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Microbiological Quality and Antimicrobial Resistance of Staphylococcus aureus in Raw Chapli Kebab, Kabul, Afghanistan

Mohammad Asif Noori[1]✉, Nasir Ahmad Sarwary[2], Mohammad Farzad Afshar[3]

[1, 2]Kabul University, Department of Food Technology and Hygiene, Faculty of Veterinary Sciences, Kabul, Afghanistan
[3]Universiti Putra Malaysia, Department of Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, 43400, Serdang, Selangor, Malaysia
✉E-mail: mohammadasifnoori48@gmail.com (corresponding author)

ABSTRACT

Foodborne illnesses remain a serious global health concern, especially in developing countries with inadequate food safety laws. In Kabul, chapli kebab, a traditional minced beef dish, is widely consumed. *Staphylococcus aureus* is a common foodborne pathogen associated with meat and meat products and can produce heat-stable enterotoxins that remain active after cooking. This study aimed to evaluate the microbiological quality of raw Chapli kebab meat and the antimicrobial resistance profile of *Staphylococcus aureus* isolated from it in local restaurants in Kabul City. Forty samples from two districts were analyzed for *S. aureus* presence and aerobic plate counts (APC) using standard microbiological and biochemical techniques. The confirmed isolates (20%) were tested against 12 commonly used antibiotics using the Kirby–Bauer disk diffusion method. The average APC for District 1 and District 3 was 7.93 $\log_{10}$ CFU/g and 7.64 $\log_{10}$ CFU/g, respectively, and the difference was significant ($p < 0.001$). Tetracycline resistance (87.5%), penicillin resistance (75%), and multidrug resistance (MDR) to 8 antibiotic classes (37.5%) were all high among the isolates. These findings indicate that Chapli kebab meat harbors S. aureus with multidrug antimicrobial resistance and poor microbiological quality. There is an urgent need to improve the quality.

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INTRODUCTION

Meat and its products are regarded as vital elements in humans' diets owing to their high nutritional value and high protein content. Nevertheless, these products can serve as vital media for the transmission of foodborne pathogens if proper hygiene standards during handling and production are not maintained. One of the most common meat products in Afghanistan is raw minced meat. These kinds of meat products are very popular among Afghan citizens, especially those living in Kabul, as they are widely prepared and sold in restaurants, street vendors, and butchers there. Due to frequent handling, grinding, contact with contaminants, and improper storage temperatures, minced meat products are easily infected with microorganisms (Rajaei et al., 2021). Meat is frequently linked to the development of foodborne illnesses and is also extremely prone to spoilage. Raw meat contamination is a major cause of foodborne disease (Bhandare et al., 2007). All potentially edible tissues are contaminated by many sources both within and outside the animal during slaughter and processing. Numerous microorganisms are present on the surfaces of living things that are in contact with their surroundings. The animal's hide is the primary source of the contaminated organisms, which also include organisms from both excrements.

Furthermore, during processing, beef products are more likely to become contaminated with harmful microorganisms (Datta et al., 2012). Foodborne illnesses remain a significant health issue worldwide, especially in developing nations, where food safety controls and enforcement are often insufficient (Bintsis, 2017). Foodborne outbreaks involving meat and meat products are common because they are highly moist, contain high nutrient levels, and are prone to microbial contamination during slaughtering, processing, handling, and storage (Almashhadany, 2021). This is particularly susceptible in ground meat products, since the grinding process exposes more surface area, spreads microorganisms throughout the product, and, in most instances, handling is manual, thereby increasing the risk of contamination (ICMSF, 2011).

Chapli kebab is a traditional meat dish made with raw ground beef seasoned with spices and herbs, and is widely eaten in Afghanistan and surrounding areas. Chapli Kebab is a popular dish widely cooked and sold in local restaurants in Kabul City because it is cheap, delicious, and acceptable to the culture. Nevertheless, the conditions of preparation, lack of temperature control, and inconsistent hygienic standards can lead to a decline in microbiological quality and an increased risk of foodborne diseases. The same traditional meat products produced in small-scale or restaurant settings have been reported to harbor high microbial loads when good hygiene practices are not observed (Siluma et al., 2023).

Staphylococcus aureus is one of the foodborne pathogens considered a significant threat in meat and meat products. This organism is often associated with food poisoning because it can produce various heat-stable enterotoxins that retain their activity after cooking (Argudin et al., 2010; Kadariya et al., 2014). *S. aureus* contamination of meat is mainly caused by human contact since the bacterium is a normal part of the skin flora, nasal passages, and hands of food handlers. The main factors contributing to the growth and toxin production of S. aureus in meat products are poor personal hygiene, inadequate sanitation of food-contact surfaces, and poor storage temperatures (Kadariya et al., 2014).

Staphylococcus aureus is a Gram-positive bacterium that can be found living on the skin, nostrils, and mucous membranes of both humans and animals. It can be introduced into food products at various stages of production, such as slaughtering, processing, preparation, or distribution, via infected individuals or contaminated tools, as well as through unsanitary conditions. There are several public health issues related to *S. aureus*, as it produces enterotoxins that can withstand cooking temperatures and cause staphylococcal food poisoning. Common symptoms include vomiting, nausea, abdominal pain, and diarrhea shortly after consumption of affected food. Therefore, *S. aureus* contamination of food products is a serious issue (Can et al., 2017).

Besides its enterotoxinogenic capacity, S. aureus has been of growing interest due to the development of antimicrobial resistance (AMR) worldwide. The presence of antimicrobial-resistant S. aureus in animal food is a significant societal health concern because resistant strains can be transferred to humans through the food chain and compromise the efficacy of the most commonly used therapeutic agents (Almansour et al., 2023). AMR in *S. aureus* is a new threat that has been recently reported as a global health issue. The extensive misuse of antibiotics in the human and animal sectors has led to the emergence of antibiotic-resistant strains, such as Methicillin-resistant *Staphylococcus aureus* (MRSA). AMR strains have restricted the treatment options for infections, leading to high mortality and morbidity rates. Food-producing animals and meat products have been found to harbor antibiotic-resistant bacteria that can infect humans through the food chain (Lika et al., 2021). The widespread and frequent misuse of antimicrobial agents in food-producing animals and human medicine has led to the rapid development of multidrug-resistant (MDR) *S. aureus*, which is very difficult to control and treat (Laxminarayan et al., 2013; WHO, 2017).

Aerobic plate count (APC) is commonly used as a measure of the general microbiological quality and hygiene condition of meat products. High APC values are indicative of unsanitary processing conditions and inappropriate handling or storage procedures and are commonly associated with a higher probability of pathogen presence (Jay et al., 2005; ICMSF, 2011). Therefore, assessing APC levels in raw ground meat products, such as Chapli kebab, is essential for evaluating potential public health risks and identifying gaps in food hygiene practices.

Microbiological analysis of raw minced meat from Afghanistan, particularly Kabul, is poorly documented. Clinical research conducted in Kabul hospitals identified methicillin-resistant and multidrug-resistant S. aureus isolates in patients, underscoring that antimicrobial resistance is a growing concern in the region (Naimi et al., 2017). Recent research on meat-based ready-to-eat products in Kabul found that they contained substantial levels of S. aureus, which could be highly resistant and pose severe health risks to consumers (Ahmadi et al., 2025).

Although traditional meat products play a significant role in public health, there is a significant gap in published information on the microbiological quality and antimicrobial resistance profile of *S. aureus* in Afghanistan. Specifically, there is very little information available on the contamination status of Chapli kebab meat sold in the local restaurants in Kabul City. Such a knowledge gap prevents the use of evidence-based risk assessment and the formulation of effective food safety interventions.

This study aimed to address the following research questions:

- What is the microbiological quality of raw chapli kebab meat served in local restaurants in selected areas of Kabul City based on aerobic plate counts (APC)?
- What is the prevalence of *Staphylococcus aureus* in raw chapli kebab samples?
- What are the antimicrobial susceptibility patterns of *S. aureus* isolates?
- To what extent do *S. aureus* isolates exhibit multidrug resistance (MDR)?

METHODS AND MATERIALS

In the present cross-sectional study, 40 samples of raw Chapli kebab meat (ground beef mixed with spices) were collected from local restaurants of Districts 1st and 3rd of Kabul City, between June and July 2024 (Figure 1). 100 grams of each sample was collected under aseptic conditions, refrigerated (4-8 °C), placed in sterile bags, and transported within 1–2 hours to the Animal Health Laboratory located in Darulaman (CVDRL), Kabul, for immediate processing.

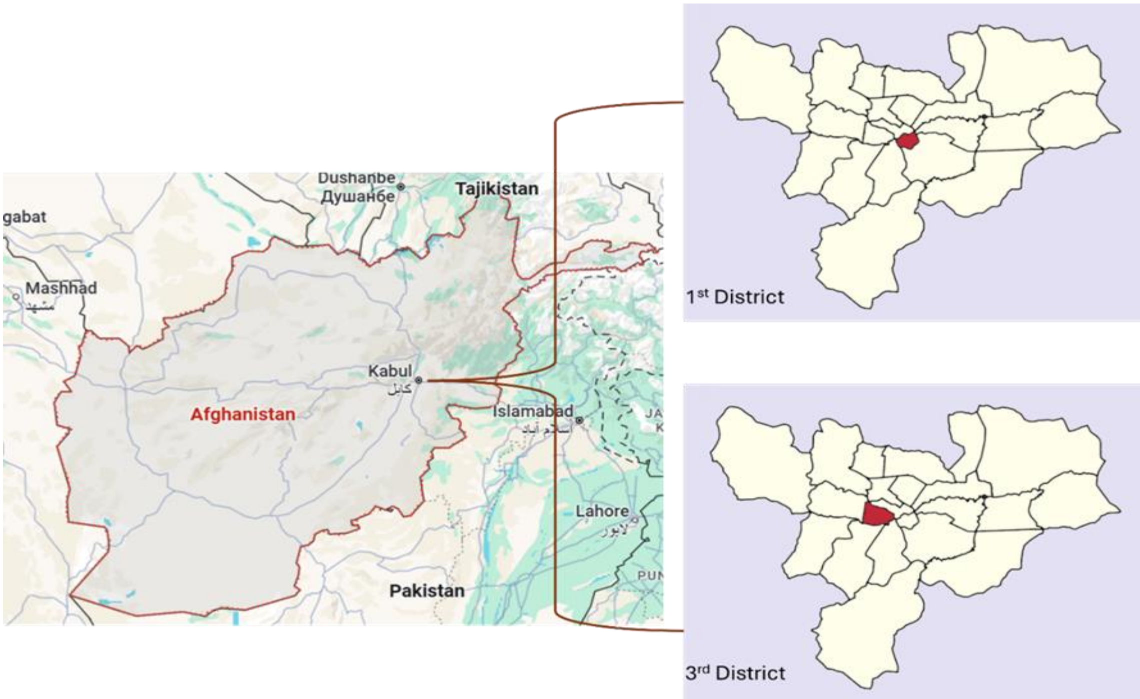

***Figure 1**. Shows the map of Afghanistan and the targeted districts in Kabul City*

Culture, Isolation and Identification

10 g of each sample was taken from the icebox using sterile forceps and weighed on an electronic balance (Sartorius Entris 224, Germany). Each sample was homogenized with 90 mL of 0.1% peptone water using a stomacher (IUL Instruments, Spain). The homogenate was then incubated in an incubator (Memmert IN55, Germany) at 37 °C for 18–24 h.

After incubation, a loopful of the culture was streaked onto blood agar plates (Oxoid, Thermo Scientific, USA) using a sterile wire loop and the quadrant streak method. The plates were incubated at 37 °C for 24 h, after which colonies were examined using a colony counter (Stuart SC6, UK).

Colonies of *S. aureus* were identified on blood agar by their round shape, hemolytic activity, variable size (large, medium, small), smooth surface, and mucoid consistency. Suspected colonies were further subcultured on mannitol salt agar (HiMedia M118, India), where *S. aureus* appeared as round, golden-yellow colonies.

Final confirmation was performed using catalase and coagulase test kits (Sigma-Aldrich, USA) and Gram staining.

Aerobic Plate Count (APC)

Seven test tubes were each filled with 9 mL of buffered peptone water (Oxoid, Thermo Scientific, USA) and labeled 1-7. One gram of raw meat sample (Chapli kebab) was added to the first tube and mixed using a vortex mixer (Heidolph Reax Top, Germany). From the first tube, 1 mL was transferred sequentially to the next tube, up to tube 7, to prepare serial dilutions. From each dilution, 1 mL was inoculated into sterile Petri dishes (Corning, USA), followed by the addition of plate count agar (HiMedia M091, India). Plates were gently swirled five times in a figure-eight motion for uniform mixing. The Petri dishes were incubated in an incubator (Memmert IN55, Germany) at 30 °C for 72 hours. After incubation, colonies were counted using a digital colony counter (Stuart SC6, UK), and visible colonies were marked with a laboratory marker. Each sample was run in duplicates (Buzón-Durán et al., 2017).

Antimicrobial Susceptibility Testing

The Kirby–Bauer disk diffusion test was used for antimicrobial susceptibility testing against twelve agents: Ciprofloxacin, 5 µg, Azithromycin, 15 µg, Gentamicin, 10 µg, Co-trimoxazole, 25 µg, Erythromycin, 15 µg, Oxacillin, 1 µg, Penicillin G, 10 units, Tetracycline, 30 µg, Clindamycin, 2 µg, Kanamycin, 30 µg, Norfloxacin, 10 µg, Nitrofurantoin, 300 µg (Oxoid™, UK). Staphylococcus aureus ATCC 25923 served as the positive control. A standardized inoculum (0.5 McFarland's standard) of the test organism was spread on Mueller–Hinton agar, and antibiotic-impregnated disks were placed on the surface. After incubation at 35–37 °C for 16–18 hours, zones of inhibition were measured. The diameters were compared with CLSI standards, and the organism was classified as susceptible, intermediate, or resistant (CLSI, 2018; CLSI, 2016).

Statistical Analysis

Descriptive statistics were used to report the percentage of positive *S. aureus* samples from each district and the percentage of resistance to each antimicrobial agent. Aerobic plate count (APC) data were $\log_{10}$-transformed before analysis. Since the data were not normally distributed, comparisons of microbial loads between District 1 and District 3 were performed using the nonparametric Mann-Whitney U test. Descriptive statistics were expressed as means and standard deviations (STD). All analyses were conducted in SPSS (version 27, IBM Corp., USA), and statistical significance was set at $p < 0.05$.

FINDINGS

Prevalence of S. aureus and Overall Microbiological Quality

The culture and biochemical tests revealed that, in general, the prevalence of S. aureus was 20% (8/40), and 4 (20%) samples in each district were positive. The aerobic plate counts (APC, log10 cfu/g) of raw ground meat samples from Kabul in two districts were compared. District 1 (n = 20) samples had a mean APC of 7.93 log whole cfu/g, and District 3 (n = 20) samples had a mean APC of 7.64 log whole cfu/g. Their difference was statistically significant (Mann-Whitney U-test, $p < 0.001$) (Table 1).

***Table 1**. Illustrates the microbial counts (log10 cfu/g; mean ± STD) in Chapli kebab samples*

District	No. of samples	APCs ±STD	*p*-value
1st	20	7.93±310	**<0.001***
3rd	20	7.64±030	

*The difference between the two districts was statistically significant (Mann–Whitney U: $p < 0.001$)

Antibiotic Resistance Profile of S. aureus Isolates

The antimicrobial susceptibility testing showed inconsistent resistance patterns among the isolates. Tetracycline (87.5%), penicillin (75%), azithromycin (62.5%), and gentamicin (62.5%) showed the greatest resistance, respectively. Ciprofloxacin, erythromycin, kanamycin, and nitrofurantoin (all 50%) had moderate resistance, and oxacillin, co-trimoxazole, clindamycin, and Norfloxacin (all 37.5% each) had lower resistance rates (Figure 2).

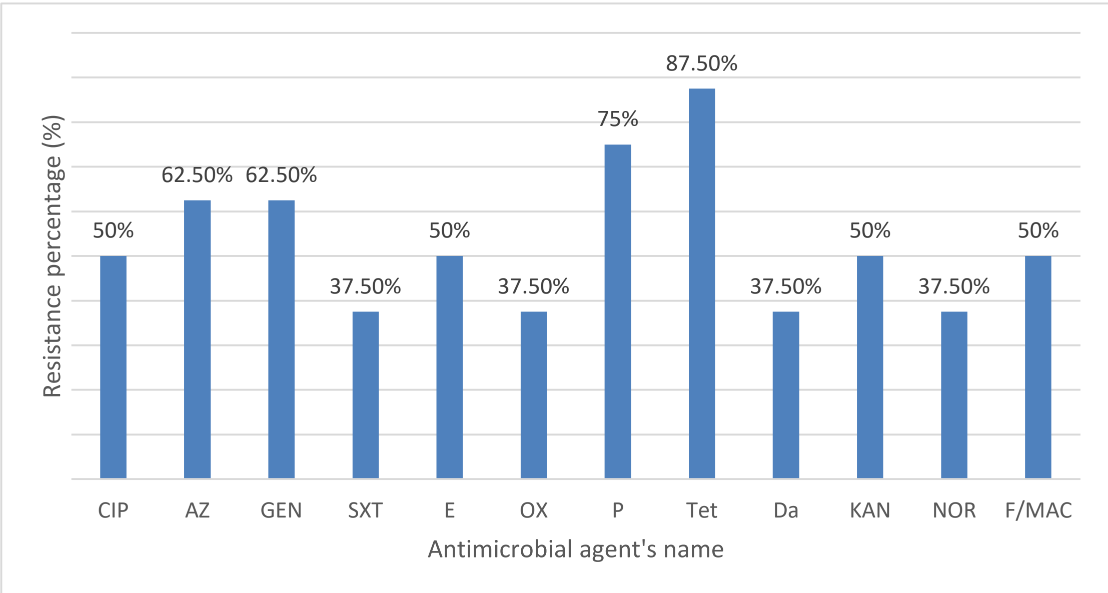

***Figure 2**. Illustrates the resistance profile of Staphylococci isolates from both districts*

CIP: ciprofloxacin (5 µg); AZ: azithromycin (15 µg); GEN: gentamicin (10 µg); SXT: Co-trimoxazole (25 µg); E: erythromycin (15 µg); OX: oxacillin (1 µg); P: penicillin G (10 units);

Tet: tetracycline (30 µg); Da: clindamycin (2 µg); KAN: kanamycin (30 µg); NOR: norfloxacin (10 µg); F/MAC: nitrofurantoin (300 µg).

Multidrug resistance was analyzed, indicating that 12.5% of the isolates were resistant to three classes of antimicrobials, with another 12.5% resistant to four classes. Five or six classes were resisted in 25 and 12.5%, respectively. The largest percentage, 37.5%, was resistant to eight antimicrobial classes (Figure 3).

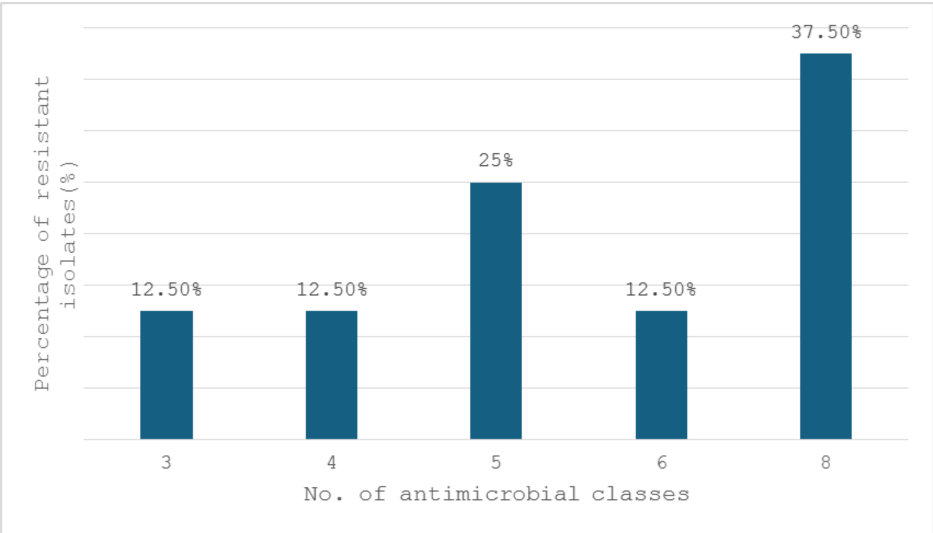

Figure 3. *Illustrates the percentage of multidrug resistance in S. aureus isolates across various antimicrobial classes*

DISCUSSION

The present study revealed that 20% of raw Chapli kebab samples collected from local restaurants in Kabul City were contaminated with *S. aureus*. It is similar to the 19.7% of retail raw meat reported by Zhao et al. (2025) and slightly smaller than the 21.23% of raw red meat reported by Şanlilbaba (2022). Nevertheless, it is still lower than the 31.25% contamination rate reported by Ahmadi et al. (2024) for Chapli kebab in Kabul and much lower than the 60.3% prevalence in raw meats in Iran (Rahimi et al., 2013). Such differences can be explained by differences in sampling, hygiene conditions during slaughter and processing, climatic conditions, and laboratory procedures.

Further evidence that the dispersion of microbes increases with grinding is provided by the high contamination rate reported in Iran in minced beef (76.3) (Rahimi et al., 2013). Chapli kebab is made from ground beef; hence, it can be handled roughly and mixed with spices, potentially cross-contaminating it. Additionally, Thwala et al. (2021) noted that *S. aureus* is commonly found in meat and meat products in African nations, specifically beef and poultry, and indicated that meat products contaminated by this pathogen are a global rather than a local problem.

The results of the aerobic plate counts (APC) in the current study (7.93 and 7.64 log 10 CFU/g) were quite high, indicating low levels of hygienic practices and a high risk of temperature abuse. These high bacterial counts indicate systemic contamination of the preparation and storage process. Even though APC values may not be easily compared across studies, comparable issues regarding hygiene and meat handling have been highlighted in other studies in Africa and Asia (Thwala et al., 2021; Taddese et al., 2025), in which meat products were found to be a major source of bacterial contamination because of poor maintenance of the cold chain and manual meat processing.

The patterns of antimicrobial resistance in the present study are a matter of concern. The highest resistance rates were to tetracycline (87.5%) and penicillin (75%), which align with the results reported by Thwala et al. (2021), who observed significant resistance to beta-lactams and tetracyclines in meat isolates from Africa. On the same note, Taddese et al. (2025) reported high resistance to tetracycline and kanamycin in animal feed. The resistance to these widely used antibiotics may indicate their extensive and uncontrolled use in food-producing animals.

Even though the current study found a lower percentage of resistance to oxacillin (70.5%) than in Egypt (Shaltout et al., 2019), the occurrence of resistant isolates is alarming. Both Thwala et al. (2021) and Zhao et al. (2025) reported the presence of MRSA strains in retail meats, as evidenced by the *mec*A gene. Although molecular confirmation of MRSA was not performed in the current study, the observed phenotypic resistance indicates the potential circulation of methicillin-resistant strains and genes along the meat production chain in Kabul.

One of the most worrying aspects of this study was the high prevalence of multidrug-resistant (MDR) isolates, with 37.5% being resistant to eight antimicrobial classes. Although the MDR rate is lower in comparison with the very high 96.87% presented by Şanlilbaba (2022), it is still quite high, and it is comparable to the results in Egypt (42.8%) (Shaltout et al., 2019). On the same note, Zhao et al. (2025) reported that MDR was high among retail raw meat isolates, further validating the mounting global alarm about resistant *S. aureus* in the food chain.

Notably, several local and international encyclopedias have identified enterotoxin genes in meat-associated *S. aureus* isolates. According to Rahimi et al. (2013), the prevalence of classical enterotoxin genes was 13.5% among isolates, whereas Şanlilibaba (2022) found a high rate of enterotoxin genes, especially in isolates from raw red meat. The presence of toxin genes, along with multidrug resistance, makes the impact on the health of the population extremely high, as even with a decrease in bacterial counts due to cooking, toxigenic strains can still cause food poisoning.

The much greater APC in District 1 than in District 3 ($p < 0.001$) indicates a pattern of unequal access to hygienic facilities and safe food-handling practices across different parts of Kabul City. This kind of city-wide variability indicates that a different approach is warranted, especially in safety inspections and food handler training. As with the study, the evidence from Africa, Asia, and the Middle East overwhelmingly points to raw meat products as carriers of antimicrobial-resistant and possibly toxigenic *S. aureus*. The lethal combination of high microbial load, resistance to most, if not all, of the critical cease-and-desist antimicrobials, and multi-drug resistance calls for urgent action to ensure effective food hygiene practices, One Health-based antimicrobial resistance (AMR) stewardship programs, and in-depth molecular monitoring of the AMR (antimicrobial resistance) status of the meat in the entire production and distribution processes in Afghanistan.

This study provides vital evidence to support the existing literature, but several limitations need to be addressed. First, the sample size (n = 40) is small and only encompasses two districts of Kabul City; consequently, the findings of this study may have limited generalizability to the entire Chapli kebab market in Kabul City. The role of *S. aureus* in food poisoning is well-known, but its specific genetic and resistance characteristics are not. Due to a lack of data on enterotoxin genes (sea, seb, sec, sed) and the mecA resistance gene, the overall virulence and genetic profile of these isolates remain undocumented. Furthermore, the cleanliness of food-handling personnel, monitoring of storage temperatures, and direct assessment of cross-contamination were not addressed. It is highly recommended that future researchers use a larger sample size and incorporate molecular techniques to provide a more robust analysis of antimicrobial resistance in *S. aureus* in traditional meat products in Afghanistan.

Concerns regarding the food chain serving as a reservoir and a pathway for the spread of antibiotic resistance are also raised by the existence of isolates resistant to several antimicrobial classes. Treatment options for staphylococcal infections may be compromised if resistant bacteria spread resistance determinants to the human microbiome. The potential of further resistance increases in environments with inadequate or unevenly applied veterinary medication restrictions and antimicrobial stewardship initiatives.

The implementation of national antimicrobial stewardship policies in the human and veterinary sectors under a One Health concept, as well as the reinforcement of food safety inspection systems, the enhancement of food handler training, and the enforcement of cold chain management, are therefore urgently needed.

CONCLUSION

According to the current study, high aerobic plate counts and the presence of Staphylococcus aureus indicate that the raw Chapli Kebab meat sold in specific Kabul City neighborhoods has low microbiological quality. Multidrug resistance, including resistance to common antibiotics such as penicillin and tetracycline, was observed in a significant proportion of isolates.

These results demonstrate that traditional ground meat products may serve as conduits for the spread of antibiotic-resistant microorganisms in Afghanistan. To protect public health and stop the emergence of antibiotic resistance in the area, it is crucial to implement ongoing microbiological surveillance, enhance sanitary handling procedures, enforce regulations, and use antibiotics sensibly.

Author Contributions

Mohammad Asif Noori contributed to the conceptualization of the study, development of the methodology, data collection, analysis, and preparation of the manuscript. Nasir Ahmad Sarwary was involved in methodological design, supervision of the research process, and critical review and editing of the manuscript. Mohammad Farzad Afshar participated in data analysis, validation of results, and manuscript revision. Shamshad Haidari contributed to sample collection, laboratory procedures, and data curation. All authors have read and approved the final version of the manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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