi avaliada em cepas γ - hemolíticas. **Resultados:** Um total de 6 *E. coli* foram isolados. As cepas apresentaram alta capacidade de resistência a antibióticos. A maior prevalência de resistência foi contra Amicacina (20%), Ceftiofur (20%), Fosfomicina (20%), Ciprofloxacina (40%), Gentamicina (40%), Florfenicol (80%), Enrofloxacina Baytril (80%), Norfloxacina (80%), Apramicina (100%), Ampicilina (100%) e Doxiciclina (100%). **Conclusões:** Portanto, cepas comensais de *E. coli* de leitões representam um alto risco epidemiológico para a conservação e disseminação de genes de resistência na cadeia produtiva de suínos. Além disso, essas cepas apresentam um risco potencial de presença na cadeia alimentar.

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

Introduction

Some strains of *E. coli* can carry pathogenic characteristics and cause life-threatening diseases (Zhong *et al.*, 2012; Torres-León *et al.*, 2022). Hemolytic capacity is a pathogenicity characteristic of *E. coli*. The ability of a bacterium to lyse red blood cells is associated with the presence of oligomeric toxins capable of producing pores in the cell membrane. In livestock production, *E. coli* causes great economic losses (Torres-León *et al.*, 2022). In humans, *E. coli* causes urinary and skin infections, meningitis, myositis, osteomyelitis, and orchiepididymitis (Vila *et al.*, 2016).

Antibiotics such as aminoglycosides, penicillin, streptomycin, cephalosporins, sulfonamides, tetracycline, and quinolones are used to treat diseases caused by *E. coli* (Younis *et al.*, 2017). However, many *E. coli* are resistant to these antibiotics (Roth *et al.*, 2019). Approximately 20-45% of *E. coli* are resistant to first-line antibiotics such as tetracycline, penicillins, and sulfonamides (Pitout, 2012). Although humans are the major carriers of antibiotic-resistant *E. coli* strains, several strains of *E. coli* resistant to antibiotics have been reported in species such as cattle, pigs, and poultry (Alonso *et al.*, 2017). The presence of microorganisms resistant to antibiotics causes a negative impact on the health and welfare of animals; this also affects public health; 60.3% are of animal origin (Mcarthur, 2019).

Antibiotic resistance is caused by various natural or artificial factors, such as the high adaptability of microorganisms (Rodríguez *et al.*, 2020), the capacity for genetic exchange, the use of antibiotics without medical prescription, incomplete treatments, incorrect diagnoses (Torres-León *et al.*, 2021). Some resistance mechanisms are the inactivation of the antibiotic by enzymes, bacterial modifications, and the alteration of the target point (Reygaert, 2018; Van Boeckel *et al.*, 2015).

The high adaptability of bacteria to extrinsic factors causes multi-resistance (Mendoza *et al.*, 2019). Bacteria can exchange resistance genes through conjugation, translation, and transformation. Bacteria transfer genetic material through plasmids, integrons, and transposons (Pérez, 2017). The transmission of resistance genes is a dynamic process produced by the movement of mobile genetic elements (Murray *et al.,* 2022).

In animal production systems, the use of antimicrobials generates selection pressure on microorganisms that leads to the accumulation of resistance genes. E. coli can acquire and carry resistance genes. Therefore, *E. coli* can be considered as an indicator for monitoring the presence of antimicrobial resistance in different animal and human populations (Loayza *et al.,* 2020).

Antibiotic-resistant *E. coli* is considered one of the main public health problems in the world (Jang *et al.*, 2017). The World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE) have declared antibiotic resistance a threat to humanity (WHO, 2014). Resistance to antibiotics decreases the possibilities of treatment for different diseases (caused by microorganisms) and increases the risk of transmission (Mcarthur, 2019; Pérez, 2017). In developing countries, the occurrence of diarrhea caused by *E. coli* has decreased. However, this disease continues to mainly affect the child population (Anderson et al., 2019). The objective of this study was to evaluate the resistance to antibiotics of non-pathogenic *E. coli* strains obtained in pig farms.

Materials and Methods

Bacterial strains

Non-hemolytic *E. coli* strains (γ–hemolysis) were obtained by rectal swabbing 77 piglets randomly chosen from six intensive swine farms in Valle del Cauca, Colombia. The selection was based on records indicating the presence of diarrhea in piglets. Samples were collected in the towns 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 to 11.2 kg, corresponding to the lactating or nursery phase, were selected from each farm, as described in previous studies published by Pabón *et al*, (2023). The samples were named strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12.

Hemolytic activity

The strains were cultured in triplicate on blood agar plates spiked with 5% defibrinated sheep red blood cells. The plates were incubated for 24 h at 37°C. γ–hemolytic strains were defined as strains that do not lyse red blood cells around the colonies.

Molecular identification of strains

γ-hemolytic *E. coli* strains were confirmed by evaluating the 16S ribosomal gene using primers 27F and 1492R. The identification was made by PCR tests.

Gene amplification LT, STa and STb

The presence of thermolabile (LT) and thermostable (STa and STb) toxin genes of the *E. coli* strains were measured by the PCR methodology. *E. coli* strains were reactivated, and the deep freeze (-60°C) vial was brought to room temperature (25°C). Briefly, 1 ml of this sample was taken and added to a test tube containing 4 ml of soy broth; Subsequently, the sample was incubated for 24 h at 37 ºC and cultured on Mac Conkey agar. *E. coli* strain positive for γ – hemolysis and negative for the presence of LT, STa, or STb genes was considered non-pathogenic.

The presence of the genes was identified by observing the amplification bands of the gene of interest, with an expected size of STa: 163 bp, STb: 368 bp, and LT: 275 bp. The result of the amplification was mounted on 2% agarose gels in an electrophoresis chamber and was read in a photo documenting equipment.

Antibiotic resistance of E. coli strains

The determination of the antibiotic resistance of the *E. coli* strains (molecularly identified) was carried out according to the standards for disk antimicrobial susceptibility tests (assessment of the diameters of inhibition) (Begum *et al.*, 2016). Briefly, 50 µL of inoculum of each of the *E. coli* strains was cultured on Mueller Hinton agar (by surface, with a sterile swab). Antibiotic discs ampicillin (10 ug), enrofloxacin (5 ug), ciprofloxacin (5 ug), gentamicin (10 ug), florfenicol (30 ug), fosfomycin (50 ug), doxycycline (30 ug), norfloxacin (10 ug), amikacin (30 ug), ceftiofur (30 ug), and apramycin (15ug) were placed on the surface; the Petri dishes were incubated at 37ºC for 24 h. At the end of the incubation, standardized photographs of the boxes were taken, and images were analyzed in Image J software (Valencia-Hernández *et al.*, 2016). Each antibiotic resistance test was performed in triplicate.

Results

Hemolytic activity

The *E. coli* strains did not show a halo around their colonies, indicating the absence of hemolysis. This is due to the absence of an enzyme system capable of destroying 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 *hly*CABD operon (Henrique *et al.*, 2022). Hemolysis is a

characteristic of pathogenicity (Sarowska *et al.*, 2019). However, according to our results, pathogenic *E. coli* can carry virulence factors without having hemolytic capacity.

Molecular identification of strains

A fragment of the 16s ribosomal gene of the *E. coli* strains was amplified through a PCR test and subsequently sequenced. Figure 1 shows the amplification bands of the 16s rRNA gene of the strains isolated by rectal swabs of piglets. Sequencing was performed with a reverse primer to obtain two sequences for each strain, avoiding forming false polymers. The bands in lanes 1,2,3,4,5,6 are at a higher level than the molecular marker lane (Mm), with a size of 1000bp. Consequently, the observed band size is approximately 1500 bp, which is consistent with the expected size for the 16s rRNA gene of isolates 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12 using molecular markers of 100 bp and kb. In addition, the reactions show no contamination. The use of growth promoters, antibiotics, and environmental conditions in animal husbandry influence the structure and quantity of microbial strains; these strains are identified using microbial variation in the identification of the 16s rRNA gene (Mantilla and Torres Sáez, 2019).

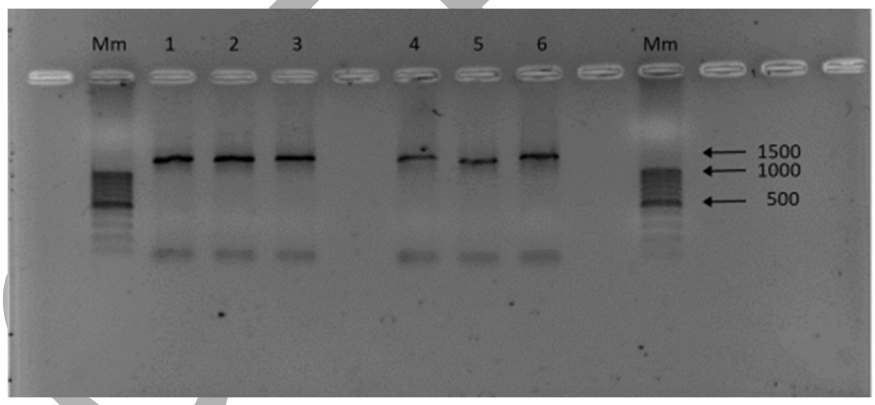

Figure 1. Polymerase chain reaction amplification gene 16s of *E. coli* strains isolated from piglets: Lanes (1-6) corresponding to strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12 and lane (Mm) 100 bp molecular marker.

Table 1 shows the molecular identification of strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12. This corresponds to partial identification of the 16s gene of the strains. The results show a percentage of identity between 94 and 98% with *E. coli* strains. Therefore, this agrees with the results of microbiological cultures.

191 **Table 1.** Genus and species of PCR test fragments from *E. coli* strain isolated by rectal swabs
192 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

193

194 *Amplificación de los genes LT, STa y STb*

195 The strains of *E. coli* 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12 did not present amplification of the
196 corresponding genes evaluated thermolabile (LT), thermostable type A (STa), and thermostable
197 type B (STb); therefore, these strains are not pathogenic. Enterotoxigenic *E. coli* strains are the
198 main cause of illness and death in neonatal pigs. Post-weaning diarrhea has two types of
199 virulence factors: adhesins (K88 (F4), K99 (F5), 987P (F6), F41 (F7), and F18) and enterotoxins
200 (LT, STa, and STb).

201 *Antibiotic resistance of E. coli strains*

Table 2 shows the response of the *E. coli* strains 1:2, 1:6, 2:1, 2:11, 3:2, and 3:12 to the different antibiotics (resistant, intermediate, and sensitive). The antibiotic resistance pattern of the study strains was amikacin (20%), ceftiofur (20%), fosfomycin (20%), ciprofloxacin (40%), gentamicin (40%), florfenicol (80%), enrofloxacin Baytril (80%), norfloxacin (80%), apramycin (100%), ampicillin (100%) and doxycycline (100%). Therefore, the study strains presented resistance to multiple antimicrobial drugs. The strain with the highest resistance capacity was 1:2, with a resistance capacity to 10 types of antibiotics.

Table 2. Antibiotic resistance of *E. coli* strains 1:2, 1:6, 2:1, 2:11, 3:2 y 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 *E. coli* to fluoroquinolones (nalidixic acid, cinoxacin, pipemidic acid, enoxacin, ofloxacin, ciprofloxacin, pefloxacin, norfloxacin, lomefloxacin, levofloxacin, sparfloxacin, tosufloxacin, gatifloxacin, trovafloxacin, clinafloxacin, and moxifloxacin) is possibly due to the acquisition and modification of plasmids that harbor genetic material (Singh *et al.*, 2022). These results agree with those reported previously by Begum *et al.* (2016), who reported resistance to fluoroquinolones in 84% of *E. coli* strains. This behavior is reported in studies in China and Japan (Terahara and Nishiura, 2020; Zeng *et al.*, 2020).

Previously, antibiotic-resistant strains of E. coli isolated from pigs have been reported in Colombia (Mantilla *et al*, 2022). The resistance percentages observed were: 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 are in accordance with those reported in the present work: ampicillin (100%) and florfenicol (80%). Additionally, the γ-hemolytic E. coli strains were compared with a group of pathogenic β-hemolytic *E. coli* strains isolated from piglets and reported by Pabón *et al*, (2023); the two strains were 100% resistant to

the antibiotics ampicillin, apramycin and doxycycline, and presented high resistance to the antibiotics enrofloxacin (80%) and florfenicol (80%).

The resistance of non-pathogenic *E. coli* strains to ampicillin (as well as other antibiotics) reflects the prevalence of multidrug-resistant strains in the Colombian pork industry. This phenomenon is not surprising given the wide availability and use of antibiotics in livestock production, which generates selective pressure on bacterial populations and promotes the development and spread of resistance (Monger *et al.*, 2021). The problem is due to administering subtherapeutic doses in the animals' food and drinking water. This scenario creates an environment conducive to developing and spreading resistant strains (Garcias *et al.,* 2024).

Resistance to multiple antibiotics in Colombia indicates the urgent need to implement effective strategies to control and regulate antimicrobials in the pork industry (in treating diseases and as growth promoters) (Mantilla *et al.,* 2022). This regulation is crucial to minimize the risk of disease outbreaks caused by resistant bacteria and to safeguard public health (Monger *et al.*, 2021; Nowaczek *et al.*, 2021; Daneman *et al.,* 2022; Magiorakos *et al.,* 2011).

According to the results of this study, stricter supervision and implementation of responsible antibiotic management practices on pig farms are necessary to mitigate the risk of outbreaks and the spread of antibiotic-resistant bacteria. Therefore, regulation by organizations such as the Colombian Agricultural Institute (ICA) is essential to ensure that the use of antibiotic medications in pork production is carried out under adequate supervision (Arenas and Melo, 2018). The transmission of resistance genes can occur between different bacterial populations. Therefore, the ability of *E. coli* to acquire and transfer resistance genes makes it an important indicator of the spread of antimicrobial resistance (in animals and humans) (Loayza *et al.,* 2020; O'Neill *et al.*, 2023).

In Colombia, where the Colombian Agricultural Institute (ICA) regulates the use of antibiotic medications in livestock production; these regulations must be strengthened and rigorously applied to safeguard public health and ensure the long-term sustainability of the pork industry.

Conclusions

This study demonstrated that strains of *E. coli* isolated from pig farms are resistant to antibiotics. The strains were molecularly identified as non-hemolytic and lacking thermolabile (LT) and thermostable toxins (STa and STb). The resistance of these bacteria represents a risk to animal

and human health. Therefore, controls and regulations must be implemented. The identification of these *E. coli* strains, and their molecular identification can contribute to the national antimicrobial resistance response plan of Colombian ministry of health. Our findings show that antibiotics used in porcine production should be moderated.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare they have no conflicts of interest with regard to 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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