



## Hygienic Status of Modern Animal Slaughterhouse in Sulaymaniyah Province, Iraq



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### Abstract

**E**NSURING meat hygiene is critical for public health, particularly in resource-limited settings where infrastructure and safety standards are suboptimal. This study aimed to assess the hygienic status and microbial contamination at the modern slaughterhouse in Sulaymaniyah Province, Iraq, with a focus on identifying *E. coli* and extended-spectrum  $\beta$ -lactamase (ESBL)-producing strains. Over a six-month period, 250 samples were collected from various critical points, including carcasses, tools, water, and workers' hands. Hygienic practices were evaluated across 16 standard indicators. Microbiological analyses involved, Total bacterial count, conventional culturing, biochemical identification using the VITEK® 2 system, and ESBL screening. The hygienic evaluation revealed severe deficiencies, with 15 out of 16 criteria rated as unsatisfactory. *E. coli* was detected in 94.86% of all samples, with 100% positivity in samples from workers' hands, knives, and inner carcass surfaces. Chi-square analysis revealed no statistically significant difference in *E. coli* presence across most sample types ( $p = 0.077$ ), although water samples showed significantly lower contamination rates ( $p = 0.002$ ). Total bacterial count was significantly reduced post-washing ( $p < 0.05$ ), yet residual contamination remained high. ESBL-producing *E. coli* was found in 186 of 237 isolates (68.13%), with the highest prevalence in outer carcasses (96.2%), showing a significant distribution across sample sources ( $p < 0.05$ ). These findings highlight critical lapses in hygiene and the presence of ESBL-producing *E. coli*, underscoring the urgent need for policy interventions. Enhanced sanitation practices, and staff training are essential to mitigate public health risks and align practices with international food safety standards.

**Keywords:** ESBL *E. coli* produce, *Escherichia coli*, Meat hygiene, Slaughterhouse contamination.

### Introduction

The worldwide rise in human population correlates with a heightened demand for animal-derived consumables [1]. Thus, guaranteeing the security, quality, and safety of food is a global problem [2]. Because animals and goods produced there are frequently produced in less-than-ideal hygienic circumstances, it is an especially serious issue in poor nations [3, 4]. The majority of meat-borne bacterial outbreaks are typically linked to supply chain contamination brought on by careless handling procedures [5]. Animals that produce food are the main source of many foodborne infections and can contaminate meat, which can cause foodborne

diarrhoeal illnesses in humans to spread widely [6, 7]. The abattoir is a critical point in the supply chain, as it is a source from which foodborne diseases can disseminate through the processing and distribution network, including retail outlets, ultimately affecting the end consumer. Consequently, ensuring the quality and safety of meat to safeguard public health necessitates stringent hygienic protocols at slaughterhouses, throughout distribution and storage at retail establishments, and during sales [2]. Insufficient infrastructure and negligent animal management at slaughterhouses intensify microbial contamination of cattle, potentially leading to the transmission of foodborne illnesses to humans [8, 9].

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Numerous research studies examined the prevalence of illnesses along the cattle supply chain. [10]. Others identified particular levels of pollution at abattoirs [11] across various nations. Cross-contamination and contamination from raw meat are significant contributors to foodborne illnesses, especially in developing nations [12].

Interventions are necessary to tackle food safety concerns throughout the cattle supply chain. A comprehensive understanding of the local factors contributing to microbial meat contamination across the meat production, processing, and distribution chain is essential for identifying specific intervention targets in particular contexts. Meat serves as an optimal environment for the proliferation of several pathogenic and non-pathogenic bacteria that people may acquire, including *Escherichia coli* (*E. coli*), which can generate multiple diarrhoeal pathogens referred to as Diarrheagenic *Escherichia coli* (DEC) [13]. *E. coli* is another principal facultative anaerobic microorganism present in the intestines of both humans and animals. This bacterium has evolved into a highly perilous infection capable of inducing harmful enteric and extra-intestinal diseases in the presence of immunosuppressive conditions, intestinal barrier compromise, or other forms of debilitation. [14]. The opportunistic bacteria *E. coli* can enter the body whenever an opportunity arises, leading to many disorders, including diarrhoea, meningitis, septicaemia, and bacteraemia [15]. *E. coli*, which generates extended-spectrum  $\beta$ -lactamase (ESBL), presents considerable risks to public health. These microbes produce enzymes that hydrolyse and inactivate several  $\beta$ -lactam antibiotics, including monobactams and third- and fourth-generation cephalosporins. Multidrug-resistant (MDR) phenotypes arise from ESBL genes, which are generally located on plasmids that also harbour other resistance genes. ESBL-producing *E. coli* is increasingly prevalent in both clinical and non-clinical environments, including food, water, and animals. These strains are now often identified in livestock and other food-producing animals, no longer confined to hospitals. The overapplication of antibiotics in veterinary and agricultural contexts correlates with their widespread occurrence beyond hospital environments. Moreover, integrons and virulence factors, which facilitate the horizontal transfer of resistance genes among bacterial populations, are often associated with ESBL-producing *E. coli*. They endure and multiply in the environment and food chain as a consequence of this. The emergence of resistant strains jeopardizes the health of humans and animals by diminishing treatment options and increasing the likelihood of therapeutic failure [16].

The purpose of the study is to monitor the hygiene and safety of the slaughter process in the Sulaymaniyah slaughterhouse from the beginning to the end of the slaughter process by checking and following the scientific principles for slaughtering animals until they reach the consumer. Also identify a type of bacteria called *Escherichia coli* because there is a direct relationship between the number of *Escherichia coli* bacteria and food hygiene, and investigate the ESBL-producing *E. coli*.

## **Material and Methods**

### *Study Design*

Samples were taken from the modern slaughterhouse in Sulaymaniyah city's northeastern region in order to evaluate the microbiological contamination of meat with *Escherichia coli*. The sampling was carried out over a six-month period, from October 2024 to March 2025, focusing on crucial checkpoints throughout the slaughtering process.

Sulaymaniyah is a city in the east of the Kurdistan region of Iraq. The new slaughterhouse in Sulaimani is one of the main slaughterhouses in the province, where 78% of animals such as sheep, goats, cattle, and cows are slaughtered. In generally 70 to 80 tonnes of red meat are imported into the Sulaimani market daily through the Sulaymaniyah animal slaughterhouse. However, the local domestic livestock resources do not meet more than 40 % of the domestic needs, so 60 % of the meat needs should be met by imports from abroad through official and smuggled animals coming to Kurdistan. The research study was conducted out at the Sulaimani University Research Centre New Campus Microbiology Laboratory.

### *Sample Collection*

A total of 250 different samples were collected from various sources. The samples were distributed as follows: 60 samples from the body cavity of the animal carcass, 60 samples from the surface of the animal carcass, 40 samples from workers hands, 40 samples from the slaughter knife, 40 samples from the meat hook, and 10 samples from water sources. Samples were obtained by sterile swabs (Huachenyang Biotechnology, China) pre-hydrated with 10 mL buffered peptone water (BPW; Liofilchem, Italy). 100 ml of water from the source was gathered in sterile containers [17]. All samples were tagged with location, time, and stage identifiers and stored in insulated cold boxes with ice packs (0–4°C) to inhibit bacterial proliferation during travel. The samples were subsequently transferred to the University of Sulaimani Research Centre for bacteriological investigation.

### *Hygienic status*

To determine the hygienic status of the slaughter process from the beginning to the last stage, we identified 16 points that are used as a standard to determine the hygiene of the animal slaughterhouse [18]. Throughout my research, all 16 points were monitored and evaluated daily during the six-month period (October 2024–March 2025). At each sampling visit the results were recorded.

### *Total Bacterial Count (TBC)*

Serial decimal dilutions ( $10^{-1}$  to  $10^{-6}$ ) were performed in sterile 0.1% peptone water following the homogenization of samples via vortexing at 3000 rpm for 1 minute. To mitigate thermal shock to bacterial cells, 1 mL aliquots from the appropriate dilutions were aseptically transferred into sterile Petri dishes (90 mm in diameter) and mixed with 15–20 mL of molten Plate Count Agar (PCA; HiMedia, India), pre-cooled to 40–45°C. Plates were inverted and incubated aerobically for 48 hours at 37°C following solidification. Each dilution was subjected to triplicate plating to ensure statistical robustness [19].

### *Isolation and Identification of Escherichia coli*

After swab collection, the samples are put in 10 mL of broth made with peptone water (Liofilchem, Italy) to maintain bacterial activity until return to the microbiology laboratory. Then took a full loop from the buffered peptone water and put it in 10 mL of MacConkey broth and then put it in an incubator for 24 hours at 37°C for enrichment of *E. coli* (Liofilchem, Italy). After enrichment, each sample was streaked onto MacConkey agar and then incubated for 24 hours at 37°C (Liofilchem, Italy). After 24 hours in the incubation, soft, pink, round colonies with a smooth morphology appear on the MacConkey agar. These colonies indicate the presence of lactose-fermenting bacteria, especially *E. coli*, which are typically characterised by their distinct colour on this selective medium, lactose-positive colonies were isolated using the streak plate method and subsequently re-cultured on eosin-methylene blue (EMB) agar (Liofilchem, Italy) for 24 hours at 37°C. Following incubation, the colonies showed varied levels of pigmentation, indicating lactose fermentation. This enabled us to distinguish between lactose-positive and lactose-negative bacteria successfully. After 24 hours of incubation at 37°C, colonies with a distinctive green metallic sheen on EMB agar were identified as *E. coli* [20]. *E. coli* was identified using the VITEK® 2 compact system (BioMérieux, Marcy l'Etoile, France). The system cards were automatically filled, sealed, and put into the VITEK® 2 compact system instrument for reading and incubation in accordance with the manufacturer's instructions.

### *Extended-spectrum $\beta$ -lactamase Screening of the isolates*

The Double-Disc Synergy Test (DDST) was utilized to confirm the presence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, employing the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA; Oxoid). Bacterial inoculated were prepared by adjusting each test strain, as well as both positive and negative controls, to a turbidity corresponding to the 0.5 McFarland standard using 3 mL of sterile saline solution. The prepared suspensions were inoculated uniformly onto MHA plates with sterile swabs and permitted to dry for 3 to 5 minutes. Antibiotic discs of amoxicillin-clavulanic acid (AMC, 20/10 µg or 30/10 µg; Oxoid), ceftazidime (CAZ, 30 µg), and cefotaxime (CTX, 30 µg) were positioned on the agar surface with a 15 mm separation between the amoxicillin-clavulanic acid disc and the cephalosporin discs (center to center). The plates were incubated at  $35 \pm 2$  °C for a duration of 16 to 24 hours. The augmentation of the inhibitory zone between cefotaxime or ceftazidime and amoxicillin-clavulanic acid revealed synergy, hence establishing the presence of ESBL formation [21].

### *Statistical analysis*

All data underwent a one-way analysis of variance (ANOVA) utilizing SPSS version 19.2.2 and the XLSTAT software for Windows. Discrepancies in the means were analyzed with Duncan's multiple range tests. The findings are presented as the mean, with a significance threshold established at  $p < 0.05$ . A chi-square test of independence was employed to evaluate the distribution of *E. coli* contamination across the different samples. This non-parametric statistical test is commonly employed to ascertain whether observed frequencies significantly diverge from expected frequencies, assuming independence, and is appropriate for categorical data [22].

## **Results**

### *Hygienic status*

Fifteen of the sixteen evaluated indicators received a low rating. 100% non-compliance was found with critical measures such as disinfecting the premises, using footbaths, cleaning hands, and disposing of condemned corpses. Additionally, 85–90% of workers did not wash their hands before and after slaughter, wash their blades and equipment, or wear protective clothes. In more than 70% of cases, surface disinfection, drainage, and ventilation failed. In the majority of sites, only the structural condition—cracks in the walls, floors, and pillars (Table 1).

### *Total Bacteria Count (TBC)*

The bacterial load on the inside surfaces of corpses taken from a slaughterhouse was assessed in this section of the investigation. To evaluate the effect of washing on microbiological contamination, samples were collected both before and after the washing procedure. Each group in the chart titled "Total Count," which summarizes the results of the measurement of the total bacterial count (TBC), reflects a particular combination of sample day, body area, and treatment condition. Internal carcass samples that had not been cleaned consistently had the highest levels of bacterial contamination. D is taking swabs from the inside of the animals' carcasses. B means before washing the animal with water. A means after the same animal has been washed with water. The statistical category "A" includes groups like D1B1, D4B2, and D3B2. In both cases, I took samples to see if washing had any effect on reducing TBC. Which all had the highest LS mean values and showed no discernible differences from one another. Usually, these numbers were higher than 18,000 CFU/cm<sup>2</sup>. On the other hand, cleaned carcasses exhibited noticeably lower bacterial counts, especially those in D1A2, D4A2, and D2A2. These were classified as "B" because their means frequently fell below 10,000 CFU/cm<sup>2</sup> (Fig. 1).

### *Isolation and Identification of E coli*

A total of 250 samples were taken from a variety of surfaces in the new animal abattoir in Sulaymaniyah, including worker hands, slaughter knives, meat hooks, corpses, and water sources. According to the findings, 237 (94.86%) of the 250 samples had positive *E. coli* tests. 100% of the samples tested positive, with worker hands and slaughter knives showing the greatest contamination rates. While the body cavity of the animal carcass had 100% *E. coli* positivity, the meat hooks and surface of the animal had a somewhat lower contamination rate (95% and 93.3%, respectively). Remarkably, there was no contamination found in the water source samples (Table 2). The statistical analysis indicates that, overall, *E. coli* contamination is not significantly associated with the type of sample ( $p = 0.077$ ). However, water source samples showed a significantly lower contamination rate than expected ( $p = 0.002$ ), suggesting effective separation or treatment protocols for water compared to other environmental surfaces and carcass sites. The consistently high contamination observed in hand, knife, and carcass samples underscores the need for stringent hygiene and sanitation measures throughout the slaughtering and processing chain.

The medium-sized, smooth, shiny colonies are flat on MacConkey agar, dry, doughnut-shaped, and

pink to dark pink as a result of lactose fermentation. The colonies on EMB had a metallic green shine (Fig. 2). As demonstrated by the Gram stain, the isolates were rod-shaped, purple, Gram-negative, and present singly or in clusters. The VITEK® 2 system's dependability in bacterial identification is highlighted by the high confidence scores (above 99% for all isolates). All the results confirmed that it was *E. coli* positive 100%.

### *Phenotypic screening for ESBL production*

The Double-Disk Synergy Test (DDST) analyzed all 237 *E. coli* isolates and identified 186 (68.13%) bacteria as ESBL producers (Table 3, Figure 3).

### **Discussion**

The slaughter process in slaughterhouses from beginning to end plays an important role in providing citizens with clean and safe meat because the animal carcass after slaughter is a very favorable environment for the growth of microorganisms, especially *E. coli* bacteria. *E. coli* is a major cause of customer infections such as GIT, UTI, and many infections in other parts of the body [23].

According to the study's findings, the Sulaymaniyah modern slaughterhouses' hygiene assessment revealed serious non-compliance with globally recognized hygienic standards. Significant shortcomings in preserving hygienic conditions during slaughtering operations are shown by the widespread disregard for basic hygiene precautions, such as inadequate disinfection, a lack of protective gear, and inappropriate waste disposal procedures. Given how important abattoir hygiene is to maintaining the safety and quality of meat products as well as public health, the results are alarming. Similar reports from other regions are consistent with the reported low compliance with disinfection measures (100% non-compliance with sanitizing footbaths, premises, and condemned carcass disposal). Additionally, recent research has shown that improper disinfection practices greatly increase the risk of meat product contamination, with pathogens like *Escherichia coli* being a serious problem [18, 24]. Lack of hygiene in slaughterhouses can lead to the growth of infections, which increases the hazards to food safety and the spread of zoonotic illnesses.

Furthermore, the difficulty of attaining microbiological cleanliness with water alone is shown by the high bacterial counts discovered on the internal surfaces of carcasses, especially those that had not been regularly cleaned. Although research has indicated that water spraying might lessen bacterial contamination, a variety of techniques, including the application of disinfectants and appropriate hygiene practices, are necessary for full

decontamination [25]. This is in line with the results of this investigation, which showed that significant bacterial loads were still present in even cleaned corpses. The research has stressed the necessity of more effective decontamination methods, such as the application of antimicrobial agents [26].

A serious problem in the slaughterhouses is shown by the fact that *E. coli* was isolated and identified in 93.6% and 96% of the samples from the large and small animal departments, respectively. Given that *E. coli* is a well-known pathogen linked to foodborne diseases such as hemolytic uremic syndrome and haemorrhagic colitis, this high contamination rates are especially worrisome [27]. The prevalence of *E. coli* on surfaces, including worker hands, slaughter knives, and carcasses, shows that hygiene precautions have not been effective and emphasizes the need for improved sanitation practices.

This study determined that samples taken from several abattoir sources had a significant incidence of isolates that produce extended-spectrum beta-lactamase (ESBL). 186 (68.13%) of the 237 isolates in total were found to produce ESBL. The samples from the surface of animal carcasses (96.2%) and body cavity of the animal carcasses (66.67%) had the highest percentages of ESBL producers, followed by those from worker hands (87.5%), slaughter knives (80%), and meat hooks (74.4%). Significant contamination during processing is suggested by the preponderance of ESBL-producing isolates in carcass samples, most likely as a result of poor sanitation and equipment-carcass contamination. This result is in line with a study by [28] that found high concentrations of *Escherichia coli* that produce ESBL on the carcasses of slaughtered pigs and in

meat processing settings in Spain. The study also found that personnel hands and food processing surfaces are important sites for the spread of bacteria. In the same way, [29] found ESBL-producing *E. coli* in retail meat in the Netherlands and linked the infection to unsanitary slaughter and evisceration procedures. The current study's high rate of hand isolation (18.83%) highlights the importance of rigorous personal hygiene and efficient hand sanitation procedures.

### **Conclusion**

To sum up, the study's results highlight how urgently the Sulaymaniyah slaughterhouses must enhance their cleanliness procedures in order to comply with international food safety regulations. Comprehensive sanitary measures, such as improved disinfection procedures, the need for workers to wear protective gear, and stricter worker training, should be the focus of efforts. Furthermore, in order to protect public health and food safety, the problem of antimicrobial resistance must be addressed by more prudent use of antibiotics and improved surveillance.

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This study didn't receive any funding support.

### ***Declaration of Conflict of Interest***

The authors declare that there is no conflict of interest.

### ***Ethical of approval***

This study was ethically approved by the University of Sulaimani's College of Agricultural Engineering Sciences' institutional animal care and use committee (UV.AGR.2024.1).

**TABLE 1. Items seen at slaughterhouses under the hygiene section**

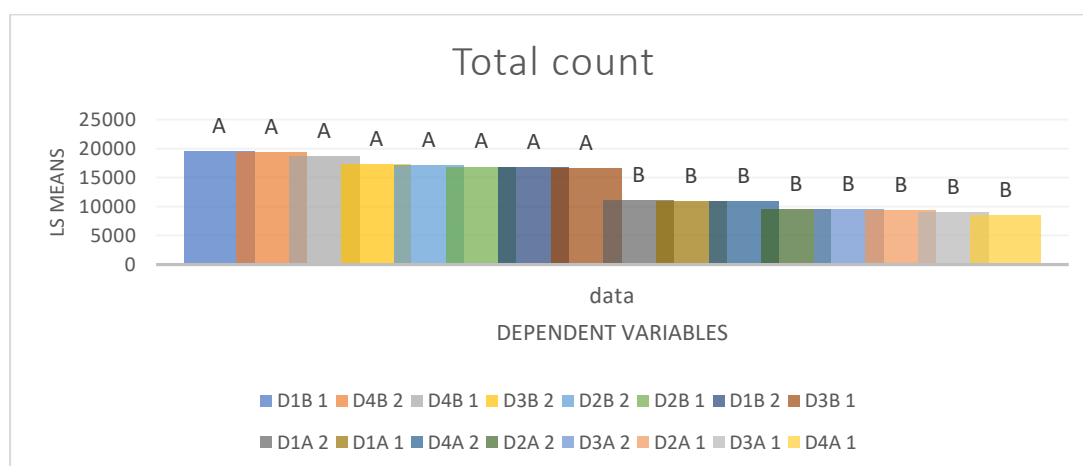
No.		%Not Meeting Standard	% Meeting Standard	Remark
1	Environmental cleanliness and disinfection	95%	5%	Poor
2	Disinfection of premises	100%	0%	Poor
3	Use of footbath	100%	0%	Poor
4	Hygiene before entry	95%	10%	Poor
5	Ventilation system	70%	30%	Poor
6	Disinfection of surfaces	80%	20%	Poor
7	Crack in floor/walls/pillars	10%	90%	Good
8	Waterlogging	30%	70%	Poor
9	Handwashing before slaughter	90%	10%	Poor
10	Hand disinfection before slaughter	100%	0%	Poor
11	Gloves, mask, head cover, boots, protective clothes	85%	20%	Poor
12	Washing slaughtering equipment	85%	15%	Poor
13	Drainage system	80%	20%	Poor
14	Disposal of condemned carcass	100%	0%	Poor
15	Handwashing after slaughter	90%	10%	Poor
16	Washing knife after each slaughter	90%	10%	Poor

**TABLE 2.** The new Sulaymaniyah animal slaughterhouse's surface *E. coli* contamination prevalence

Type of Sample	No. of Samples	No. of Positive <i>E. coli</i>	%
Hand worker	40	40	100
Slaughter knife	40	40	100
Meat hook	40	39	97.5
Surface of animal carcasses	60	58	96.7
Body cavity of animal carcass	60	60	100
Water sources	10	0	0
Total	250	237	94.8

**TABLE 3.** Extended spectrum beta- lactamases producing isolates from various samples using phenotypic methods DDST

Source	Total Isolate No.	ESBL. Producer No. (%)
Hand worker	40	35(87.5)
Slaughter knife	40	32 (80)
Meat hook	39	29 (74.4)
Surface of animal carcasses	58	50 (96.2)
Body cavity of animal carcass	60	40 (66.67)
Total	237	186 (68.13)

**Fig. 1.** Total bacterial count of cattle and sheep in the Sulaymaniyah slaughterhouse indifferent day of the week.**Fig. 2.** Colony patterns of *E. coli*, A: MacConkey agar and B: Eosin Methylene Blue.

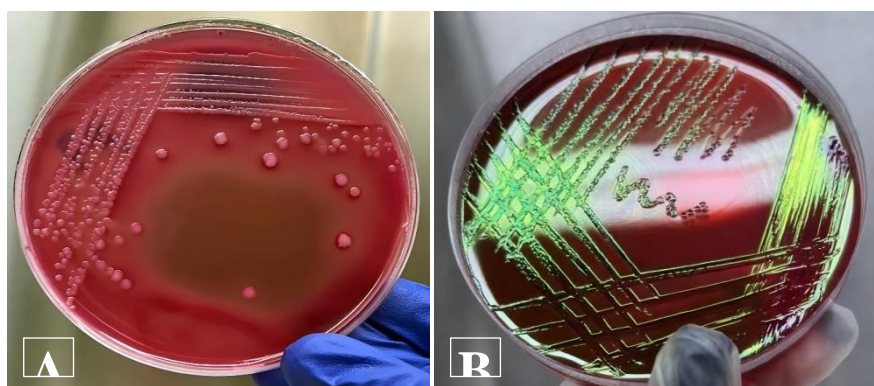


Fig. 3. Extended-spectrum  $\beta$ -Lactamase positive isolates by double-disc synergy test

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## الحالة الصحية لنظافة المسلخ الحيواني الحديث في محافظة السليمانية، العراق

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### الملخص

تُعدّ ضمان سلامة اللحوم أمراً حاسماً للصحة العامة، ولا سيما في البيئات محدودة الموارد حيث تكون البنية التحتية ومعايير السلامة دون المستوى المطلوب. هدفت هذه الدراسة إلى تقييم الحالة الصحية والملوثات الميكروبية في المجزرة الحديثة بمحافظة السليمانية، العراق، مع التركيز على الكشف عن بكتيريا *E. coli* والسلالات المنتجة للبيتا-لاكتاماز واسعة الطيف (ESBL) وخلال فترة ستة أشهر، جُمعت 250 عينة من نقاط حرجة متعددة، شملت الذبائح، الأدوات، المياه، وأيدي العاملين. كما تم تقييم الممارسات الصحية وفق 16 مؤشراً قياسياً. شملت التحاليل الميكروبيولوجية العدّ الكلي للبكتيريا (TBC)، والزراعة التقليدية، والتعريف البيوكيميائي باستخدام نظام VITEK® 2، إضافة إلى فحص إنتاج الـ ESBL عبر اختبار التآزر بالقرص المزدوج. (أظهرت نتائج التقييم الصحي وجود قصور حاد، إذ صُنّف 15 من أصل 16 معياراً على أنه غير مرضٍ. تم الكشف عن *E. coli* في 94.86% من جميع العينات، مع نسبة إيجابية بلغت 100% في عينات أيدي العاملين، السكاكين، والأسطح الداخلية للذبائح. كشف اختبار كاي-تربيع عن عدم وجود فرق معنوي في وجود *E. coli* بين معظم أنواع العينات ( $p = 0.077$ )، في حين أظهرت عينات المياه معدلات تلوث أقل بشكل معنوي ( $p = 0.002$ ) كما انخفض العدد الكلي للبكتيريا بعد الغسل ( $p < 0.05$ )، إلا أن التلوث المتبقي ظل مرتفعاً. وُجدت السلالات المنتجة للـ ESBL في 186 من أصل 237 عينة (68.13%)، مع أعلى نسب انتشار في الأسطح الخارجية للذبائح (96.2%)، وقد أظهر توزيعها اختلافاً معنوياً باختلاف مصادر العينات ( $p < 0.05$ ). تُبرز هذه النتائج ثغرات جسيمة في إجراءات النظافة وجود بكتيريا *Escherichia coli* المنتجة للبيتا-لاكتاماز واسعة الطيف، ما يؤكد الحاجة الملحة إلى تدخلات تنظيمية. وتُعدّ تحسينات ممارسات التعقيم، وتدريب الكوادر ضرورية للحد من المخاطر الصحية العامة ومواءمة الممارسات مع معايير سلامة الغذاء الدولية.

**الكلمات الدالة:** إنتاج الإشريكية القولونية لبيتا لاكتاماز واسع الطيف، الإشريكية القولونية، صحة اللحوم، تلوث المجازر.