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Molecular detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus* isolated from dairy mastitis in Nineveh governorate, Iraq

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Abstract

Staphylococcus (*S.*) *aureus* is universally the leading aetiologic cause of dairy mastitis. Additionally, methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogenic bacterium in veterinary medicine and public health. Sixty-six cattle's milk samples were collected randomly from different areas of the Nineveh Province from November 2018 to February 2020. In this study, the classical and molecular biology methods had used to identify the MRSA and detect the target genes. The results revealed that *S. aureus* was isolated and identified based on classical methods such as catalase, clumping factors, and coagulase test. In addition, the *nuc* gene was detected in all the positive *S. aureus* isolates 23 (34.8%), while the *mecA* gene was detected in 12 (52.2%) MRSA isolates by using polymerase chain reaction (PCR) assay. The present work emerged that the results of classical methods and the PCR technique were similar. MRSA is regarded as a significant causative agent of various types of bovine mastitis in Iraq, and it can to resist all types of beta-lactams. MRSA isolated from different regions in Mosul city. PCR assay is a powerful method for detecting the different genes based on the target sequence of the specific gene.

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Introduction

Mastitis is considered a primary distributed disease in dairy herds and it is a frequent infection in dairy cows and ruminants. Mastitis causes a significant economic loss via direct and indirect costs (1). The direct costs of bovine mastitis are veterinary treatment costs and require more of the labor requirements (2). The indirect cost of clinical and subclinical bovine mastitis is the significant economic losses are reduced milk yield and quality due to mastitis (3). More than 100 types of bacteria have been detected in the mammary glands of cows, but a small number of these microorganisms cause mastitis (4). The etiology microorganisms of bovine mastitis are divided into two groups: the first group is a contagious bacterium, and the second group is an environmental bacterium according to their reservoir, source, and mode of transmission (5). Contagious or environmental bacteria infect the mammary gland because of the transmission of this bacteria from the source of contamination such as a contaminated milking machine, towels, the hands of milkers, bedding, soil, and the feces from the udder of cattle (6). Successful programs are applied to control mastitis based on the cleaning and disinfecting of all the machines, and utensils that direct contact with the surface of the teats and udder (5). *Staphylococcus* is frequently present in various habitats such as humans, animals, and plants (6). Also, it is considered a highly significant cause that triggers clinical and primarily subclinical mastitis in cattle herds (7). Furthermore, *S. aureus* is considered an essential food-borne pathogen, which is mainly responsible for the cause of food poisoning cases, and outbreaks worldwide (8).The infected cow may be a primary reservoir and source of contamination of the raw milk with *S. aureus* (9). *S. aureus* possesses more than 30

different virulence factors (10). *S. aureus* can produce different types of exotoxins (11). Contaminated milk and dairy products with *S. aureus* are seen as the primary sources to trigger food poisoning to consumers (12). *S. aureus* can resist various types of antibiotics and disinfectants (13). This variation is based on the geographical territories, the genetic characteristics of the isolates, and the type of samples (14). Moreover, the *S. aureus* isolates showed resistance to all types of β-lactam antibiotics. However, yet are applied to cure of bovine mastitis (15). Methicillin-resistant *Staphylococcus aureus* (MRSA) was documented in the 1970s and isolated from bovine mastitis (16). MRSA has the gene (*mecA*), which codes penicillin-binding protein (PBP 2a), which has an alow affinity for β-lactam antibiotics (17). Many reports showed that the MRSA strains had been isolated from animals and humans causing infections, and MRSA has been able to transfer between dairy cows and the person milking them (18). The veterinarians, farm workers, and farm animals are exposed to the potential risk by direct contact with the infected cattle by the presence of MRSA in bovine milk (19). The previous studies declared that MRSA had been isolated from domestic animals such as veal calves (20), poultry (21) , horses (22), and camel (23).

This study aimed to detect *S. aureus* isolates using the traditional and molecular techniques on a specific gene *nuc* and investigate methicillin-resistant *S. aureus* by detecting the specific *mecA* gene for MRSA.

Materials and methods

Sampling

Sixty-six samples were collected from the sub-clinical mastitis from November 2018 to February 2020 from different areas of the Nineveh Governorate. Milk samples were obtained after cleaning the teat, ant-dipping the teat, and discarding the first few streams of milk. The milk samples collected from subclinical bovine mastitis were 10 ml. All milk was placed into sterile glass vials. After dipping the teat detergent agents such as 70% alcohol were used to disinfect the teat from bacteria (24). The samples were kept on an icebox and transported to the Research Center and Laboratories, College of Veterinary Medicine, Mosul University. The milk samples were streaked onto Blood media (Lab M limited Topley house, Lancashire, United Kingdom) and Mannitol salt media 7.5% plates (118 g/L) (Lab M limited Topley house, Lancashire, United Kingdom). The culture plates were incubated at 37°C for 24 h.

Isolation and Identification of *S. aureus*

The typical *S. aureus* colonies were examined by gram staining and the traditional biochemical methods (catalase and coagulase test), and morphology (25).

DNA isolation

All the positive *S. aureus* isolates were grown on mannitol salt media for 24 h at 37°C. Genomics DNA of *S. aureus* was isolated based on manufacturer's protocol for Gram-positive bacteria, DNA of *S. aureus* isolated with the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). The amount of DNA extracted was weighted using Nanodrop (Biodrop, United Kingdom), and the DNA was stored at -20°C.

PCR Reaction

Based on the PCR assay, the specific-species *nuc* gene of *S. aureus* and the *mecA* gene of MRSA was detected. PCR interaction was implemented by the 200µl tube (Biozym, Oldenhorf, Germany) together the whole volume mishmash was 25 µL, consisting of 1 µL of each F and R primer (each ten pmol/µL), (Eurofins Genomics, Ebersberg, Germany) (Table 1). The molecular weight of the *nuc* gene is 166 bp (26), while the molecular weight of the *mecA* gene is 533 (27), 12.5 µL of 2×Go Taq Green Master Mix, and eight µL of double-distilled water (Promega). Eventually, a 2.5 µL DNA template of *S. aureus* or MRSA was added to each 200 µl tube. The amplicons were determined using gel electrophoresis with a 100 bp ladder in 2% agarose gel (Peqlab, Erlangen, Germany).

Table 1: PCR programs and Primers in detecting of *nuc* and *mecA* of *S. aureus* and MRSA

Gene	Primer	Sequence (5- 3)	Amplicon Size [bp]	Programme*	Reference
nuc	nuc-1	5-CCTGAAGCAAGTGCATTTACGA-3	166	I	(26)
	nuc-2	5-CTTTAGCCAA GCCTTGACGAACT-3			
mecA	MEC A-1	5-AAAATCGATGGTAAAGGTTGGC-3	533	II	(28)
	MEC A-2	5-AG TTCTGCAGTACCGGATTTGC-3			

PCR program: I: 35 times (94°C - 30s, 55°C - 30s, 72°C - 30s), II: 35 times (94°C - 30s, 54°C - 30s, 72°C - 30s).

Results

The number of samples used in the current study collected from different regions in the Nineveh Governorate was 66. *S. aureus* isolated from the milk samples was 23 (34.8%). All the positive *S. aureus* isolates had appeared the positive results with Gram, catalase, and coagulase tests. In addition, *S. aureus* was detected as round, golden-yellow clusters on mannitol salt media and hemolysis on the blood media. Furthermore, the study results demonstrated the *nuc*

gene, which was detected in 23 (34.8%) of *S. aureus* (Figure 1). PCR assay was similar to the result of the phenotypic determination tests. In terms of the presence and absence, the *mecA* gene was seen in 12 (52.2%) of the 23 *S. aureus* isolates (Figure 2).

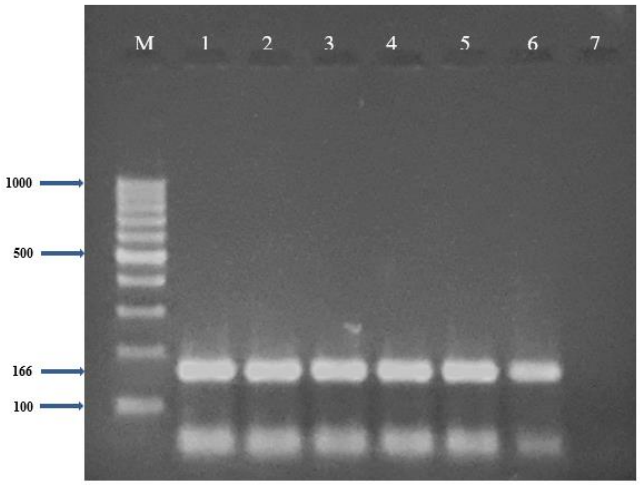

Figure 1: 2% of Agarose gel electrophoresis shows the product size of the *nuc* gene of the *S. aureus* isolates (166 bp).

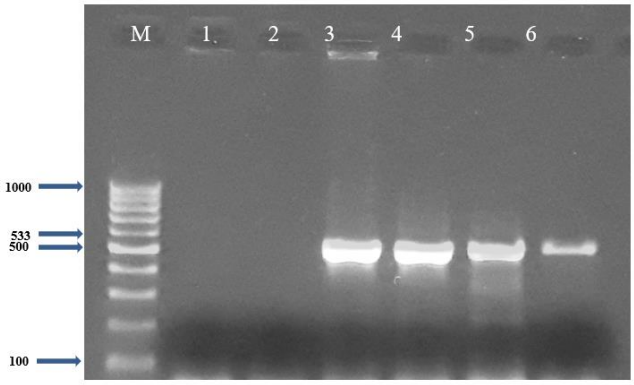

Figure 2: 2% of Agarose gel electrophoresis shows an amplicon of the *mecA* gene product of the methicillin-resistant *S. aureus* isolates (533 bp).

Discussion

For several decades, *S. aureus* has been emergency pathogenic bacteria in human and animal importance fields. The current study used classical and molecular biological methods to isolate and identify MRSA in bovine milk. This study showed that the prevalence rate of *S. aureus* isolated from milk samples was 23 (34.8%). The result of the present study was nearest to the previous studies, which reported that the prevalence rate of *S. aureus* isolated from dairy mastitis milk was 35.9% and 36.3% in Egypt (27, 28). While the result of this study was lower than other studies that reported the prevalence rate of *S. aureus* isolated from bovine mastitis milk was 74% in Egypt, 70% in Hungary, 43% in the USA (29, 30, 31). In addition, the result of the present study was higher than other studies that reported the spread rate of *S. aureus* isolated from mastitis milk was 21.8% in Germany (32), and 5.6% in Korea (33). The prevalence rate of *S. aureus* is different based on the geographical distribution and the sanitary conditions in dairy farms, dairy plants. Many previous studies appeared that the *S. aureus* has isolated from skin of udder, bedding, workers' hands, insects, and dust that played an essential role in the transmission of *S. aureus* among cattle and contaminated milk (34). In addition, the isolation of *S. aureus* from the different organs of infected cows such as vagina, muzzle, skin wounds participates in the transmission of *S. aureus* between body sited in one hand and the environments on the other hand, as well as dairy herds, moreover, from cows to their calves by way of the ventilation or by feeding of milk having *S. aureus* (35).

Furthermore, the prevalence rate of MRSA isolated from bovine mastitis milk was 12 (52.2%). The result of the current study was higher than other studies that reported the spread rate of MRSA isolate was 28.2% in Egypt (36), 15% in Belgium (37), and 0.18% in Korea (38). MRSA is colonized and proliferated in the udder leading to cause the subclinical and clinical mastitis, which causes enormous economic loss (39). MRSA is transmitted from infected cattle to the calf (20). MRSA has isolated from humans, which contact domestic animals such as veterinarians, workers, owners (19). The molecular biology techniques were used to detect the mecA gene in *S. aureus* isolate, which was encoded for the synthesis to synthesize Penicillin-binding protein based on the (PCR) assay to detect and identify the target gene in the isolates (40).

Conclusion

S. aureus is regarded as a significant pathogenic microorganism to humans and ruminants. It can cause mastitis in mammals. MRSA possesses the *mecA* gene, causing problems to humans and animals during treatment because MRSA can resist the different types of beta-lactams antibiotics. PCR is an essential method for identifying the bacteria isolates and detecting the specific gene, and PCR is faster, simpler, and more accurate.

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Conflict of interest

There is no conflict of interest.

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التشخيص الجيني لجين *mecA* في المكورات العنقودية الذهبية المقاومة للميثيسيلين المعزولة من التهاب الضرع في محافظة نينوى، العراق

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الخلاصة

تعتبر المكورات العنقودية الذهبية هي العامل المسبب الرئيسي لالتهاب الضرع البقري في جميع أنحاء العالم. بالإضافة إلى ذلك ، فان المكورات العنقودية الذهبية المقاومة للميثيسيلين هي بكتيريا ممرضة رئيسية في مجال الطب البيطري والصحة العامة. تم جمع ستة وستين عينة من حليب الأبقار بشكل عشوائي من مناطق مختلفة من محافظة نينوى خلال الفترة من تشرين الثاني ٢٠١٨ إلى شباط ٢٠٢٠. في هذه الدراسة ، تم استخدام طرق البيولوجي الجزيئية والكلاسيكية لتحديد جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين واكتشاف الجينات المستهدفة . أوضحت نتائج الدراسة الحالية أن جراثيم المكورات العنقودية الذهبية تم عزلها وتحديدها على أساس الطرق التقليدية مثل الكاتلاز وعوامل التكتل واختبار تجلط الدم. بالإضافة إلى ذلك ، تم الكشف عن جين *nuc* في جميع عزلات المكورات العنقودية الذهبية الإيجابية ٢٣ (٣٤,٨٪)، بينما تم الكشف عن جي*mecA* في ١٢ عزلة (٥٢,٢٪) من جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين باستخدام تقنية تفاعل البلمرة المتسلسل .أظهرت الدراسة الحالية أن نتائج الطرق التقليدية وتقنية تفاعل البلمرة المتسلسل كانت متشابهة. تعتبر جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين في العراق عاملاً مسببًا رئيسيًا لأنواع مختلفة من التهاب الضرع في الابقار ولها القدرة على مقاومة جميع انواع مجموعة بيتا لاكتام. تم عزل جراثيم المكورات العنقودية الذهبية المقاومة للميثيسيلين من مناطق في مدينة الموصل. تعد تقنية تفاعل البلمرة المتسلسل طريقة رئيسية للكشف عن الجينات المختلفة بناءً على التسلسل المستهدف للجين المحد.

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