Iraqi Journal of Veterinary Sciences

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Isolation and molecular diagnosis of *Staphylococcus aureus* from eye infections in domestic cats in Mosul city

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Article information

Article history:
Received 24 June, 2024
Accepted 29 December, 2024
Published online 01 January, 2025

Keywords:
S. aureus
Cats
Eye infections
PCR

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Abstract

Bacterial eye infections caused by *Staphylococcus aureus* are considered one of the most important infections that affect cats and cause serious health problems. Accordingly, this research was designed to diagnose *S. aureus* from eye infections in cats by bacteria isolation and molecular methods in Mosul City, Iraq. One hundred eye swabs were collected from cat species that attended the pet clinics and were subjected to standard bacterial isolation and identification. Also, a disc diffusion method for antibiotic susceptibility was performed to determine antibiotic resistance. Further confirmation was done using polymerase chain reaction (PCR) targeting the *nuc* gene of *S. aureus* as molecular confirmation. This study showed that *S. aureus* was successfully isolated from 41/100 (41%) of the infected cats. In addition, the male cats represented 23/41 (56.1%), while the females represented 18/41 (43.9%). Furthermore, the cats aged ≥ 6 months were more susceptible to eye infections and recorded 29/41 (70.7%) than cats aged ≤ 6 months and recorded 12/41 (29.3%). The AST results showed significant antibiotic resistance, mainly against ampicillin and cefotaxime. Contrarily, all the isolates were completely susceptible to ciprofloxacin followed by levofloxacin. Also, PCR confirms *S. aureus* in all isolates with 279 bp for the *nuc* gene. In conclusion, *S. aureus* can cause eye infections in pet cats with significant importance, and the development of antibiotic resistance might have a human health impact, especially when these cats have close contact with humans.

DOI: 10.33899/ijvs.2025.151178.3741,

Introduction

Cats are one of the most common pets kept in homes worldwide. However, these animals are susceptible to different bacterial infections, including eye infections (1,2). *Staphylococcus* spp. are well-known as common pathogens isolated from different animals, including humans (3-5). Previous studies showed that cats with healthy and ocular diseases have predominating Gram-positive bacteria, especially *Staphylococcus* spp. (3). *Staphylococcus aureus* belongs to the coagulase-positive group, considered the most pathogenic species of *Staphylococci* spp. (6,7). *Staphylococcus* spp. especially coagulase-negative Staphylococci, commonly classified as opportunistic pathogens, cause no health problems under normal conditions (8). However, eye tissue trauma or damage can predispose to further infections (9). Also, viral infections such as feline herpes virus (FHV), feline panleukopenia virus (FPV) and infection with *Mycoplasma* spp. can predispose to bacterial infections (9-12). Cats' eye infections with *S. aureus* have great importance as the infection might transmitted to humans with significant public health issues (2). On the other hand, antibiotic resistance become an increasing public health concern worldwide (13-15). Pet cats are usually raised inside homes with very close contact with their owners; therefore, besides their zoonosis, they can easily transfer antibiotic resistance to close contact with humans, including veterinarians, especially when these cats

become infected with different bacterial pathogens (16). Nowadays, pet trading has flourished in Mosul city, with many cat species introduced from various sources to local animal markets, which complicates the scene. Nevertheless, few studies have been conducted to determine bacterial eye infections in cats in Mosul. To the best of our knowledge, the only conducted study related to cat bacterial eye infections in Mosul city was done by Hussein (17). She reported the isolation of different bacterial species, including *Staphylococcus* spp. using standard bacterial culture.

However, focusing on advanced molecular diagnosis tools such as polymerase chain reaction (PCR) was not introduced in such studies; also, focusing on *S. aureus* as a main cause of bacterial eye infections in pet cats did not take much importance. Accordingly, this study was designed to isolate and molecularly identify *S. aureus* from eye infections in cats in Mosul city.

Materials and methods

Ethical approval

Before starting the sample collection, an ethical approval letter (Ref, UM.VET.2024.016) was obtained. The Institutional Animal Care and Use Committee, College of Veterinary Medicine, University of Mosul, acquired the agreement.

Samples collection

One hundred eye swabs were collected from cats who attended private veterinary clinics in different areas of Mosul City, the Veterinary Teaching Hospital, the College of Veterinary Medicine, and the University of Mosul from September 2023 to March 2024. Included criteria were cats with clinically noticed eye infections with secretions. Also, a questionnaire was designed to collect all the related information, including species of cats, gender, age (two categories less and more than 6 months ago), type of infection (unilateral or bilateral) and antibiotic administration. Using disposable sterile swabs with transport media, samples were collected from the affected eyes and transferred under refrigerated conditions using a cooling ice box to the Bacteriology Laboratory for further processing.

Bacterial isolation and identification

Standard bacterial isolation protocol was followed to isolate and identify *S. aureus* from cat eye infections (18). Eye swabs were first cultured on 10% sheep blood agar (Himedia, India) and incubated at 37°C for 24 h. After that, suspected colonies were examined by Gram stain (Atom Scientific, UK) and then subcultured on Mannitol salt agar (MSA) (Himedia, India) for further confirmation. To confirm the diagnosis, positive colonies on MSA were additionally subjected to biochemical tests, including catalase, oxidase and coagulase.

Antibiotic sensitivity tests (AST)

The identified isolates were further tested for antibiotic resistance using the Kirby-Bauer disc diffusion method for antibiotic sensitivity tests (19). Briefly, 0.5 McFarland standards from each isolate were prepared using overnight bacterial culture and inoculated by sterile swab on Mueller-Hinton agar plates. The respective discs were then placed on the plates. The antibiotic discs include (gentamycin (CN 10 µg), tetracycline (TE 10 µg), amoxicillin (AX 10 µg), ampicillin (AM 25 µg), ciprofloxacin (CIP 10 µg), methicillin (ME 10 µg), doxycycline (DO 10 µg), azithromycin (AZM15 µg), vancomycin (VA 30 µg), clarithromycin (CLR 5 µg), amikacin (AK 10 µg), tobramycin (TOB 10 µg), cefotaxime (CTX 30 µg) and levofloxacin (LEV 5 µg). The plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured according to CLSI (19).

DNA extraction

All the confirmed isolates of *S. aureus* were subjected to DNA extraction using an AddPrep Bacterial Genomic purification kit (Addbio, Korea). Following the manufacturer procedure, Briefly, a few single pure colonies of an overnight bacterial culture of each *S. aureus* isolate were subjected to lysis by Lysozyme (50 mg/ml) with incubation at 37 ℃ in the water bath (Gemmy, Taiwan) for 60 minutes. After that, the tubes were subjected to centrifuge at 13,000 rpm for 4 minutes, followed by discarding the supernatant and keeping the remaining sediment. A lysis solution containing Proteinase K Solution (20 mg/ml) was added and incubated at 56 ℃ for 15 minutes. Binding Solution 200 µl and 200 µl of absolute ethanol were added respectively and mixed well, then centrifuged at 13,000 rpm for 4 minutes. The supernatant was transferred to the spin column with the collection tube and centrifuged at 13,000 rpm for 1 min. the column was washed with 500 µl of Washing 1 Solution and Washing 2 Solution, respectively, using microcentrifuge at 13,000 rpm for 1 min. Finally, the spin column was dried by additional centrifugation at 13,000 rpm for 1 min. DNA was eluted by adding 100 µl of Elution Solution to the spin column and centrifugation at 13,000 rpm for 1 min. The extracted DNA was kept at -20 °C for further assays.

Polymerase chain reaction

The *nuc* gene was used to identify the positive isolates of *S. aureus* by PCR following Rahman *et al*. (20). The primers were ordered from (Macrogen Co, Korea). The forward primer NucF: GCGATTGATGGTGATACGGTT and the reverse primer NucR: AGCCAAGCCTTGACGAACTAAAGC gave 279 bp product size. The PCR was set using 2X Master Mix (Addbio, Korea). The PCR was done using (T100 BioRad Thermocycler, USA). The conditions included polymerase activation at 95 °C for 10 min, then 38 cycles of denaturing

at 94 °C for 45 seconds, annealing at 60 °C for 45 seconds, and extension at 72 °C for 1 min. Final extension for one cycle at 72 °C for 5 min followed by holding step at 4 °C. After amplification, the PCR product was subjected to gel electrophoresis using 1.5% agarose (Addbio, Korea) and 6 µl of the DNA marker (AddBio, Korea) was used as ladder. After electrophoresis, the gel was documented to determine the expected bands.

DNA sequencing

The positive samples were sequenced according to the Sanger sequencing procedure. Four samples were also submitted to GenBank (NCBI) to obtain the accession number.

Statistical analysis

The chi-square test compared different groups using the Sigma Stat software program version 4.

Results

Eye infections in different can species were recorded among the cats who attended veterinary pet clinics (Figure 1). The bacterial isolation and identification showed successful isolation and identification of *S. aureus* using blood and MAS agar (Figure 2). The results showed that *S. aureus* recovered from 41/100 (41%) of the infected cats. Most were male cats and represented 23/41 (56.1%), while the females represented 18/41 (43.9%). Also, cats aged ≥6 months were more susceptible to eye infections and recorded 29/41 (70.7%) than cats aged ≤ 6 months and recorded 12/41 (29.3%). In addition, bilateral eye infection was predominant and recorded 26/41 (63.4%). However, unilateral infection represented 15/41 (36.6%). The AST results showed the development of significant antibiotic resistance against most of the tested antibiotics, with very high resistance against ampicillin and cefotaxime, followed by gentamycin, amoxicillin, meropenem, amikacin, azithromycin and clarithromycin. On the other hand, the isolates showed absolute sensitivity to ciprofloxacin, followed by levofloxacin (Table 1 and Figure 3). On the other hand, the results of molecular diagnosis confirm all 41/41(100) isolates as *S. aureus* with 279 bp for the *nuc* gene (Figure 4). Also, sequencing of the nuc gene of *S. aureus* (n=4) confirmed the PCR results and the four sequences were submitted successfully to the GenBank under accession no. PP836109, PP836110, PP836111, PP836112. The phylogenetic analysis of the partial sequencing of the *nuc* gene showed that our isolates had 100% similarity with Belgium isolates of *S. aureus* of human origin (Figure 5).

Figure 1: Different cat species attended pet clinics with prominent eye infections.

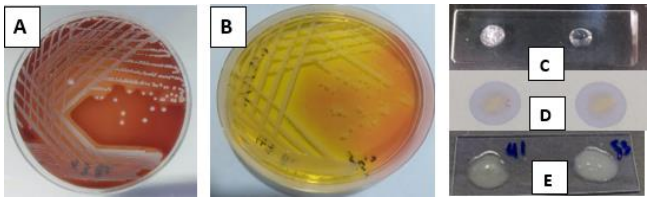

Figure 2: Bacterial isolation and identification of *S. aureus*. A: is growth on sheep blood agar. B: is growth on MSA ?C: is catalase test. D: is oxidase test. E: is slide coagulase test.

Table 1: Antibiotic resistance percentage of *S. aureus* isolates

Antibiotic (Concentration)	Resistance n (%)
Gentamycin (10 µg)	20 (48.8)
Tetracycline (10 µg)	15 (36.6)
Amoxicillin (10 µg)	22 (53.7)
Ampicillin (25 µg)	34 (82.9)
Ciprofloxacin (10 µg)	0.0 (0.0)
Methicillin (10 µg)	19 (46.3)
Doxycycline (10 µg)	12 (29.3)
Azithromycin (15 µg)	17 (41.5)
Vancomycin (30 µg)	10 (24.4)
Clarithromycin (5 µg)	17 (41.5)
Amikacin (10 µg)	19 (46.3)
Tobramycin (10 µg)	14 (34.2)
Cefotaxime (30 µg)	32 (78.1)
Levofloxacin (5 µg)	3 (7.3)

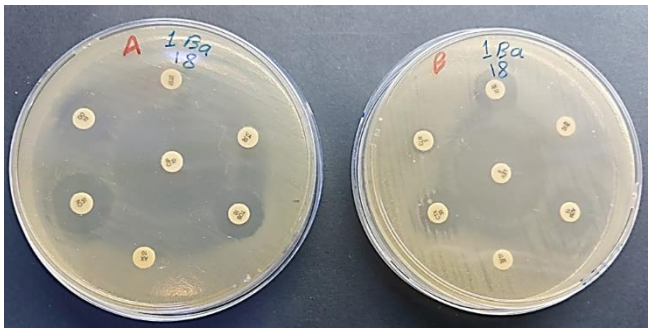

Figure 3: Antibiotic sensitivity tests of *S. aureus*.

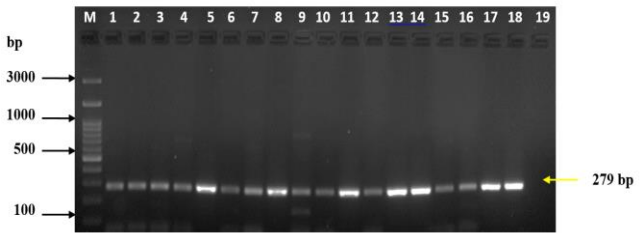

Figure 4: Gel electrophoresis for the *nuc* gene of *S. aureus* showed positive samples at wells 1-18 and well 19 negative controls.

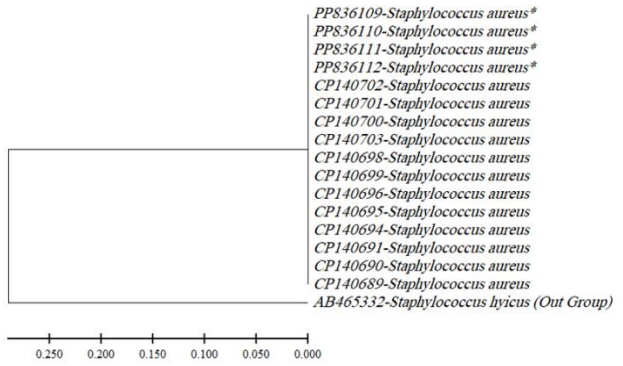

Figure 5: Phylogenic tree of *S. aureus* from Iraq (*). The phylogenic tree was constructed using MEGA11 software.

Discussion

Staphylococcus spp. is the most Gram-positive bacteria commonly isolated from feline normal eye flora (21,22). However, under certain conditions, when tissue damage occurs, the entire eye or part of it, such as the cornea or conjunctiva, or the eyelids get infected (1,23). In this study, *S. aureus* was recovered from 41/100 (41%) of the infected cats, and this was higher than the results of a previously conducted study in Mosul city by Hussein (17), where *Staphylococcus* spp isolated from 12/47 (25.5%), which may be due to close contact of the cats with their owners without proper sanitation and hygienic regime. Also, many of these pet cats were kept in small cages without properly changing the litter, which may be a potential source of infection (8). However, in a recent study by Radhy (24), conducted in Baghdad city to detect some causes of feline eye infections, he reported the presence of *S. aureus* only 4/40 (10%). Also, Cengiz (6) reported a low prevalence rate of *S. aureus* 6/200 (3%) of cats and dogs with eye discharge. The differences between our results and other studies may be due to different predisposing conditions related to eye infection, such as breed susceptibility, immune status such as compromised immune system, viral infection such as feline herpes virus (FHV) and feline calicivirus, and infection with *Chlamydophila* spp., *Mycoplasma* spp. (24-26). Also, irritation caused by allergies or overcrowded environments when cats are kept in small cages can significantly increase infection rates. Male cats represented 56.1% while the females represented 43.9% with no significant differences $p \leq 0.05$, which agreed with other studies (6,24). However, cats aged ≥6 months were more susceptible to eye infections and recorded 29/41 (70.7%) than younger cats aged ≤ 6 months and recorded 12/41 (29.3%), and this may be due to the reason that small-aged cats received more intense care during the first months rather than old-aged cats. In addition, bilateral eye infection was more predominant than unilateral infection, mainly due to the eye irritation that caused the cat to spread the infection from the affected eye to the non-affected one (20,23). This study also showed increased levels of resistance to the most common antibiotics used, especially cefotaxime, meropenem, azithromycin and clarithromycin, and these antibiotics are considered the most common antibiotics used in the treatment of different human infections (27-30). Hence, increasing antibiotic resistance of the pathogenic bacteria will limit the options for treating more complicated cases (31-33). Additional confirmation was done in this study by PCR targeting the *nuc* gene of *S. aureus* with a band size of 279 bp. Also, sequencing of the *nuc* gene of *S. aureus* confirms the PCR results are highly similar to other deposit sequences in the GenBank, mainly from humans. Tracking the source of infection using molecular tools has been adapted by different studies (34-36). Accordingly, sequencing data analysis could help understand the epidemiology of such zoonotic infections (37-41).

Conclusion

In conclusion, eye infections caused by *S. aureus* are significant in pet cats, and the development of antibiotic resistance might impact human health, especially when these cats have close contact with humans.

Conflict of interest

The authors assert the absence of any conflicts of interest.

Acknowledgements

The authors express gratitude to the members of the College of Veterinary Medicine, University of Mosul, for their collaboration.

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العزل والتشخيص الجزيئي لجراثيم المكورات العنقودية الذهبية من التهابات العين في القطط المنزلية في مدينة الموصل

نغم دريد النجماوي و إحسان منير احمد

[1]طبيب بيطري، قطاع خاص، [2]فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تعتبر التهابات العين الجرثومية الناتجة عن المكورات العنقودية الذهبية من أهم الالتهابات التي تصيب القطط وتسبب مشاكل صحية خطيرة. وعليه فقد صممت هذه الدراسة لتشخيص جراثيم المكورات العنقودية الذهبية من التهابات عيون القطط باستخدام العزل الجرثومي والطرق الجزيئية في مدينة الموصل، العراق. تم جمع ما مجموعه ١٠٠ مسحة عيون من أنواع مختلفة من القطط الواردة إلى عيادات الحيوانات الأليفة وتم إجراء العزل الجرثومي القياسي والتوصيف المظهري. كما تم إجراء اختبار الحساسية للمضادات الحيوية لتحديد مقاومة المضادات الحيوية. وتم تأكيد التشخيص أيضاً باستخدام تفاعل البلمرة المتسلسل الذي استهدف الجين *nuc* كتأكيد جزيئي. أظهرت نتائج الدراسة أنه تم عزل المكورات العنقودية الذهبية بنجاح من ١٠٠/٤١ (٤١%) من القطط المصابة. بالإضافة إلى ذلك، شكلت القطط الذكور ٤١/٢٣ (٥٦,١%) بينما شكلت القطط الإناث ٤١/١٨ (٤٣,٩%). علاوة على ذلك، كانت القطط بعمر ٦ ≤ أشهر أكثر عرضة للإصابة بالتهابات العين وسجلت ٤١/٢٩ (٧٠,٧) من القطط بعمر ٦ ≤ أشهر وسجلت ٤١/١٢ (٢٩,٣%). وأظهرت نتائج اختبار فحص الحساسية تطور مقاومة كبيرة للمضادات الحيوية بشكل رئيسي ضد الامبسلين، والسيفوتوكسيم. من جانب اخر فقد اظهرت جميع العزلات حساسية كاملة ضد السايبروفلوكساسين تليه الليفوفلوكساسين. كما أكد تفاعل البلمرة المتسلسل وجود المكورات العنقودية الذهبية في جميع العزلات بحجم ناتج ٢٧٩ كيلو دالتون للجين *nuc*. في الختام، فإن التهابات العين التي تسببها المكورات العنقودية الذهبية لها أهمية كبيرة في القطط الأليفة، وقد يكون لتطور مقاومة المضادات الحيوية تأثير على صحة الإنسان، خاصة عندما تكون هذه القطط على اتصال وثيق بالإنسان.

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