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Distribution of Antibiotic Resistance Genes in Gram Negative Bacteria Isolated from Contact Surfaces at Slaughterhouses and Butcheries

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Abstract

Background: Cross contamination of contact surfaces in slaughterhouses and meat shops (butcheries) may contain antibiotic resistance genes that pose a serious public health challenge.

Aims: The present study aimed to assess prevalence of antibiotic resistance genes in Gram-negative strains isolated from contact surfaces at slaughterhouses and meat shops**.**

Methods: A total of 71 swab samples were collected from different contact surfaces at slaughterhouses and meat shops and cultured for bacterial identification, antibiotic resistance testing, and resistance gene detection. Polymerase chain reaction (PCR) was used to detect *strA-strB, sul2, aadA1, tet(B), tet(A), cat, sul3,* and *blaSHV* resistant genes in Gram-negative bacteria.

Results: The results showed that the most common Gram-negative bacteria were *Klebsiella pneumoniae* (41.2%) and *Escherichia coli* (35.3%) followed by *Enterobacter cloacae* (5.9%). Resistance was most prevalent against trimethoprim-sulfamethoxazole (88.1%), ampicillin (85.3%), and cefuroxime (70.6%). The most prevalent antibiotic resistance gene was aadA1 (88.2%), followed by *tet* (A) (73.5%), and *sul3* (70.6%). The results also indicated that 29.4% of the bacteria carried one resistance gene, 44.1% carried two genes, 20.6% carried more than two genes.

Conclusions: The findings of this study emphasize the critical role of hygiene and antimicrobial stewardship in slaughterhouses and butcheries. The high prevalence of Gram-negative bacteria and their resistance to multiple antibiotics highlight the urgent need for improved sanitation practices and responsible antibiotic usage. Future studies should focus on whole-genome sequencing to further investigate resistance mechanisms and explore alternative disinfection strategies to reduce bacterial contamination in meat-processing facilities.

Keywords: Resistance Genes, Contact surfaces, Meat, Gram negative bacteria, PCR

1. Introduction

Contamination of meat sold in slaughterhouses and meat shops (butcheries) may arise from biological sources such as bacteria, viruses, and parasites, as a result from multiple factors associated with production, transport, storage (Casalinuovo et al., 2016). Contamination of meat contact surfaces at various slaughterhouses and sales points can occur at various stages, including production, transportation, storage, and display (Mashak 2018). It may originate from multiple sources, such as contaminated equipment, unclean water, or surfaces used for processing (Todd et al., 2008; Das et al., 2019). The risk of meat contact surfaces contamination can significantly rise due to the high volume of slaughtering, handling, and distribution (Schwab & Armah, 2019). The surge in animals slaughtered for sacrifice places added strain on resources, often leading to rushed processing and a greater chance of compromised hygiene practices. (Danforth 2014) Bacteria found on contact surfaces in slaughterhouses and butcheries can possess various virulent factors that contribute to their survival, persistence, and potential to cause foodborne illnesses (Darwish et al., 2016). Many bacteria, such as *Listeria monocytogenes* and *Salmonella*

spp., can form biofilms on surfaces like stainless steel, plastic, and cutting boards, making them resistant to cleaning and disinfection. Surface proteins (e.g., fimbriae, pili, adhesins) enable bacteria like *Escherichia coli* and *Staphylococcus aureus* to attach to surfaces, facilitating their persistence in the environment. Bacteria on surfaces may carry antibiotic resistance genes, such as methicillin-resistant Staphylococcus aureus (MRSA), making infections harder to treat. Some pathogens like Salmonella and Listeria possess cold and desiccation resistance, allowing them to survive in refrigerated slaughterhouses and butcher shops (Cossi et al., 2013). Bacteria may secrete proteases and lipases to degrade cleaning agents, helping them survive sanitation processes. Understanding these virulent factors is crucial for preventing bacterial contamination in meat-processing environments.

However, the high demand for rapid processing may lead to inadequate refrigeration or improper handling, heightening the risk of contamination (Adamson et al., 2023). Crowded conditions in slaughterhouses and markets, due to the influx of visitors, make it challenging to uphold sanitary standards, increasing the chances of cross-contamination between animals, surfaces, and handlers. Traditional slaughtering and processing methods may not always meet modern food safety standards, which can increase contamination risks (Conficoni et al., 2022). Additionally, using shared equipment and surfaces for different animals or types of meat without adequate cleaning can spread pathogens. In some instances, slaughtering and processing occur in less-regulated environments with limited hygiene enforcement, raising the likelihood of bacterial contamination (Sujarwanta et al., 2024). Moreover, consumers may lack knowledge about proper handling, storage, and cooking practices for meat, potentially leading to improper practices at home that allow bacteria to thrive (Noviyanti, 2017). Bacterial contamination in marketed meat can involve different types of bacteria, either Gram-positive or Gram-negative bacteria. Both types can pose health risks when they contaminate meat, especially if the meat is not handled properly or thoroughly cooked. Among the primary gram-positive bacteria that cause meat contamination are *Staphylococcus aureus* and *Listeria monocytogenes*, Additionally, methicillin-resistant *Staphylococcus aureus* (MRSA) can cause skin infections and severe diseases if there are open wounds (Sumner et al., 2003). Gram-negative bacteria commonly found in meat contamination include *Escherichia coli* (E. coli), which is particularly dangerous as some strains, like O157, produce toxins that cause severe food poisoning (Nagarajan et al., 2018). This group also includes *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, and other enteric bacteria (Jordan et al., 2007). As a result, these genes can be transferred among bacteria, promoting the creation of multi-drug-resistant strains that pose a significant public health threat and may impact sheep health and productivity. The spread of antibiotic resistance genes in slaughtered meat is a major concern due to its potential impact on public health, as it can lead to the transfer of resistance to human pathogens (Baah et al., 2022). Horizontal gene transfer of resistance genes between different bacterial species could result in the creation of more challenging strains. Examples include genes conferring resistance to aminoglycosides, such as expression of the *strA-strB* streptomycin resistance genes (Zhu et al., 2020). Additionally, tetracycline resistance genes, such as *tet (A)*, *tet (B)*, and *tet (M)*, resist tetracycline antibiotics like doxycycline and tetracycline. Sulfonamide resistance genes, including sul1, sul2, and sul3, confer resistance to sulfonamides such as sulfamethoxazole.

The main cause of resistance to chloramphenicol is the enzyme chloramphenicol acetyltransferase (*cat*), which acetylates the antibiotic and inactivates it due to acquiring plasmids encoding or the lower outer membrane permeability (Sunde & Norström, 2006; Abdalhamed et al., 2021; Argudín et al., 2017). Studying the prevalence of antibiotic resistance genes in slaughterhouses and meat shops (butcheries) is essential to protect public health, ensure food safety, and promote the responsible use of antibiotics in both animal and human healthcare. The present study aimed to assess prevalence of *strA-strB, sul2, aadA1, tet(B), tet(A), cat, sul3,* and *blaSHV* resistant genes in multidrug gram-negative strains isolated from contact surfaces at slaughterhouses and meat shops (butcheries) in Makkah city, Saudi Arabia.

2. Materials and Methods

2.1 Sample Collection

A total of 71 wet swab samples in Buffered Peptone Water (BPW) were collected from 30 contact surfaces at slaughterhouses and butcher shops in Makkah city, Saudi Arabia during June 2024. A mix of large commercial slaughterhouses and small-scale butcheries were included to assess contamination levels across different operational scales. Facilities were selected from various locations to ensure a representative sampling of different environmental and hygiene conditions. High-traffic and granted permission for sample collection facilities with a large volume of meat processing were prioritized. The contact surfaces swabbed in slaughterhouses and butcheries were chosen based on their frequent exposure to raw meat, handling, and potential contamination risks, such cutting boards (15), knives and meat cleavers (20), grinding and slicing machines (15), scales and weighing trays (71). Swabs were taken from designated surface area 10 cm^2 of each surface using a zigzag motion while applying

light pressure. The swab was then placed in a sterile transport tube containing transport medium after proper labeling. Samples were preserved in sterile containers and transported in coolers within 2–4 hours to the Microbiology Laboratory at the Custodian of the Two Holy Mosques Institute for Hajj and Umrah Research at Umm Al-Qura University, where they were stored for 24 hours at a low temperature (approximately 4°C) until preparation and analysis could begin.

2.2 Isolation and Identification

Bacterial suspensions were prepared from the swabs by immersing each swab in Nutrient Broth. The samples were then cultured on Nutrient Agar (Oxoid) and MacConkey Agar (Oxoid). Each swab tube was vortexed for homogenization and streaked the swab directly onto Nutrient Agar (Oxoid) and MacConkey Agar (Oxoid). All the plates were incubated at 35-37 °C for 24–48 hours under aerobic conditions. Colony morphology and color were observed, Gram Staining and common biochemical tests (Oxidase Test, Triple Sugar Iron (TSI) Test, IMViC Test (Indole, Methyl Red, Voges-Proskauer, Citrate), and Urease etc.) were used for initial differentials of Gram-negative bacteria. The identification and antibacterial sensitivity of bacteria growing on MacConkey Agar were confirmed using the MicroScan technology along with their antibiotic susceptibility profile (Abdel-razik et al., 2022; CLSI 2022). Sixteen antibiotics were tested, including amikacin, amoxicillin-clavulanate, ampicillin, ampicillin/sulbactam aztreonam, cefazolin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, gentamicin, imipenem, piperacillin/tazobactam, tobramycin, and trimethoprim-sulfamethoxazole.

2.3 Detection of Resistance Genes

DNA Extraction: DNA was extracted from Gram-negative bacteria using the boiling method as described by Ahmed and Dablool (Ahmed & Dablool 2017). Bacterial colonies were collected from the agar plate with a sterile loop and added to a tube with phosphate-buffered saline (PBS) and then placed in boiling water at 95-100°C for 5-10 minutes and then cooled in 4-6 °C for 5 minutes. The supernatant was gently collected into a new sterile tube and immediately used for PCR analysis, otherwise would be stored at -20°C.

Polymerase Chain Reaction (PCR): PCR was used to detect *strA-strB, sul2, aadA1, tet(B), tet(A), cat, sul3,* and *blaSHV* resistant genes specific genes associated with antibiotic resistance using primers (from Integrated DNA Technologies, Leuven, Belgium) listed in table 1. Three microliters of quantified DNA and one microliter of each primer were added to a 25-microliter PCR mixture (Taq PCR Master Mix from Qiagen, USA). PCR products were analyzed by agarose gel electrophoresis, with positive and negative controls included in the experiments. The PCR conditions were 94 °C for 4 minutes followed by 35 cycles of 94 °C for 1 minute, 55 °C for 40 seconds and 72 °C for 1 minute followed by final extension at 72 for 10 minutes. PCR products were observed under UV light using the UVP BioDoc-It imaging system after staining with ethidium bromide.

2.4 Statistical Analysis

All statistical analyses were conducted using SPSS version 25. Pearson's Chi-square test was used to examine correlations between resistance genes and the types of isolated bacterial strains, with statistical significance set at $P < 0.05$.

Table 1. PCR analysis protocols for detecting antibiotic resistance genes

Primer	Sequence	Gene	Size (bp)	Ref.
strAstrB-F	TATCTGCGATTGGACCCTCTG	*strA-strB*	538	(Sunde & Norström, 2006)
strAstrB-R	CATTGCTCATCATTTGATCGGCT			
sul2-F	GCGCTCAAGGCAGATGGCATT	*sul2*	293	(Sunde & Norström, 2006)
sul2-R	GCGTTTGATACCGGCACCCGT			
aadA-F	GAGAACATAGCGTTGCCTTGG	*aadA1*	198	(Kerrn et al., 2002)
aadA-R	TCGGCGCGATTTTGCCGGTTAC			
tetB-F	CTCAGTATTCCAAGCCTTTG	*tet*(B)	435	(Perreten & Boerlin 2003)
tetB-R	CTAAGCACTTGTCTCCTGTT			
tetA-F	TTGTTCCTGAAGTGCCAGTAA	*tet*(A)	370	(Sunde & Norström, 2006)
tetA-R	GACGTCGTTCGAGTGAACCAGA			

cat-F	GGTGAGCTGGTGATATGG	*cat*	209	19
cat-R	GGGATTGGCTGAGACGA			(Guardabassi et al., 2000)
sul3-F	GAGCAAGATTTTTGGAATCG	*sul3*	789	(Brinas et al., 2002)
sul3-R	CATCTGCAGCTAACCTAGGGCTTTGGA			
SHV-F	CACTCAAGGATGTATTGTG	bla_{SHV}	885	(Sørum et al., 2003)
SHV-R	TTAGCGTTGCCAGTGCTCG			

3. Results

This study aimed to assess bacterial contamination on contact surfaces in slaughterhouses and butcheries by isolating and identifying potential pathogens. Table 2 illustrates the main findings from meat swabs taken from various selling points and slaughterhouses. Out of 71 swabs, 49 (69%) were positive for culture, while 22 samples (31%) were negative. Out of 49, the number of Gram-positive bacteria was 15 (30.6%), while the Gram-negative bacteria was 34 (69.4%). The most common Gram-negative bacteria species was *Klebsiella pneumoniae* (41.2%), followed by *Escherichia coli* (35.3%) and *Enterobacter cloacae* (5.9%). Table 3 shows bacterial antibiotic resistance and prevalence of their resistant genes, the highest bacterial resistance rate (94.1%) was against trimethoprim-sulfamethoxazole (88.1%), ampicillin (85.3%), and cefuroxime (70.6%) as shown in figure 1. The most prevalent antibiotic resistance gene was aadA1 (88.2%), followed by *tet(A)*, *sul3* (70.6%), *sul2* (35.3%), *strA-strB* (23.5%), *cat* (5.9%) and *tet* (B) (5.9%) (figure 2). The results also indicated that 29.4% of the bacteria carried one resistance gene, 44.1% carried two genes, 20.6% carried more than two genes. The study demonstrated a strong association ($p < 0.05$) between the genes *aadA*1, *tet* (A) *tet* (B), and *cat*, and the bacterial strain type as shown in table 4.

Table 2. Microbiological results of swab samples of meat contact surfaces

Variable	Result	No	%
Culture results	Positive	49	69
	Negative	22	31
	Total	71	100
Bacterial group	Gram positive bacteria	15	30.6
	Gram negative bacteria	34	69.4
	Total	49	100
Gram negative Species	*Klebsiella pneumoniae*	14	41.2
	Escherichia coli	12	35.3
	Enterobacter cloacae	2	5.9
	Acinetobacter baumanniic	1	2.9
	Bordetella bronchiseptica	1	2.9
	Enterobacter Aerogenes	1	2.9
	Kluwera ascorbata	1	2.9
	Klebsiella oxytoca	1	2.9
	Pseudomonas agarici	1	2.9
	Total	34	100

Table 3. Results of antibiotic resistance of the isolated Gram-negative bacteria and their resistant genes

Variable	Result	No	%
Antibiotic resistance	Amikacin	11	32.4
	Amoxicillin/clavulanic acid	20	58.8
	Ampicillin/sulbactam	22	64.7
	Ampicillin	29	85.3
	Aztreonam	5	14.7
	Cefazolin	11	32.4
	Cefepime	17	50.0
	Cefotaxime	4	11.8
	Cefoxitin	21	61.8
	Ceftazidime	1	2.90
	Cefuroxime	24	70.6
	Gentamicin	22	64.7
	lmipenem	8	23.5
	Piperacillin/tazobactam	0.0	00.0
	Tobramycin	19	55.9
	Sulfamethoxazole/trimethoprim	30	88.2
Antibiotic resistance genes	*strA-strB*	8	23.5
	aadA1	30	88.2
	sul2	12	35.3
	sul3	24	70.6
	tet (A)	25	73.5
	tet (B)	2	5.9
	bla_{SHV}	0	0.0
	cat	2	5.9
Gene Multiplicity	None	2	5.9
	One gene	10	29.4
	Two genes	15	44.1
	More than two genes	7	20.6
	Total	34	100

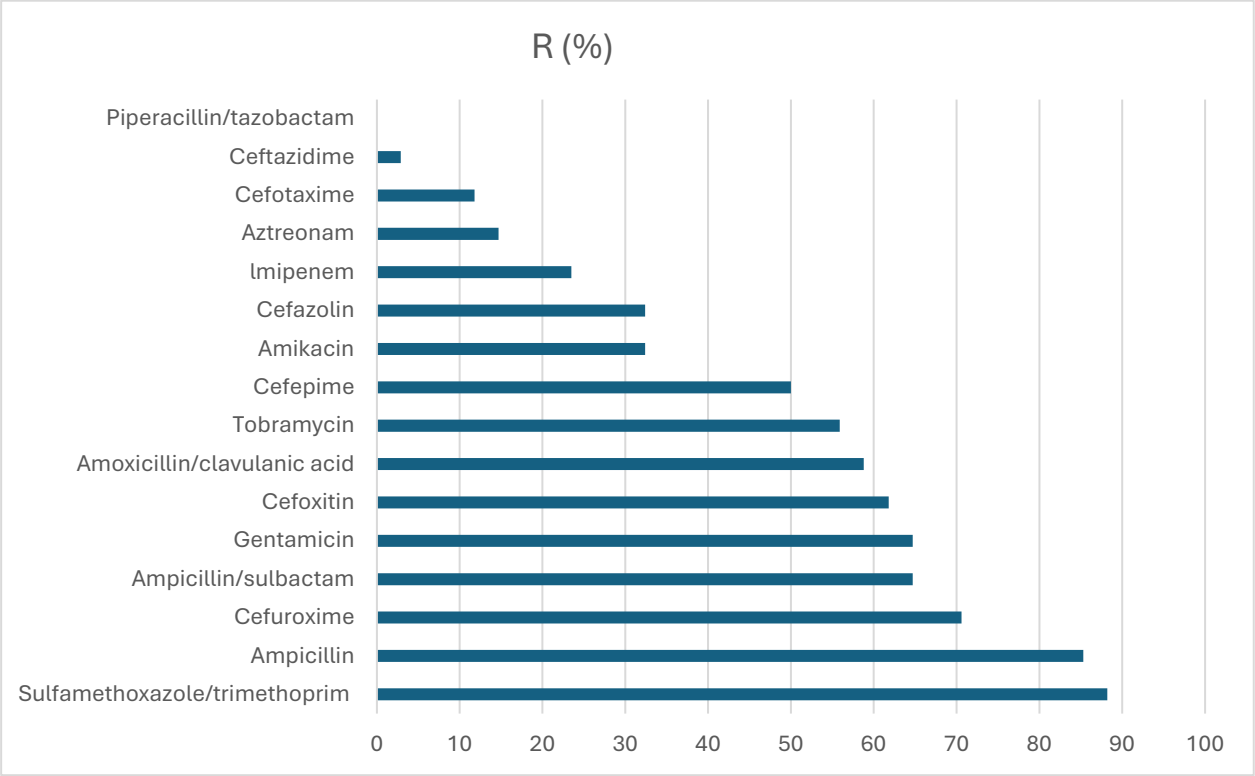

Figure 1. Antibiotic resistance of the isolates to the commonly used antibiotics

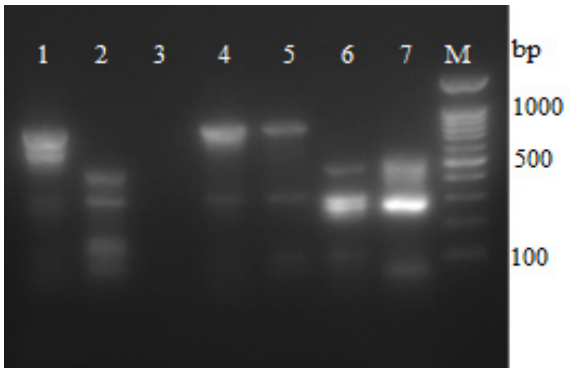

Figure 2. PCR analysis on gel electrophoresis, Lanes: 1, 2, 4-7: Multiple resistance genes, lane 3: Negative control, M=Marker (100bp).

Table 4. The correlation between gene type and species among the studied samples

Test	Genes	*p*-value
Pearson chi square (between gene type and species)	*strA-strB*	0.889
	aadA1	0.022
	sul2	0.173
	sul3	0.058
	tet (A)	0.009
	tet (B)	0.026
	cat	0.002

4. Discussion

The current study aimed to investigate the prevalence of MDR Gram negative bacteria and their antibiotic resistance genes in the contact surfaces at various slaughterhouses and sales points. This study highlights the microbiological contamination of meat contact surfaces in slaughterhouses and butcheries, revealing a high prevalence of Gram-negative bacteria, with *Klebsiella pneumoniae* and *Escherichia coli* being the dominant species. The presence of these opportunistic pathogens raises significant public health concerns, as they are commonly associated with foodborne illnesses and antimicrobial resistance. This prevalence of Gram-negative bacteria is concerning due to their known resistance mechanisms and association with severe infections. The high positivity rate (69%) of bacterial cultures from meat surface swabs suggests inadequate hygiene practices, improper handling, and possible cross-contamination in slaughterhouses and butcheries. The predominance of Gram-negative bacteria (69.4%) over Gram-positive bacteria (30.6%) aligns with previous studies, as Gram-negative species are more adaptable to moist environments commonly found in meat processing areas. *Klebsiella pneumoniae* (41.2%) and *Escherichia coli* (35.3%) were the most frequently isolated pathogens, both of which are known to cause gastrointestinal infections and opportunistic infections in immunocompromised individuals. The detection of *Acinetobacter baumannii* and *Pseudomonas agarici*—both of which are associated with multidrug resistance—further emphasizes the potential risks of exposure to contaminated meat. Generally, bacterial contamination in meat can lead to health issues if improper handling occurs within slaughterhouses or retail settings Holzapfel, 1998). Studies show that *Pseudomonas* species, *E. coli,* and *Staphylococcus* species are the most common isolates found on meat processing surfaces along the production chain (Lerma et al., 2013). Globally, research has found that *Escherichia coli* and *Klebsiella pneumoniae* are among the most common Gram-negative bacteria in meat, both of which are naturally present in the intestines of animals and humans and can transfer to meat during processing (Iroha et al., 2011; Dubey et al., 2018). For instance, contamination with *Klebsiella pneumoniae* can cause food poisoning, leading to severe diarrhea, nausea, vomiting, and abdominal pain. In more serious cases, this can result in dehydration and shock (Davis & Price 2016). *Escherichia coli* may also transfer to meat during slaughter or due to contaminated water or surfaces (Barco et al., 2015). Certain strains, such as *Escherichia coli O157*, produce toxins that can cause acute cramps, bloody diarrhea, and even kidney failure in severe cases. These bacteria can lead to health issues, including diarrhea, intestinal cramps, and fever, with potentially serious complications in some cases (Rasschaert et al., 2020). The resistance patterns observed in this study demonstrate a concerning level of antimicrobial resistance among bacterial isolates. High resistance rates were recorded for commonly used antibiotics, particularly ampicillin (85.3%), sulfamethoxazole/trimethoprim (88.2%), and cefuroxime (70.6%), indicating that many isolates possess resistance to β-lactams and sulfonamides. The moderate resistance to amoxicillin/clavulanic acid (58.8%) and gentamicin (64.7%) suggests that extended-spectrum β-lactamase (ESBL)-producing strains may be present, contributing to the global challenge of AMR. However, no resistance was observed for piperacillin/tazobactam, highlighting its potential effectiveness against these pathogens. These high resistance rates suggest that treating infections with these antibiotics could be challenging if similar bacteria infect humans. Interestingly, *blaSHV*, a gene commonly associated with ESBL production, was not detected in any isolates, suggesting that other β-lactamase genes may be responsible for the observed β-lactam resistance. The multiplicity of resistance genes was also notable, with 44.1% of isolates harboring two resistance genes and 20.6% carrying more than two, indicating a high level of genetic exchange and horizontal gene transfer among bacterial populations in meat-processing environments. A similar study showed that over 50% of the strains isolated from meat samples exhibited resistance to streptomycin (54.9%), gentamicin (74.5%), tetracycline (82.4%), and trimethoprim (62.7%) (Askari et al., 2019). In another study, 75 *E. coli* isolates

(12.1%) were identified from 620 samples of cooked meat products in Henan, China, with high resistance rates to tetracycline (56.0%), trimethoprim/sulfamethoxazole (41.3%), streptomycin (29.3%), ampicillin (26.7%), and nalidixic acid (14.7%) (Jiang et al., 2014). Another investigation found that among 106 bacteria from 14 different species, high resistance was observed to tetracycline, and trimethoprim; *E. coli* strains, which constituted 49% of isolates, showed 90% resistance to nalidixic acid, 94% to moxifloxacin, 78% to levofloxacin, and 77% to tetracycline (Ayandiran et al., 2018). Overuse of trimethoprim-sulfamethoxazole in agriculture and healthcare, both for humans and animals, has likely driven this resistance. A key public health implication of this trend is the reduced effectiveness of available treatments, complicating infection management. Moreover, increased resistance can lead to higher healthcare costs due to the need for more expensive and sometimes less safe alternatives. The transmission of resistant bacteria through the food chain to humans raises the risk of infection with resistant strains and increases selective pressures, further intensifying bacterial resistance (Mashak 2018). The genetic analysis revealed the presence of multiple resistance genes, with *aadA1* (88.2%), *sul3* (70.6%), and *tet(A)* (73.5%) being the most prevalent. These genes contribute to resistance against aminoglycosides, sulfonamides, and tetracyclines, respectively, all of which are widely used in both human and veterinary medicine. The detection of *sul2* (35.3%) and *tet(B)* (5.9%) further reinforces the spread of sulfonamide and tetracycline resistance. The gene *strA-strB*, encoding resistance to streptomycin, was present in 23.5% of isolates. This high level of multidrug resistance complicates treatment and may increase the risk of treatment failure. Ayandiran et al. reported that the genes *tetA*, *tetB*, and *qepA* were commonly found in *Escherichia coli* isolated from meat samples (Ayandiran et al., 2018). In a separate study, out of 75 isolates, resistance genes were detected in 13.3% of the samples, with *aac(6')-Ib-cr* identified individually or together in 6.7% and 10.7% of isolates, respectively (Jiang et al., 2014). Another investigation revealed that *sul* genes were prevalent in sulfonamide-resistant *Escherichia coli* isolates from *P. vannamei* and pork samples, showing rates of 90.0% and 88.6%, with significantly higher occurrences of *sul1* and *sul2* than *sul3* ($p < 0.05$) (Jiang et al., 2019). In another study, 313 *E. coli* samples were isolated using standard culture techniques, and 98% of resistant isolates were found to carry resistance genes as confirmed by PCR. Detected resistance genes included *tet*(A) and *tet*(B) for tetracycline resistance, *strA* and *aad*A1 for streptomycin resistance, *sul*I and *sul*II for sulfonamide resistance, *dfr* and *aph*A for kanamycin resistance, and *bla*TEM for ampicillin resistance. One *stx1*-harboring *Escherichia coli* strain from pork carried the *tet(A)* gene and belonged to phylogenetic group B2, while another *stx1*-positive isolate from beef was multi-resistant, carrying *bla*TEM, *aph*A, *str*A–B, *sulII*, and *tet*(A) and belonged to phylogenetic group A (Sacher-Pirklbauer et al., 2021). A similar study highlighted that the most frequent resistance genes found in antibiotic-resistant bacteria from surfaces of meat samples included those conferring beta-lactam resistance, followed by fluoroquinolone resistance genes, aminoglycoside resistance genes, sulfonamide resistance genes, and tetracycline resistance genes (*tetA*, *tetB*) (Racewicz et al., 2022). Moreover, there was a significant correlation ($p < 0.05$) between the presence of specific resistance genes (*aadA1*, *tet (A*), *tet (B),* and *cat*) and particular bacterial strains, indicating a strong association between certain bacterial types and resistance to aminoglycosides, tetracyclines, and chloramphenicol. This relationship underlines the risk of cross-resistance, where bacteria resistant to one antibiotic class may carry resistance to others. The presence of resistance genes confirms that these bacteria have evolved to resist aminoglycosides (such as streptomycin and spectinomycin), tetracycline, and sulfonamides (such as sulfamethoxazole), which are antibiotics commonly used in veterinary treatments. Findings from similar studies indicate that many bacterial strains possess a high number of resistance genes, rendering them resistant to numerous antibiotics commonly used in both human and veterinary medicine (Lavilla Lerma et al., 2014; Sunde & Norström, 2006). The combined presence and spread of these genes present critical public health challenges, including treatment difficulties, increased healthcare burdens, and rising healthcare costs (Friedman et al., 2016; Serwecińska 2020)). Furthermore, the risk of resistance transmission between humans and animals poses significant concerns, as antibiotic-resistant bacteria can transfer from contaminated meat to humans through the food chain. This can potentially lead to higher mortality rates, particularly in cases where infections fail to respond to standard treatments (Founou et al., 2016; Kumar et al., 2020). These findings highlight the need for strict hygiene measures in meat handling, careful monitoring of antibiotic resistance patterns, and consideration of alternative options to combat these resistant strains. The presence of antibiotic-resistant bacteria on meat contact surfaces poses a major food safety challenge. Poor hygiene during meat processing can facilitate the transmission of these pathogens to consumers, leading to foodborne outbreaks. Strict hygiene measures, proper sanitation of equipment, and controlled antibiotic use in livestock farming are essential to mitigating these risks. Additionally, surveillance programs should be implemented to monitor the spread of AMR in meat production environments.

5. Conclusion

The findings of this study emphasize the critical role of hygiene and antimicrobial stewardship in slaughterhouses

and butcheries. The high prevalence of Gram-negative bacteria and their resistance to multiple antibiotics highlight the urgent need for improved sanitation practices and responsible antibiotic usage. Such measures will help safeguard public health and ensure the safe consumption of meat during these significant occasions. Future studies should focus on whole-genome sequencing to further investigate resistance mechanisms and explore alternative disinfection strategies to reduce bacterial contamination in meat-processing facilities.

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Competing Interests

The authors declare no conflicts of interest.

References

Abdalhamed, A. M., Ghazy, A. A., & Zeedan, G. S. G. (2021). Studies on multidrug-resistance bacteria in ruminants with special interest on antimicrobial resistances genes. *Adv. Anim. Vet. Sci*, *9*(6), 835-844. http://dx.doi.org/10.17582/journal.aavs/2021/9.6.835.844

Abdel-razik, N. E., Alfifi, R. A., Ageel, H., Gobri, R., Alfaifi, M., Nassib, Z., Almalki, W., Awaji, O., & Bantun, F. (2022). Surveillance Study on Bacterial Contamination Associated With Automated Teller Machines of Different Banks in Jazan Region. *Journal of Complementary Medicine Research*, 14(1), 16. https://doi.org/10.5455/jcmr.2022.13.05.03

Adamson, J., Sawyer, C., Hobson, G., Clark, E., Fina, L., Orife, O., ... & Thomas, D. R. (2023). An outbreak of Salmonella Typhimurium following an Eid al-Adha celebration barbecue in Wales (UK), July 2021. *medRxiv*, 2023-01. https://doi.org/10.1101/2023.01.03.22283848

Ahmed, O. B., & Dablool, A. S. (2017). Quality improvement of the DNA extracted by boiling method in gram negative bacteria. *International Journal of Bioassays*, *6*(4), 5347-5349. https://doi.org/10.21746/ijbio.2017.04.004

Argudín, M. A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Denis, O., ... & Roisin, S. (2017). Bacteria from animals as a pool of antimicrobial resistance genes. *Antibiotics*, *6*(2), 12. https://doi.org/10.3390/antibiotics6020012

Askari, N., Momtaz, H., & Tajbakhsh, E. (2019). Acinetobacter baumannii in sheep, goat, and camel raw meat: virulence and antibiotic resistance pattern. *AIMS microbiology*, *5*(3), 272. https://doi.org/10.3934/microbiol.2019.3.272

Ayandiran, T. O., Falgenhauer, L., Schmiedel, J., Chakraborty, T., & Ayeni, F. A. (2018). High resistance to tetracycline and ciprofloxacin in bacteria isolated from poultry farms in Ibadan, Nigeria. *The Journal of Infection in Developing Countries*, *12*(06), 462-470. https://doi.org/10.3855/jidc.9862

Baah, D. A., Kotey, F. C., Dayie, N. T., Codjoe, F. S., Tetteh-Quarcoo, P. B., & Donkor, E. S. (2022). Multidrug-resistant gram-negative bacteria contaminating raw meat sold in Accra, Ghana. *Pathogens*, *11*(12), 1517. https://doi.org/10.3390/pathogens11121517

Barco, L., Belluco, S., Roccato, A., & Ricci, A. (2015). A systematic review of studies on Escherichia coli and Enterobacteriaceae on beef carcasses at the slaughterhouse. *International journal of food microbiology*, *207*, 30-39. https://doi.org/10.1016/j.ijfoodmicro.2015.04.027

Brinas, L., Zarazaga, M., Sáenz, Y., Ruiz-Larrea, F., & Torres, C. (2002). β-Lactamases in ampicillin-resistant Escherichia coli isolates from foods, humans, and healthy animals. *Antimicrobial agents and chemotherapy*, *46*(10), 3156-3163. https://doi.org/10.1128/AAC.46.10.3156-3163.2002

Casalinuovo, F., Ciambrone, L., Cacia, A., & Rippa, P. (2016). Contamination of bovine, sheep and goat meat with Brucella spp. *Italian Journal of Food Safety*, *5*(3). https://doi.org/10.4081/ijfs.2016.5913

CLSI. Performance standards for antimicrobial susceptibility testing. In: CLSI supplement M100. 32nd ed. Wayne, PA: Clinical and Laboratory Standards Institute. (2022)

Conficoni, D., Zaghi, M., Rossin, T., Brscic, M., & Giaccone, V. (2022). Meeting religious requirements and food safety during ritual slaughter: a case study on how Italian authorities handle the issue. *Animal Frontiers*, *12*(1), 25-34. https://doi.org/10.1093/af/vfac011

Cossi, M. V. C., Burin, R. C. K., Lopes, D. A., Dias, M. R., de Castilho, N. P. A., de Arruda Pinto, P. S., & Nero,

L. A. (2013). Antimicrobial resistance and virulence profiles of Salmonella isolated from butcher shops in minas gerais, Brazil. *Journal of food protection*, *76*(9), 1633-1637. https://doi.org/10.4315/0362-028X.JFP-13-119

Danforth, A. (2014). *Butchering poultry, rabbit, lamb, goat, and pork: the comprehensive photographic guide to humane slaughtering and butchering*. Storey Publishing.

Darwish, W. S., Tharwat, A. E., & Atia, A. S. (2016). Studies on the hygienic status of animal carcasses and their contact surfaces in some butchery shops. *Benha Veterinary Medical Journal*, *31*(2), 289-296. https://doi.org/10.21608/bvmj.2016.31316

Das, A. K., Nanda, P. K., Das, A., & Biswas, S. (2019). Hazards and safety issues of meat and meat products. In *Food safety and human health* (pp. 145-168). Academic Press. https://doi.org/10.1016/B978-0-12-816333-7.00006-0

Davis, G. S., & Price, L. B. (2016). Recent research examining links among Klebsiella pneumoniae from food, food animals, and human extraintestinal infections. *Current environmental health reports*, *3*, 128-135. https://doi.org/10.1007/s40572-016-0089-9

Dubey, S., Saini, P., Singh, D., & Singh, P. (2018). Incidence of enterobacteriaceae in retail meat samples and their antibiotic susceptibility. *Microbiology Research Journal International*, *23*(5), 1-9. https://doi.org/10.9734/MRJI/2018/40767

Founou, L. L., Founou, R. C., & Essack, S. Y. (2016). Antibiotic resistance in the food chain: a developing country-perspective. *Frontiers in microbiology*, *7*, 1881.

Friedman, N. D., Temkin, E., & Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical microbiology and infection*, *22*(5), 416-422. https://doi.org/10.3389/fmicb.2016.01881

Guardabassi, L., Dijkshoorn, L., Collard, J. M., Olsen, J. E., & Dalsgaard, A. (2000). Distribution and in-vitro transfer of tetracycline resistance determinants in clinical and aquatic Acinetobacter strains. *Journal of medical microbiology*, *49*(10), 929-936. https://doi.org/10.1099/0022-1317-49-10-929

Holzapfel, W. H. (1998). The Gram-positive bacteria associated with meat and meat products. *The microbiology of meat and poultry*, *31*, 35-74.

Iroha, I. R., Ugbo, E. C., Ilang, D. C., Oji, A. E., & Ayogu, T. E. (2011). Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. *Journal of public health and epidemiology*, *3*(2), 49-53.

Jiang, H., Cheng, H., Liang, Y., Yu, S., Yu, T., Fang, J., & Zhu, C. (2019). Diverse mobile genetic elements and conjugal transferability of sulfonamide resistance genes (sul1, sul2, and sul3) in Escherichia coli isolates from Penaeus vannamei and pork from large markets in Zhejiang, China. *Frontiers in Microbiology*, *10*, 1787. https://doi.org/10.3389/fmicb.2019.01787

Jiang, X., Yu, T., Wu, N., Meng, H., & Shi, L. (2014). Detection of qnr, aac (6′)-Ib-cr and qepA genes in Escherichia coli isolated from cooked meat products in Henan, China. *International journal of food microbiology*, *187*, 22-25. https://doi.org/10.1016/j.ijfoodmicro.2014.06.026

Jordan, D., Phillips, D., Sumner, J., Morris, S., & Jenson, I. (2007). Relationships between the density of different indicator organisms on sheep and beef carcasses and in frozen beef and sheep meat. *Journal of applied microbiology*, *102*(1), 57-64. https://doi.org/10.1111/j.1365-2672.2006.03060.x

Kerrn, M. B., Klemmensen, T., Frimodt-Møller, N., & Espersen, F. (2002). Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *Journal of antimicrobial chemotherapy*, *50*(4), 513-516. https://doi.org/10.1093/jac/dkf164

Kumar, S. B., Arnipalli, S. R., & Ziouzenkova, O. (2020). Antibiotics in food chain: The consequences for antibiotic resistance. *Antibiotics*, *9*(10), 688. https://doi.org/10.3390/antibiotics9100688

Lavilla Lerma, L., Benomar, N., Knapp, C. W., Correa Galeote, D., Gálvez, A., & Abriouel, H. (2014). Diversity, distribution and quantification of antibiotic resistance genes in goat and lamb slaughterhouse surfaces and meat products. *PloS one*, *9*(12), e114252. https://doi.org/10.1371/journal.pone.0114252

Lerma, L. L., Benomar, N., Gálvez, A., & Abriouel, H. (2013). Prevalence of bacteria resistant to antibiotics and/or biocides on meat processing plant surfaces throughout meat chain production. *International Journal of Food Microbiology*, *161*(2), 97-106. https://doi.org/10.1016/j.ijfoodmicro.2012.11.028

Mashak, Z. (2018). Prevalence and antibiotic resistance of Escherichia coli O157: H7 isolated from raw meat samples of ruminants and poultry. *Journal of Food and Nutrition Research*, *6*(2), 96-102. https://doi.org/10.12691/jfnr-6-2-5

Nagarajan, V., Wahab, A., Shivraj, S., & Alex, L. (2018). Study of bacterial contamination of raw meat in Hyderabad. *MOJ Proteomics Bioinform*, *7*(1), 46-51. https://doi.org/10.15406/mojpb.2018.07.00212

Noviyanti, N. (2017). Implementing social marketing strategies to improve food-safety awareness during Eid-Al Adha festival in Indonesia. *Proceedings of Indonesia Focus*, *1*(1).

Perreten, V., & Boerlin, P. (2003). A new sulfonamide resistance gene (sul3) in Escherichia coli is widespread in the pig population of Switzerland. *Antimicrobial agents and chemotherapy*, *47*(3), 1169-1172. https://doi.org/10.1128/AAC.47.3.1169-1172.2003

Racewicz, P., Majewski, M., Biesiada, H., Nowaczewski, S., Wilczyński, J., Wystalska, D., ... & Madeja, Z. E. (2022). Prevalence and characterisation of antimicrobial resistance genes and class 1 and 2 integrons in multiresistant Escherichia coli isolated from poultry production. *Scientific Reports*, *12*(1), 6062. https://doi.org/10.1038/s41598-022-09996-y

Rasschaert, G., De Zutter, L., Herman, L., & Heyndrickx, M. (2020). Campylobacter contamination of broilers: the role of transport and slaughterhouse. *International journal of food microbiology*, *322*, 108564. https://doi.org/10.1016/j.ijfoodmicro.2020.108564

Sacher-Pirklbauer, A., Klein-Jöbstl, D., Sofka, D., Blanc-Potard, A. B., & Hilbert, F. (2021). Phylogenetic groups and antimicrobial resistance genes in escherichia coli from different meat species. *Antibiotics*, *10*(12), 1543. https://doi.org/10.3390/antibiotics10121543

Schwab, B., & Armah, R. (2019). Can food safety shortfalls disrupt 'Ag for Nutrition'gains? Evidence from Eid al-Adha. *Food Policy*, *83*, 170-179. https://doi.org/10.1016/j.foodpol.2019.01.002

Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. *Water*, *12*(12), 3313. https://doi.org/10.3390/w12123313

Sørum, H., L'Abée-Lund, T. M., Solberg, A., & Wold, A. (2003). Integron-containing IncU R plasmids pRAS1 and pAr-32 from the fish pathogen Aeromonas salmonicida. *Antimicrobial agents and Chemotherapy*, *47*(4), 1285-1290. https://doi.org/10.1128/AAC.47.4.1285-1290.2003

Sujarwanta, R. O., Afidah, U., Suryanto, E., Rusman, Triyannanto, E., & Hoffman, L. C. (2024). Goat and Sheep Meat Production in Indonesia. *Sustainability*, *16*(11), 4448. https://doi.org/10.3390/su16114448

Sumner, J., Petrenas, E., Dean, P., Dowsett, P., West, G., Wiering, R., & Raven, G. (2003). Microbial contamination on beef and sheep carcases in South Australia. *International Journal of Food Microbiology*, *81*(3), 255-260. https://doi.org/10.1016/s0168-1605(02)00220-9

Sunde, M., & Norström, M. (2006). The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products. *Journal of Antimicrobial Chemotherapy*, *58*(4), 741-747. https://doi.org/10.1093/jac/dkl294

Todd, E. C., Greig, J. D., Bartleson, C. A., & Michaels, B. S. (2008). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 5. Sources of contamination and pathogen excretion from infected persons. *Journal of food protection*, *71*(12), 2582-2629. https://doi.org/10.4315/0362-028x-71.12.2582

Zhu, X., Li, P., Qian, C., Liu, H., Lin, H., Zhang, X., ... & Li, K. (2020). Prevalence of aminoglycoside resistance genes and molecular characterization of a novel gene, aac (3)-IIg, among clinical isolates of the Enterobacter cloacae complex from a Chinese teaching hospital. *Antimicrobial agents and chemotherapy*, *64*(9), 10-1128. https://doi.org/10.1128/AAC.00852-20