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Direct detection of *Staphylococcus aureus* in camel milk in the Nineveh governorate by using the PCR technique

O.H. Sheet[1], Dh. M. Jwher[1], R.A. Al-Sanjary[1] and A.Dh. Alajami[2]

[1]Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, [2]Central Veterinary Research Laboratories, Public Authority of Agriculture Affairs and Fish Resources, Kuwait

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Correspondence:
O.H. Sheet
omar.sheet@uomosul.edu.iq

Abstract

Staphylococcus (*S.*) *aureus* is the main facultative organism of contagious intramammary infections from lactating animals. It is considered a major foodborne organism that can cause food poisoning conditions around the world. Camels are very important to the lifestyle of many countries because they can produce milk that contains the major components such as proteins, energy, vitamins, and minerals. The present study used a polymerase chain reaction (PCR) method on a base of the *nuc* gene as a target gene, which is a specific gene that recognizes the *S. aureus* amongst other microorganisms. Fifty milk samples have been collected from camels from different areas of the Nineveh Governorate, Iraq. According to the phenotypic characteristics, isolation and identification of *S. aureus* have been accomplished by characterizing the shape of the colonies, painting the suspected isolates by gram stain, using the biochemical tests such as coagulase and catalase. In this study, *S. aureus* was isolated from 70% (35/50) camel milk samples. The classical method of identifying the *S. aureus* isolated from camel milk was consistent with the PCR method. The PCR technique indicated that all positive *S. aureus* possessed the *nuc* gene. The increased percentage of *S. aureus* isolated from the camel milk has a relationship with the type of farm management, poor nutrition, and/or environmental conditions, rather than treatment of the infected camel. The PCR method is considered one of the best-used techniques to identify the *S. aureus* isolated from camel milk by detection of *nuc* gene, the specific gene of *S. aureus*.

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Introduction

Staphylococcus. aureus is the major pathogenic bacteria isolated from various hosts, including humans, and different mammals as well as birds. *S. aureus* may be present as a commensal bacterium, which has the ability to colonization and growth in both human and veterinary medicine that lead to multiple infectious diseases outbreak (1). In addition, *S. aureus* has been able to cause mastitis in camels and other mammals. Furthermore, *S. aureus* is considered a foodborne pathogen that can cause food poisoning conditions around the world as a function of several types of virulence factors such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) (2). Dairy products are considered important vehicles that can transfer different types of pathogens such as *S. aureus* (3). Camels are able to live under the poor/harsh conditions in the desert. Camels are considered as a major vehicle transmission and a source of meat and milk for the humans in different areas around the world. Additionally, camels are the source of nutrition to humans by providing meat and milk, which has energy, proteins, vitamins, and minerals (4). In the past, camels were thought to be unaffected with any type of disease that infects animals. However, several studies showed that camel milk contained a high number of pathogens. Camels play an important role as a reservoir and the carrier of various infectious pathogens and zoonotic diseases that distribute them among the livestock (5). There

are various methods, including the classical and modern methods, used to detect *S. aureus* (6). The classical methodology used for isolating and identifying pathogenic bacteria such as *S. aureus* includes microbiological media, then by biochemical tests and antimicrobial susceptibility examining. However, the classical methods may need from 48 to 72 hours to provide the final results (7). Many molecular identification methods have been utilized to recognize *S. aureus* isolated from mastitis milk. These methods are characterized as more accurate and rapid, and they identify the final decisions concerning the appropriate antimicrobial therapy (8). There are several molecular identification methods including polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), and loop-mediated isothermal amplification (LAMP). PCR has been used in identification of different pathogens (9). In addition, PCR has been applied to reveal the species-specific gene acting as the pathogenic organism in clinical microbiology central laboratories (10). The specific gene of *S. aureus* is the *nuc* gene and the molecular methods for detecting this gene helped the scientists to rapidly identify *S. aureus* in clinical samples (11).

The objective of the current study was to isolate *S. aureus* from camel milk from different areas in the Nineveh Governorate. In addition, *S. aureus* isolates were detected based on the *nuc* gene by using oligonucleotides primer sets.

Materials and methods

Camel milk samples

A total of 50 milk samples were obtained from camels randomly collected from different areas around Nineveh Governorate between May and June 2019. The samples were collected directly from the udder as following: first streams were discarded, and the subsequent milk was placed in sterile tubes and stored in the cool box (12) and transported to the Department of Veterinary Public Health, College of Veterinary Medicine, Public Health Laboratory. All milk samples were inoculated on blood agar and Mannitol salt agar 7.5% plates 118 g/L. All the culture plates were aerobically incubated at 37ºC for overnight.

Identification of bacterial isolates

Bacterial isolates were microscopically identified on a base of gram staining and cell morphology (13). Gram - positive cocci and gram - negative bacteria were biochemically identified as previously described (14).

DNA extraction and template preparation

The suspected *S. aureus* isolates were streaked on sheep blood agar. Extraction of the DNA for *S. aureus* isolates was performed using DNeasy blood and tissue kit (Qiagen, Hilden, Germany). According to the instructions of the manufacturer, mature *S. aureus* colonies were lysed in 180 µl lysis buffer consisted of lysozyme 20 mg μL^{-1} and lysostaphin 3 µl 1 mg mL^{-1}; for 30 minutes at 37ºC. Subsequently, 25µL proteinase K 20 mg mL^{-1} and 200 µL AL buffer (Qiagen) were added to the mixture, bended by the vortexer, and incubated for 30 minutes at 56ºC. Later on, the suspension was placed in DNeasy Mini spin column, 200 µl ethanol was added, and the mixture was centrifuged at 6200 × g for 1 minute. Next, the DNA in the spin column was rinsed two times: first by use of 500 µL AW1 buffer with centrifugation at 6200 × g for 1 minute, and next by 500 µL AW2 buffer with centrifugation at 17,000 × g for 3 minutes. The column spin was transfer to a 1.5 ml microcentrifuge tube, where the DNA was harvested by adding 200 µL AE buffer (Qiagen). Finally, DNA concentration was measured by use of micro-volume measurement platforms (Biodrop, United Kingdom) and stored at -20ºC.

PCR reaction

The species-specific *nuc* gene was amplified by using the PCR assay to identify *S. aureus*. Extracted *S. aureus* DNA was amplified to detect the species-specific *nuc* gene with molecular weight of 166 bp (15). The total volume of the mixture was 25 µL and consisted of: 1 µL forward primer 5-CCTGAAGCAAGTGCA TTTACGA-3 10 pmol/µL (Eurofins Genomics, Ebersberg, Germany), 1 µL of reverse primer 5-CTTTAGCCAAGCCTTGACGAACT-3 10 pmol/µL (Eurofins Genomics, Ebersberg, Germany), 12.5 µL of 2×Go Taq Green Mix Master containing (1 unit GoldStar DNA polymerase, 400 µM dNTPs, 3 µM $MgCl_2$, 20 µM $(NH4)_2SO4$, 75 µM TrisHCl (pH 8.5), yellow and blue dyes which function as loading dye (Promega Corporation, USA), 8 µL of nuclease-free water (Promega Corporation, USA), and 2.5 µL DNA template of *S. aureus*. The mixture was placed in PCR reaction tube (Biozym, Oldenhorf, Germany). The thermocycler program was set as the following: 5 minutes at 95ºC for the denaturation, 35 cycles, where each cycle consisted of denaturation 30 seconds at 95ºC; annealing 30 seconds at 54ºC; and extension 30 seconds at 72ºC, and 5 minutes at 72ºC for the final extension. Finally, the amplicons were determined by gel electrophoresis together with DNA marker 100 bp ladder in 2% agarose gel (Peqlab, Erlangen, Germany).

Results

In this study, *S. aureus* was isolated from 35 (70%) samples. All positive *S. aureus* isolates were round, golden-yellow colonies, when grown on mannitol salt agar. Furthermore, the isolates appeared hemolysis, when grown on blood agar plates. All positive isolates were, gram-positive, catalase-positive, and coagulase-positive. The concentration of extracting DNA from the *S. aureus* isolates ranged from 75 to 125 ng/µL. The PCR result indicated that the *nuc* gene was identified in 35 (70%) of the isolates (Figure 1). The results of the microbiological method for identifying *S. aureus* isolates in camel milk has correlation of 100% with the result of the PCR assay.

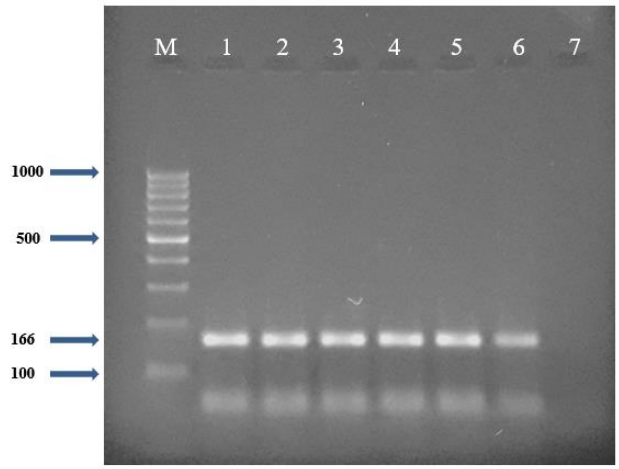

Figure 1: Identification of *nuc* gene (166 bp) in *S. aureus* using PCR technique.

Discussion

S. aureus is considered as a major cause of mastitis in camel and other mammary animals. In addition, it has the ability to produce several types of Staphylococcal Enterotoxins and thus is responsible for many food poisoning cases worldwide (16). Furthermore, *S. aureus* causes a huge economic loss which includes: inferior quality milk and decreased production, plus the therapeutic cost of treating the animals (17). There are many methods used to detect *S. aureus*, including classical methods that require about 24 hours to provide the final result. By comparison, the PCR technique is considered much faster and more accurate compared with the traditional methods. The PCR technique requires about 4 hours to identify the *S. aureus* isolates instead of 24 hours (18).

The present study revealed that 70% (35/50) of isolates were *S. aureus*. These results were in line with the result of a previous study which revealed that 75% (9/12) of *S. aureus* isolated from the camel milk was positive (19). The high incidence of mastitis could be attributable to the weak effect of the protective proteins (lysozyme, lactoferrin, lactoperoxidase, and immunoglobulin G, A) in camel milk on this bacterium (20,21). In addition, the reason for the increased rate of *S. aureus* isolated from camel milk is the presence of *S. aureus* inside the udders or on the skin. *S. aureus* was also found in soil, bedding, water, manure, and calving pads (18). In addition, the milking utensils, the workers, and their clothes can have a function in spreading *S. aureus* between camels in the herd. Isolation of *S. aureus* from the milk is considered a public health concern (22).

The results of this study showed higher *S. aureus* percentages in comparison to previous studies: where *S. aureus* was isolated from camel milk at 61.2, and 38.9% (23,24). Utilizing good management practice with proper sanitation and monitoring udder health status, in coordination with the tick control measures, can lead to the elimination of existing infections in camels (25). Furthermore, the high percentage of *S. aureus* 70% in camel's milk may be due to camel farms' failure to adopt modern camel breeding programs. Good breeding management, better living conditions, culling suspected infected camels in the herd, and prompt treatment controls reduce spreading the pathogen among the herd and transferring it to other herds in the region. Additionally, the modern technique used for detecting the pathogens that will help to rapid diagnosis the disease and the decrease the spread of the disease among the camel herds.

Conclusion

The present study recommends that camel milk is tested frequently by using the classic or the modern assays to help prevent the spread of the pathogen within camel herds. More frequent testing can be achieved utilizing the PCR assay since it is more rapid and accurate than other methods.

Acknowledgments

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Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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الكشف المباشر عن المكورات العنقودية الذهبية في حليب الإبل في محافظة نينوى باستخدام تقنية تفاعل البلمرة المتسلسل

عمر هاشم شيت١، ضياء محمد طاهر جوهر١، رعد عبد الغني بشير السنجري١ و احمد ضيدان علي العجمي٢

١فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق، ٢مختبرات البحوث البيطرية المركزية، الهيئة العامة لشؤون الزراعة والثروة السمكية، الكويت، الكويت

الخلاصة

تعد جراثيم المكورات العنقودية الذهبية المسبب الرئيسي للإصابة بالتهاب الضرع المعدية في العديد من الحيوانات المرضعة. إضافة إلى ذلك، فإنها تعتبر من العوامل المسببة للأمراض التي تنتقل عن طريق الأغذية وكذلك فإنها مسؤولة عن حالات التسمم الغذائي في جميع أنحاء العالم. تعتبر الإبل من الحيوانات المهمة جدا لنمط الحياة في العديد من البلدان وذلك لقدرتها على إنتاج الحليب الذي يعتبر مصدرا للبروتينات والطاقة والفيتامينات والمعادن. في هذه الدراسة، تعتمد تقنية تفاعل البلمرة المتسلسل على الجين *nuc*، وهو جين خاص يستعمل لتمييز والتعرف على المكورات العنقودية الذهبية عن بقية الكائنات الحية الدقيقة الأخرى. جمعت خمسون عينة من حليب الإبل من مناطق مختلفة من محافظة نينوى، العراق. وفقا لخصائص والصفات المظهرية، تم عزل المكورات العنقودية وتحديدها عن طريق المظهر الخارجي لمستعمرة المكورات العنقودية، صبغة غرام، واختبارات الكيمياء الحيوية مثل تجلط الدم واختبار الاختزال. أظهرت نتائج هذه الدراسة بان عدد جراثيم المكورات العنقودية المعزولة من حليب الإبل ٥٠/٣٥ عينات وبنسبة ٧٠٪. كانت الطرق الكلاسيكية لتحديد المكورات العنقودية الذهبية المعزولة من حليب الإبل متوافقة مع طريقة تفاعل البلمرة المتسلسل. وأظهرت تقنية تفاعل البلمرة المتسلسل أن كل المكورات العنقودية الذهبية الإيجابية كانت تمتلك الجين النووي. زيادة النسبة المئوية جراثيم المكورات العنقودية المعزولة من حليب الإبل بسبب عدم تطبيقها برامج الإدارة الجيدة، التغذية السيئة، الظروف البيئية السيئة، وكذلك عدم علاج الإبل المصابة. تعتبر طريقة تفاعل البلمرة المتسلسل واحدة من أفضل التقنيات المستخدمة للتعرف على عزلات جراثيم المكورات العنقودية في حليب الإبل من خلال الكشف عن الجين *nuc*، وهو الجين المحدد لـ جراثيم المكورات العنقودية