Molecular Characterization of *S. Aureus* Isolated from Cases of Bovine Mastitis

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Abstract

The purpose of the investigation was to find *S. aureus* that had been isolated from raw cow's milk. 152 milk samples (42 clinical mastitis and 110 apparently healthy milk) were collected from 152 dairy cows from different regions at Damietta governates. Subclinical mastitis was found using the California mastitis test. Prevalence of mastitis was 59% (90/152). Moreover, prevalence of clinical mastitis was 21.1% (32/152) and subclinical mastitis 48.3% (58/120). To isolate bacteria, a loopful of suspicious milk was cultivated on various culture media. Following biochemical testing, PCR, and an examination of culture characteristics on several selective media, the isolated bacteria were identified. Fifty of the 152 samples tested positive for *S. aureus*. The isolated *S. aureus* was verified by PCR. The information gathered for this study helps to clarify how common *S. aureus* is in the Damietta Governorate as a cause of mastitis.

Key words: Mastitis, *S. aureus*, PCR

Introduction

Mastitis is a globally prevalent disease among dairy animals, posing serious zoonotic threats and economic burdens. It is a multifactorial condition that can manifest either clinically or sub clinically, depending on a variety of factors involving the host animal, environmental conditions, and the infectious agent ***(Tesfaye et al., 2021).*** In dairy cows, mastitis is mainly categorized into two forms: clinical mastitis, which shows evident symptoms such as abnormal milk and udder changes, and subclinical mastitis, which is harder to detect due to the absence of visible signs. Clinical mastitis is associated with noticeable inflammation in the mammary glands, often resulting in altered udder appearance and milk consistency. Clinical mastitis typically presents with redness, swelling, increased temperature, and pain in the udder, along with visible changes in the milk. In contrast, subclinical mastitis is marked by inflammation at the cellular level

without external signs or lesions, but is usually diagnosed through an increase in milk somatic cell count using the California Mastitis Test (CMT) ***(Abutarbush, 2010).*** *S. aureus*, a Gram-positive, catalase- and coagulase-positive facultative anaerobe, is one of the most common pathogens implicated in bovine mastitis due to its high contagiousness and ability to establish persistent infections ***(Perez et al., 2020; Levison et al., 2016).*** The infection process starts when *S. aureus* enters the mammary gland through the teat canal, colonizes the tissue, and spreads throughout the ductal system ***(Zecconi et al., 2006).*** The release of microbial products known as microbial-associated molecular patterns (MAMPs) triggers an immune response from mammary epithelial cells (MECs), which act as local sentinels by producing inflammatory mediators ***(Gilbert et al., 2013).***

This study was performed to determine the prevalence of both clinical and sub clinical mastitis in dairy cows in Damietta governorate using the California Mastitis Test (CMT) with isolation and Biochemical identification of *S. aureus.* All isolates were confirmed by PCR

Materials and Methods

A total of 152 milk samples (42 clinical mastitis and 110 apparently healthy milk) were collected from 152 dairy cows from different regions at Damietta Governorate. Cows were clinically examined and to find subclinical mastitis, the California Mastitis Test (CMT) was used. Before sample collection, the udders were properly cleaned and disinfected with 70% ethanol, and milk was aseptically collected from each quarter.

Isolation of *S. aureus*

Sediments from the milk samples were streaked onto Nutrient Agar, Blood Agar, Mannitol Salt Agar, and Baird Parker Agar, followed by aerobic incubation at 37°C for 24 to 72 hours. Suspected colonies of *S. aureus* were sub cultured for further biochemical and molecular identification. Final identification of suspected isolate was done based on Gram staining, catalase test, sugar fermentation and coagulase test and PCR.

Gram's staining

Gram-positive cocci appearing in grape-like clusters were presumed to be Staphylococcus species.

Catalase test

Bubbles were observed upon mixing isolates with 3% hydrogen peroxide, confirming catalase positivity.

Oxidation and fermentation test

Acid production resulting in a yellow color indicated glucose metabolism under both aerobic and anaerobic conditions, confirming facultative anaerobic nature.

Slide coagulase test (Clumping factor) (Bound coagulase):

A positive result was indicated by white clumping within 15–20 seconds upon mixing bacterial

suspension with plasma.

Molecular detection of *S. aureus* strain using multiplex PCR according to *Mehrotra et al., (2000)*

Extraction of DNA

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract DNA from samples, with some adjustments made based on the manufacturer's instructions. Oligonucleotide Primers used for confirmation of *S. aureus* by amplification of 23 srRNA (F' TATACCGGTAAGGAGTGCTGGAG' R, F' ATCAATTAACCTTCGAGCACCG' R)

Amplification via PCR

Primers were used for PCR in a 25 µl reaction that included 6 µl of DNA template, 4.5 µl of water, 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), and 1 µl of each primer at a concentration of 20 pmol.

Results

1. Prevalence of *S. aureus* in bovine mastitis

Overall prevalence of mastitis in bovine from dairy farms under investigations at Damietta Governorate was 59.2 % (90/152). Moreover, the prevalence of clinical mastitis was 21.1% (32/152) and subclinical mastitis in the examined milk sample from different quarters using CMT was 48.3% (58/120).

The total prevalence of *S. aureus* in the examined milk samples was 32.9% (50/152). All isolates were confirmed to be *S. aureus* based on conventional bacteriological tests in additionto detection of *S. aureus* 23S rRNA gene.

2.* Morphological and biochemical characters of the isolated *S. aureus

S. aureus appeared as Gram positive cocci arranged in irregular clusters (bunches of grapes). Colonies of *S. aureus* performed golden yellow color on nutrient agar also were β-hemolytic on blood agar. While give yellow colonies on mannitol salt agar

3. Coagulase activity of *S. aureus*

The development of a curd-like clotting in comparison to the negative control validated the coagulase test's positive result.

The result of coagulase activity of 50 isolated *S. aureus* showed that:

All isolates were tested by both tube and slide coagulase tests. Most of isolates gave positive reaction with both slide and tube methods (41) while some isolates were positive for one test only.

Molecular typing of retrieved *S. aureus*

The target 23SrRNA gene (1250 bp fragment) could be successfully amplified from the DNA templates of every isolated *S. aureus* strain using the improved PCR test (fig 1).

Table 1 Prevalence of clinical and subclinical mastitis based on CMT.

NO. of samples	Clinical Mastitis		Subclinical mastits	
	NO.	%	NO.	%
	32	21.1	58	48.3

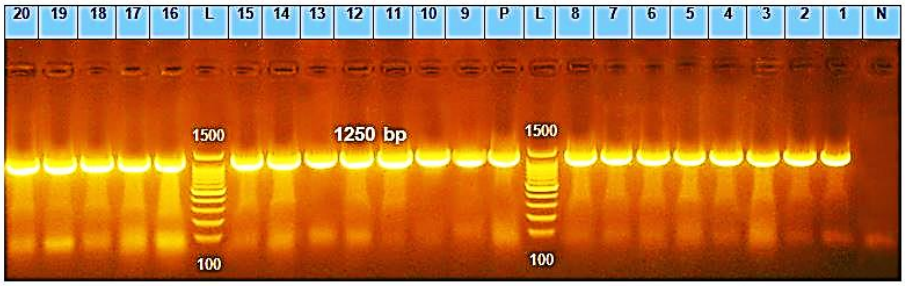

Figure (1): Electrophetic pattern of PCR amplified by 23SrRNA gene primer targting 1250bp.

Discussion

An extremely serious disease, bovine mastitis results in a marked decrease in milk output and quality, as well as financial losses for the livestock industry both in Egypt and globally ***(stevens et al., 2016).*** Affection with mastitis need collaboration between the management processes (mainly the milking) and infectious agents that widely distributed in the environment of dairy farms ***(Khairullah et al., 2020).*** Overall prevalence of mastitis in bovine from dairy farms under investigations at Damietta Governorate was 59.2 % (90/152). Moreover, the prevalence of clinical mastitis was 21.1% (32/152) and subclinical mastitis in the examined milk sample from different quarters using CMT was 48.3% (58/120). These results are closed to that recorded in other previous studies as in Nigeria (48.8%, ***Suleiman and Nahed, 2018),*** Ethiopia, (39.2%, ***Adane et al., 2017)*** and (43.6%, ***Girma and Tamir, 2022),*** and (44%, ***Algammal et al., 2020)*** in Ismailia Governorate. Meanwhile, the prevalence of mastitis in this study was lower than studies conducted in Uganda (86.2%, ***Abrahmsen et al., 2014),*** and Egypt (87.5%, ***Elsayed et al., 2015).***

One of the most common bacteria linked to both clinical and subclinical mastitis globally is *S. aureus* ***(Rainard et al., 2018; Santos et al., 2020).*** The prevalence of *S. aureus* recovered from total mastitis cases was 26.3% (40/152), and this result is nearly similar to the findings of studies conducted in Africa (36.8%, ***Suliman and Nahed, 2018).*** Meanwhile, high incidence of *S. aureus* conducted in India (79.71%, ***Neelam et al., 2022),*** Brazile (100%, ***Teixeira et al., 2014)*** and Bangladesh (100%, ***Jahan et al., 2015).*** On the other side, low

incidence was reported by ***(Alemu et al., 2013; Shen et al., 2021)*** as (13.8%) and (16.3%) respectively. Also, Iran (20.1%, ***Jamali et al., 2014).*** One important phenotypic characteristic that is used to identify *S. aureus* is coagulase production. Nearly identical results were found in the current investigation, which shows a mixed pattern with a substantial number of coagulase-positive *S. aureus* isolates (85%) and a small number of coagulase-negative isolates (15%) ***(Neelam et al., 2022).***

Conclusion

This study highlights the high prevalence of both clinical and subclinical mastitis among dairy cows in Damietta Governorate, with *S. aureus* being a major causative agent. The integration of clinical examination, CMT screening, bacteriological culture, and molecular diagnostics such as PCR provided reliable detection and confirmation of *S. aureus*. The high occurrence of coagulase-positive isolates confirms the pathogenic potential of the strains involved. These findings emphasize the significance of early detection and effective control strategies to limit the economic losses and public health risks associated with bovine mastitis.

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الملخص العربي

التوصيف الجزيئي لبكتيريا المكورات العنقودية الذهبية المعزولة من حالات التهاب الضرع البقري

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أُجريت هذه الدراسة للكشف عن المكورات العنقودية الذهبية المعزولة من حليب الأبقار الخام .جُمعت 152 عينة حليب (42 عينة التهاب ضرع سريري و110 عينات حليب سليمة ظاهريًا) من 152 بقرة حلوب من مناطق مختلفة بمحافظة دمياط .استُخدم اختبار كاليفورنيا للكشف عن التهاب الضرع دون السريري .بلغ معدل انتشار التهاب الضرع 59 % (90/152) علاوة على ذلك، بلغ معدل انتشار التهاب الضرع السريري 21.1% (152/32)، والتهاب الضرع دون السريري 48.3 % (58/120) زُرعت عينة من الحليب المشتبه به على أوساط زراعة مختلفة لعزل البكتيريا .حُددت البكتيريا المعزولة من خلال دراسة الخصائص على أوساط زراعة انتقائية مختلفة، والاختبارات الكيميائية الحيوية، وأخيرًا عن طريق اختبار البلمرة المتسلسل (PCR) .من بين 152 عينة، كانت 50 عينة إيجابية لبكتيريا المكورات العنقودية الذهبية .استُخدم تفاعل البلمرة المتسلسل (PCR) لتأكيد عزل المكورات العنقودية الذهبية .تُسهم البيانات المُستقاة من هذه الدراسة في فهم أفضل لانتشار المكورات العنقودية الذهبية كعامل مُسبب لالتهاب الضرع في محافظة دمياط.

الكلمات المفتاحية:

التهاب الضرع البقري – بكتيريا المكورات العنقودية – اختبار البلمرة المتسلسل