

Article

Prospective Study of the Relative Abundance of Antimicrobial Resistance Genes in *Escherichia coli* O157:H7 Obtained from Chicken Carcasses from Local Markets in Lima, Peru

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Abstract

Objective: This study addresses antimicrobial resistance (AMR), a growing public health threat, by evaluating the role of chicken carcasses as possible vehicle for the spread of *Escherichia coli* O157:H7 and antimicrobial resistance genes (ARGs), with the aim of analyzing the association between bacterial load and the relative abundance of ARGs in samples obtained from an open and an enclosed market in Lima, Peru. **Methods:** SYBR Green qPCR was used to analyze 28 chicken carcasses from two local markets in the Lima metropolitan area (Enclosed market $n = 13$, and Open Market $n = 15$), detecting *Escherichia coli* O157:H7 and ARGs like *bla*_{CTX-M}, *bla*_{TEM}, and *strA*. **Results:** The bacterial load was higher in the enclosed market (5.062 log CFU/mL) than in the open market (3.875 log CFU/mL). *E. coli* O157:H7 was detected in 76.9% and 86.6% of samples, with average loads of 1.676 and 1.251 log CFU/mL, respectively. The relative abundance of *bla*_{CTX-M} and *bla*_{TEM} showed greater dispersion in the open market, whereas *strA* was more homogeneous in both markets. Significant positive correlation was found between *E. coli* load and ARGs abundance, stronger in the enclosed market ($r = 0.904\text{--}0.945$) and moderate to high in the open market ($r = 0.794\text{--}0.920$). **Conclusions:** The results demonstrate a significant correlation between *E. coli* O157:H7 load and ARGs, highlighting the need for a comprehensive approach within the framework of the “OneHealth” initiative.

Keywords: antimicrobial resistance; chicken meat; qPCR; bacterial pathogens; resistance genes



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1. Introduction

A poorly explored sector in the development of antimicrobial resistance (AMR) is the unregulated use of antibiotics in livestock production, particularly in animals and their derived products intended for human consumption [1]. In recent decades, a significant increase in the prevalence of AMR in animal-derived food has been documented [2]. This phenomenon is particularly relevant for meat and its derivatives, which, in addition to being an essential source of proteins, vitamins, and minerals in the human diet, have exerted increasing pressure on production systems [3]. The sustained rise in meat demand has driven the unregulated use of antibiotics in animals, both for therapeutic purposes and as antibiotic growth promoters (AGPs), a practice still common in low- and middle-income countries. This has led to the evolution and spread of high levels of bacterial resistance, which must be addressed within the framework of the “One Health” approach [4].

The transfer of AMR bacteria from animal-based food products to humans, either through the consumption of contaminated food or through handling and marketing, represents a serious food safety issue [5]. Recent studies confirm the hypothesis that foodborne bacteria transfer antimicrobial resistance genes (ARGs) to the human gut microbiota [6,7]. Due to the high demand for meat products intended for human consumption, AGPs have been used indiscriminately, progressively contributing to the acquisition of AMR within the animal microbiota [8]. According to official data from the Ministry of Agrarian Development and Irrigation (2024), the average per capita consumption of chicken meat in Peru reached 52 kg in 2023 [9].

In this context, the commercialization of fresh chicken in local markets represents a high risk of bacterial contamination, as the handling and hygiene conditions vary considerably across different sales environments. In many cases, the lack of proper cold chain management and prolonged exposure of the meat favor cross-contamination, increasing the likelihood of pathogen transmission to consumers [10]. Additionally, in intensive farming systems, poultry vendors often offer chickens raised under suboptimal conditions, resorting to the indiscriminate use of antibiotics to maximize productivity and growth. This practice increases the risk of consumers being exposed to pathogenic bacteria carrying ARGs [11]. Given this scenario, it is necessary to investigate the presence of them directly associated with pathogenic bacteria in foods like chicken, a widely consumed product in the Peruvian population that, due to its production, marketing, and handling chain, constitutes a potential transmission vehicle to humans.

In this regard, we propose the quantification of the bacterial pathogen *Escherichia coli* O157:H7, recognized as one of the most relevant variants of enterohemorrhagic *E. coli* and a producer of Shiga toxins, which is an important causative agent of foodborne diseases (FBD). Its presence and load in chicken meat constitute a risk to public health, as this product could act as a vehicle of transmission to consumers and has been implicated in foodborne outbreaks worldwide [12]. This reinforces the need for constant surveillance of high-turnover poultry products such as chicken.

Additionally, this risk takes on greater significance when strains simultaneously carry antimicrobial resistance genes, such as *bla*_{TEM} and *bla*_{CTX-M} (related to resistance to β -lactam antibiotics) and *strA* (associated with resistance to aminoglycosides such as streptomycin), whose relative burden or abundance is also sought to be determined, mainly favoured by the indiscriminate use of antimicrobials during intensive poultry farming. The combination of virulence, resistance and high bacterial load not only increases the spread of these pathogens but also limits the available treatment options, making the monitoring and quantification of both bacteria and antibiotic resistance genes an essential component of food safety [13].

In order to achieve this, real-time quantitative PCR (qPCR) will be used, a sensitive and accurate technique that allows for the simultaneous identification and quantification of both pathogens and ARGs. Unlike conventional methods based on bacterial enrichment and culturing, which are often labor-intensive and limit the detection of certain foodborne microorganisms, qPCR offers a more efficient alternative for pathogen detection, generating key scientific evidence for epidemiological surveillance and antibiotic use control in poultry production [14]. Furthermore, the distribution of both resistance genes and the target pathogen will be assessed in samples of chicken carcasses sold in two marketing contexts: open markets (i.e., street vendors or informal stalls) and enclosed markets (i.e., permanent retail establishments). In both types of markets, processing and sanitation practices differ significantly, potentially increasing the risk of bacterial contamination and ARG abundance, influenced by environmental factors. Consequently, the aim of this study is to detect and quantify, using total DNA, both bacterial pathogens and antimicrobial resistance genes in

chicken carcasses to analyze their relative abundance distribution across different marketing contexts, and to provide relevant scientific evidence to improve food safety regarding the AMR challenge.

2. Results

2.1. Organoleptic Characteristics of the Samples

The organoleptic characteristics of each sample were recorded, including colour, odour, texture, and visible fat content, which allowed for a comparison of the apparent quality of the product between the two local markets prior to the total dressing of the carcass. In terms of colour, a yellowish tone predominated in the enclosed market (46.1%) and a greyish/yellow tone in the open market (64.3%). A fresh odour was more common in the enclosed market (61.5%), while a rancid odour predominated in the open market (73.3%). A rough texture predominated in both markets, especially in the open market (80%). Figure 1 shows the relative frequency of each characteristic by market, highlighting the predominant patterns in the samples analyzed.

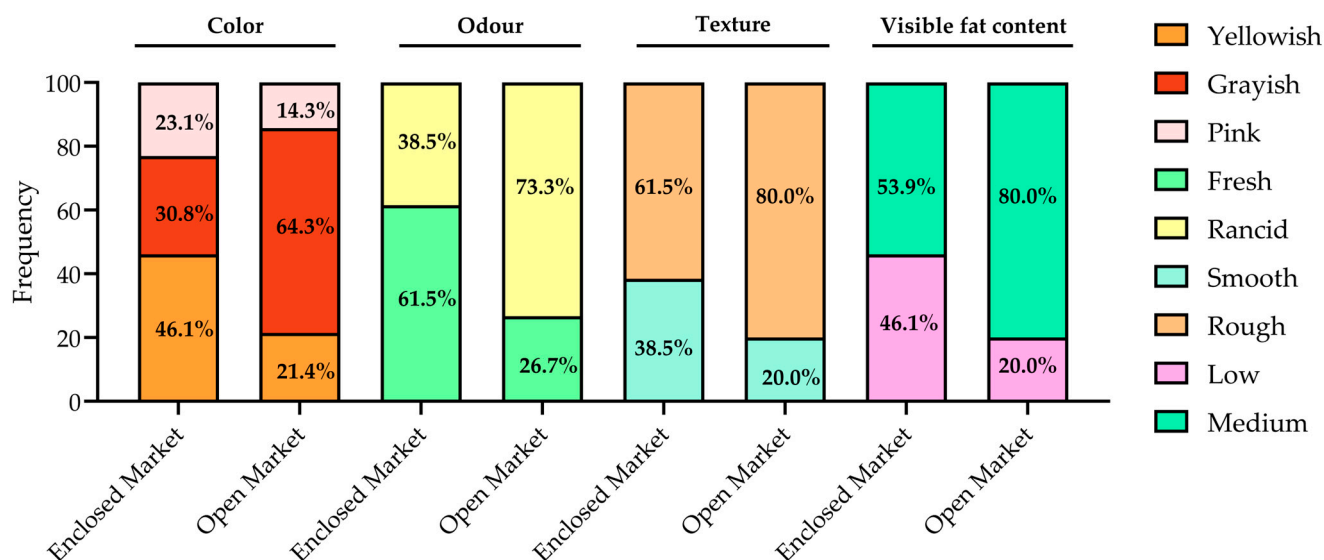


Figure 1. Frequency of organoleptic characteristics (color, odour, texture, and visible fat content) in chicken carcasses by local markets. Bars represent the frequency of each organoleptic characteristic for chicken carcasses from the enclosed market ($n = 13$) and open market ($n = 15$), highlighting differences between them.

2.2. Analytical Performance of 16S rRNA and *Escherichia coli* O157:H7 qPCR Assays

Standard curves were established for the quantification of the total bacterial population by qPCR using the control strain ATCC 25922 and primers targeting the 16S rRNA gene. This step was performed solely to construct and validate the 16S rRNA standard curve, ensuring precise and consistent amplification before quantifying the total bacterial population in chicken carcass samples. The assay was previously performed in the laboratory using available strains from the genera *Raoultella*, *Salmonella*, *Pseudomonas*, and *Klebsiella*, showing no significant variations in 16S rRNA gene amplification (Figure 2a).

The Ct values obtained from the serial dilutions allowed a linear regression curve to be generated ($y = -3.2863x + 40.3701$; $R^2 = 0.99$), with a lower detection limit of Ct 38.8 and an upper limit of 11.3, calculating an amplification efficiency of 95.3%. Figure 2 shows the behaviour of each dilution with respect to bacterial log(CFU/mL).

For the absolute quantification of *Escherichia coli* O157:H7, the *fliC* gene was used, obtaining the following standard curve parameters (Figure 3): slope -3.316 , intercept

37.969, efficiency 100.25%, coefficient of determination $R^2 = 0.9984$, lower detection limit Ct 38.0, and upper limit 17.63. The variability of the unknown samples ranged from 0.007 to 0.445. The specificity of the amplified products was verified using melting curves. The positive and negative controls included in each run corroborated the validity of the tests, while the linearity of the curves and the consistency of the Ct values in the serial dilutions confirmed the reproducibility of the methods and the high reliability of the quantification of the total bacterial population and *E. coli* O157:H7 in chicken carcasses.

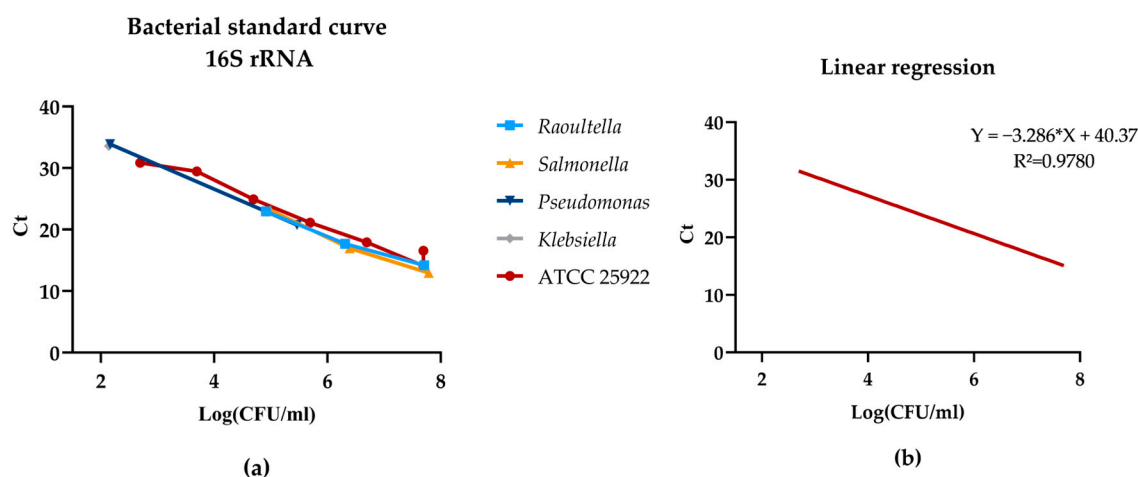


Figure 2. Standard curve for the absolute quantification of the bacterial population. Figure (a) shows the evaluation of representative strains of the genera *Raoultella*, *Salmonella*, *Pseudomonas*, *Klebsiella*, and *Escherichia coli* ATCC 25922, demonstrating the correspondence between serial dilutions and Ct values. (b) Shows the linear regression analysis for *Escherichia coli* ATCC 25922, including its linear equation and R^2 value, used to calculate reaction efficiency and estimate the bacterial population load.

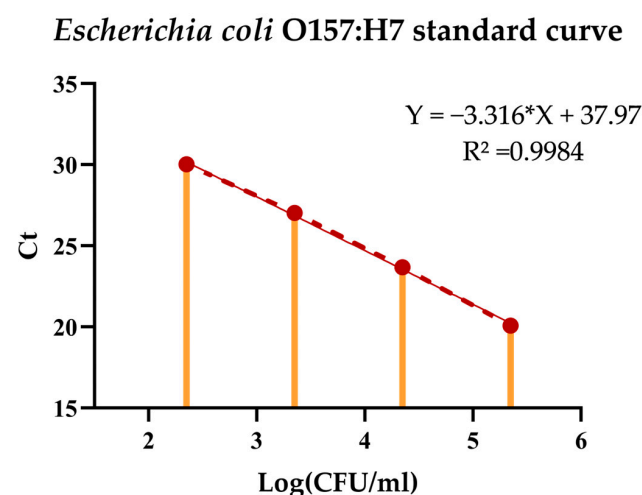


Figure 3. Standard curve for the absolute quantification of *Escherichia coli* O157:H7. The points corresponding to each dilution relative to their Ct values are shown, as well as the standard curve equation and R^2 value used to calculate efficiency and estimate the bacterial load of *Escherichia coli* O157:H7.

2.3. Detection and Quantification of Total Bacterial Load and *Escherichia coli* O157:H7

The absolute load of the total bacterial population (16S rRNA) and *E. coli* O157:H7 (*fliC*) was quantified from the chicken carcasses collected. Descriptive statistics for positive samples are presented in Tables S1 and S2, available in the Supplementary Material.

A high bacterial load was observed in both markets, with an average of 5.062 Log CFU/mL in the enclosed market and 3.875 Log CFU/mL in the open market, the latter showing the greatest variability. With regard to *E. coli* O157:H7, the open market had a higher percentage of positive samples (86.6%), but with a slightly lower concentration (1.251 Log CFU/mL) than the enclosed market (1.676 Log CFU/mL).

Overall, the results indicate that the total bacterial population remained high in both markets, although with greater variability in the open market (Figure 4).

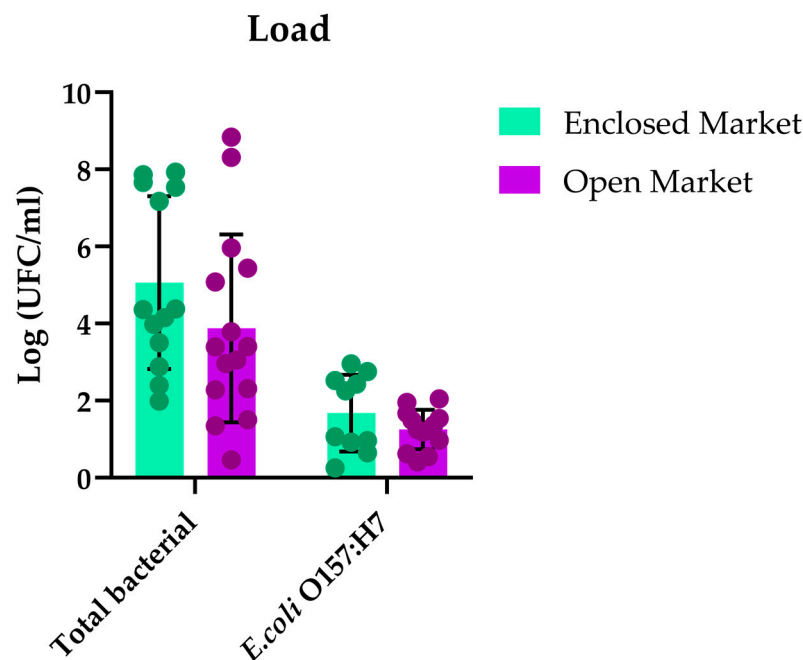


Figure 4. Comparison of total bacterial load and *Escherichia coli* O157:H7 load in analyzed samples.

Additionally, the graphical amplification analyses and melting curves corresponding to representative samples from each market are shown. In Figure 5, panel (a1,a2) presents the results obtained for the 16S rRNA, showing the detection of the total bacterial population together with a single peak in the dissociation curve, with a T_m value of 86.53 ± 0.23 °C, confirming the specificity of the reaction. Panel (b1,b2) shows the results of the amplification of the *fliC* gene of *Escherichia coli* O157:H7, whose specific product showed a T_m of 83.69 ± 0.27 °C, also with a single peak in the dissociation curve, corroborating the absence of non-specific products.

2.4. Distribution of ARGs in Chicken Carcasses

The average relative abundance of each AMR gene was estimated (Table S3), and the differences between chicken carcasses from the enclosed and open markets were compared (Figure 6). The *bla*_{CTX-M} gene showed a higher average relative abundance in samples from the open market (0.107 Log₁₀(AR)) compared to the enclosed one (−1.205 Log₁₀(AR)). Likewise, *bla*_{TEM} and *strA* presented greater values in open-market samples (1.199 and 0.847, respectively) than in those from the enclosed market (−0.464 and −0.072).

These results show a consistent trend towards higher relative expression of AMR genes in carcasses from the open market. Full details of relative abundance values and their variability are presented in the Supplementary Material, which complements the general comparison summarized in the main tables and figures.

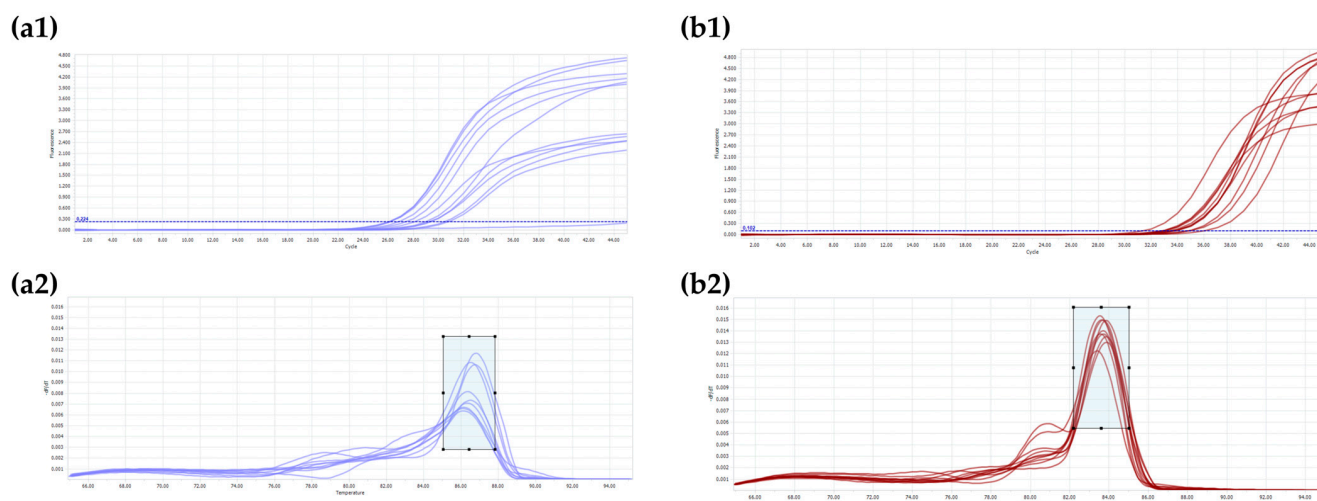


Figure 5. Representative chicken samples amplified by qPCR. Panel (a1,a2) shows the amplification of the 16S rRNA in selected samples from both markets, accompanied by their melting curves, which support the specificity of the products generated. Panel (b1,b2) shows the detection of the *fliC* gene of *Escherichia coli* O157:H7, together with the corresponding dissociation curve, which confirms the absence of non-specific amplifications and the validity of the results obtained.

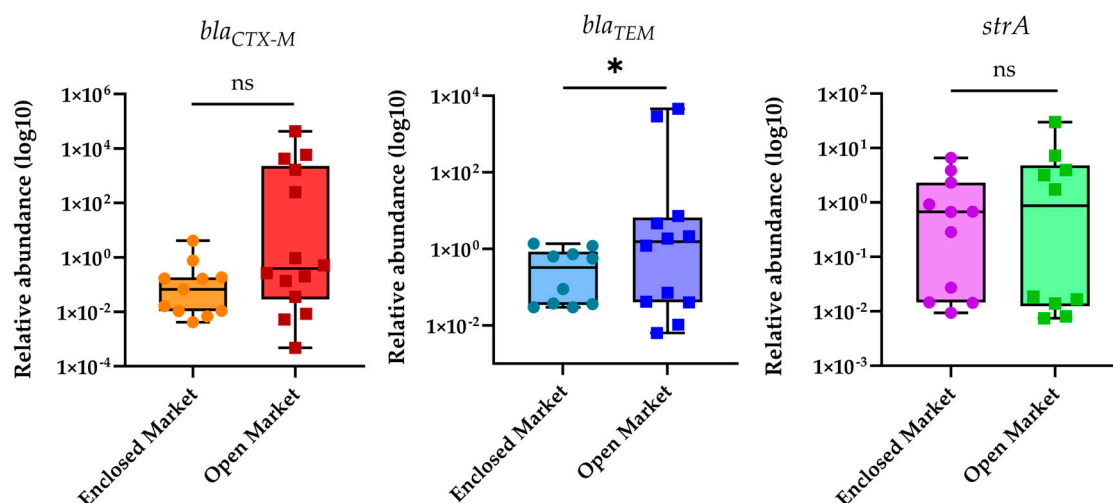


Figure 6. Comparison of the relative abundance of resistance genes in chicken carcasses at the enclosed and open markets. The graphs show the differentiation in relative abundance, represented by boxplots. Boxplots represent the distribution of data, with individual data points shown as dots. The black line within each box indicates the median. Statistical analysis was performed using Student's *t*-test, with *p*-values of *bla*_{CTX-M} = 0.08, *bla*_{TEM} = 0.03, and *strA* = 0.26. (*) indicates statistical significance at *p* < 0.05, while "ns" denotes a non-significant result.

Furthermore, Figure 7 shows the representation of the amplifications obtained by qPCR for each of the AMR genes analysed (*bla*_{CTX-M}, *bla*_{TEM}, and *strA*). Complementarily, the associated melting curves present a single defined peak, confirming the specificity of the amplicons.

2.5. Relational Analysis of Bacterial Load and Antimicrobial Resistance Genes

In the enclosed market, the *E. coli* O157:H7 load showed significant correlations with *bla*_{CTX-M} (*r* = 0.927), *bla*_{TEM} (*r* = 0.945) and *strA* (*r* = 0.904). Likewise, a strong association was found between *bla*_{CTX-M} and *bla*_{TEM} (*r* = 0.982, *p* < 0.05), indicating possible co-selection of these genes.

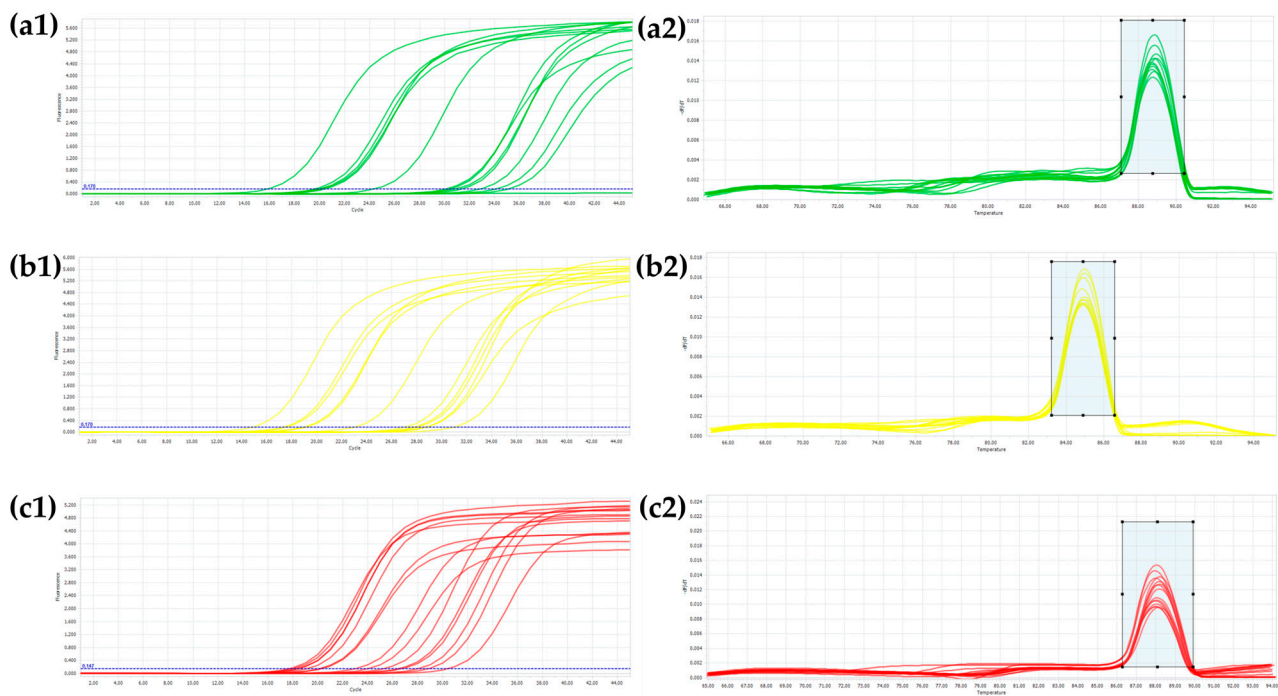


Figure 7. ARG qPCR amplifications and melting curves. (a1,a2) The T_m value of the specific amplicons of the *bla*_{CTX-M} gene was 88.78 ± 0.13 °C. (b1,b2) The T_m value for the detection of the *bla*_{TEM} gene was 84.82 ± 0.12 °C. (c1,c2) The T_m value for the detection of the *strA* gene was 87.90 ± 0.21 °C.

Similar patterns were observed in the open market: positive correlations between *E. coli* O157:H7 load and *bla*_{CTX-M} ($r = 0.794$), *bla*_{TEM} ($r = 0.844$) and *strA* ($r = 0.920$). In this case, the correlation between *bla*_{CTX-M} and *bla*_{TEM} was even higher ($r = 0.995$, $p < 0.05$), reinforcing the hypothesis of genetic co-occurrence and possible joint transfer.

Taken together, the results show that a higher burden of *E. coli* O157:H7 is consistently associated with a higher abundance of AMR genes, suggesting that these pathogens may act as key reservoirs in the spread of bacterial resistance in chicken meat.

3. Discussion

In the present study, a total of 28 chicken carcasses from the enclosed and open markets were analyzed. Total DNA was extracted from each sample, and qPCR was employed as a detection method for both bacterial pathogens and ARGs. This approach aligns with the “One Health” framework, which emphasizes the close relationship between human health, animal welfare, and environmental balance. In this context, poultry products can serve as a potential channel for the transmission of antibiotic-resistant bacteria to consumers [15]. The application of molecular tools in this research aims to optimize monitoring and surveillance processes of AMR, as such resistance can go undetected using conventional microbiological methodologies. It has been reported that various microorganisms present in food matrices and the environment, when found in low concentrations (CFU/mL) or in a viable but non-culturable (VBNC) state, may not be detected using conventional culture techniques, which can lead to false-negative results and pose a potential risk of exposure to public health [16].

Initially, the organoleptic characteristics of the samples collected from each market were evaluated (Figure 1). It was observed that the enclosed market had a higher proportion of carcasses in acceptable conditions of freshness, whereas the open market showed signs of deterioration, suggesting a direct impact of environmental conditions and product handling in each setting. This contrast is consistent with [17], which identified a higher frequency

of bacterial contamination in meat sold in informal and open markets, where exposure to the environment favours both sensory deterioration and microbiological risk. Likewise, other studies reinforce the idea that informality and lack of sanitary control at points of sale contribute not only to the physical deterioration of meat but also to the proliferation of pathogens and the spread of antimicrobial resistance genes [18]. These conditions not only result in greater physical deterioration of food, as initially evident in organoleptic characteristics, but also favour the proliferation of bacterial pathogens, thereby increasing the risk of exposure to contaminated food.

Similar studies have been reported in other countries. For example, in China, poultry products were analyzed in seven provinces, specifically in outdoor stalls. It was determined that establishments without a continuous refrigeration chain and with constant product handling favored bacterial proliferation and, therefore, posed a significant risk to food safety [19].

The difference between the two sampling points suggests variations in the handling or storage of chicken carcasses, as well as possible differences in the initial microbial load of the product (Figure 4). The higher bacterial load observed in the enclosed market may be attributed to environmental and management conditions, as limited air circulation and higher humidity inside the facility could favor microbial proliferation. In contrast, the open market, characterized by greater air flow and outdoor exposure, may experience reduced surface bacterial accumulation. Based on the results presented in Table S1, the average absolute quantification of the bacterial population was 5.06 log CFU/mL for the enclosed market and 3.87 log CFU/mL for the open market. This was higher than the 3.3 log CFU/mL previously reported in a study using the carcass rinsing method and conventional microbiological methodologies in two slaughterhouses in the Huancavelica region, Peru [20].

The *E. coli* O157:H7 bacterial load calculated in Table S2 for both markets was considered moderate to low, with an average range of 1.25–1.67 log CFU/mL. According to NTS No. 071, approved by R.M 591-2008/MINSA/DIGESA-V-01, which establishes the permitted microbiological limit for *E. coli* in raw chopped and ground meat ($50-5 \times 10^2 \sim 0.7-1.70$ log CFU/mL), the results obtained reflect a low frequency of bacterial load of this pathogen in most samples (Figure 4). However, even at low bacterial loads, the detection of *E. coli* O157:H7 is of public health importance due to its low infectious dose and potential to disseminate extended-spectrum β -lactamase (ESBL)-producing enterobacteria. In line with this, Cortez-Sandoval et al. (2022) [21] identified the presence of resistance genes such as *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-9}, *bla*_{TEM}, and *SHV* in *E. coli* strains obtained from chicken meat samples sold in supply markets in the Santiago de Surco district of Metropolitan Lima. Despite the low bacterial counts, these isolates carried antimicrobial resistance genes, emphasizing the need for further studies in Peru to explore the association between bacterial pathogens and AMR genes [21]. The slightly higher values observed in this study compared with those reported previously may be related to differences in sampling conditions, analytical methods (qPCR vs. culture-based), or environmental exposure at the retail level. Despite the moderate bacterial counts, these findings highlight the epidemiological relevance of continuous monitoring of pathogenic *E. coli* and associated AMR genes in food products in Peru.

The detection frequency of *E. coli* O157:H7 was 76.9% and 86.6% of the total samples analyzed in the enclosed and open markets, respectively. This high occurrence, consistent with other qPCR-based reports, poses a public health concern given the low infectious dose of this pathogen. Contamination may occur during slaughter, handling, or sale due to inadequate hygiene or temperature control, underscoring the need for improved sanitary practices in the poultry supply chain. For instance, Alhadlaq et al. (2023) [22] reported

that imported meat could act as a carrier of *E. coli* O157:H7. In their study conducted in Saudi Arabia, meat products accounted for 15.71% of all imported foods, within which the presence of this pathogen was confirmed [22].

The relative abundance of the evaluated ARGs—*bla*_{CTX-M}, *bla*_{TEM}, and *strA*—was calculated using the 16S rRNA gene as a normalizer, establishing the minimum and maximum values for each quantified gene (Table S3). It was observed that the relative abundance of *bla*_{CTX-M} had a lower standard deviation in the enclosed market (0.9) compared to the open market (2.3), suggesting greater dispersion of relative abundance in open environments. Similarly, for *bla*_{TEM}, the relative abundance showed greater variability in the open market.

According to the literature, the diversity in resistance gene abundance is influenced by external environmental conditions, with open spaces being more prone to heterogeneity. In a comparative study of open and enclosed poultry farms in China, open farms were found to harbour a greater variety and diversity of AMR genes [23]. This supports the present findings, indicating that open environments promote greater dispersion and heterogeneity of resistance genes, reflected in the variability of relative abundance in the markets analyzed.

Additionally, the differences in variability between the two markets (Figure 6) showed statistically significant behaviour ($p < 0.05$) for *bla*_{TEM}, while for *bla*_{CTX-M} and *strA*, the differential pattern was non-significant, reinforcing the influence of structural conditions of the chicken meat retail environment on the distribution of AMR genes.

On the other hand, the relative quantification of *strA*, belonging to the aminoglycoside resistance family frequently used in poultry farming, did not show high variability between the two markets. This suggests a more homogeneous distribution, possibly due to the widespread and systematic use of aminoglycosides in intensive poultry farming, with a higher prevalence and abundance as previously reported [24]. As a result, no statistically significant differences were observed in the relative abundance of *strA* between the two markets.

The correlation between *E. coli* O157:H7 bacterial load and the relative abundance of AMR genes was evaluated using Pearson's correlation coefficient. The results showed a significant positive association ($r > 0.5$) in both markets, indicating that an increase in bacterial load is directly related to a greater abundance of the AMR genes analyzed.

As observed in the correlation for the enclosed market (Figure 8a), there was a strong positive correlation ($r > 0.9$) between bacterial load and the relative abundance of AMR genes. In addition, strong correlations were evident among the resistance genes themselves, suggesting possible co-abundance and co-occurrence of these determinants in bacteria present in chicken carcasses. This pattern can be explained by co-selection mechanisms described in recent resistome studies, which report high connectivity between β -lactam and aminoglycoside resistance genes, favoured by mobile genetic elements [25].

In the open market (Figure 8b), a similarly significant associative pattern was observed, though with slightly lower correlation strength. Pearson coefficients ranged from 0.794 to 0.920 between ARGs and *E. coli* O157:H7 load. This reduced association strength may be attributed to environmental exposure typical of open-air spaces, as exemplified by the open market. This variability is also reflected in Figure 8c, where the distribution of relative abundance of resistance genes shows greater dispersion across most of the collected samples. Nonetheless, despite this heterogeneity, the association between variables remains statistically significant and positive, reinforcing the potential relationship between pathogen presence and the relative expression of the ARGs evaluated in this study.

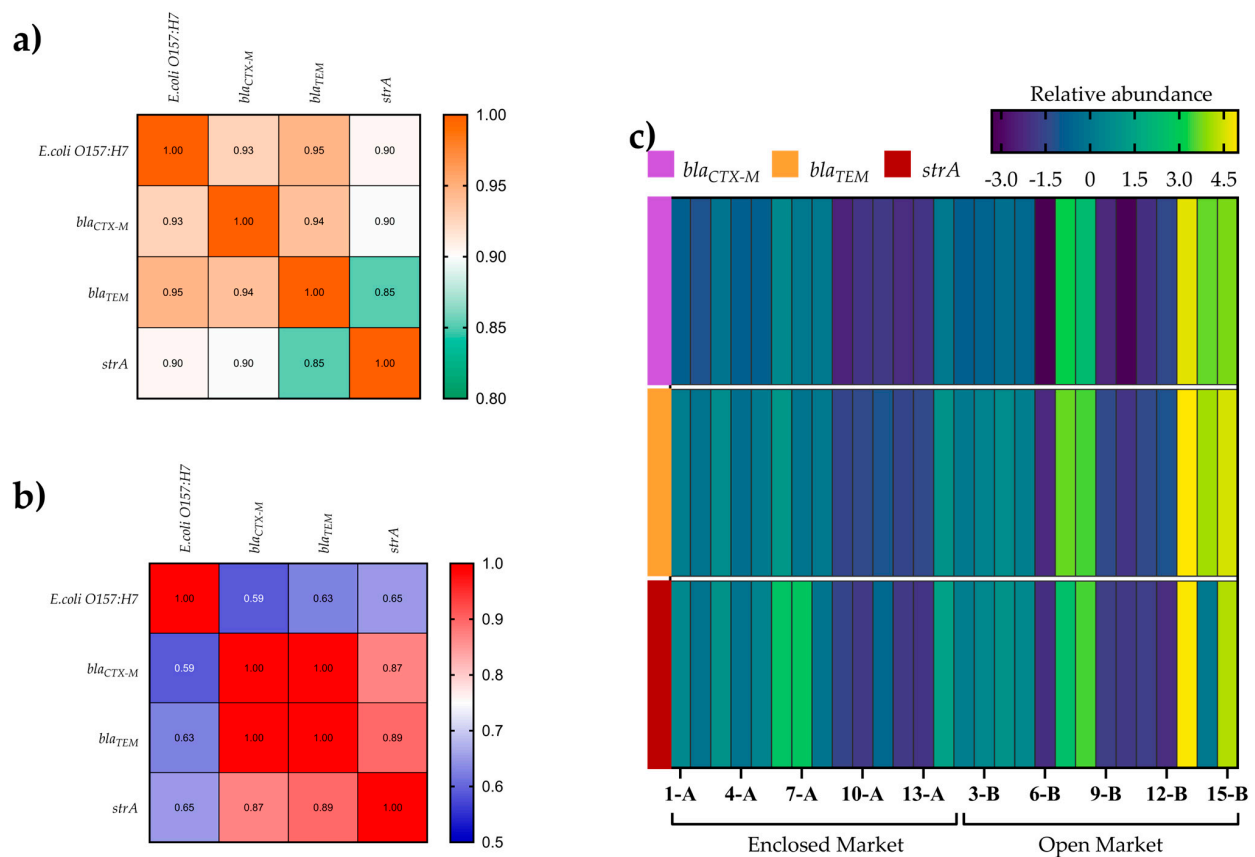


Figure 8. Positive correlations for the enclosed (a) and open (b) markets. Heat map (c) showing the distribution of the relative abundance of the antimicrobial resistance genes evaluated, based on the total number of chicken carcass samples collected in each market analyzed.

This study had limitations due to resource and time constraints. Antibiotic use practices on poultry farms were self-reported by vendors and not validated by independent inspections. Regarding the identification of *E. coli* O157:H7, although a validated molecular marker was used, it is recommended to complement this with virulence genes such as *eae*, *stx1*, and *stx2* to categorize pathogenicity. Furthermore, the analysis focused only on *bla*_{CTX-M}, *bla*_{TEM}, and *strA*, without exploring other AMR gene families such as *qnr*, *sul*, or *tet*. While qPCR detection was sensitive and specific, future studies should broaden ARG evaluation and assess phenotypic correlations to provide a more comprehensive understanding of the resistome and its relationship with pathogen load. The observed correlations should be regarded as indicative trends rather than definitive relationships, as the small sample size limits statistical power. Expanding the dataset in future studies is recommended to strengthen the validity of these associations.

4. Materials and Methods

4.1. Study Area and Sample Collection

A total of 28 chicken carcasses, each weighing approximately 400–500 g, were analyzed. Samples were randomly collected between 4 November 2024 and 6 December 2024 from two local markets, an enclosed market and an open market, located in the district of Comas (Lima, Peru). These markets were selected for their representativeness in terms of typical sales flow and daily product availability. The sample size was estimated using the proportional stratified sampling formula:

$$n_h = n \times \frac{N_h}{N}$$

assuming a 95% confidence level and a 0.05 sampling error [26]. Although the ideal sample size was calculated as 35 chicken carcasses, a total of 28 samples were obtained (13 from the enclosed market and 15 from the open market), ensuring proportional representation according to market availability and the exploratory nature of the study.

Each carcass was placed in an individual sterile bag, labelled with the origin and date of purchase, and transported in a thermal container with cold gel packs, maintaining a temperature of approximately 4 °C until arrival at the Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias Naturales y Matemática, Universidad Nacional Federico Villarreal, Lima, Peru, where they were processed immediately. In the laboratory, the organoleptic characteristics (color, odor, texture, and visible fat) were evaluated through direct observation according to the criteria established in Annex 2 of the *Reglamento Sanitario de Funcionamiento de Mercados de Abasto* (Ministerial Resolution No. 282-2003-SA/DM, Ministry of Health, Peru) [27], in order to record the general condition of the samples prior to molecular analysis.

4.2. DNA Extraction from Chicken Carcasses

Total DNA extraction was performed following a modified whole-carcass rinsing method [28]. Each whole chicken carcass was rinsed with 225 mL of peptone water for 30 min in an orbital shaker to release the bacterial DNA present. Subsequently, DNA was extracted using the Qiagen DNA extraction kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. DNA concentration was quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with the Qubit dsDNA HS Assay Kit, ensuring the recovery of high-quality DNA for subsequent qPCR analyses.

4.3. Optimization of qPCR Conditions

Quantitative real-time polymerase chain reaction (qPCR) was used to quantify the total bacterial load (16S rRNA), *Escherichia coli* O157:H7, and antimicrobial resistance genes (ARGs) in DNA extracted from chicken carcasses. Amplification was performed using a LightCycler 96 thermocycler (Roche, Mannheim, Germany). Each 20 µL reaction contained 12.5 µL of 2× SYBR Green I Master Mix, 0.5 µL of each primer, 0.3 µL of Taq polymerase, 3 µL of DNA, and nuclease-free water, following the protocol of Suria et al. (2012) [29] with minor modifications. The qPCR programme included a pre-incubation at 95 °C for 180 s, then 40 cycles of denaturation at 95 °C for 15 s, annealing at 57–60 °C for 15 s, and extension at 72 °C for 15 s. A pre-melting step at 70 °C for 180 s and a melting curve (95 °C for 10 s, 65 °C for 60 s, 90 °C for 1 s) were included to verify specificity. All reactions were performed in three biological replicates. Standard curves with 1:10 serial dilutions of bacterial DNA extracted from standardized reference strains were used to quantify total bacterial load (using strain ATCC 25922) and *E. coli* O157:H7.

Table 1 lists the primers used for 16S rRNA detection [30] and for the specific identification of *E. coli* O157:H7 through the *fliC* H7 gene, which have been previously validated in beef and chicken by other studies [30,31]. *E. coli* O157:H7 positive DNA obtained from the Clinical and Bacteriology Laboratory of the National Institute of Health (INS) in Lima, Peru, was used as a positive control.

4.4. qPCR Assay Efficiency and Validation

qPCR efficiency was evaluated using standard curves generated from serial dilutions of bacterial DNA (10^{-1} to 10^{-5}), prepared from suspensions adjusted according to 0.5 McFarland scale to obtain a defined bacterial density. Threshold cycle (Ct) values were

plotted against the logarithm of colony-forming units (CFU/mL). Amplification efficiency (E) was calculated using the formula [32]:

$$E = 10^{(-1/\text{slope})} - 1$$

Efficiencies between 90% and 110% and correlation coefficients ($R^2 \geq 0.95$) were considered acceptable. Specificity was verified by melting curves, ensuring a single peak per reaction. Each assay included positive and negative controls, and the 16S rRNA gene was used as an internal control to standardize quantification of bacterial load and ARGs (*bla*_{TEM}, *bla*_{CTX-M}, *strA*), ensuring accuracy and reproducibility.

Table 1. Primers used for the detection of bacterial species, *Escherichia coli* O157:H7 and 16S rRNA genes in qPCR assays.

Primer		Sequence (5'-3')	Amplicon Size (bp)	Identified in	References
<i>fliC</i>	F	ATAATCTACGCCGCAACT	284	<i>Escherichia coli</i> O157:H7	[29]
	R	GACTCCATCCAGGACGAAA			
16s rRNA	F	GTG CCA GCM GCC GCG GTA A	900	<i>Escherichia coli</i> str. K-12	[30]
	R	TAC GGY TAC CTT GTT ACG ACTT			

4.5. Relative Abundance of ARGs

Relative quantification of the resistance genes *bla*_{TEM}, *bla*_{CTX-M}, and *strA* was performed by qPCR using specific primers. Specificity was confirmed by melting curves. The relative abundance of each ARG was calculated using the 16S rRNA gene as a bacterial load reference, applying the ΔC_t method [33]:

$$\text{Relative abundance} = 2^{-(C_{t\text{ARGs}} - C_{t16s\text{ rRNA}})}$$

All reactions were performed in triplicate. The resulting data were used to compare ARG abundance across samples and marketing contexts. Table 2 lists the primers used.

Table 2. Primers for antimicrobial resistance genes.

Primer		Sequence (5'-3')	Amplicon Size (bp)	References
<i>bla</i> _{CTX-M}	F	CGCTTTGCGATGTGCAG	550	[34]
	R	ACCGCGATATCGTTGGT		
<i>bla</i> _{TEM}	F	ATCAGCAATAAACCCAGC	516	[34]
	R	CCCCGAAGAACGTTTTC		
<i>strA</i>	F	CCTGGTGATAACGGCAATTC	548	[35]
	R	CCAATCGCAGATAGAAGGC		

4.6. Statistical Analysis

Data were compiled in a database and analyzed using SPSS v25 and GraphPad Prism v10. For group comparisons of ARGs, parametric (*t*-test) or non-parametric (Mann–Whitney) tests were applied depending on data distribution. Associations were assessed using Pearson's correlation. A difference was considered statistically significant at $p < 0.05$. Results are presented as mean $\pm$ standard deviation or median with interquartile range, as appropriate.

5. Conclusions

Under the evaluated conditions, this comparative exploratory study, based on total DNA extraction from chicken carcasses, enabled the identification and quantification of *Escherichia coli* O157:H7 using SYBR Green-based real-time qPCR. To date, this represents one of the first reports in Peru to apply this quantitative molecular technique to chicken samples within the context of bacterial antibiotic resistance. Furthermore, the study successfully determined and quantified the antimicrobial resistance genes *bla*_{CTX-M}, *bla*_{TEM}, and *strA* with high efficiency and specificity. Differences in the relative abundance of these genes between the two markets were observed, associated with environmental exposure conditions of the carcasses, with more pronounced differences in the open market, characterized by its ambulatory nature. These findings confirm that qPCR is a reliable and robust tool for context-specific monitoring of pathogens and antimicrobial resistance genes within the food supply chain.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antibiotics15020191/s1>. Table S1: Absolute quantification of total bacterial load in chicken carcasses; Table S2: Absolute quantification of *Escherichia coli* O157:H7 bacterial load; Table S3: Quantification of the relative abundance of ARGs.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance
qPCR	Quantitative real-time polymerase chain reaction
FBD	Foodborne diseases
AGPs	Antibiotic growth promoters
ARGs	Antimicrobial resistance genes
CFU	Colony Forming Units

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