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Research Article

Detection of methicillin-resistant *Staphylococcus aureus* and enterotoxin genes in chicken meat samples

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ABSTRACT

Foodborne outbreaks remain a significant global public health concern, often linked to the consumption of contaminated animal-derived foods. Among these, chicken meat is particularly recognized as a major reservoir of *Staphylococcus aureus*, a pathogenic microorganism associated with both food intoxication and antimicrobial resistance. *S. aureus* can produce enterotoxins responsible for foodborne illness and may also exhibit methicillin resistance (MRSA), complicating treatment options. This study aims to investigate the methicillin resistance and the presence of enterotoxin genes in *S. aureus* strains isolated from retail chicken meat. Out of 200 chicken meat samples, MRSA was detected in 24% (n=48). Among these MRSA isolates, the *mec*A gene, which is associated with methicillin resistance, was identified in 66.6% (n=32) of isolates via PCR. Notably, no classical enterotoxin genes were found in any of the tested samples. Although the absence of classical enterotoxin genes suggests a low risk of staphylococcal intoxication in this sample group, the presence of MRSA strains underscores a persistent public health concern regarding antimicrobial resistance in the food chain.

Keywords: Chicken meat, Enterotoxin genes, Methicillin-resistant, *S. aureus*

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Introduction

Staphylococcus aureus is a bacterium that commonly colonizes the skin, mucous membranes, and nasal passages of healthy humans and animals. It is responsible for staphylococcal food poisoning, which occurs through the consumption of food contaminated with its toxins (15). Staphylococcal food poisoning can cause nausea, vomiting, and in some cases diarrhea and abdominal pain (12). Foods frequently implicated in staphylococcal food poisoning include meat and meat products, poultry and egg-based products, milk and dairy items, salads, bakery goods (particularly cream-filled pastries), and sandwiches. However, the specific types of implicated foods may vary across countries, primarily due to differences in dietary habits (15). In particular, food contamination by *S. aureus* primarily results from its ability to enter the food chain through contaminated raw materials, improper food processing, and failure to maintain the cold chain (25). *S. aureus* is often the etiological agent of infections associated with hospital settings.

Methicillin is a semi-synthetic antibiotic commonly used in the treatment of staphylococcal infections. In recent years, the prevalence of methicillin-resistant *S. aureus* (MRSA) strains has significantly increased, which has become a serious international concern for the treatment and control of staphylococcal infections. These strains are also resistant to multiple clinically used antibiotics and are often referred to as “antibiotic-resistant bacteria” in general discourse (11, 26, 37). Although MRSA was initially associated with hospital-acquired infections, community-associated cases have increasingly been reported. MRSA strains have been isolated from food and food-producing animals worldwide and are considered one of the most significant antibiotic-resistant

pathogens due to their capacity to cause a wide range of serious infections (12, 22, 23).

MRSA is resistant to several antibiotics, including tetracycline, amoxicillin, ampicillin, ciprofloxacin, ceftriaxone, beta-lactams, and azithromycin. Due to its widespread distribution and the severity of the infections it is associated with, the World Health Organization (WHO) has included MRSA in its list of "priority pathogens" (26). MRSA produces a modified penicillin-binding protein (PBP2a) that confers resistance to all beta-lactam antibiotics. Methicillin resistance in staphylococci is primarily attributed to the presence of the *mec*A and *mec*C genes, which are located on the staphylococcal cassette chromosome (SCC*mec*). Additionally, other resistance genes such as *mec*B and *mec*D have been identified, particularly in *Macrococcus* species (20). *S. aureus* is a common contaminant in retail chicken meat, often resulting from unhygienic processing conditions (1, 28). Moreover, various types of meat such as chicken, pork, and beef as well as ready-to-eat foods have been identified as potential reservoirs of MRSA (13, 41). Although MRSA is frequently isolated from chicken meat, the consumption of contaminated food of animal origin is increasingly recognized as an important route of exposure (21). The aim of this study was to determine the presence of methicillin-resistant *S. aureus* in chicken meat and to identify classical enterotoxin genes.

Materials and Methods

Sample Collection: A total of 200 chicken meat samples (thigh, breast, and wing) were collected from retail outlets in Konya between June and August 2024 during four independent visits (n = 50 per visit). All samples were transported to the laboratory within 2 hours under refrigerated conditions (<4°C) using insulated containers with ice packs, and microbiological analysis was initiated on the same day.

MRSA Isolation: Each 25 g chicken meat sample was aseptically transferred into sterile bags with 225 mL of Mueller Hinton Broth (MHB, 6.5% NaCl; Biolife 4017402), homogenized for 2 min, and incubated at 37°C for 18 h (3, 30). Following visible turbidity in MHB, 1 mL of the culture was transferred into 9 mL Phenol Red Mannitol Broth (PHMB+, HiMedia M570) and incubated at 37°C for 24–48 h. A color change from red to yellow indicated mannitol fermentation (31). Positive samples were streaked onto HiCrome MRSA Base Agar (HiMedia M1953) and incubated at 37°C for up to 48 h. Green colonies were presumptively MRSA and subcultured on 5% sheep blood agar. Colonies with β-hemolysis were further identified by Gram staining, catalase, DNase, and tube coagulase tests (4). DNase positivity was indicated by a clear zone after HCl, and coagulase positivity by clot formation within 4 h at 37°C. *S. aureus* ATCC 43300 was used as a positive control.

DNA Isolation: Genomic DNA was extracted from presumptive *S. aureus* isolates using the cetyltrimethylammonium bromide (CTAB) method, as previously described by Stefanova et al. (35). The bacterial cells were harvested from overnight cultures on blood agar, and lysis was performed by incubation in CTAB buffer followed by chloroform-isoamyl alcohol extraction and isopropanol precipitation. DNA quality and concentration were assessed using a NanoDrop spectrophotometer (Allsheng Nano-400A) and stored at -20°C until PCR analysis.

***PCR Analysis*:** The extracted DNA was used to detect the *mec*A gene via conventional-monoplex PCR for identifying MRSA isolates and to identify classical staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*) using multiplex PCR. Conventional PCR reactions were prepared in a total volume of 25 µL, consisting of 12.5 µL PCR Master Mix (2X) (Thermo Fisher-K0171), 1.5 µL of each primer (forward and reverse), 7.5 µL nuclease-free water, and 2 µL DNA template. Multiplex PCR was similarly conducted in a 25 µL reaction volume containing 13.75 µL nuclease-free water, 5 µL MyTaq Reaction Buffer (Bioline BIO-21126), 1 µL of each primer (forward and reverse, 10 mM) specific to each enterotoxin gene, 1.5 U Taq DNA polymerase, and 5 µL DNA template. Primer sequences, amplicon sizes, thermal cycling conditions, and corresponding literature references for both PCR protocols are presented in Table 1.

Results

Among the 200 chicken meat samples analyzed, 48 (24%) presumptive MRSA isolates were obtained based on growth on HiCrome MRSA base agar. The highest MRSA positivity rate was found in chicken breast at 42%, while the rates for chicken wings and thighs were 8% and 4%, respectively. Of these, 32 isolates (66.6%) were confirmed to be coagulase-positive and DNase-positive, suggesting phenotypic characteristics of MRSA. The presence of the *nuc* gene, a specific marker for *S. aureus*, was confirmed in these 32 isolates using monoplex PCR. The results of this amplification are presented in Figure 1. In the same isolates, the *mec*A gene, which confers methicillin resistance, was detected in 32 samples, as shown in Figure 2. The distribution of *mec*A-positive isolates across different sample types (e.g., breast, wing, thigh) is summarized in Table 2. None of the isolates tested positive for the classical staphylococcal enterotoxin genes (sea, seb, sec, sed, see).

Table 1. Primer sequences and amplification conditions used in the study.

Gene	Primer sequence (5'- 3')	Amplicon size (bp)	Amplification conditions	References
nuc	F: GCGATTGATGGTGAT ACG GTT R:AGCCAAGCCTTGACGAACTAA AGC	279	95°C 5 min, 95°C 1 min, 55°C 45 sec, 72°C 1 min (30 cycles) 72°C 5 min	18
*mec*A	F:GTAGAAATGACTGAACGTCCG ATAA R:CCAATTCCACATTGTTTCGGTCTAA	310	94°C 4 min, 94°C 45 sec, 50°C 45 sec, 72°C 60 sec (30 cycles) 72°C 2 min	14
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120	94°C 4 min, 94°C 2 min, 55°C 2 min, 72°C 1 min (30 cycles) 72°C 5 min	19, 38
seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC	478		
sec	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257		
sed	F: CTAGTTTGGTAATATCTCCT R: TAATGCTATATCTTATAGGG	317		
see	F: TAGATAAAGTTAAAACAAGC R: TAACTTACCGTGGACCCTTC	170		

Table 2. Distribution of *mec*A genes in sample groups.

Sample type	Number of samples	MRSA positive (n)	*mec*A positive isolates (n)
Chicken breast	100	42 (42%)	29 (69%)
Chicken wing	50	4 (8%)	2 (25%)
Chicken thigh	50	2 (4%)	1 (50%)
Total	200	48 (24%)	32 (66.6%)

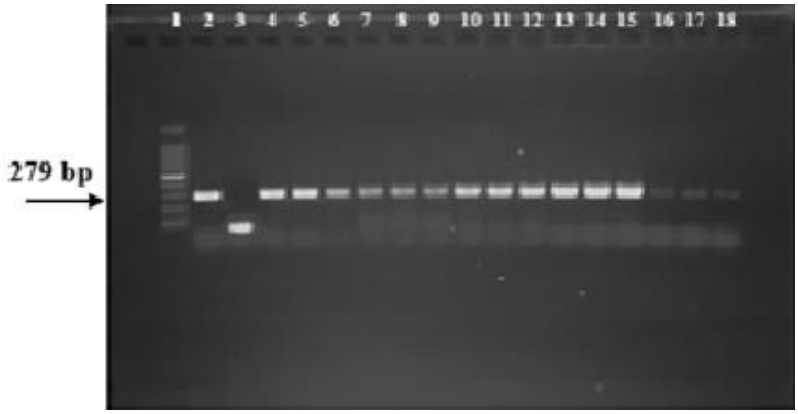

Figure 1. Positive samples containing the *S. aureus*-specific *nuc* gene region. 1: 100 bp Ladder, 2: Positive control ATCC 29213; 3: Negative control; 4-18: Positive samples.

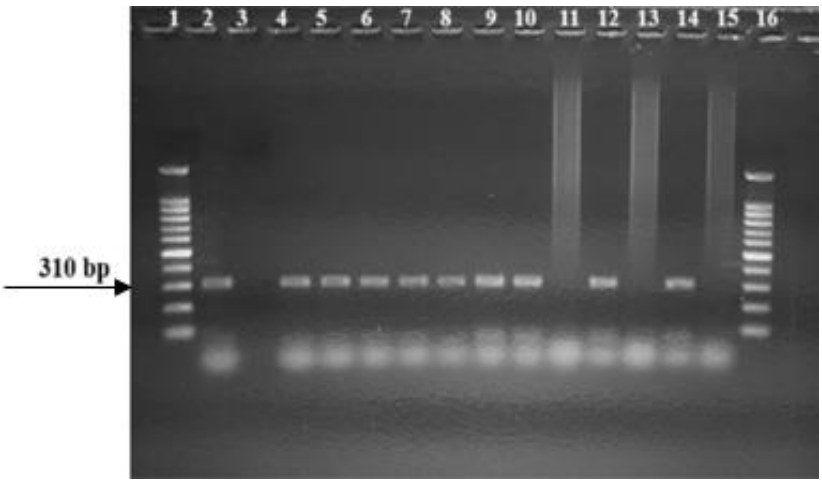

Figure 2. Positive samples with the MRSA-specific *mec*A gene region. 1, 16: 100 bp Ladder, 2: Positive control ATCC 43300; 3: Negative control ATCC 25923; 4-10, 12, 14: Positive samples; 11, 13, 15: Negative samples.

Discussion and Conclusion

Today, methicillin-resistant *S. aureus* is known to have developed resistance to numerous antimicrobial agents. The widespread and often uncontrolled use of antimicrobials in food-producing animals has significantly contributed to the emergence and dissemination of this resistance. Several studies have demonstrated that MRSA strains isolated from animal-derived food products frequently exhibit multidrug resistance and can be transmitted to humans either through direct contact or via the food chain. Various studies conducted worldwide have reported differing levels of MRSA prevalence in animal-derived foods, reflecting regional differences in antimicrobial usage and hygiene practices. The presence of the *mec*A gene in 69% of MRSA strains isolated from breast meat supports the prevalence of resistant strains in these products. These differences may be related to factors such as hygiene practices during processing, product exposure time, textural structure, and surface area. Studies have reported higher MRSA rates in breast meat compared to other cuts (5, 43). This can be explained by factors such as the breast meat having more contact surface in the processing line and being more exposed to slicing and packaging processes. For instance, Sadiq et al. (32) investigated the presence of MRSA in 300 samples in Pakistan. PCR analysis revealed that 118 of the samples were positive for the *mec*A gene. Among the sample types, the highest prevalence of MRSA was found in chicken meat (77%, 23/30), followed by beef (63%, 25/40), lamb (50%, 15/30), cutting knives (77%, 18/40), and food handlers (18%, 7/40). Overall, the prevalence of MRSA across all tested samples was reported as 79%. Similarly, Elshebrawy et al. (13) reported that 54 out of 96 whole chicken carcass samples (53.3%) marketed in Egypt were contaminated with *S. aureus*. Among these isolates, the *mec*A gene was detected in 56.3% of the strains, indicating a substantial presence of MRSA in retail poultry products. Previous studies have emphasized that high contamination rates in chicken meat are often linked to inadequate hygienic practices during slaughter, evisceration, distribution, and storage (32, 33). In addition, certain cultural habits—such as consumer preference for freshly slaughtered chicken carcasses purchased directly from live animal markets—further contribute to the microbial load observed in retail poultry products. These findings underscore the critical need for stricter hygiene protocols and targeted consumer education programs, particularly in regions where traditional marketing practices remain widespread (13).

In the current study, the prevalence rates of *S. aureus* and MRSA observed in chicken meat samples were within the range of those reported in previous studies, with some studies showing lower and others higher rates depending on the geographical region, sampling and detection method, hygiene conditions during processing, and antimicrobial usage in poultry production. For instance, a prevalence study conducted in Turkey reported the detection of *S. aureus* in 10% and MRSA in 5% of retail chicken meat samples, with all MRSA isolates carrying the *mecA* gene (37). Similarly, a study in the United States found a relatively low MRSA prevalence of 1.8% in chicken meat samples collected in Tulsa, Oklahoma (1). Another investigation conducted in Korea detected *S. aureus* in 94 of 200 samples from retail markets, including four MRSA isolates (21). Similarly, in a study conducted in Bangladesh, China and Sri Lanka, the prevalence of MRSA in chicken samples was reported as 3-10%, with significant variation observed among different parts (6). In contrast, higher contamination rates were reported in other studies; for example, a study from Egypt found MRSA in 56.3% of *S. aureus*-positive chicken carcasses (13), while Sheet et al. (33) reported MRSA contamination in 40% of broiler carcass swab samples collected from different poultry slaughterhouses. Additional studies from China observed MRSA prevalence rates of 6% and 9.9%, respectively (27, 40). A study in Denmark reported an 18% MRSA prevalence in imported chicken meat (2). Furthermore, Pauly et al. (30) found MRSA in 20.9% of chicken carcass, wing, and leg samples using chromogenic agar. These varying results underline the importance of local surveillance data and emphasize how differences in food production systems, sampling strategies, and detection methodologies can significantly influence reported MRSA prevalence rates.

The observed variations across studies can be attributed to multiple factors such as differences in sample size, regional hygiene and slaughtering practices, antimicrobial usage patterns in poultry farming, and the sensitivity and specificity of the laboratory detection methods employed. Despite variability in prevalence rates, the detection of MRSA in retail chicken meat even at relatively low levels remains a public health concern due to its antimicrobial resistance profile and zoonotic transmission potential. Therefore, ongoing surveillance, improved hygiene standards, and stringent control measures throughout the poultry production and distribution chain are essential to mitigate the dissemination of resistant strains (5, 6, 13, 17, 36). The transmission of MRSA in poultry meat can occur through multiple routes during the production and processing chain. Colonized live poultry serve as a primary reservoir, with *S. aureus* frequently inhabiting the nares, skin, cloaca, and feathers. During slaughter particularly at the defeathering and evisceration stages bacteria can be transferred from these colonized sites onto the carcass surface (29). Equipment such as scalders, defeathering machines, conveyor belts, knives, and cutting boards, if not properly sanitized, can act as persistent fomites and

facilitate cross-contamination between carcasses (39, 42). Additionally, human handlers colonized with MRSA particularly nasal carriers can transmit the pathogen via direct contact or through respiratory droplets in poorly ventilated environments (24). Aerosolized bacteria in abattoirs have also been documented as potential vectors (39). Post-slaughter handling, including cutting, packaging, and storage, can further contribute to contamination if good manufacturing practices (GMP) and hygiene protocols are not strictly observed. Furthermore, insufficient cold chain maintenance during distribution may allow bacterial survival and even proliferation under suboptimal refrigeration conditions. The complexity of these transmission routes underscores the necessity for an integrated hygiene control system at each critical control point, from farm to retail.

In the present study, MRSA-selective agar was used to isolate presumptive colonies, which were subsequently subjected to phenotypic confirmation via coagulase and DNase tests. However, not all presumptive MRSA isolates exhibited the *mec*A gene upon PCR confirmation. This discrepancy may be attributed to several factors. First, although chromogenic MRSA media are valuable for preliminary screening, they may produce false positives due to the growth of coagulase-negative staphylococci or methicillin-susceptible strains capable of surviving under selective conditions (8). Second, not all isolates that exhibit methicillin resistance phenotypically necessarily harbor detectable *mec*A or other *mec* type genes. Resistance can also arise from alternative mechanisms such as hyperproduction of β-lactamases, or mutations in native penicillin-binding proteins, leading to borderline oxacillin resistance (9, 10). Third, limitations in PCR primer specificity, low target gene expression in certain environmental isolates, or sequence variations may result in false-negative PCR results (11, 44). Furthermore, recent studies have documented phenotypic methicillin resistance in *S. aureus* strains lacking *mec*A, highlighting the role of non-mecA mediated resistance pathways. For example, BORSA (borderline oxacillin-resistant *S. aureus*) strains display elevated oxacillin MICs in the absence of *mec* genes and are increasingly recognized in foodborne isolates. Among the MRSA isolates confirmed by both phenotypic and genotypic methods, only the *mec*A gene was detected, while none of the alternative methicillin resistance genes (*mec*C, *mec*B, or *mec*D) were identified. This finding highlights the dominance of *mec*A-mediated resistance in poultry derived *S. aureus* strains and suggests a relatively uniform resistance mechanism within the studied population. The absence of additional resistance determinants may be attributed to the limited genetic exchange in poultry production environments or to regional epidemiological characteristics of circulating *S. aureus* clones. This observation is consistent with the broader understanding of methicillin resistance mechanisms in *S. aureus*. The *mec*A gene, responsible for encoding the modified penicillin-binding protein PBP2a, is located on the staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element that enables horizontal gene transfer between staphylococcal populations. To date, thirteen SCC*mec* types (I–XIII) have been identified, each with distinct structural and epidemiological features (34). While other resistance genes such as *mec*C, *mec*B, and *mec*D have been described, they are less frequently encountered and are more often associated with non-*S. aureus* species like *Macrococcus* (34). The widespread dissemination of *mec*A is largely driven by clonal expansion of MRSA strains and selection pressure resulting from the excessive or inappropriate use of β-lactam antibiotics in both human and veterinary medicine (7, 16).

Although this study focused on the classical enterotoxin genes (*sea–see*), recent evidence indicates that newly described enterotoxin genes such as *seg*, *seh*, and *sei* are increasingly detected among foodborne *S. aureus* isolates. These genes exhibit similar emetic activities and may contribute to staphylococcal food poisoning even in the absence of classical toxins. The detection of such genes is epidemiologically significant, as their distribution patterns provide valuable insight into the virulence potential, clonal diversity, and transmission dynamics of *S. aureus* strains circulating within the food chain (25). Furthermore, the presence of MRSA in chicken meat has important public health implications, since poultry products may act as reservoirs for antimicrobial-resistant bacteria capable of colonizing or infecting humans, thereby facilitating the dissemination of resistance through the food chain (29, 43).

In conclusion, this study highlights the occurrence of MRSA in retail chicken meat and underscores the persistence of antimicrobial resistance even in the absence of classical enterotoxigenic profiles. Although enterotoxin genes were not detected, the phenotypic and genotypic confirmation of MRSA in poultry meat suggests a silent but significant public health concern, particularly in terms of resistance dissemination through the food chain. The application of both culture-based and molecular techniques strengthened the diagnostic reliability, emphasizing the value of integrated methodologies in foodborne pathogen surveillance. The findings reinforce the importance of implementing rigorous hygiene standards and prudent antimicrobial stewardship along the poultry production continuum from farm to processing to retail. Considering the potential for zoonotic transmission and the role of animal-derived foods as reservoirs of resistant bacteria, further research should explore additional virulence determinants and strain-level

characterization. Strengthened monitoring and control strategies are essential to mitigate the spread of resistant *S. aureus* strains and protect public health.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

GT: Conceptualization, methodology, microbiological and PCR analysis, writing of original draft; review & editing. AET: Microbiological and PCR analysis, writing of original draft; review & editing. NT: Writing of original draft; review & editing. YB: Writing of original draft; review & editing. YD: Review & editing.

Data Availability Statement

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

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