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Molecular Characterization of Antimicrobial Resistance Genes in Processed Red Meat-Derived *Escherichia coli* Isolates

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Abstract

This study aimed to determine the presence of extended-spectrum β-lactamase (ESBL) genes (blade, blush, bloat, blast-M), sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*), and integrin genes (*int1*, *int2*, and *int3*) in *Escherichia coli* isolates isolated from processed red meat samples collected in Adıyaman Province, Türkiye. A total of 14 *E. coli* isolates were analyzed using a molecular technique. PCR results revealed that several isolates carried multiple resistance genes simultaneously. Isolates harboring both ESBL and sulfonamide resistance genes were identified as 19B, 16B, 15B, and 2B, while those carrying integrin and sulfonamide resistance genes were 14A, 19A, 19B, 13A, and 15B. Only isolates 19B and 15B contained all three gene groups. These findings indicate that foodborne *E. coli* strains may possess multiple antibiotic resistance mechanisms, posing a potential public health risk. The study highlights the importance of implementing molecular surveillance programs to monitor antibiotic resistance within the food safety framework.

Keywords: *Escherichia coli*; Antibiotic Resistance; ESBL; Sulfonamide; Integrin

1. Introduction

The widespread and uncontrolled use of antibiotics has accelerated the dissemination of resistance genes among bacteria, leading to a serious global public health issue [1]. Antimicrobial resistance (AMR) is no longer confined to hospital environments but has spread across the environment, livestock production, and the food chain [2]. The use of antibiotics as prophylactic or growth-promoting agents in animal husbandry contributes to the emergence of resistant bacterial strains. These bacteria can be transmitted to humans via food and cause infections that are difficult to treat [3].

Escherichia coli is a commensal bacterium naturally found in the intestinal flora of humans and animals. However, certain *E. coli* strains have acquired genetic determinants that enable them to resist multiple classes of antibiotics. Extended-spectrum β-lactamases (ESBLs) hydrolyze β-lactam antibiotics, such as cephalosporins and monobactams, thereby reducing the effectiveness of treatment. Consequently, ESBL-producing *E. coli* strains have become important pathogens in both community-acquired and foodborne infections [4].

Resistance to sulfonamide antibiotics is primarily mediated by *sul* genes (*sul1*, *sul2*, and *sul3*), which are often located on mobile genetic elements such as plasmids and transposons, allowing horizontal gene transfer among bacterial populations [5]. Similarly, integrins are genetic structures capable of capturing and transferring resistance gene cassettes, playing a major role in the dissemination of antibiotic resistance genes [6].

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Recent studies have reported the simultaneous presence of multiple resistance genes in foodborne *E. coli* isolates. This co-occurrence increases the potential transmission of resistant bacteria through the food chain, posing a significant threat to public health [7].

The aim of this study was to investigate the presence and co-occurrence of ESBL, sulfonamide resistance, and integron genes in *E. coli* isolates obtained from processed red meat samples in Adıyaman Province, Türkiye. The findings of this research will contribute to the understanding of antibiotic resistance mechanisms in foodborne bacteria and support regional antimicrobial resistance monitoring efforts.

2. Materials and Methods

2.1. Samples and Isolation of *E. coli* Strains

In this study, genotypic identification and molecular characterization of *Escherichia coli* strains isolated from processed red meat samples were conducted. A total of 14 red meat samples were analyzed. All samples were aseptically collected in sterile polyethylene bags, transported under cold-chain conditions, and processed within 2–4 hours of collection to minimize bacterial overgrowth. For bacterial isolation, 25 g of each meat sample was homogenized in 225 mL of phosphate-buffered saline (PBS, pH 7.6) using a stomacher homogenizer. Aliquots of 100 µL from appropriate dilutions were plated onto MacConkey agar and Eosin Methylene Blue (EMB) agar media. The inoculated plates were incubated at 37°C for 18–24 hours under aerobic conditions. After incubation, colonies with typical *E. coli* morphology were identified based on their characteristic appearance—pink to red colonies on MacConkey agar and metallic green sheen colonies on EMB agar. Representative colonies were repeatedly sub cultured to obtain pure isolates, which were preserved at −20°C for molecular analyses.

2.2. DNA Extraction

Genomic DNA was extracted from the confirmed *E. coli* isolates using the boiling method. Briefly, bacterial colonies were suspended in nuclease-free water, heated at 95°C for 10 minutes, and centrifuged. The supernatant containing DNA was collected and stored at −20°C until further use as a PCR template.

2.3. Molecular Confirmation of *E. coli* (*uspA* Gene)

Molecular confirmation of phenotypically identified isolates was performed by polymerase chain reaction (PCR) as suggested by Chen and Griffiths (1998) [8] targeting the spa gene, which encodes a universal stress protein specific to *E. coli*. The reference strain *E. coli* ATCC 25922 was used as a positive control.

PCR conditions were adapted from the method described by Chen and Griffiths (1998) and optimized for the present study. Reaction mixtures and thermal cycling parameters followed a standard PCR protocol. PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized under a UV transilluminator. Samples yielding an amplicon of 884 bp were considered molecularly confirmed as *E. coli*.

2.4. PCR Analysis of Resistance Genes

Ten target genes were investigated by polymerase chain reaction (PCR), including ESBL genes ($bla_{CTX\text{-}M}$, bla_{SHV}, bla_{OXA}, and bla_{TEM}), sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*), and integron class genes (*int1*, *int2*, and *int3*). PCR reactions were performed in a total volume of 25 µL, and the specific thermal cycling conditions for each gene are summarized in Table 3.4. Each reaction mixture contained Master Mix, forward and reverse primers, template DNA, and nuclease-free water.

Table 1 PCR amplification conditions and references for target genes used in *E. coli* isolates

Target Gene	Initial Denaturation (°C/min)	Denaturation (°C/s)	Annealing (°C/s)	Extension (°C/s)	No. of Cycles	Final Extension (°C/min)	Reference
bla$_{TEM}$	94 / 5	94 / 30	55 / 30	72 / 45	35	72 / 7	[9]
bla$_{SHV}$	94 / 5	94 / 30	56 / 30	72 / 45	35	72 / 7	
bla$_{OXA}$	94 / 5	94 / 30	52 / 30	72 / 45	35	72 / 7	
bla$_{CTX-M}$	94 / 5	94 / 30	60 / 30	72 / 45	35	72 / 7	
sul1	94 / 5	94 / 30	58 / 30	72 / 45	35	72 / 7	[10]
sul2	94 / 5	94 / 30	55 / 30	72 / 45	35	72 / 7	
sul3	94 / 5	94 / 30	54 / 30	72 / 45	35	72 / 7	
int1	94 / 5	94 / 30	56 / 30	72 / 45	35	72 / 7	[11]
int2	94 / 5	94 / 30	57 / 30	72 / 45	35	72 / 7	
int3	94 / 5	94 / 30	58 / 30	72 / 45	35	72 / 7	

All PCR reactions were performed in 25 µL volumes using gene-specific primers under the conditions described above.

2.5. Agarose Gel Electrophoresis

PCR products were separated on a 1.5% agarose gel prepared in 1X TBE buffer, stained with ethidium bromide, and visualized using a Viler Lurma UV gel documentation system. A 100 bp DNA ladder was used as a molecular marker.

2.6. Statistical Analysis

The data were analyzed descriptively, and gene distribution frequencies were expressed as percentages.

3. Results and Discussion

3.1. Detection of ESBL Genes

The molecular analysis of extended-spectrum β-lactamase (ESBL) gene variants in *E. coli* isolates obtained from processed red meat samples demonstrated a limited distribution of β-lactamase genes. Among the 14 isolates analyzed, the *bla*$_{CTX-M}$ gene was detected in only one isolate (15B), whereas the bloat gene was identified in five isolates (15A, 19B, 21A, 16B, and 2B). No amplification was observed for the *bla*$_{TEM}$ and *bla*$_{SHV}$ genes. The overall distribution of the ESBL genes detected in *E. coli* isolates is summarized in Table 2. Representative agarose gel electrophoresis images showing PCR amplification of the *bla*$_{OXA}$ and *bla*$_{CTX-M}$ genes are presented in Figure 1.

Table 2 Distribution of ESBL genes in *E. coli* isolates

Number	Isolate Code	*bla*$_{CTX-M}$	*bla*$_{OXA}$	*bla*$_{SHV}$	*bla*$_{TEM}$
1	18	-	-	-	-
2	13B	-	-	-	-
3	15A	-	+	-	-
4	14A	-	-	-	-
5	19A	-	-	-	-
6	19B	-	+	-	-
7	9B	-	-	-	-
8	13A	-	-	-	-
9	21A	-	+	-	-
10	12B	-	-	-	-
11	19D	-	-	-	-
12	16B	-	+	-	-
13	15B	+	-	-	-
14	2B	-	+	-	-

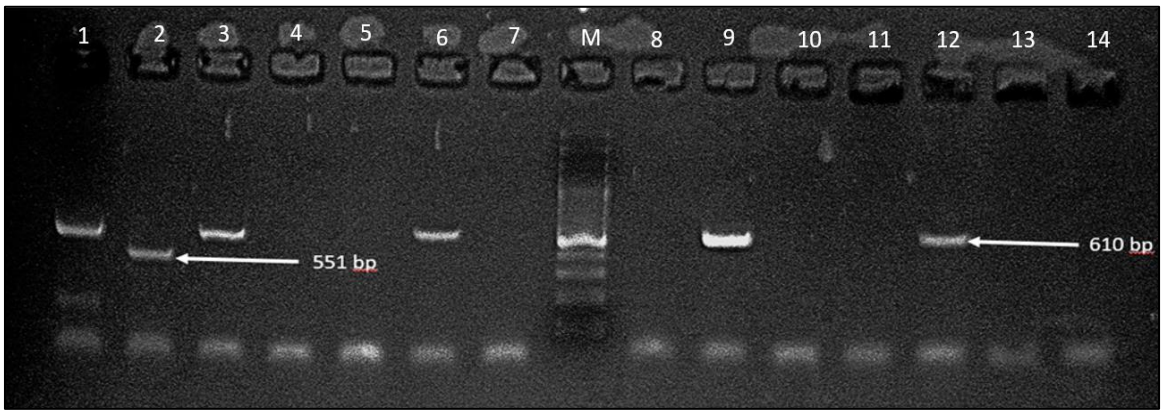

Figure 1 Agarose gel electrophoresis image showing PCR products of *bla*$_{OXA}$, *bla*$_{TEM}$, and *bla*$_{CTX-M}$ genes

The detection of *bla*$_{OXA}$ in five isolates and *bla*$_{CTX-M}$ in a single isolate indicates a relatively low prevalence of ESBL-producing *E. coli* strains in the analyzed meat samples. The absence of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes suggests that these older ESBL variants may be gradually replaced by newer β-lactamase types such as *bla*$_{CTX-M}$ and *bla*$_{OXA}$ in this geographical region.

When compared with data from other countries, notable differences can be observed. In India, *bla*$_{CTX-M}$ and *bla*$_{TEM}$ were predominant among ESBL-positive *E. coli* strains isolated from street foods, detected in 69.04% and 66.66% of isolates, respectively, while *bla*$_{OXA}$ was found in 19.04% and *bla*$_{SHV}$ was absent [12]. In Germany, 10.1% of red meat isolates carried *bla*$_{CTX-M}$, whereas *bla*$_{SHV}$ and *bla*$_{TEM}$ were detected at lower frequencies (2.0% and 0.8%, respectively) [13]. Similarly, studies in Taiwan reported *bla*$_{CTX-M}$ as the dominant ESBL gene among multidrug-resistant *E. coli* strains from bovine carcasses [14], whereas in Mexico, *bla*$_{CTX-M}$ was detected in 20% of retail meat isolates, with no detection of *bla*$_{TEM}$, *bla*$_{SHV}$, or *bla*$_{OXA}$ [15]. In addition, a study conducted in the northern regions of Egypt reported that among *E. coli* isolates obtained from raw beef and mutton, *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{OXA}$ genes were detected in 52.4%, 42.9%, and 14.3% of isolates, respectively [16]. Compared with these results, the present study showed a markedly lower prevalence of ESBL genes, suggesting geographical variations in antibiotic usage and resistance dissemination patterns across neighboring Mediterranean region.

The predominance of bla_{OXA} over bla_{CTX-M} in the present study may be related to regional differences in antibiotic usage practices, livestock management, and environmental selection pressures in Türkiye. The relatively low overall ESBL detection rate may also reflect limited use of third-generation cephalosporins in local animal farming or differences in sample type and size.

The identification of bla_{OXA} and bla_{CTX-M} positive *E. coli* isolates, even at low frequency, is of particular concern, as these genes are often plasmid-mediated and can spread rapidly among bacterial populations via horizontal gene transfer [17]. These findings highlight the need for continuous surveillance of antimicrobial resistance in foodborne bacteria and emphasize the importance of prudent antibiotic use in livestock production to minimize the risk of dissemination to humans through the food chain.

3.2. Detection and Discussion of Sulfonamide Resistance Genes

The molecular screening of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) among *E. coli* isolates obtained from processed red meat samples revealed variable gene distributions. As shown in Table 3, the *sul1* gene was detected in isolates 19A, 13A, and 12B; *sul2* was identified in isolates 18, 13B, 14A, 19A, 19B, 13A, 12B, 16B, and 2B; and *sul3* was detected in isolates 18, 14A, and 15B. Representative agarose gel electrophoresis images of *sul1*/*sul2* and *sul3* amplification products are presented in Figures 2 and 3, respectively.

Table 3 Distribution of s*ul* Genes in *E. coli* Isolates

Number	Isolate Code	*sul1*	*sul2*	*sul3*
1	18	-	+	+
2	13B	-	+	-
3	15A	-	-	-
4	14A	-	+	+
5	19A	+	+	-
6	19B	-	+	-
7	9B	-	-	-
8	13A	+	+	-
9	21A	-	-	-
10	12B	+	+	-
11	19D	-	-	-
12	16B	-	+	-
13	15B	-	-	+
14	2B	-	+	-

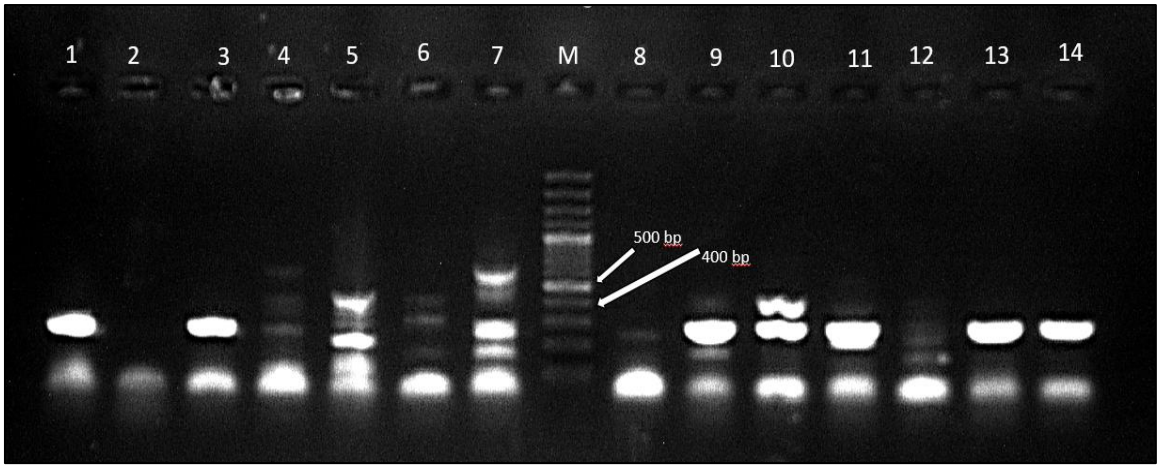

Figure 2 Agarose gel electrophoresis showing amplification of *sul1* and *sul2* genes in *E. coli* isolates

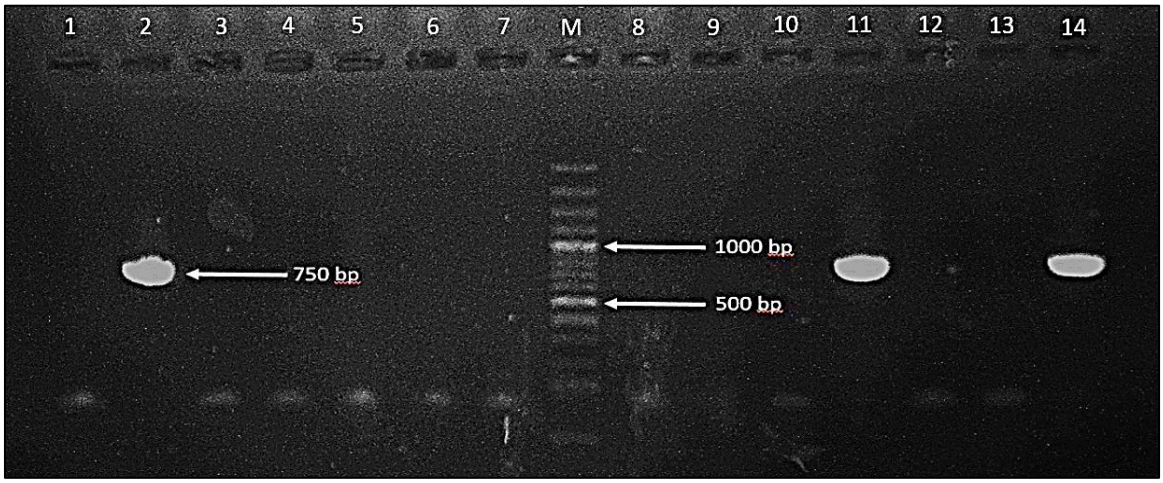

Figure 3 Agarose gel electrophoresis showing amplification of *sul3* gene in *E. coli* isolates

Multiple gene combinations were observed in several isolates. Specifically, *sul1* and *sul2* coexisted in isolates 13A, 19A, and 12B, while *sul2* and *sul3* were simultaneously detected in isolates 18 and 14A. These results indicate that some *E. coli* strains may carry multiple sulfonamide resistance determinants, potentially enhancing their ability to survive under selective antibiotic pressure.

When compared with results reported from other countries, noticeable variations in *sul* gene distribution patterns can be observed. In Spain, Ramos et al. reported that among *E. coli* strains isolated from pork meat, 23.1% harbored both *sul1* and *sul2* genes, 3.1% carried *sul2* and *sul3*, and 1.5% carried *sul1* and *sul3* together; notably, only one isolate contained all three *sul* genes simultaneously [18]. In Canada, a study investigating *E. coli* isolates from a commercial beef processing plant found that *sul1* was present in samples from hides (6%), washed carcasses (20%), conveyor belts (7%), beef trimmings (15%), and ground beef (4%), whereas *sul2* was detected in 9%, 10%, 12%, 4%, and 15% of these respective sample types, and no isolates carried *sul3* [19]. Similarly, an Austrian study analyzing *E. coli* from multiple meat sources (pork, beef, poultry, and minced meat) found that among 142 sulfonamide-resistant isolates, *sul2* (n = 113) was far more prevalent than *sul1* (n = 32), with only three isolates carrying both genes [20].

The findings of the present study are consistent with these international reports, as *sul2* was also the most frequently detected gene, followed by *sul1* and *sul3*. The detection of multiple *sul* genes in individual isolates suggests the potential for horizontal gene transfer and co-selection mechanisms under antibiotic pressure. The relatively lower detection rate of *sul3* compared with *sul1* and *sul2* aligns with previous observations that *sul3* is a less common variant, often restricted to specific plasmid types or ecological niches.

Overall, these results highlight the persistent presence of sulfonamide resistance determinants in foodborne *E. coli* and underscore the necessity of ongoing monitoring to assess their role in the broader dissemination of antimicrobial resistance within the food chain.

3.3. Detection and Discussion of Integrin Genes

The molecular screening of *E. coli* isolates obtained from processed red meat samples revealed the presence of the *int1* gene, whereas *int2* and *int3* were not detected in any of the samples. As shown in Table 4, the *int1* gene was identified in isolates 18, 14A, 19A, 19B, 13A, and 15B. Representative agarose gel electrophoresis images showing PCR amplification of the int1 gene are presented in Figure 4.

Table 4 Distribution of *int1*, *int2*, and *int3* genes among *E. coli* isolates obtained from processed red meat samples

Number	Isolate Code	*int1*	*int 2*	*int 3*
1	18	+	-	-
2	13B	-	-	-
3	15A	-	-	-
4	14A	+	-	-
5	19A	+	-	-
6	19B	+	-	-
7	9B	-	-	-
8	13A	+	-	-
9	21A	-	-	-
10	12B	-	-	-
11	19D	-	-	-
12	16B	-	-	-
13	15B	+	-	-
14	2B	-	-	-

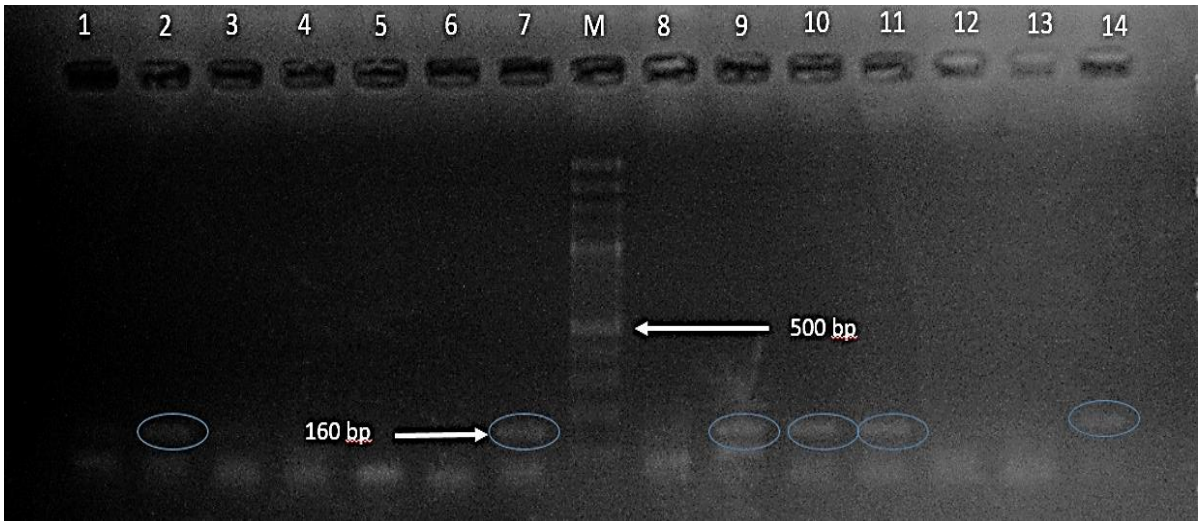

Figure 4 Agarose gel electrophoresis showing amplification of the *int1* gene in *E. coli* isolates

The detection of int1 in 6 of the 14 isolates indicates that class 1 integrons are present among *E. coli* strains isolated from processed meat products, while the absence of int2 and int3 genes suggests limited dissemination of these integron classes in the analyzed samples. Integrons play a key role in the horizontal transfer of antibiotic resistance genes, and the identification of int1-positive isolates highlights their potential role in the spread of multidrug resistance determinants.

Similar observations have been reported in previous studies conducted in different geographical regions. In Norway, class 1 and class 2 integrons were detected in 12% (*int1*) and 6% (*int2*) of 241 resistant *E. coli* strains isolated from meat products, respectively [21]. In contrast, a study conducted in South Korea identified class 1 integrons (*int1*) in 16 isolates from animal-derived foods, while class 2 and class 3 integrons were not detected, consistent with the findings of the present study [22]. Likewise, in India, 56 of 96 (58.3%) multidrug-resistant *E. coli* isolates from ready-to-eat food samples were positive for class 1 integrons, showing a significant correlation between integron occurrence and food origin [23].

Taken together, these results indicate that the presence of class 1 integrons in *E. coli* isolates from meat products is a globally observed phenomenon, although their prevalence varies by country and sample type. The detection of *int1* in this study, even at a moderate frequency, suggests that processed meat products may act as reservoirs for integron-associated resistance genes. Continuous monitoring of integron-bearing *E. coli* isolates is therefore essential to better understand their role in the dissemination of antimicrobial resistance through the food chain.

3.4. Overall Evaluation

The combined evaluation of ESBL, sulfonamide, and integron gene profiles revealed a clear indication of multidrug resistance among *E. coli* isolates obtained from processed meat samples. Although the overall detection frequency of individual genes was relatively low, several isolates harbored multiple resistance determinants simultaneously, suggesting the presence of mobile genetic elements mediating horizontal gene transfer.

The predominance of bla_{OXA} and *sul2* genes, together with *int1*-positive isolates, highlights the potential linkage between β-lactamase, sulfonamide, and integron-associated resistance mechanisms. Such co-localization increases the risk of multidrug resistance dissemination through the food chain.

These findings underline the importance of integrated molecular surveillance strategies that simultaneously target ESBLs, *sul* genes, and integron classes to provide a more complete understanding of antimicrobial resistance dynamics in foodborne *E. coli*.

4. Conclusion

This study provides a comprehensive molecular characterization of *Escherichia coli* isolates obtained from processed red meat samples, focusing on β-lactamase (ESBL), sulfonamide resistance, and integron genes. The detection of bla_{OXA} and $bla_{CTX\text{-}M}$ genes, along with the widespread occurrence of *sul2* and the presence of class 1 integrons (*int1*), indicates that foodborne *E. coli* strains may act as reservoirs of multiple resistance determinants. The coexistence of these genes in certain isolates suggests possible plasmid-mediated horizontal gene transfer events contributing to the dissemination of multidrug resistance within the food chain. Although the overall gene prevalence was moderate, these findings highlight the need for continuous molecular surveillance to monitor the spread of antimicrobial resistance. Strengthening hygiene standards, regulating antibiotic use in animal husbandry, and implementing integrated resistance monitoring systems are crucial steps to prevent further dissemination of resistant *E. coli* from food sources to humans.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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