Biofilm-forming *Staphylococcus aureus* in retail meat: A study from Anand, Gujarat, India

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Abstract

Foodborne illnesses represent significant public health challenges worldwide. Developing countries face heightened risks due to insufficient food handling and sanitation practices. Although animal tissue begins as a sterile environment, contamination can occur during the slaughtering process from microorganisms present both externally and internally. *Staphylococcus aureus* stands as a major culprit in causing foodborne diseases. It has the capability to form biofilms on various surfaces and can thrive in the diverse environmental conditions commonly encountered in food processing facilities. The capacity of *Staphylococcus aureus* to form biofilms significantly contributes to its survival, persistence, antibiotic resistance, and virulence, making infections associated with biofilms challenging to manage and control. This study sought to evaluate the prevalence, molecular detection, and biofilm production of *Staphylococcus aureus* in raw meat sourced from retail shops in Anand, Gujarat. A total of 100 samples (50 chicken and 50 chevon) underwent cultural isolation and PCR confirmation for the presence of *Staphylococcus aureus* relying on the detection of the *mecA* gene. Biofilm formation was assessed using the Congo Red Agar method. Results revealed an overall isolation rate of *Staphylococcus aureus* at 21%, with a higher prevalence observed in chicken samples (71.42%) compared to chevon samples (28.57%). Methicillin-resistant *Staphylococcus aureus* was exclusively found in chicken samples, accounting for 9.52% of isolates. Among the confirmed isolates, 57.14% exhibited biofilm production. These findings underscore the critical importance of adhering to stringent hygiene practices during meat handling and highlight the potential health risks associated with consuming meat products.

Keywords: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, chevon, chicken, PCR, biofilms

Introduction

Foodborne illnesses, stemming from a myriad of agents such as bacteria, viruses, fungi, parasites, or chemicals, present formidable challenges to public health worldwide. With over 200 diseases linked to foodborne transmission, an estimated 600 million individuals, nearly 1 in 10 people globally, suffer from illness due to consuming contaminated food annually. This results in 420,000 deaths each year, accompanied by a loss of 33 million healthy life years, with diarrheal illnesses comprising most cases (World Health Organization, 2022) [24]. Common pathogens like *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, and norovirus are often implicated in acute foodborne illnesses (Bintsis, 2017) [3]. Developing and underdeveloped nations face heightened risks of food contamination due to factors such as poor sanitation, inadequate infrastructure, and improper handling practices (Dandage *et al.*, 2017) [6]. In India, stagnant food safety standards are projected to lead to over 100 million cases of foodborne illnesses annually, a figure expected to rise to 150-177 million by 2030 (Smeets *et al.*, 2017) [21]. From 2009 to 2018, the Integrated Disease Surveillance Program documented 2,688 foodborne disease outbreaks in India, resulting in 153,745 illnesses and 572 deaths (Bisht *et al.*, 2021) [4]. The rapid globalization of food production and trade has further exacerbated the likelihood of food contamination, necessitating global collaboration among food safety authorities to effectively address these concerns (Jawad *et al.*, 2020) [11]. *Staphylococcus aureus* is characterized by irregular clusters resembling bunches of grapes, consists of spherical cells with diameters ranging from 0.5 to 1.0 μm, often occurring singly or in bunches.
These facultative anaerobic Gram-positive cocci are nonmotile and non-spore-forming, producing catalase when growing aerobically. On non-selective media, typical colonies of *S. aureus* appear yellow to golden-yellow, smooth, raised, glistening, circular, and translucent, reaching sizes of 6–8 mm in diameter. While *S. aureus* commonly colonizes the skin, anterior nares, and mouth of healthy individuals, nasal colonization is a significant source of infection and a risk factor for subsequent colonization, although most carriers remain asymptomatic (Gorwitz et al., 2008) [9]. Nonetheless, *S. aureus* is a multifaceted pathogen accountable for both hospital and community-acquired infections, encompassing staphylococcal food poisoning (SFP) and toxic shock syndrome (TSS) (Song et al., 2017) [22]. SFP, a prevalent cause of foodborne intoxication worldwide, manifests with violent vomiting shortly after ingestion and is typically self-limiting (Kerouanton et al., 2007) [12]. Symptoms of acute gastroenteritis, such as nausea, abdominal cramps, diarrhea, and fever, can appear within 2–6 hours of consuming food containing staphylococcal enterotoxins. Additionally, TSS, characterized by fever, erythema, hypotension, and multi-organ dysfunction, is associated with toxins produced by *S. aureus*, including TSST-1 and staphylococcal enterotoxins (Todd et al., 1978) [23].

Methicillin-resistant *Staphylococcus aureus* (MRSA), emerged in 1961 is a strain of the bacterium *S. aureus* known for its resistance to multiple antibiotics, presenting a significant challenge in healthcare settings. Methicillin resistance is mediated by the carriage of the staphylococcal cassette chromosome mec (SCCMec), a mobile genetic element containing the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) with decreased affinity for β-lactam antibiotics (Doulgeraki et al., 2017) [7]. Additionally, a new homologue of the *mecA* gene, designated as *mecC* gene, has also been identified. MRSA strains also produce β-lactamase encoded by *blaZ*, contributing to decreased activity of β-lactam antibiotics. Epidemiologically and molecularly, MRSA is categorized into hospital-associated MRSA (HA-MRSA), livestock-associated MRSA (LA-MRSA), and community-associated MRSA (CA-MRSA). Its ability to acquire multiantibiotic resistance poses challenges for future chemotherapy against multiresistant pathogens (Dahms et al., 2014) [5]. LA-MRSA strains have been detected in pork and chicken products. These findings highlight the potential for meat products, to serve as a source of food poisoning.

Biofilm serves as a prevalent mechanism adopted by bacteria to endure diverse adverse environmental conditions, facilitating the formation of cellular communities encased within extracellular polymeric substances (EPS). Bacterial biofilms, pose challenges due to their resilience against antibiotic treatments and host immune defenses. These infections, often chronic and prone to relapse, account for a substantial portion of human bacterial infections and present significant challenges in both human and veterinary medicine (Hall-Stoodley et al., 2004) [10]. *S. aureus* demonstrates notable biofilm formation abilities, particularly evident in various food-related settings such as raw materials, processing environments, equipment surfaces, and commodity circulation (Miao et al., 2017) [13]. In the case of *S. aureus*, biofilm development progresses through attachment, microcolony formation, maturation, and detachment stages, with EPS playing a pivotal role in cellular encasement and community function across these phases (Rosenthal et al., 2014) [18].

Given the increasing importance of raising public awareness about food safety and quality, understanding the prevalence of *S. aureus* and methicillin-resistant strains (MRSA) in food animals is crucial. This study aimed to assess the prevalence of *S. aureus* and detect the presence of the methicillin resistance gene (*mecA*) in meat sold in retail outlets in Anand, using both conventional and molecular techniques.

**Materials and Methods**

**Collection and processing of samples**

Total 100 meat samples (50 chicken & 50 chevon) were collected from the retail meat shops in and around the Anand city (Gujarat, India). Samples were collected aseptically in pre-sterilized container. They were then immediately transferred on ice at 4°C to the laboratory of the Department of Veterinary Public Health & Epidemiology, College of Veterinary Science and Animal Husbandry, Anand for further processing.

**Isolation and identification of *Staphylococcus aureus***

All samples were enriched in Peptone Water broth at 37 °C for 24 hours. For the isolation of *S. aureus*, Mannitol Salt Agar (MSA) and Baird Parker Agar (BPA) were used as selective media. A loopful of inoculum from the enrichment broth was streaked onto both MSA and BPA supplemented with Egg Yolk Emulsion and 3.5% Potassium Tellurite solution. The inoculated plates were then incubated at 37 °C for 24–48 hours. Presumed *S. aureus* isolates were subsequently confirmed through Gram's staining using the HiMedia Gram Staining Kit.

**Molecular detection of *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus***

Culturally positive *S. aureus* isolates were subjected to molecular identification using PCR targeting the *nuc* and *mecA* gene, respectively. The snap chill process was used to obtain template DNA from isolates. The PCR reaction was set up in 200 μL PCR tubes on ice, with a total volume of 25 μL. The amplification aimed to screen the genes using a Thermocycler PCR machine (Eppendorf Mastercycler gradient). The reaction mixture comprised 12.50 μL of PCR master mix (2X), 1 μL each of forward and reverse primers (10 pmol each), 5.50 μL of nuclease-free water, and 5 μL of template. The specific oligonucleotide primer sequences for the *nuc* gene and *mecA* gene and the PCR thermal cycling conditions are outlined in Table 1 and Table 2, respectively. The resulting amplified product was subsequently analyzed via agarose gel electrophoresis using a 1% agarose gel, and the bands were visualized using a gel documentation system.

**Evaluation of biofilm production by *Staphylococcus aureus* isolates**

The isolates identified morphologically and molecularly were streaked on Congo Red Agar as described by Freeman et al. (1989) [8]. The plates were incubated at 37°C for 24 h.

**Results and Discussions**

Out of a total of 100 samples, 24% were isolated as *S. aureus*, with 66.66% (16/24) originating from chicken samples and 33.33% (8/24) from chevon samples. These...
isolates exhibited distinctive characteristics: jet-black colonies on BPA and golden-yellow colonies on MSA, along with a gram-positive cocci arrangement in clusters (Fig 1, 2, 3). In a study conducted by Owuna et al. (2015) [17], it was found that out of 40 poultry meat samples, 29 samples (72.50%) tested positive for the presence of S. aureus. Sangeetha et al. (2020) [19] reported, 55% prevalence rate of S. aureus in chevon whereas chicken samples had 27% prevalence rate.

Fig 1: Jet black colonies of S. aureus on Baird Parker Agar

Fig 2: Yellow colonies of S. aureus on Mannitol Salt Agar

Fig 3: Gram positive cocci in bunches

Fig 4: Agarose gel Electrophoresis of PCR products for detection of nuc gene (L: 100 bp DNA ladder; P: Positive control; Lane 1-5: Positive samples; N: Negative control)

Fig 5: Agarose gel Electrophoresis of PCR products for detection of meca gene (L: 100 bp DNA ladder; P: Positive control; Lane 1&2: Positive samples; N: Negative control)

<table>
<thead>
<tr>
<th>Name of primers</th>
<th>Primer Sequence (5’–3’)</th>
<th>Amplicon Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuc</td>
<td>F: GCTGGCATATGTATGGAATT R: GCTTCCAGCACATTTCTCTAC</td>
<td>385</td>
<td>Shylaja et al. (2010) [20]</td>
</tr>
<tr>
<td>meca</td>
<td>F: AAA ATC GAT GGT AAA GGT TGG C R: AGT TCT GCA GTA CCG GAT TGG C</td>
<td>533</td>
<td>Lee (2003) [14]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primers (Forward and Reverse)</th>
<th>Cycling Conditions</th>
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<tbody>
<tr>
<td></td>
<td>Initial Denaturation</td>
</tr>
<tr>
<td>nuc</td>
<td>94 °C</td>
</tr>
<tr>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>meca</td>
<td>94 °C</td>
</tr>
<tr>
<td>4 min</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td>35 cycles</td>
</tr>
</tbody>
</table>
Of the 24 \( S. \) \textit{aureus} isolates identified via culture technique, PCR confirmed 21 samples out of the total isolates. Specifically, 15 chicken samples tested positive, accounting for 71.42\%, while 6 chevon samples were positive, constituting 28.57\% (Fig 4). Additionally, 2 isolates of \( S. \) \textit{aureus} from chicken samples were confirmed as MRSA (Fig 5). Zehra \textit{et al.} (2019) \[25\] and Latha \textit{et al.} (2017) \[13\] reported 17.70\% and 40\% prevalence of \( S. \) \textit{aureus} from chevon samples. Regarding chicken samples, Naas \textit{et al.} (2019) \[10\] and Zehra \textit{et al.} (2019) \[25\] reported a prevalence of 40\% and 26.50\%. Zehra \textit{et al.} (2019) \[25\] reported 2.72\% prevalence of MRSA from chicken samples. These variations in findings underscore potential differences in prevalence rates across diverse geographic regions or sample populations.

Isolates confirmed with PCR were subjected to streaking on CRA, which revealed 57.14\% (12/21) biofilm producers as they produced black colour colonies on CRA, whereas no change in colouration was observed in non-biofilm producing isolates (Fig 6). According to Ballah \textit{et al.} (2022) \[2\], among 100 \( S. \) \textit{aureus} isolates, 89\% exhibited characteristic colonies on Congo Red Agar (CRA) and were classified as biofilm producers. In contrast, Acheh \textit{et al.} (2020) \[11\] reported that all \( S. \) \textit{aureus} isolates (8) from food samples were biofilm producers, totalling 100\%. The overall results of this study are compiled in Table 3.

The significance of \( S. \) \textit{aureus} and MRSA and their role in human infection through consumption of contaminated food are emphasised in this work. We hope that this study will serve as a guide for other scientists working in related domains.

### Table 3: Overall Prevalence (%) of \( S. \) \textit{aureus} observed by Biochemical and Molecular methods

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples examined</th>
<th>No. of positive samples for ( S. ) \textit{aureus} (culturally)</th>
<th>No. of positive samples for ( S. ) \textit{aureus} (PCR)</th>
<th>Biofilm producers (CRA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>50</td>
<td>16</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Chevon</td>
<td>50</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Overall</td>
<td>100</td>
<td>24</td>
<td>21</td>
<td>12</td>
</tr>
</tbody>
</table>

The current research reveals a moderate occurrence of \( S. \) \textit{aureus} and methicillin-resistant \( S. \) \textit{aureus} in meat samples, suggesting a need for enhanced sanitary and hygienic measures in meat handling practices. Moreover, the formation of biofilms in settings associated with food processing is notably significant, as they can serve as a continuous reservoir of microbial contamination, leading to food spoilage or the transmission of diseases.

Conclusions
The current research reveals a moderate occurrence of \( S. \) \textit{aureus} and methicillin-resistant \( S. \) \textit{aureus} in meat samples, suggesting a need for enhanced sanitary and hygienic measures in meat handling practices. Moreover, the formation of biofilms in settings associated with food processing is notably significant, as they can serve as a continuous reservoir of microbial contamination, leading to food spoilage or the transmission of diseases.

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References


