# Study of *Staphylococcus aureus* isolated from the mouth of canary

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**Abstract**

The study was done for described genotypically characterize of Staph. aureus isolated from the oral cavity of canary birds in Mosul city using polymerase chain reaction technique which was achieved by amplifying of the thermonuclear nuc gene specialized with *Staph. aureus.* Sixty birds were examined from variable ages of both sexes from different regions of Mosul city for the period of 1/5/2018-1/6/2019 was carried out. The results indicate that 35 samples gave *Staph. aureus with* the percentage of 58.4%. These isolates are positive for pigmentation of mannitol salt agar, hemolysis on blood agar, catalase and coagulase-positive, gram staining and oxidase negative. PCR technique indicate that all 35 isolates were positive for the nuc gene and produce amplicon of 166 bp. These results considered positive and it is very specific for bacterial isolates of *staph aureus* as well as may be used for strain isolation, characterization, and differentiation from other types of bacteria.

***Keywords***: Cultural methods, PCR, Pet birds

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# دراسة جراثيم المكورات العنقودية المعزولة من فم الكناري

**صبا عبد الرحيم حسين**

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

**الخلاصة**

أنجزت الدراسة الحالية لوصف مميزات الأنماط الوراثية لجراثيم المكورات العنقودية المعزولة من التجويف الفموي لطيور الكناري في مدينة الموصل وذلك باستخدام تقنية تفاعل البلمرة المتسلسل والتي اجريت من خلال تضخيم جين النواة الحراري الخاص بجراثيم المكورات العنقودية. نفذت الدراسة بفحص ٦٠ طائر بمختلف الأعمار ولكلا الجنسين ومن مناطق مختلفة من مدينة الموصل للفترة بين ٢٠١٨/٥/١- ٢٠١٩/٦/١. شخصت النتائج ٣٥ عزلة من جراثيم المكورات العنقودية وبنسبة ٥٨,٤%، إذ كانت العزلات موجبة لإنتاج الخضاب على وسط أكار ملح المانيتول، محللة للدم على وسط أكار الدم، موجبة الكتاليز، موجبة لاختبار التجلط، موجبة لصبغة كرام وسالبة لاختبار الاوكسديز. أكدت نتائج تقنية تفاعل البلمرة المتسلسل على ان جميع العزلات المشخصة ٣٥ كانت موجبة لإنتاج جين النواة الحراري، فضلا عن إنتاج المضخم ١٦٦ زوج قاعدي واعتبرت هذه النتائج موجبة لاختبار تفاعل البلمرة المتسلسل، كما انها خاصة لعزلات جراثيم المكورات العنقودية وممكن استخدامها في العزل والتمييز والتفريق عن الأنواع الأخرى من الجراثيم.

## Introduction

Canaries was first bred in captivity in 17th century, then brought by sailor, became expensive and fashionable to breed, also breeding by rich only, thus made them very popular (1,2). In canary's disease, the diagnostic character was that the bird was very good at hiding their illness from owner; this is a part of hereditary trait, until reach the last stage of disease (3). Previously Staphylococcal infection in canary was only described in general terms of bird diseases books, but present studies thought that Staph. aureus could cause death in canaries kept in aviaries (4). Staphylococcal

food- borne infections resulting from ingestion of contaminated food with bacterial toxins of *Staph. aureus* is a great cause of food-borne disease (5, 6). Gonzales (7) showed that Staphylococcus is very serious infected disease in canary. Staphylococcal infection in bird was differ according to the site and route of infection also when enter the blood stream cause systemic infection in many organs leading to breakdown and finally sever economic losses (8). The most common infection includes those that affect the sinuses or upper respiratory tract, intestinal tract and liver, urinary and reproductive tract, and the skin including feather follicles. Polymerase chain reaction (PCR) is a rapid method for determination of *Staph. aureus*. Hence, it is considering the method of choice, in comparison with other method, which are time consuming, susceptible to contamination due to manual method and unsuitable for routine diagnostic laboratories (9).

The aim of this work is to describe genotypically characterize of *Staph. aureus* isolated from oral cavity of canary birds in Mosul city using PCR technique.

## Material and methods

### Bacterial isolation and identification

Sixty swabs were taken from oral cavity of canary bird of both sex and different ages. The sample collected from bird shops in Mosul city. The swab was transported immediately to laboratory of Microbiology, College of Veterinary Medicine, University of Mosul. Under sterile condition, cotton swabs were placed in nutrient broth for 24 hours at 37°C, A loopful from incubated broth were cultured on blood agar, milk agar and mannitol salt agar and incubated at 37°C for 24 hours, pure single colony were examined for gram staining, morphological and biochemical tests (10-12).

### DNA extraction

Isolates were cultured on blood agar for 24 hrs at 37°C. DNA extraction were carried out according to the manufactured company (Geneaid, Biotech Ltd., Registration No QAIC/TW/50077, Korea). The colonies were suspended in 200 µl of RBC lysis in water both at 60ºC overnight, Vortex 1-2 min, added 200 µl of FABG buffer, vortex 1-2 min, added 200 µl of ethanol in column and filter centrifuge 14000 rpm at 1 min, added 400 µl of wash buffer 1, centrifuge 14000 rpm at 1 min, added 600 µl of wash buffer 2, centrifuge 14000 rpm at 1 min, drying the sample by centrifugation, finally added 100 µl of Elution buffer, centrifuged for 5 min, the bacterial DNA obtained from isolates was found in supernatant which was placed into a new tube and keep under freezing until used as DNA template (9,13).

### PCR Detection of *Staph. aureus*

For PCR amplification, the mixture 20 µl which consist of 10 µl Master Mix, 2 µl $MgcL_2$, 2 µl of primer 1 (*nuc* 1), 2 µl, of primer 2 (*nuc* 2), and 4 µl of distilled water. Primers was shown in table 1 (14).

Table 1: Primers used in study

| primer | Nucleotides | |
|---|---|---|
| *nuc*-1 | 5-CCTGAAGCAAGTGCATTTACGA-3 | 166 |
| *nuc*-2 | 5-CTTTAGCCAAGCCTTGACGAACT-3 | |

This mixture was added to 4 µl of prepared DNA. The tube placed in to thermal cycling (15), with programmer of 90 minutes (denaturation at 95ºC, 30 second; annealing at 50 ºC, 30 second; extension at 72ºC, 30 second and final extension at 72°C, 5 minutes) (16). Electrophoresis were determined by adding the mixture, which consist of 10 µl of prepared DNA and 2 µl of loading buffer. This mixture added to 2 % an agarose gel containing ethidium bromide along with 3 µl of standard molecular marker (Biozym Diagnostic) (17). Finally, we saw the band under UV transilluminator (18,19). All equipment, which was used in present study, belong to one system (Biometry, Rudolf-Wisselt - Str. 30, 3707g Gottingen, Germany).

## Result

Our study indicates that, *Staphylococcus aureus* was identified as positive for pigmentation on mannitol salt agar, hemolysis on blood agar, catalase and coagulase positive, oxidase negative, and positive for gram staining. From the total of 60 swabs we were found that only 35 isolates were *Staph. aureus* 58.4%. The result of RCR revealed that the amplification of thermonuclear *nuc* gene and produce an amplicon of 166 bp, which is expected PCR product size in the isolates of *Staph. aureus*. Amplicon of *nuc* gene are shown in figure 1.

## Discussion

Staphylococci spp.cause varies infections in farm workers and broilers (22) , so the ratio of bacterial isolation of our study are nearly compatible to that of reported (23) who recorded that 53.33% from oral swabs of caged parrots, and faraway from data recorded (24) from the oral swabs of water birds 37.5%. The difference in percentage of isolation indicate the unhygienic breeding of bird and opportunistic behavior of *Staph. aureus* (25), in addition the bacteria owns many virulence factors which contribute the bacterial adhesion, invasion and colonization in bird (26-29). Others factors, including cellular immunity inhibition and condition

of nutrition (30). Production of amplicon of 166 bp, which consider a positive isolate, this reflects the suggestion of a great role of this element in the pathogenicity of *Staph. aureus* in canary.

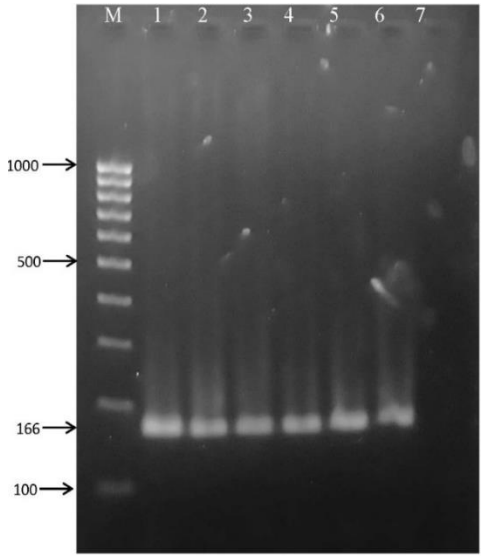


Figure 1: Agarose gel electrophoresis (2%) showing the typical amplicon of the *nuc* gene product of *Staph. aureus* isolates. The amplification of DNA appears as a ladder-like pattern. Lane 1 is the positive control, Lanes 2-6 represent positive isolates, and Lane 7 is a non-template control. Lanes M are DNA Marker 100 bp ladder (Biozym Diagnostic).

At present, the information is little about use of PCR technique for detection of *Staph. aureus* isolated from canary bird origin, so a comparable study for PCR Identified of *Staph. aureus* from bird had been determined in (6) who recorded various virulence determinant of *Staph. aureus* in captive birds. Genotypically, others (31) who isolated *Staph. aureus* from lame birds and used Random amplification of polymorphic DNA.

We wanted to be achieved a new study for identified of *Staph. aureus* genotypically in canary bird because it is very necessary to show the properties of strains in order to look more detailed about epidemiological spreading of genetic patterns which is an effect on animal and human health (32).

## Conclusion

The results of our study may help pet bird clinicians to interpret the microbiological culture and PCR results especially in canary bird, so the use of molecular methods for detection of Staphylococcal diseases in canary was very little or rare, thus we need further studies to know the sequencing of DNA and to detect the virulence determinant of *Staph. aureus* in canary bird by using PCR technique to prevent the spreading of pathogenic strain of bacteria between birds.

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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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