

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

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Abstract

In most developing countries, rainwater is considered as a major source of water for drinking, washing, bathing, and cooking purposes, on the another hand, this water is the medium for transporting different types of microorganisms such as bacteria, viruses, parasites, and fungi to humans and animals. Most domestic and wild animals drink this kind of water-borne illnesses that leads to various types of diseases which causes huge economic losses. The current study was aimed to isolate *Staphylococcus (S.) aureus* from the swamps and ponds in various areas included (Qawseat, Kukagle, Besan, Al-Arabi, and Al-Shlalat) that surrounding the Mosul city during the rainfall season. A one hundred samples of rainwater were collected from swamps and ponds in various areas the Mosul city. 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 that has been exposed to contamination by *S. aureus* which posed in the ground and it is not fit for the drinking of animals and humans.

Keywords: rainwater, swamps and ponds, *S. aureus*, PCR technique

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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في معظم البلدان النامية، تُعتبر مياه الأمطار مصدرًا رئيسيًا 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 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٪ (١٠٠/١٢) بناءً 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، فإن نتائج الطرق الكلاسيكية كانت مشابهة لنتائج اختبار تفاعل البلمرة المتسلسل. استنتجت هذه الدراسة إلى مياه البرك والمستنقعات تشكل من مياه الأمطار التي تعرضت للتلوث بجراثيم المكورات العنقودية الذهبية الموجودة في الأرض وأن مياه تلك البرك والمستنقعات لا تصلح لشرب الحيوانات والإنسان.

Introduction

Water is an inorganic compound, it is characterized as transparent, odorless, and tasteless. Water caps approximately 70.9% of the Earth's flatness, mostly in rivers, lakes, seas, and oceans. In developing countries, many people and animals drink and use unhygienic water which lacks sanitary conditions due to distribute the disease between humans and animals (1). Water-borne 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 to 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 microorganism (6). *S. aureus* is regarded as one of the most important emergent zoonotic pathogen which is responsible for mastitis in ruminants and food poisoning in human (7, 8). *S. aureus* has ability to produce different types of exotoxins, such as exfoliative toxins A (ETA) and exfoliative toxins B (ETB), toxic shock syndrome toxin-1 (TSST-1), panton-valentine leukocidin (PVL), and staphylococcal enterotoxins (SEs) (9). Staphylococcal enterotoxins are responsible to cause of staphylococcal food poisoning in human (10). *S. aureus* is a major 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 as one of the main causes of mastitis in ruminants, which are very problematic to curing (12). In dairy manufacturing, ruminants mastitis is an important disease that causes the enormous economic losses which includes the 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 use 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 samples of water were collected in the present study from the swamps and ponds which 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 by using sterile containers and then transmitted them directly to laboratories of the College of Science and College of Veterinary Medicine, Mosul University, Iraq for identifying the phenotypic characterizations of *S. aureus* and extracting 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 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* were 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), DNA of *S. aureus* was isolated by using the protocol for Gram-positive bacteria.

PCR reaction

The PCR assay was used in the present study 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), 1 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), 8 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 the following: at 95°C for 5 minutes to the denaturation, 35 cycles, where each cycle consisted of: I. denaturation at 95°C for 30 sec.; II. annealing at 54°C for 30 seconds; III. extension at 72°C for 30 sec., and 5 min. at 72°C for the final extension. Finally, the amplicons were determined by using the gel electrophoresis together with 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 that appeared 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 were concorded with the result of the PCR technique.

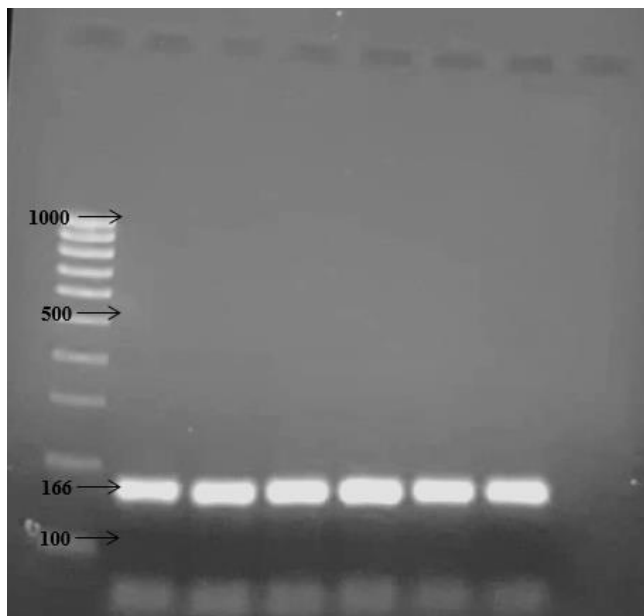


Figure 1: Agarose gel electrophoresis of PCR products: 100 bp DNA ladder, positive result at 166 bp for *nuc* gene of *S. aureus* isolates

Discussion

Animals need water continuously to fulfill their vital works every day. The constituent mostly of the bodies of animals is water so that all the reactions in all systems of the body of animal used water which is required as a media. In addition, water saves the temperature of the animal's bodies during drinking and sweating through the skin. The most importance reservoirs of zoonotic pathogen bacteria which may be the origin of water-borne bacteria are a waste from humans and animals, during

1986-1998 there are many of waterborne disease outbreaks had occurred due to pasturing animals near from the sources of water (16). Many of the previous studies referred to the agreeable levels of microbiological quality in the rainwater by using the classical methods to detect the pathogenic bacteria (17). Recent years, other studies employed the PCR technique to direct monitoring of microorganisms in environmental water, the PCR technique has several features such as it is 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, in the USA and Nigeria showed that the most pathogenic bacteria isolated from water of swamps and ponds was *S. aureus* (20), (21). Additionally, the previous study appeared that the prevalence rate of *S. aureus* isolated from the well water which 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 of farmers had used the wastewater to plants irrigation for conserving hydrological resources that may be helped to spread the pathogenic bacteria in the environment (24). Suspended sediments may play an important 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 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 not use the water of the swamps and ponds for animal consumption to avoid spread of any zoonotic diseases among the human and animal population.

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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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Article Highlights

1. The research highlights through detection of *S. aureus* from the water of swamps and ponds.
2. Detection the specific - species *nuc* gene in *S. aureus*.
3. PCR assay had used in this project, which is considered as more specific, rapid, simple, and accurate.
4. The result showed the spread of *S. aureus* in the water of swamps and ponds and the PCR method is the most important for detecting of *S. aureus*.

الملاحظات التي يتوجب عليكم تعديلها والتي لا يتم قبول البحث بدون إجرائها (عندما يتم تعديل أية فقرة أو كلمة يجب كتابتها باللون الأحمر):

- ١- يتوجب إضافة **أربعة مصادر** على الأقل من البحوث المنشورة في المجلة العراقية للعلوم البيطرية للسنتين ٢٠٢٠ و ٢٠٢١ حصراً ضمن قائمة مصادر البحث الحالي، ويمكن إدراجها في جزء المواد وطرائق العمل الخاصة بالتقنيات المتشابهة التي استخدمت في البحث الحالي (**يعد هذا المطلوب مهم ويتوجب العمل به لقبول البحث**).
- ٢- يرجى كتابة رقم ORCID للباحثين وهو مطلب أساسي والباحث الذي لا يمتلك هذا الرقم، يجب أن يسجل ويرسل الرقم مع البحث.

- ٣- إضافة البريد الإلكتروني لجميع الباحثين مع تحديد من المسؤول عن المرسلّة والذي يعرف باسم corresponding author.
- ٤- إعادة تنسيق المصادر على نسق المجلة العراقية للعلوم البيطرية، ويمكنكم العودة إلى موقع المجلة الإلكتروني من اجل الاطلاع على كيفية كتابة المصادر وتنسيقها، مع تقليل المصادر لتكون بين ٢٠ و ٢٥ مصدراً في اقصى حالة عملاً بتعليمات المجلة.

ملاحظة مهمة: يرجى إكمال التصحيحات وخلال يومين من استلامكم البحث لغرض المضي في خطوات قبول النشر اللاحقة

Galley Proof