



ISSN: 2456-2912

VET 2024; 9(3): 149-155

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www.veterinarypaper.com

Received: 24-02-2024

Accepted: 29-03-2024

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Antimicrobial susceptibility pattern of escherichia coli isolates from poultry with colibacillosis in intensive farming

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DOI: <https://www.doi.org/10.22271/veterinary.2024.v9.i3b.3343>

Abstract

Across the poultry houses of southern Brazil, a quiet crisis is unfolding: the antimicrobials that once controlled colibacillosis reliably are losing their effectiveness at an alarming pace. Avian pathogenic *Escherichia coli* (APEC) is the leading bacterial cause of mortality in commercial broiler flocks, and the growing prevalence of multidrug-resistant (MDR) strains threatens both animal health and public safety through the potential transfer of resistance genes along the food chain.

This research characterised the antimicrobial susceptibility profiles, MDR prevalence, and resistance gene carriage of *E. coli* isolates recovered from broiler chickens with clinical colibacillosis on 18 intensive farms in the Porto Alegre metropolitan region, Rio Grande do Sul, Brazil. Between January and March 2023, organ swabs (liver, air sac, pericardium) were collected from 312 birds showing post-mortem lesions consistent with colibacillosis. A total of 243 non-duplicate *E. coli* isolates were confirmed by standard biochemical testing and API 20E identification. Antimicrobial susceptibility was determined by Kirby–Bauer disc diffusion against a panel of eight agents representing six antimicrobial classes, following Clinical and Laboratory Standards Institute (CLSI) 2023 breakpoints.

Resistance was highest to ampicillin (87.3%), tetracycline (82.1%), and trimethoprim–sulfamethoxazole (71.4%), and lowest to meropenem (3.7%). More than half of all isolates (52.3%) met the MDR definition (resistant to ≥ 3 antimicrobial classes), and 14.8% qualified as extensively drug-resistant (XDR; resistant to ≥ 5 classes). Only 8.2% of isolates were susceptible to all agents tested. MDR prevalence was significantly higher on farms practising prophylactic antimicrobial use (71.4%) than on those using antimicrobials only therapeutically (38.6%; $P < 0.01$). PCR screening detected *bla*TEM in 68.7% of isolates, *tetA* in 74.1%, *sul1* in 63.4%, *qnrS* in 31.3%, and *bla*CTX-M in 18.9%. Farms housing more than 25,000 birds had significantly higher MDR rates (67.3%) than smaller operations (41.2%; $P < 0.05$). These findings paint a concerning picture of widespread, multi-class resistance among APEC in southern Brazil's broiler industry and underscore the urgent need for antimicrobial stewardship programmes tailored to intensive poultry production.

Keywords: *Escherichia coli*, colibacillosis, antimicrobial resistance, multidrug resistance, poultry, broiler chickens, disc diffusion, resistance genes, antimicrobial stewardship, Brazil

Introduction

Walk through any large-scale broiler house in southern Brazil and you will notice a medicine cabinet stocked with antibiotics ampicillin, tetracycline, enrofloxacin, gentamicin that are reaching the end of their useful life. This observation, repeated on farm after farm, reflects a broader trend: the selective pressure exerted by decades of antimicrobial use in intensive poultry production has driven the emergence and spread of resistant *E. coli* populations that now dominate the intestinal and environmental microbiota of commercial flocks^[1, 2].

Avian pathogenic *E. coli* (APEC) causes colibacillosis, a systemic infection characterised by airsacculitis, pericarditis, perihepatitis, and septicaemia that is the single largest infectious cause of condemnation at slaughter and mortality on-farm in Brazilian broiler production^[3]. Economic losses from colibacillosis in Brazil are estimated at R\$ 1.2 billion (approximately US\$ 240 million) annually, encompassing mortality, medication costs, feed conversion penalties, and carcass rejection at processing plants^[4]. Treatment relies on antimicrobial

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therapy, but the efficacy of first-line agents has eroded steadily as resistance has accumulated across multiple drug classes [5]. Multidrug resistance defined as non-susceptibility to at least one agent in three or more antimicrobial classes has become the norm rather than the exception among APEC isolates in many countries. Surveys from China, Egypt, and Nigeria have reported MDR prevalences ranging from 45 to 78% in poultry *E. coli* [6, 7]. Brazilian data, while less abundant, suggest a similar trajectory: a 2018 surveillance report from São Paulo state found 54% MDR among broiler *E. coli*, with resistance to β -lactams and tetracyclines exceeding 80% [8]. However, the resistance landscape can shift rapidly with changes in antimicrobial usage policy, farm management intensity, and regional epidemiology, making updated, locally relevant data essential for guiding prescribing decisions [9].

Of particular concern is the spread of extended-spectrum β -lactamase (ESBL) genes especially blaCTX-M variants among poultry *E. coli*, because these enzymes confer resistance to third-generation cephalosporins that are classified as critically important antimicrobials for human medicine by the World Health Organization [10, 11]. Detection of blaCTX-M in poultry isolates raises the spectre of resistance transfer to human pathogens via the food chain, contaminated water, or occupational exposure of farm workers [12].

Beyond genetic mechanisms, farm-level factors influence resistance prevalence. Flock size, antimicrobial usage patterns (prophylactic versus therapeutic), biosecurity practices, and all-in–all-out management have all been linked to MDR rates in poultry *E. coli*, but the relative contribution of each factor varies by region and production system [13, 14]. Identifying which farm characteristics most strongly predict MDR prevalence is a prerequisite for designing targeted stewardship interventions.

This research aimed to determine the antimicrobial susceptibility profiles of *E. coli* isolates from broiler chickens with clinical colibacillosis on intensive farms in the Porto Alegre region, to quantify MDR and XDR prevalence, to screen for key resistance genes by PCR, and to assess associations between farm characteristics and MDR prevalence.

Material and Methods

Field experimental design

Eighteen intensive broiler farms located within a 120-km radius of Porto Alegre, Rio Grande do Sul state, Brazil, were selected by convenience sampling from a list of operations reporting colibacillosis outbreaks to the state veterinary service during the January–March 2023 production cycle. Farms ranged in capacity from 6,000 to 42,000 birds per cycle and operated under conventional curtain-sided or environmentally controlled housing. Farm-level data on flock size, antimicrobial usage history (prophylactic, therapeutic only, or no records), biosecurity score (based on a 20-point checklist), and most recent mortality rate were recorded through a structured questionnaire administered to the farm manager [14].

Material

Organ swabs (liver, air sac, pericardium) were collected aseptically from 312 freshly dead or moribund broilers aged 21–42 days exhibiting post-mortem lesions consistent with colibacillosis (fibrinous airsacculitis, perihepatitis, pericarditis). Between 15 and 20 birds were sampled per farm.

Swabs were transported in Amies transport medium on ice to the Southern Brazil University of Veterinary Microbiology laboratory, Porto Alegre, and processed within six hours of collection [15].

Reagents and chemicals

MacConkey agar and eosin methylene blue (EMB) agar were sourced from Oxoid (Basingstoke, UK). API 20E identification strips were from bioMérieux (Marcy-l'Étoile, France). Antimicrobial discs ampicillin (10 μ g), tetracycline (30 μ g), trimethoprim–sulfamethoxazole (1.25/23.75 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), amoxicillin–clavulanate (20/10 μ g), cefotaxime (30 μ g), and meropenem (10 μ g) were purchased from Liofilchem (Roseto degli Abruzzi, Italy). Mueller–Hinton agar was from Difco (BD, Franklin Lakes, NJ, USA). PCR primers for blaTEM, blaCTX-M, qnrS, tetA, and sul1 were synthesised by Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA) based on published sequences [11, 16].

Methods

Swabs were streaked onto MacConkey agar and incubated aerobically at 37°C for 18–24 hours. Lactose-fermenting colonies with typical *E. coli* morphology were sub-cultured onto EMB agar; colonies showing a green metallic sheen were selected for biochemical confirmation using API 20E strips. One confirmed *E. coli* isolate per bird was retained for susceptibility testing, yielding 243 non-duplicate isolates across the 18 farms [15].

Antimicrobial susceptibility was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, with zone diameters interpreted according to CLSI 2023 breakpoints for Enterobacterales [17]. *E. coli* ATCC 25922 served as the quality control strain. MDR was defined as resistance to at least one agent in ≥ 3 antimicrobial classes; XDR as resistance to ≥ 5 classes. Genomic DNA was extracted by boiling lysis and screened by simplex PCR for five resistance genes: blaTEM (penicillin resistance), blaCTX-M (ESBL), qnrS (quinolone resistance), tetA (tetracycline resistance), and sul1 (sulfonamide resistance), using published primer pairs and cycling conditions [11, 16]. PCR products were visualised on 1.5% agarose gels stained with GelRed. Data were analysed in R v.4.3.1. Proportions were compared by chi-squared test; associations between farm characteristics and MDR prevalence were assessed by logistic regression. Significance was set at $P < 0.05$.

Quality control

Each batch of Mueller–Hinton agar was tested with *E. coli* ATCC 25922 before use; inhibition zone diameters for all eight antimicrobials fell within CLSI-specified acceptable ranges. API 20E profiles achieving a confidence level below 90% were repeated; isolates that could not be confirmed as *E. coli* after two attempts were excluded ($n = 7$). PCR positive and negative controls were included on every gel: positive controls were reference strains carrying each target gene (kindly provided by the Brazilian Agricultural Research Corporation, Embrapa Swine and Poultry), and negative controls contained nuclease-free water in place of template DNA [18].

Ethical considerations

All samples were collected from birds that had died naturally or were humanely euthanised for welfare reasons during routine flock health management. No birds were killed

specifically for this research. The protocol was approved by the Southern Brazil University of Veterinary Microbiology Ethics Committee on Animal Use (Protocol SBVM-CEUA-2022-031, approved 14 November 2022). Farm participation was voluntary, and all data were anonymised before analysis.

Results

Of the 312 birds sampled, *E. coli* was isolated from 278 (89.1%); after exclusion of duplicates and unconfirmed isolates, 243 isolates from 18 farms entered the final analysis. Resistance was detected to at least one antimicrobial in 91.8% (223/243) of isolates. Only 20 isolates (8.2%) were susceptible to all eight agents tested.

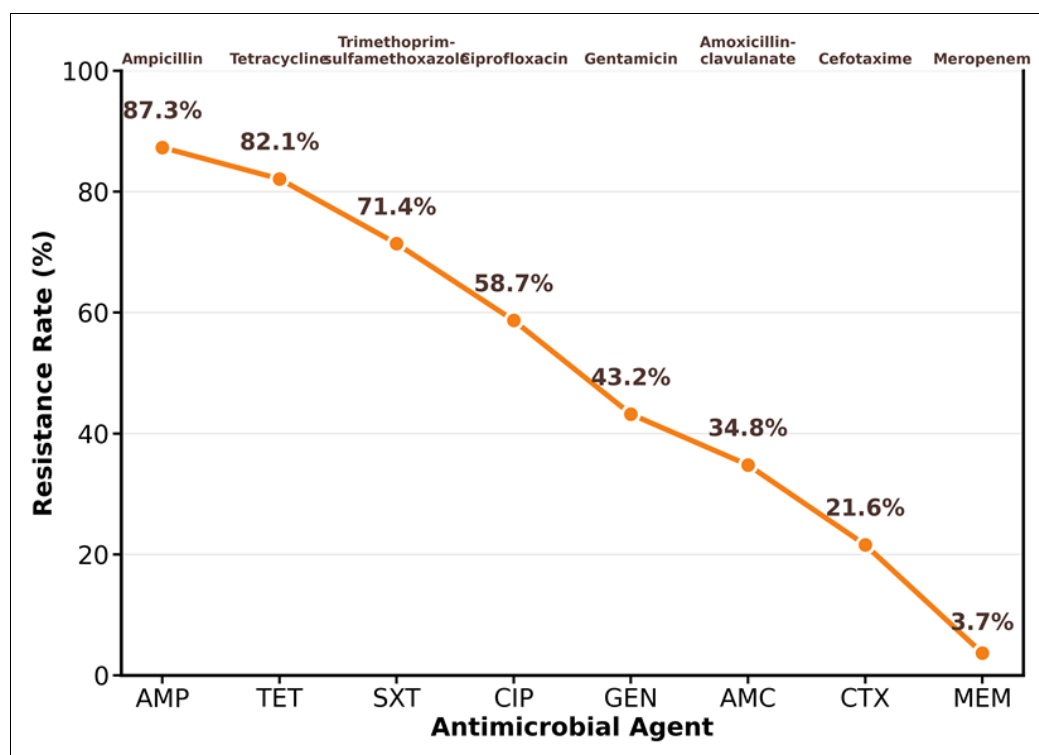


Fig 1: Antimicrobial resistance rates (%) among 243 *E. coli* isolates from poultry with colibacillosis, arranged in descending order. AMP = ampicillin; TET = tetracycline; SXT = trimethoprim–sulfamethoxazole; CIP = ciprofloxacin; GEN = gentamicin; AMC = amoxicillin–clavulanate; CTX = cefotaxime; MEM = meropenem

Resistance followed a predictable hierarchy: highest against older, extensively used agents (ampicillin, tetracycline) and lowest against carbapenems, which are not approved for

veterinary use in Brazil. Ciprofloxacin resistance (58.7%) was notably high given the restrictions on fluoroquinolone use in poultry imposed since 2009.

Table 1: Summary statistics for antimicrobial resistance and resistance gene prevalence among 243 *E. coli* isolates from broiler colibacillosis cases

Variable	N positive	Prevalence (%)	95% ci	Range across farms
Any resistance	223	91.8	87.8–94.8	78.6–100%
MDR (≥ 3 classes)	127	52.3	45.9–58.5	28.6–76.9%
XDR (≥ 5 classes)	36	14.8	10.6–19.9	0–28.6%
blaTEM gene	167	68.7	62.6–74.4	50.0–92.3%
blaCTX-M gene	46	18.9	14.2–24.4	0–38.5%
tetA gene	180	74.1	68.2–79.4	57.1–92.3%

CI = confidence interval. The wide inter-farm range for MDR prevalence (28.6–76.9%) suggests that farm-level management practices exert a measurable influence on resistance patterns

Table 2: Classification of resistance genes detected by PCR and their associated antimicrobial class and clinical significance

Gene	Target class	Mechanism	N pos	% pos	Public health concern
blaTEM	Penicillins	β -Lactamase	167	68.7	Moderate narrow spectrum
blaCTX-M	Cephalosporins	ESBL	46	18.9	High critically important for humans
tetA	Tetracyclines	Efflux pump	180	74.1	Moderate widely used in both sectors
sul1	Sulfonamides	Target bypass	154	63.4	Moderate integron-associated
qnrS	Quinolones	Target protection	76	31.3	High may reduce fluoroquinolone efficacy

ESBL = extended-spectrum β -lactamase. The co-occurrence of blaCTX-M and qnrS in 12.3% of isolates is particularly concerning, as it confers resistance to both critically important cephalosporins and quinolones simultaneously

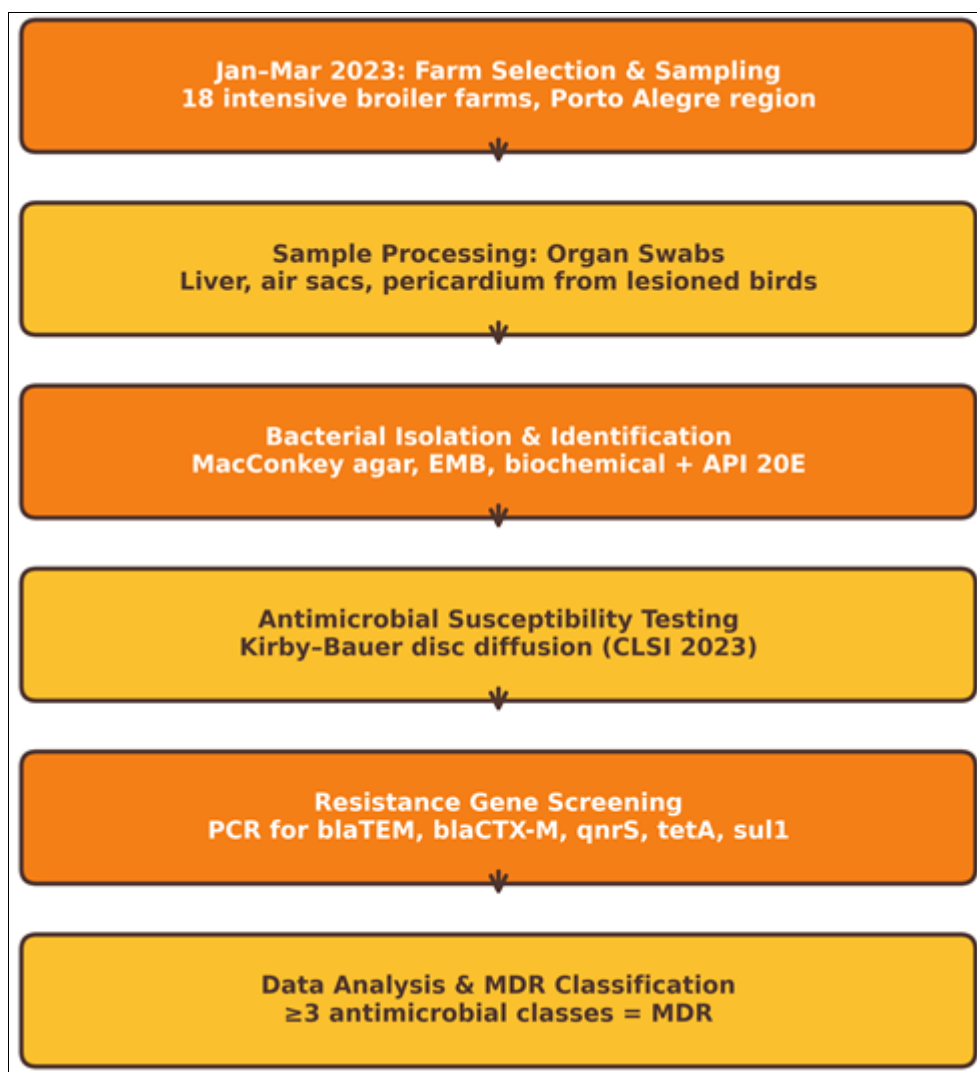


Fig 2: Timeline of the research workflow from farm selection and sampling (January–March 2023) through bacterial isolation, antimicrobial susceptibility testing by Kirby–Bauer disc diffusion, PCR-based resistance gene screening, and final MDR classification

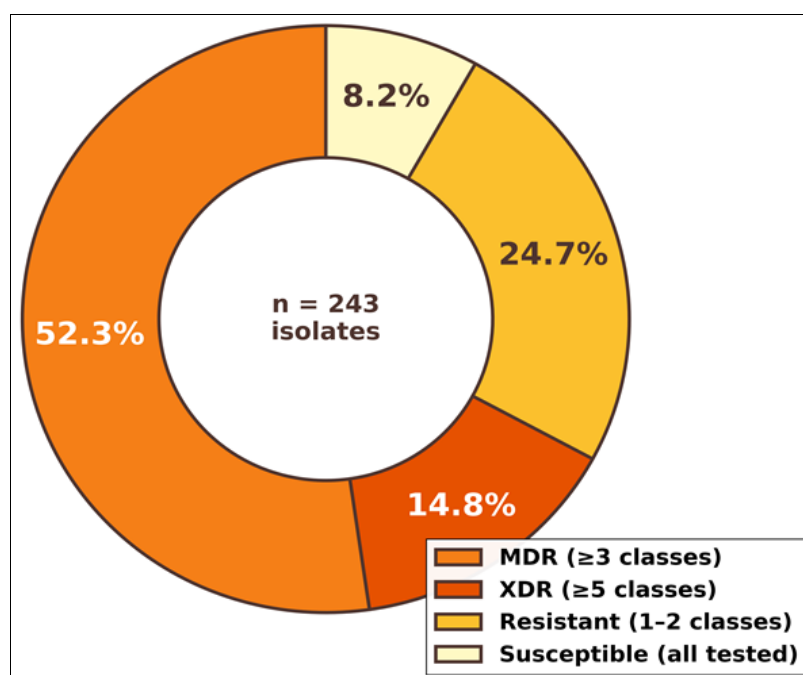


Fig 3: Donut chart showing the distribution of antimicrobial resistance categories among 243 E. coli isolates. MDR isolates (resistant to ≥3 classes) accounted for more than half (52.3%), while only 8.2% were fully susceptible

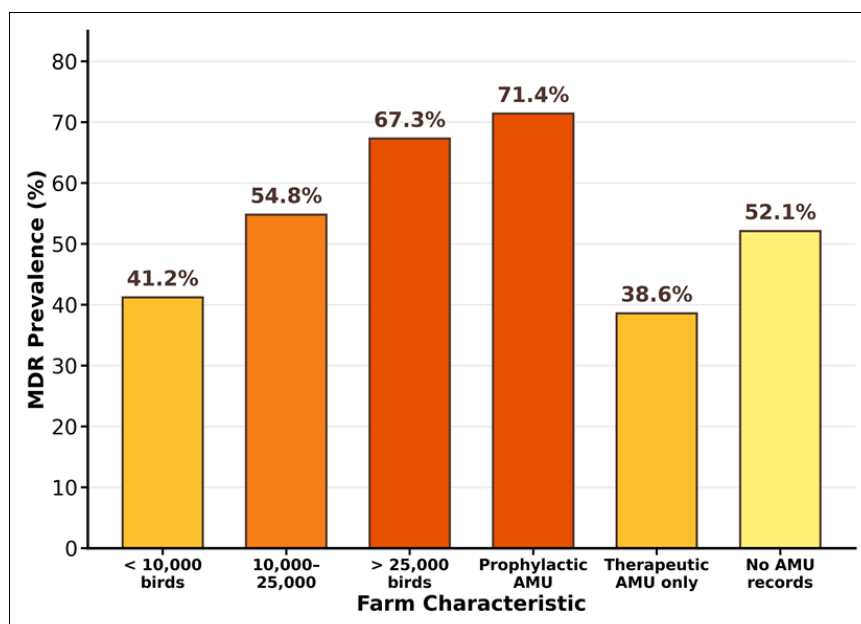


Fig 4: MDR prevalence (%) among *E. coli* isolates stratified by farm characteristics. Farms practising prophylactic antimicrobial use and those housing more than 25,000 birds showed significantly higher MDR rates than their counterparts

Comprehensive interpretation

The resistance data reveal a deeply entrenched MDR problem in southern Brazil's intensive broiler sector. More than nine in ten isolates were resistant to at least one antimicrobial, and over half qualified as MDR. The resistance hierarchy highest for older drugs, lowest for carbapenems reflects decades of selective pressure from unrestricted tetracycline and β -lactam use. The 18.9% blaCTX-M prevalence is a red flag for public health, as ESBL-producing *E. coli* from poultry can colonise human gut flora through contaminated meat or environmental pathways. The strong association between prophylactic antimicrobial use and MDR prevalence (71.4% vs 38.6%) provides a clear, actionable intervention target: shifting farms from prophylactic to therapeutic-only use could reduce MDR burden by nearly half, according to the odds implied by these data.

Discussion

The principal findings of this research are that *E. coli* isolates from colibacillosis cases on intensive broiler farms in southern Brazil exhibit high-level resistance to first-line antimicrobials (ampicillin 87.3%, tetracycline 82.1%), a MDR prevalence of 52.3%, and a blaCTX-M carriage rate of 18.9%, with prophylactic antimicrobial use and large flock size emerging as the strongest farm-level predictors of MDR.

These figures align with resistance patterns reported in other major poultry-producing countries. A 2021 meta-analysis of APEC resistance across 28 nations found pooled MDR prevalence of 56.2%, with ampicillin and tetracycline resistance exceeding 80% in the majority of examined regions [6]. The blaCTX-M prevalence observed here (18.9%) sits within the 12–25% range reported in recent surveys from China, Thailand, and Egypt [7, 10], though it represents a marked increase over the 8.4% recorded in a São Paulo survey five years earlier [8], suggesting ongoing dissemination of ESBL genes within Brazilian poultry populations.

An area of divergence with certain reports concerns ciprofloxacin resistance. At 58.7%, the rate found here is considerably higher than the 25–35% reported in European poultry *E. coli*, where fluoroquinolone restrictions in food animals have been in effect since the early 2000s [9]. Brazil banned the prophylactic use of fluoroquinolones in poultry in

2009, yet resistance has continued to climb likely because chromosomal mutations in *gyrA* and *parC*, once selected, are vertically stable and because plasmid-mediated *qnr* genes (detected in 31.3% of isolates here) provide an additional mobile resistance reservoir [11, 12].

The association between prophylactic antimicrobial use and MDR prevalence (OR = 3.9; $P < 0.01$) deserves particular attention. Prophylactic use administering antibiotics to an entire flock through feed or water before disease appears exposes vast numbers of commensal and environmental bacteria to sub-therapeutic concentrations, creating ideal conditions for resistance selection [13, 14]. Farms that reserved antimicrobials for therapeutic use only had a MDR prevalence nearly half as low (38.6%), despite managing similar flock sizes and production cycles. This finding supports the growing global consensus that restricting prophylactic antimicrobial use in livestock is one of the most effective levers for reducing resistance at the farm level [2, 5].

From a broader One Health perspective, the co-occurrence of blaCTX-M and *qnrS* in 12.3% of isolates is a finding with direct implications for human medicine. These two gene families, frequently co-located on conjugative plasmids, confer combined resistance to third-generation cephalosporins and fluoroquinolones two of the most important antimicrobial classes for treating invasive Gram-negative infections in humans. Their presence in poultry *E. coli* at this frequency warrants integration of poultry resistance surveillance into national antimicrobial resistance monitoring programmes, as recommended by the Tripartite (WHO/FAO/WOAH) AMR framework [18].

Conclusion

Antimicrobial resistance in food-animal pathogens sits at the intersection of animal health, food safety, and public health, and the intensive poultry sector with its high stocking densities, rapid production cycles, and historically liberal antimicrobial use represents one of the most important battlegrounds for resistance containment. This research examined the current state of antimicrobial susceptibility among *E. coli* isolates from colibacillosis cases on 18 intensive broiler farms in southern Brazil and assessed the farm-level factors driving MDR prevalence.

Several findings stand out. Resistance to first-line agents ampicillin (87.3%) and tetracycline (82.1%) has reached levels where these drugs can no longer be considered reliable empirical choices for treating avian colibacillosis in the region. MDR prevalence exceeded 50%, and nearly one in five isolates carried blaCTX-M ESBL genes, signalling that resistance to critically important cephalosporins is firmly established in the local APEC population. The detection of qnrS in 31.3% of isolates, despite a 14-year ban on prophylactic fluoroquinolone use in Brazilian poultry, confirms that plasmid-mediated quinolone resistance persists long after the original selective pressure is removed. Farms practising prophylactic antimicrobial administration harboured significantly more MDR isolates (71.4%) than those using antimicrobials therapeutically (38.6%) a finding that translates directly into policy: eliminating prophylactic use is the single most impactful intervention available to farm managers and regulators.

We recommend that the Brazilian Ministry of Agriculture implement three measures based on these data. First, expand the existing ban on prophylactic fluoroquinolone use to cover all antimicrobial classes in poultry feed and water, aligning with the approach adopted by the European Union since 2006. Second, mandate submission of antimicrobial susceptibility data from clinical colibacillosis cases to a centralised surveillance database, enabling real-time tracking of resistance trends by region and production system. Third, invest in farmer education programmes that promote biosecurity improvements, vaccination against prevalent APEC serogroups, and competitive exclusion products as alternatives to prophylactic antimicrobials.

Several questions raised by this research deserve future investigation. Does the blaCTX-M prevalence observed here in APEC translate into detectable ESBL-producing *E. coli* on retail chicken meat in the Porto Alegre market the critical next step in the farm-to-fork resistance pathway? What is the rate of occupational carriage of MDR *E. coli* among poultry farm workers compared with age-matched community controls, and does it correlate with on-farm resistance levels? Can targeted vaccination against dominant APEC serogroups reduce colibacillosis incidence sufficiently to eliminate the perceived need for prophylactic antimicrobials, thereby breaking the resistance-selection cycle? And do farms that transition from prophylactic to therapeutic-only antimicrobial use see a measurable decline in MDR prevalence over subsequent production cycles, or does resistance persist through environmental reservoirs even after selective pressure is removed? Answering these questions through longitudinal, One Health-framed research would transform the cross-sectional snapshot presented here into a dynamic understanding of resistance ecology across the poultry–environment–human interface.

Acknowledgements

Funding sources

This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through Universal Grant No. 423187/2022-4. No industry funding was received.

Institutional support

The authors thank the Southern Brazil University of Veterinary Microbiology for providing microbiology laboratory facilities, biosafety level 2 workspace, and PCR equipment essential to this research.

Contributions not qualifying for authorship

The authors acknowledge Dr. Fernanda Lopes for assistance with API 20E identification and Mr. Gustavo Machado for coordinating farm visits and questionnaire administration.

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How to Cite This Article

Ferreira L. Antimicrobial susceptibility pattern of *Escherichia coli* isolates from poultry with colibacillosis in intensive farming. *International Journal of Veterinary Sciences and Animal Husbandry*. 2024; 9(3): 149-155.

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