Iraqi Journal of Veterinary Sciences

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Detection of the *nuc* gene in *Staphylococcus aureus* isolated from swamps and ponds in Mosul city by using PCR techniques

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Article information

Article history:
Received November 15, 2021
Accepted January 19, 2022
Available online June 17, 2022

Keywords:
Rainwater
Swamps and ponds
S. aureus
PCR technique

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Abstract

In most developing countries, rainwater is considered a significant water source for drinking, washing, bathing, and cooking. On the other hand, this water is the medium for transporting microorganisms such as bacteria, viruses, parasites, and fungi to humans and animals. Most domestic and wild animals drink this kind of waterborne illness that leads to various types of diseases, which causes enormous economic losses. The current study was aimed to isolate *Staphylococcus* (*S.*) *aureus* from the swamps and ponds in various areas, including (Qawseat, Kukagle, Besan, Al-Arabi, and Al-Shlalat) that surrounding the Mosul city during the rainfall season. One hundred rainwater samples were collected from swamps and ponds in various Mosul city areas. The classical method and polymerase chain reaction (PCR) technique had used to identify *S. aureus* isolates. The present study showed that the prevalence rate of *S. aureus* isolated from swamps and ponds was 12% (12/100) based on the classical and PCR methods used. All the positive *S. aureus* isolates possess the specific-species *nuc* gene. In addition, the results of the classical methods are similar to the results of the PCR technique. The present study concludes that the water of swamps and ponds is formed by rainwater exposed to contamination by *S. aureus*, which posed in the ground and is not fit for the drinking of animals and humans.

DOI: 10.33899/ijvs.2022.173276.2069,

Introduction

Water is an inorganic compound. It is characterized as transparent, odorless, and tasteless. Water caps approximately 70.9% of the Earth's flatness, mainly in rivers, lakes, seas, and oceans. In developing countries, many people and animals drink dirty water, which lacks sanitary conditions due to distributing the disease between humans and animals (1). Waterborne pathogenic agents are transferred by a fecal-oral route (2). Feces excreted from infected animals may be transported through overland flow the rainwater to the swamps and ponds (3). Water plays a critical role in spreading the various types of microorganisms (pathogenic and non-pathogenic bacteria)that threaten the lives of humans and animals worldwide during drinking the contaminated water (4). Most bacterial pathogens are potentially transmitted by infected water to the gastrointestinal tract and are excreted in the feces of infected animals such as *Acinetobacter*, *Bacillus*, *Campylobacter*, *Escherichia coli, and Staphylococcus aureus* (5).

Staphylococcus (*S.*) *aureus* is a gram-positive bacterium, a facultative, unable to motile, unable to produce-spore, catalase- coagulase-positive, and usually arranged in grapelike irregular clusters. *S. aureus* was discovered in 1880 by Alexander Ogston, which isolated *S. aureus* in all wounds infected with microorganisms (6). *S. aureus* is one of the most essential emergent zoonotic pathogens responsible for mastitis in ruminants and food poisoning in humans (7,8). *S. aureus* can produce different types of exotoxins such as exfoliative toxins A (ETA), exfoliative

toxins B (ETB), toxic shock syndrome toxin-1 (TSST-1), panton-valentine leukocidin (PVL), and staphylococcal enterotoxins (SEs) (9). Staphylococcal enterotoxins are responsible for to cause of staphylococcal food poisoning in humans (10). *S aureus* is a primary causative agent of various types of disease in humans, such as skin lesions, osteomyelitis, endocarditis, urinary tract infections, and nosocomial infection of surgical wounds (11). *S. aureus* is considered one of the leading causes of mastitis in ruminants, which are very problematic to cure (12). In dairy manufacturing, ruminants mastitis is a significant disease that causes enormous economic losses, including inferior goodness and less milk production, incomplete butchery, veterinary and medicine costs, and loss of genetic capability (13).

The current study aims to isolate *S. aureus* from swamps and ponds from different districts in Mosul city, to assess the hygienic water for animals' consumption via the classical methods and the molecular diagnosis and characterization method of *S. aureus* isolated from swamps and ponds.

Materials and methods

Sample collection

One hundred water samples were collected in the present study from the swamps and ponds that accumulated through the rainwater season in the various regions in Mosul city (Qawseat, Kukagle, Besan, Al-Arabi, and Al-Shlalat) during the period from November 2019 to February 2020. The samples were obtained from the swamps and ponds formed by rainwater within 24 h. All samples were collected using sterile containers and then transmitted directly to laboratories of the College of Science and College of Veterinary Medicine, Mosul University, Iraq, to identify the phenotypic characterizations of *S. aureus* and extract DNA. All the samples were streaked onto Blood agar (Lab M limited Topley house, United Kingdom), and two selective media: Mannitol salt agar (Lab M limited Topley house the United Kingdom) and Vogel-Johnson agar (Lab M limited Topley house, United Kingdom). All the plates were placed into the incubator at 37°C for 24 h.

Confinement and testimony of *S. aureus*

The gram stain and the traditional biochemical methods (catalase and coagulase test) were used to identify the suspected *S. aureus* colonies and their morphology (14).

DNA extraction and Template Preparation

The purified *S. aureus* was prolefeed on the mannitol salt agar for 24 h at 37°C. Based on the manufacturer's instructions of the DNeasy blood and tissue kit (Qiagen, Hilden, Germany), the DNA of *S. aureus* was isolated by using the protocol for Gram-positive bacteria.

PCR reaction

The present study used the PCR assay to distinguish *S. aureus* by detecting the species-specific *nuc* gene (166 bp) (15). The whole volume of the mixture was 25 µL and consisted of: 1 µL primer F 5-CCTGAAGCAAGTGCA TTTACGA-3 10 pmol/µL (Eurofins Genomics, Germany), one µL of primer R 5-CTTTAGCCAAGCCTTGACGAACT-3 10 pmol/µL (Eurofins Genomics, Germany), 12.5 µL of 2×Go Taq Green Mix Master containing (Promega Corporation, USA), eight µL of nuclease-free water (Promega Corporation, USA), and 2.5 µL DNA template of *S. aureus*. The mixture was posed in a PCR reaction tube (Biozym, Oldenhorf, Germany). The thermocycler program was placed as follows: at 95ºC for 5 minutes to the denaturation, 35 cycles, where each cycle consisted of I. denaturation at 95ºC for 30 sec.; II. annealing at 54ºC for 30 seconds; III. extension at 72ºC for 30 sec., and 5 min. At 72ºC for the final extension. Finally, the amplicons were determined using gel electrophoresis and DNA marker 100 bp marker in 2% agarose gel (Peqlab, Erlangen, Germany).

Results

In the current study, the prevalence rate of *S. aureus* in swamps and ponds was 12% (12/100). The positive *S. aureus* isolates on mannitol salt agar were round and golden-yellow colonies. In addition, the positive isolates of *S. aureus* were declared hemolysis on blood agar plates and black colored colonies on Vogel-Johnson agar. Furthermore, the isolates were positive with gram stain, catalase test, and coagulase test. The PCR result showed that the *nuc* mRNA was identified in 12% of the isolates (Figure 1). The results of the classical methods for identifying *S. aureus* isolated from the water of swamps and ponds concurred with the result of the PCR technique.

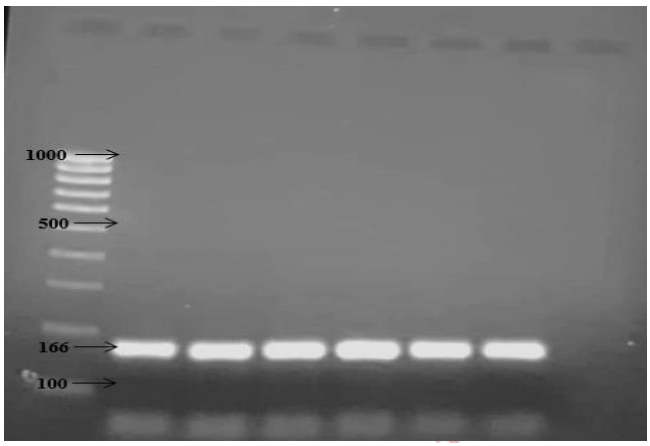

Figure 1: Agarose gel electrophoresis of PCR products: 100 bp DNA ladder, positive result at 166 bp for *nuc* gene of *S. aureus* isolates.

Discussion

Animals need water continuously to fulfill their vital works every day. Animal bodies are mostly water, so all the reactions in all systems of animals use water required as a medium. In addition, water saves the temperature of the animal's body during drinking and sweating through the skin. The most important reservoirs of zoonotic pathogen bacteria, which may be the origin of waterborne bacteria, are waste from humans and animals. During 1986-1998, many waterborne disease outbreaks had occurred due to pasturing animals near the sources of water (16). Many previous studies referred to the agreeable levels of microbiological quality in the rainwater by using the classical methods to detect the pathogenic bacteria (17). In recent years, other studies employed the PCR technique to direct the monitoring of microorganisms in environmental water. The PCR technique has several features, such as it is the more rapid, simple, cheap, and accurate approach to detect many types of pathogenic bacteria in rainwater (18). In the present study, the prevalence rate of *S. aureus* isolated from water of swamps and ponds was 12% (12/100). A previous study in Southeast Queensland, Australia, showed that the prevalence rate of *S. aureus* isolated from the rainwater tanks is 15% (19). While, the USA and Nigeria showed that the most pathogenic bacteria isolated from water of swamps and ponds were *S. aureus* (20,21).

Additionally, the previous study appeared that the prevalence rate of *S. aureus* isolated from the well water used for drinking animals and humans was 6.25% (20/144) (22). Feces excreted from infected animals by pathogen bacteria may be transported directly toward water sources through overland flow such as rainwater and sewage water (23). Moreover, most farmers had used the wastewater to plant irrigation for conserving hydrological resources that may be helped to spread the pathogenic bacteria in the environment (24). Suspended sediments may play an essential role in transporting fecal bacteria into the water (25).

Conclusion

The study concludes that the water of the swamps and ponds formed during the rainfall in the winter season in different districts of Mosul city was contaminated by *S. aureus* as a result of its transportation via rainwater from contaminated to non-contaminated areas making this water is not suitable for both animal and human consumption. Our recommendations to the owners are not to use the water of the swamps and ponds for animal consumption to avoid the spread of any zoonotic diseases among the human and animal population.

Acknowledgments

The authors acknowledge the efforts of the University of Mosul, College of Veterinary Medicine, College of Environmental Sciences and Technology, and College of Sciences for providing all the facilities to carry out the project in their laboratories.

Conflict of interest

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

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الكشف عن جين *nuc* في جراثيم المكورات العنقودية الذهبية المعزولة من البرك والمستنقعات في مدينة الموصل باستخدام تقنية تفاعل البلمرة المتسلسل

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الخلاصة

في معظم البلدان النامية، تُعتبر مياه الأمطار مصدرا رئيسيا للشرب والغسيل والاستحمام والطبخ، ومن جهة أخرى تكون هذه المياه وسيلة لنقل أنواع مختلفة من الكائنات الحية الدقيقة مثل البكتيريا والفيروسات والطفيليات والفطريات إلى الإنسان والحيوان. تشرب معظم الحيوانات الداجنة والبرية الأمراض المياه الملوثة والتي تؤدي إلى الإصابة بأنواع مختلفة من الأمراض والتي تسبب خسائر اقتصادية فادحة. هدف الدراسة الحالية هو عزل جراثيم المكورات العنقودية الذهبية من البرك والمستنقعات في مناطق مختلفة من مدينة الموصل أثناء هطول الأمطار. تم جمع مئة عينة من مياه الأمطار من البرك والمستنقعات في مناطق مختلفة شملت (القوسيات، كوكجلي، بيسان، العربي، الشلالات) التي تحيط بمدينة الموصل. تم استخدام الطريقة التقليدية وتفاعل البلمرة المتسلسل للتعرف على جراثيم المكورات العنقودية الذهبية. أوضحت الدراسة الحالية أن معدل انتشار جراثيم المكورات العنقودية الذهبية المعزولة من الأحواض والمستنقعات كان ١٢٪ (١٢/١٠٠) بناء على نتائج الطرق التقليدية وطريقة تفاعل البلمرة المتسلسل المستخدمة. تمتلك جميع عزلات المكورات العنقودية الذهبية الموجبة الجين *nuc* الخاص بجراثيم المكورات العنقودية الذهبية. بالإضافة إلى ذلك، فان نتائج الطرق الكلاسيكية كانت مشابهة لنتائج اختبار تفاعل البلمرة المتسلسل. استنتجت هذه الدراسة إلى مياه البرك والمستنقعات تتشكل من مياه الأمطار التي تعرضت للتلوث بجراثيم المكورات العنقودية الذهبية الموجودة في الأرض وان مياه تلك البرك والمستنقعات لا تصلح لشرب الحيوانات والإنسان.

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