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Exploring S*taphylococcus aureus* Prevalence and Antimicrobial Resistance in Ready-to-Eat Meat Products in Kabul City

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Abstract

THE current study aimed to evaluate the prevalence of *Staphylococcus aureus* and its antibiotic resistance profile in ready-to-eat meat and meat products of animal origin in various locations within Kabul City, Afghanistan. A total of 120 meat and meat product samples were collected from six locations in Kabul City from January to May 2023. These products were tested in the laboratory using culture, Gram's staining and conventional biochemical tests. Antimicrobial susceptibility testing was performed using the disk diffusion method. Out of the 120 samples tested, only 16 samples presented *S. aureus*. Chapli kebab and liver kebab were among the foods with the highest prevalence. The results of the antimicrobial susceptibility test revealed the highest resistance to chloramphenicol and ciprofloxacin followed by tetracycline. Half of the isolates were multidrug-resistant and 5 multidrug-resistant *S. aureus* turned out to be methicillin-resistant. The study revealed that contamination of ready-to-eat animal-origin foods with *S. aureus* is a concern. Foodborne diseases caused by microbiological agents pose significant challenges for developing countries such as Afghanistan, which is a public health problem.

Keywords: Multidrug resistance, methicillin resistance, contamination, food safety, *S. aureus*.

Introduction

Ready-to-eat (RTE) foods are food items that are ready for consumption without the need for further heat treatment or washing, often sold at the point of purchase. These include various forms often referred to as suitable, ready, instant, and fast foods [1]. The popularity of RTE foods varies by country and depends on the local staple diet [2].

In recent years, concerns about food safety have intensified among scientists specializing in food toxicology and microbiology due to the high prevalence of food-borne diseases [3]. While food supplies in industrialized countries are generally considered safe, evidence indicates that foodborne illnesses are still prevalent, with certain pathogens on the rise [3,4]. In lower-and middle-income countries, food-borne illnesses are high; this is because RTE food vendors often have limited literacy, which can lead to a lack of knowledge about proper hygiene and food handling practices [3,5]. Furthermore, some RTE foods are prepared, stored, and served under unsanitary conditions, and outdoor vending exposes the food to contaminants such as aerosols, insects, and rodents, which carry dangerous bacteria causing food-borne illnesses [6,7].

Staphylococcus aureus, a recognized opportunistic pathogen transmitted through food, is implicated in numerous outbreaks both within healthcare facilities and in community settings around the globe [4]. Besides, *S. aureus* can often colonize the human skin and may cause opportunistic infections in the host [8], which can contaminate or re-contaminate cooked foods by workers' hands, equipment, or utensils [9]. Recent studies from neighboring countries reveal the presence of *S. aureus* with different prevalences in RTE foods such as Pakistan (28.57%), Iran (15.42%), India (15%), and China (2.1%) [10–13]. Moreover, the prevalence of *S. aureus* has also been reported in several Asian

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and European countries which illustrates the presence of *S. aureus* as a threat to public health [3,14].

As Afghanistan imports foods including RTE foods from neighbouring countries, and hygienic practices are less followed by food vendors in Kabul City, a variety of microorganisms including *S. aureus* are likely to be detected in meat and meat products [15,16]; however, to the best of our knowledge, very few studies have been done in Kabul on the microbiological safety of RTE foods. Additionally, as Ready-to-Eat (RTE) foods don't require heat treatment before consumption, it has the potential to act as a carrier for the transmission of antibiotic-resistant microorganisms. Ultimately, it is crucial to screen ready-to-eat foods in Kabul City to detect harmful bacteria, thereby controlling and preventing instances of food poisoning. Therefore, this study aims to determine the prevalence of *S. aureus* and its antibiotic resistance in ready-to-eat meat and meat products sourced from various locations within Kabul City.

Material and Methods

Sample Collection

Samples for this study were ready-to-eat foods of animal origin. A total of 120 meat and meat product samples of animal origin (see Table 1) were randomly collected from various restaurants in six locations in Kabul city (1st, 2nd, 3rd, 5th, 8th, and 13th districts). The sample collection and testing were performed from January to May 2023. Each of the 120 samples was aseptically collected in sterilized tubes and bags; then immediately transferred to an icebox and taken to the laboratory for further processing.

Bacterial Isolation and Identification

Ten grams of food samples were mixed thoroughly with 90 mL of buffered peptone water (HiMedia®, Mumbai, India). This mixture was then incubated overnight at a temperature of 37 ℃. After incubation, the samples were cultured onto *Staphylococcus* Medium 110 agar (Oxoid, Basingstoke, England) and Mannitol Salt Agar (HiMedia®, Mumbai, India), which are selective media for *Staphylococcus* growth and then incubated at 37°C for up to 48 hours. Yellow-orange colonies, and yellow colonies on *Staphylococcus* Medium 110 agar and Mannitol Salt Agar respectively, were regarded as *S. aureus* (Fig. 1). Morphological confirmation was performed using Gram's staining; biochemical tests such as catalase and coagulase were also performed for additional identification (Figure 1). Gram-positive round clusters that were catalase and coagulase-positive were further sub-cultured on blood agar containing 5% sheep blood, and those with β-hemolytic activity were identified as *S. aureus* [17].

Antimicrobial Sensibility Test

The disk diffusion method, outlined by the Clinical and Laboratory Standards Institute (CLSI) [18], was utilized to assess the sensitivity of all coagulase-positive colonies to antimicrobial agents. The following antimicrobial agents were included in the test: levofloxacin (5 µg), oxacillin (5 µg), ciprofloxacin (5 µg), tetracycline (30 µg), erythromycin (15 µg), and chloramphenicol (30 µg), (Oxoid™, Basingstoke, England). The inhibition zones and their diameters were measured and interpreted according to the standards of the Clinical and Laboratory Standards Institute (CLSI) (Table 2) [18].

Statistical Analysis

Statistical analysis was performed using SPSS 26 (IBM Inc., Chicago). Descriptive statistics were used to show the antimicrobial resistance and prevalence of isolates. Fisher's Exact test was used to determine the association between type of food and carriage of *S. aureus*. A p value less than 0.05 was considered statistically significant.

Results

A total of 16 samples (13.33%) of different meat and meat products tested positive for *S. aureus*. Chapli kebab had the highest *S. aureus* contamination rate (31.25%), followed by liver kebab, beef burgers, and chicken kebabs, while sausages had a lower contamination rate, and Shami steak showed no contamination. There was not a statistically significant association between type of food and *S. aureus* carriage ($p = 0.158$) (Table 1).

These samples showed the highest resistance rate to chloramphenicol and ciprofloxacin, followed by tetracycline. A lower resistance rate was detected against oxacillin and levofloxacin, and the lowest resistance was against erythromycin (Table 2). Eight (50%) isolates showed multidrug resistance, and from 8 multidrug-resistant isolates 5 (62.5%) isolates were methicillin-resistant.

Discussion

Contaminated samples of ready-to-eat food, particularly those derived from animals, are thought to likely contribute to the transmission of *S. aureus* to humans [11]. The current study's findings revealed a relatively low prevalence of RTE foods (16%), in which Chapli kebab (31.25%) and liver kebab (25%) had the highest prevalence of *S. aureus* followed by beef burger and chicken kebab (18.75%). The higher prevalence of *S. aureus* in the Chapli kebab and liver kebab can be due to cross-contamination; this is because these products are prepared by hand and instruments. Moreover, cross-contamination can happen due to long storage of these products in ambient air. Another contributing factor to the high prevalence of *S. aureus* in Chapli

kebab and liver kebab is inadequate cooking time and temperature. Similar results have been reported in Iran [19] with a 10.19% prevalence in cutlet samples. Reports of kofta (minced meat) as the highest contaminated RTE food (5.2×10^5 CFU/g) from Egypt is also following the findings of this study [9,14]. Furthermore, *S. aureus* has been isolated from liver, chicken meat, minced beef and pork, which further supports the findings of this study [20,21]. Furthermore, a study conducted in Iran revealed that 18.89% of kebab samples were contaminated with *S. aureus* [19]. Moreover, investigations have revealed that 7.5% and 12.5% of beef burgers and chicken burgers, respectively, harbour strains of *S. aureus* displaying resistance to antimicrobial agents [22]. The findings of the present study underscore the potential risk of foodborne illness associated with consuming such products from markets or restaurants that neglect hygienic practices and allow meat to remain at ambient temperatures for extended periods. It is imperative that restaurants and supermarkets adhere strictly to hygienic regulations. This necessitates proactive oversight from the Ministries of Public Health and Agriculture to monitor compliance among eateries and fresh markets and enforce regulations rigorously. Through consistent monitoring, significant strides can be made in enhancing public health and curbing the prevalence of diseases within society.

The results of antimicrobial susceptibility testing showed that 100% of the *S. aureus* isolates were resistant to chloramphenicol, while 50% and 43.75% were resistant to ciprofloxacin and tetracycline, respectively. It turned out that 31.25% of the isolates were resistant to methicillin and levofloxacin. Chloramphenicol, ciprofloxacin, and tetracycline are the antimicrobial agents that are used in both human and veterinary medicine. Naimi et al., [23] reported 34.7% and 55% of resistance to chloramphenicol and ciprofloxacin in Kabul City; the isolates carried resistance genes and 66.3% were methicillin-resistant. Moreover, the previous study in Kabul City revealed 44.44% and 22.22% resistance to ciprofloxacin and oxacillin [24]. These results highlight the presence of methicillin-resistant *S. aureus* in different products including meat in Kabul city. The presence of resistance could potentially stem from inadequate sanitation and hygiene practices. Furthermore, since *S. aureus* is abundant in the air and dust, exposure of meat and its products to air may have facilitated contamination by resistant strains of *S. aureus*. The presence of antimicrobial-resistant clones in the study of Naimi et al., [23], also implies a remarkable prevalence of MRSA in Kabul City (66.3%), which may be the reason behind the high resistance rate towards antimicrobial agents in this study.

Various resistance rates to chloramphenicol have been reported from neighbouring countries; for instance, 14.28% from Iran [11], and 0% from Pakistan and Bangladesh [25,26]. The low prevalence in these reports may be due to the lack of use of chloramphenicol in food animals in these countries as its use is banned in food animals [27]. Unfortunately, the use of chloramphenicol in food of animal origin in Kabul may indicate the illegal use of it in meat, which should be banned.

Similar resistance rates against ciprofloxacin and tetracycline were reported from neighbouring countries: 49% and 20% in Bangladesh [25] and 50% and 85.93% in Iran [11]. Furthermore, 76% resistance to ciprofloxacin in India has been recorded from RTE foods [28].

It has been reported that 30% of *S. aureus* isolates in Bangladesh were resistant to oxacillin [25]. Resistance to levofloxacin and oxacillin has been reported to be 37% and 46%, respectively, in Iran [11,22]. However, studies in Saudi Arabia and Pakistan revealed that there was no resistance to levofloxacin [26,29]. The presence of resistance to oxacillin and levofloxacin can be due to the presence of *mec*A, *gyr*A and *grl*A in the current and mentioned studies. The presence of methicillin-resistant and multidrug-resistant strains of *S. aureus* in the current study may be attributed to their existence as commensals or in the environment, a finding supported by other studies conducted in Kabul City [23,24]. Excess use of antimicrobial agents in both veterinary and human medicine, migrations, travels, commerce and international military actions are among other reasons behind the presence of antimicrobial-resistant *S. aureus* isolates in the current study [23]. These findings suggest that insufficient cleaning, inadequate handling, and contamination during post-processing due to the polluted environment in and around shops may lead to health risks for consumers. The presence of *S. aureus* and being resistance to multiple drugs in raw meat and its products pose dangers such as toxin-mediated virulence and invasiveness to individuals who consume the products. Additionally, it might lead to the shedding of antimicrobial-resistant *S. aureus* into the environment, potentially spreading antimicrobial resistance within society and complicating treatment options for infections caused by such bacteria.

To the best of the authors' knowledge, this study represents the first investigation into the microbiological safety and antimicrobial resistance profiles of *S. aureus* in meat and meat products within Kabul City. However, due to limited resources and laboratory equipment, the detection of virulence and antimicrobial resistance genes carried by the strains was not possible. Additionally, increasing the sample size and detecting significant associations between the type of food and *S. aureus* carriage were not feasible. These aspects should be further investigated in future studies.

Conclusion

The high prevalence of *S. aureus*, particularly in Chapli kebab and liver kebab samples, as well as the identification of MRSA in prepared foods, underscores the critical need for enforcing strict hygienic practices in the kitchens of fast-food establishments and restaurants. To the best of our knowledge, this is the first study that addressed the presence of *S. aureus* in RTE foods found in Kabul City markets; in addition, the study revealed that there are methicillin-resistant and multidrug-resistant strains of *S. aureus* in RTE foods in Kabul City markets and restaurants, which is the background information for further research. At the same time, this is a warning for public to be more cautious when preparing, buying and eating such foods. Authorities must develop food safety procedures for the public and those who sell these products. They must also constantly monitor fresh markets, restaurants, eateries, and food stalls to retrieve expired and unhygienic products. Future studies should aim to ecologically characterize the isolated MRSA strains to trace the sources of contamination. Food handlers must be better educated about the importance of strict hygiene during the collection of raw materials, food preparation, holding, storage, and serving processes. The indiscriminate use of antibiotics in veterinary and human medicine may have contributed to the presence of antibiotic-resistant strains of *Staphylococcus* in the meat samples sold in parts of Kabul City, which poses a significant public health concern.

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Conflicts of interest

The authors declare no conflict of interest.

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Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; 49/11/2023).

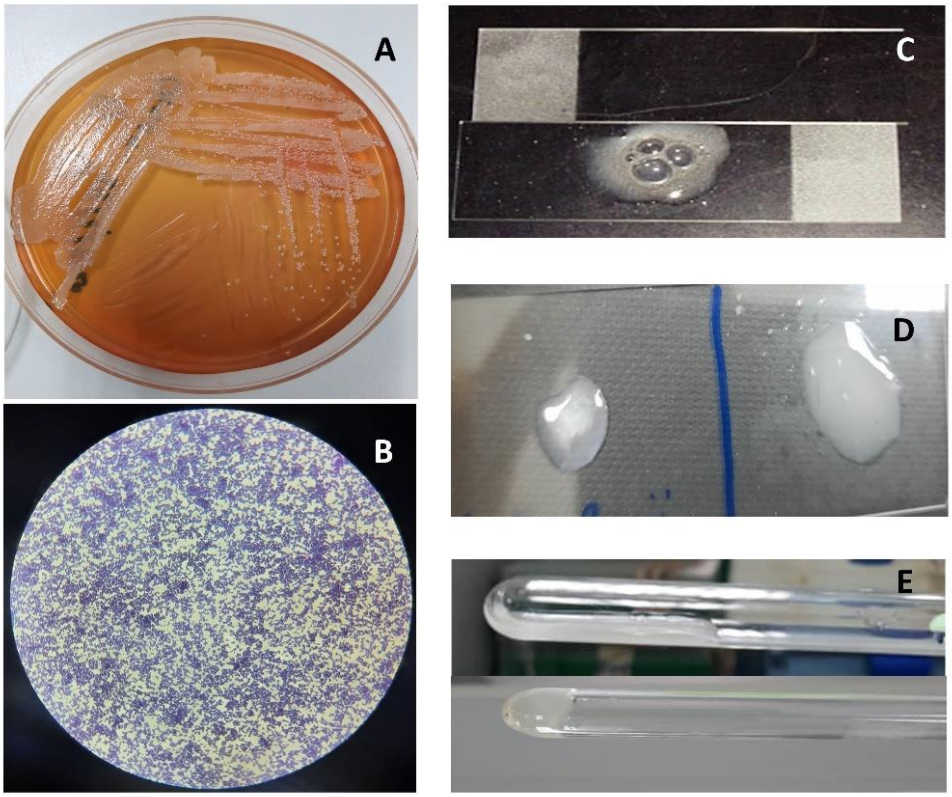

Fig. 1. Morphology and biochemical characteristics of *S. aureus*. A: the yellow colonies of *S. aureus* on mannitol salt agar; B: Gram positive colonies of *Staphylococci*; C: results of catalase test (bubble formation); D: results of slide coagulase test (clump formation); E: results of tube coagulase test (clot formation).

TABLE 1. Prevalence of *S. aureus* in ready-to-eat foods of animal origin collected from different districts of Kabul City.

No.	Types of Food Items	No. of Samples Collected	No. of Samples Positive for *Staphylococcus* N (%)
1	(Cutlet) Chapli kebab	20	5 (31.25%)
2	Shami steak	20	0 (0%)
3	Liver kebab	20	4 (25%)
4	Beef Burger	20	3 (18.75%)
5	Chicken Kebab	20	3 (18.75%)
6	Sausage	20	1 (6.25%)
	Total	**120**	**16 (13.33%)**

TABLE 2. Results of antimicrobial susceptibility testing for isolated *S. aureus.*

Antimicrobial patterns	Numbers of isolates with their antimicrobial susceptibility pattern (%)		
	Resistant	Intermediate	Sensitive
Chloramphenicol	16 (100%)	Nil	Nil
Levofloxacin	5 (31.25%)	4 (25%)	7 (43.75%)
Erythromycin	4 (25%)	2 (12.5%)	10 (62.5%)
Oxacillin	5 (31.25%)	3 (18.75%)	8 (50%)
Ciprofloxacin	8 (50%)	2(12.25%)	6 (37.5%)
Tetracycline	7 (43.75%)	3 (18. 75%)	6 (37.5%)

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