Original Article

Feline conjunctivitis in Sleman Region Indonesia: Clinical characteristics, bacteriological isolation of *Staphylococcus aureus*, and antimicrobial susceptibility

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Abstract

Conjunctivitis is a common ocular condition in domestic cats, often associated with *Staphylococcus aureus*. Accurate detection and resistance profiling are essential for appropriate therapy. This study aimed to characterize clinical features of feline conjunctivitis, identify *S. aureus* using phenotypic and genotypic methods, and assess its antibiotic susceptibility. Twenty conjunctivitis-suspected cats in Sleman, Indonesia, underwent clinical examination and conjunctival swabbing. Bacterial isolates were cultured on Mannitol Salt Agar and identified by Gram stain, catalase, coagulase, biochemical tests, and PCR targeting 23S rRNA. Susceptibility to gentamicin, chloramphenicol, ciprofloxacin, and amoxicillin was tested by Kirby-Bauer. Predominant signs included excessive tearing (95%), hyperemia (85%), and inflammation (80%). Staphylococcus spp. was detected in 95% of samples; 75% were molecularly confirmed as *S. aureus*. All isolates were sensitive to gentamicin, chloramphenicol, and ciprofloxacin, while 52.63% showed intermediate resistance to amoxicillin. *Staphylococcus aureus* is a significant bacterial agent in feline conjunctivitis in Sleman, Indonesia. Combined phenotypic–genotypic identification and susceptibility testing are vital for targeted management.

Keywords: antimicrobial resistance, bacterial identification, feline conjunctivitis, *Staphylococcus aureus*

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Introduction

Conjunctivitis is among the most commonly diagnosed ocular disorders in domestic cats and is characterized by inflammation of the conjunctiva, leading to clinical signs such as conjunctival hyperemia, chemosis, ocular discharge, excessive tearing, and discomfort. It may occur as an acute, subacute, or chronic disease and can substantially affect animal welfare (Gelatt, 2014; Stiles, 2014; Hashmi *et al.,* 2025). Bacterial involvement is frequently reported in feline conjunctivitis, with one study detecting bacterial agents in 50.75–67.65% of affected cats (Bierowiec *et al.,* 2019).

Although multiple etiologies including viral infections, environmental irritants, and allergens can trigger conjunctival inflammation, bacterial pathogens, particularly *Staphylococcus aureus*, are consistently implicated (Espínola and Lilenbaum, 1996; Hindley *et al.,* 2016; Bierowiec *et al.,* 2019; Bierowiec *et al.,* 2021; Arefin *et al.,* 2025; Miszczak *et al.,* 2025). *S. aureus* is a facultative anaerobic, Gram-positive coccus that normally inhabits the skin and mucosal surfaces of mammals and birds, but may become opportunistic under stress or immunosuppression (Thomson *et al.,* 2022). Its pathogenicity is mediated by virulence factors such as Protein A, coagulase, capsular polysaccharides, and biofilm formation, which facilitate immune evasion, tissue damage, and antibiotic resistance (Cheung and Otto, 2023; Touaitia *et al.,* 2025). The increasing emergence of antimicrobial-resistant strains, including methicillin-resistant *S. aureus* (MRSA), further elevates clinical and public health concerns due to zoonotic transmission and therapeutic challenges (Algammal *et al.,* 2020; Alghamdi *et al.,* 2023).

Accurate identification and antimicrobial susceptibility profiling are therefore critical to guide rational therapy and prevent the spread of resistant strains. Conventional phenotypic tests, such as Gram staining, catalase, coagulase, and biochemical assays, remain useful but may lack precision, whereas molecular approaches, such as PCR targeting the 23S rRNA gene, offer higher specificity and sensitivity (Abdel-moein and Samir, 2011; Aziz *et al.,* 2016).

This study aimed to (1) characterize the clinical and hematological features of affected cats; (2) isolate and identify *Staphylococcus aureus* using phenotypic and genotypic methods; and (3) determine the antibiotic resistance profile of the isolates to support informed therapeutic decision-making and to guide evidence-based antimicrobial therapy.

Materials and Methods

Study design and ethical approval: This study employed a descriptive cross-sectional design conducted from September to November 2024 in Sleman, Yogyakarta, Indonesia. Ethical clearance for the research was obtained from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (No. 85/EC-FKH/int./2024).

Sampling technique, inclusion and exclusion criteria, and sample size determination: A purposive sampling approach was applied to recruit all cats that presented clinical signs of conjunctivitis at veterinary clinics and hospitals in the Sleman region during the study period. This non-probability sampling method was deemed appropriate because the target population of cats was limited and required a clinical diagnosis by a veterinarian before inclusion. The inclusion criteria were: (1) domestic cats of any breed, sex, or age clinically diagnosed with conjunctivitis based on visible signs such as conjunctival hyperemia, ocular discharge, excessive tearing, or inflammation; and (2) cats that had not received systemic or topical antibiotic therapy within the previous 14 days. The exclusion criteria were: (1) cats with other systemic diseases that could influence ocular conditions (e.g., upper respiratory infection, dermatophytosis); (2) cats with traumatic ocular injury; and (3) uncooperative animals that could not be safely handled during sampling. Only cats with localized conjunctivitis and without evidence of systemic disease based on physical examination findings were included in this study. Hematological parameters were evaluated as part of the research analysis to characterize systemic responses in affected cats.

All ophthalmic examinations were performed by a licensed veterinarian experienced in small animal internal medicine and ophthalmology. Each cat was examined under adequate ambient light using both direct focal illumination (penlight) to evaluate the conjunctiva, cornea, and eyelids. The eyes were assessed for conjunctival hyperemia, chemosis, ocular discharge, corneal opacity, and third eyelid protrusion. Ocular reflexes were systematically evaluated, including the menace response, pupillary light reflex and palpebral reflex, to assess the integrity of ocular nerves and corneal sensitivity. The severity of conjunctivitis was graded semi-quantitatively as mild, moderate, or severe, based on the extent of conjunctival redness, chemosis, and discharge observed during clinical inspection.

During sampling, cats were gently restrained by an assistant using towel-wrapping or manual restraint to expose the affected eye, ensuring minimal stress and safe handling. When needed, topical local anesthesia (Proparacaine HCl 0.5% ophthalmic solution, two drops) was administered to reduce discomfort. Conjunctival swabs were collected aseptically using sterile cotton-tipped applicators that were gently rubbed across the lower conjunctival fornix, which is the standard site for ocular specimen collection in feline patients. Each swab was immediately placed into a sterile conical tube containing nutrient broth and transported to the laboratory for incubation and culture. A total of 20 cats were included in this study based on their availability and fulfillment of the inclusion criteria. The sample size was not determined using a formal statistical formula because the study aimed to provide a descriptive and exploratory overview, rather than inferential statistical generalization. The number of animals represented all eligible cases of feline conjunctivitis encountered during the study period (total sampling).

Clinical examination and hematology: Each cat underwent physical and ophthalmic examinations.

Clinical signs including hyperemia, inflammation, ocular discharge, lacrimation, and ocular damage were recorded. Conjunctival swab samples and blood were collected aseptically. Local anesthesia was administered when necessary to ensure the welfare of the animals during sample collection. Blood samples (0.5 mL) were collected into EDTA tubes for complete blood count (CBC), which were then analyzed using an automated hematology analyzer (BC 2800, Mindray, China).

Bacterial isolation and phenotypic identification: Conjunctival swabs were first enriched in nutrient broth (NB) and tryptic soy broth (TSB) and then incubated at 37°C for 24 h. Subcultures were made on Mannitol Salt Agar (MSA) and incubated for another 24 h at 37°C. Colonies showing mannitol fermentation were subjected to phenotypic characterization, including Gram staining; Catalase test (using 3% H_2O_2 to observe bubble formation), slide and tube coagulase tests (using rabbit plasma to detect bound and free coagulase), Sugar fermentation tests (using glucose and sucrose media), Voges-Proskauer (VP) test (to assess acetoin production). Bacteria showing Gram-positive cocci morphology in grape-shape clusters, catalase-positive, and coagulase-positive reactions were suspected to be *S. aureus*.

Genotypic identification by PCR: Genotypic confirmation of *S. aureus* was performed using PCR amplification targeting the 23S rRNA gene. Bacterial genomic DNA was extracted from suspected colonies using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taipei, Taiwan), following the manufacturer's instructions.

The PCR reaction was carried out using specific primers for the *Staphylococcus* genus and *S. aureus* species. Each reaction mixture consisted of 12.5 µL of master mix (MyTaq™ HS Red Mix, Bioline, North America, USA), 1 µL each of forward and reverse primers (10 µM), 2 µL of DNA template, and nuclease-free water to a final volume of 25 µL.

The thermal cycling conditions are shown in Table 1. PCR amplification was performed using a SelectCycler II thermal cycler (Select Bio Product, Taipei, Taiwan). The resulting PCR products were separated by electrophoresis on a 1.5% agarose gel stained with FluoroSafe DNA stain, and they were visualized under a BluPAD Dual LED Blue Light Transilluminator (Biohelix, New Taipei City, Taiwan). The primers were specifically designed to detect both the *Staphylococcus* genus and *S. aureus* species based on the 23S rRNA gene sequence.

Antibiotic susceptibility testing: Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK). The research was performed at Veterinary Technology facility, Vocational College, Universitas Gadjah Mada, Yogyakarta, Indonesia. Antibiotic disks tested included: amoxicillin (AMX), gentamicin (GEN), chloramphenicol (CHL), and ciprofloxacin (CIP). After incubation at 37°C for 24 h, the diameter of inhibition zones was measured and interpreted based on the CLSI guidelines (2022).

Data analysis: Descriptive data, including clinical signs, hematological parameters, identification results, and antibiotic susceptibility patterns, were presented as percentages and summarized in Tables. All data were analyzed using Microsoft Excel and presented in both narrative and visual formats.

Table 1 Oligonucleotide primers and PCR thermal program for amplification of *S. aureus* 23S rRNA gene.

Target Organism	Primer Name	Primer Sequence (5′-3′)	Amplicon Size	PCR Conditions
Staphylococcus spp.	Staph-F	AGG GAT GTG TTA GCG GGA A	1250 bp	94°C 5 min; 30 cycles of (94°C 1 min, 55°C 1 min, 72°C 1 min); 72°C 5 min
	Staph-R	CGC TCG TGA AGT TCT GGC TCC		
Staphylococcus aureus	Saureus-F	GCT TTA ACA CGT TGT GGT G	1070 bp	(Same as above)
	Saureus-R	AGA GTT TCA GTT CGT CCC G		

Results

Clinical findings: The clinical findings in this study are listed in Table 2. Among the 20 cats diagnosed with conjunctivitis, the most common clinical signs were excessive lacrimation (95%), conjunctival hyperemia (85%), and inflammation (80%). Ocular damage was noted in 35% of cases, while a purulent discharge was observed in 20%. However, the specific localization of these lesions, whether affecting the right eye (OD), left eye (OS), or both eyes (OU), was not documented in the clinical records, and therefore could not be analyzed in this study. To minimize discomfort during examination and sampling, two drops of topical anesthetic proparacaine hydrochloride solution (Alcaine, 0.5%; Alcon Canada, Mississauga, Ontario) were administered when necessary.

Additionally, no adverse effects or complications related to the use of this topical anesthetic were observed during sampling. Therefore, we do not expect the application of this drug to have influenced the clinical findings or the overall interpretation of the study results.

Hematological profiles: Table 3 shows the hematological examination results of the 20 cats with conjunctivitis, indicating that all parameters were within the physiological reference range, with no evidence of systemic hematological abnormalities. This finding suggests that the conjunctivitis cases were localized and did not induce systemic inflammation in the majority of cats. The hematologic values remained within normal limits, supporting the interpretation that the condition represented a localized ocular infection rather than a secondary manifestation of systemic disease.

***Phenotypic and genotypic identification of Staphylococcus* spp.:** From 20 conjunctival swab samples, 19 (95%) were positive for *Staphylococcus* spp. based on phenotypic characteristics (yellow colonies on MSA, Gram-positive cocci in clusters, catalase-positive, and coagulase-positive). Further genotypic confirmation using PCR targeting the 23S rRNA gene revealed that 15 (75%) of the isolates were *S. aureus* (Table 4).

Genotypic identification of Staphylococcus aureus: Out of 19 isolates that were phenotypically identified as *Staphylococcus* spp., 15 (75%) were confirmed as *S. aureus* using PCR targeting the 23S rRNA gene. As shown in Fig. 1, the PCR assay successfully amplified approximately 1,250 base pairs (bp) fragment specific for *S. aureus*, as visualized on a 1.5% agarose gel electrophoresis.

Antibiotic susceptibility: All 19 *Staphylococcus* spp. isolates tested for antibiotic susceptibility are listed in Table 5. All isolates were sensitive to gentamicin, chloramphenicol, and ciprofloxacin. However, 11 isolates (57.89%) showed intermediate susceptibility to amoxicillin (AMX)

Table 2 Clinical signs observed in cats with conjunctivitis (n = 20).

Clinical Sign	Number of Cases	Percentage (%)
Excessive lacrimation	19	95.0
Conjunctival hyperemia	17	85.0
Inflammation	16	80.0
Ocular damage	7	35.0
Purulent discharge	4	20.0

Table 3 Hematological profiles of cats with conjunctivitis (n = 20).

Parameter	Mean ± SD	Reference Range*
Erythrocytes ($\times 10^6/\mu L$)	8.42 ± 0.73	6 – 10
Hemoglobin (g/dL)	12.6 ± 1.02	9.5 – 15
Hematocrit (%)	35.2 ± 3.5	29 – 45
MCV (fL)	42.1 ± 2.3	41 – 54
MCH (pg)	14.8 ± 1.1	13.3 – 17.5
MCHC (g/dL)	35.2 ± 1.5	31 – 36
Leukocytes ($\times 10^3/\mu L$)	13.2 ± 2.1	5.5 – 19.5
Lymphocytes ($\times 10^3/\mu L$)	3.38 ± 0.46	1.5 – 7
Monocytes ($\times 10^3/\mu L$)	0.39 ± 0.07	0 – 0.85
Granulocytes ($\times 10^3/\mu L$)	6.23 ± 0.54	2.5 – 12.5

*Reference ranges adapted from standard feline hematology values (Tilley *et al.,* 2021).

Table 4 Identification results of bacterial isolates (n = 20).

Identification Method	*Staphylococcus* spp. Positive	*S. aureus* Positive
Phenotypic	19/20	–
PCR (23S rRNA gene)	19/20	15/20

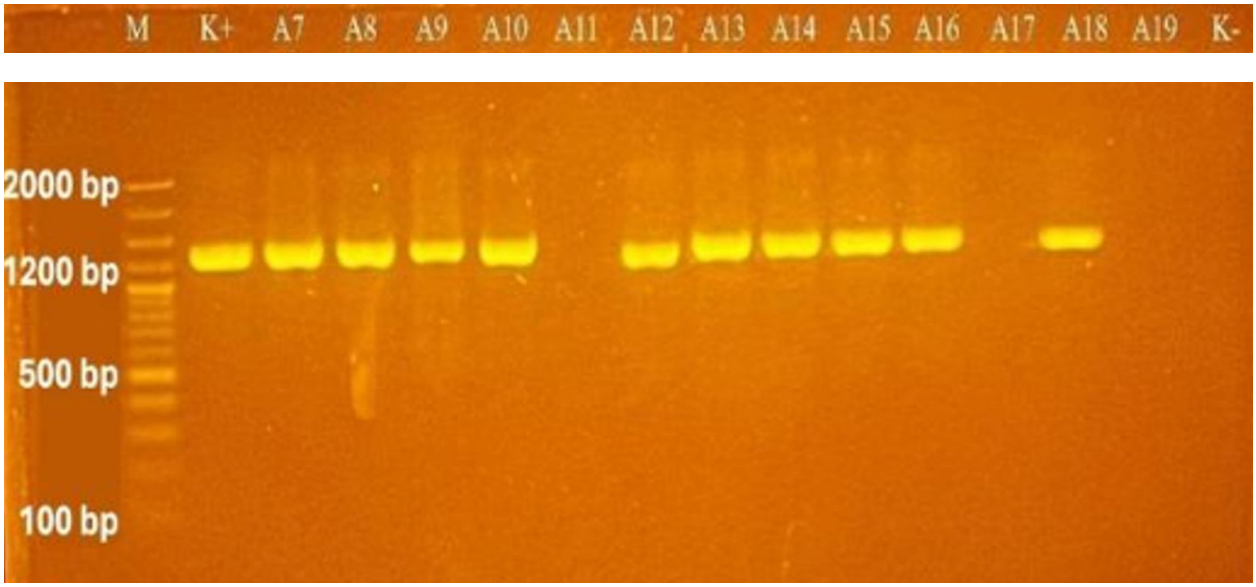

Figure 1 PCR amplification results of the 23S rRNA gene from *S. aureus* isolates. Lane M: DNA marker (100–2000 bp); K+: positive control; A7–A19: *S. aureus* isolates from cats in Sleman, Yogyakarta.

Table 5 Antibiotic susceptibility of *S. aureus* isolates from conjunctivitis cases (n = 19).

Antibiotic	Sensitive (n)	Intermediate (n)	Resistant (n)	Sensitivity Rate (%)
Gentamicin	19	0	0	100.0
Chloramphenicol	19	0	0	100.0
Ciprofloxacin	19	0	0	100.0
Amoxicillin	8	11	0	42.1 (fully sensitive)

Discussion

This study confirms *S. aureus* as one of the bacterial pathogens that can be isolated in feline conjunctivitis in the Sleman region, Indonesia. The dominant clinical signs such as excessive lacrimation (95%), conjunctival hyperemia (85%), and inflammation (80%), are consistent with those described in previous reports of bacterial conjunctivitis in cats (Bierowiec *et al.,* 2019; Miszczak *et al.,* 2023). These findings reinforce that *S. aureus*, although a commensal in healthy animals, can become opportunistic and pathogenic under certain host or environmental conditions (Espínola and Lilenbaum, 1996). However, it is important to interpret these findings cautiously, as other well-known causes of feline conjunctivitis such as *Mycoplasma* spp., *Chlamydia felis,* and *Feline herpesvirus type-1* (FHV-1) were not specifically excluded in this study.

The isolation of *Staphylococcus* spp. in 95% of cases and the molecular confirmation of *S. aureus* in 75% aligns with global trends indicating the high prevalence of this organism in feline ocular infections (Bierowiec *et al.,* 2016; Drougka *et al.,* 2016; Locke *et al.,* 2025). Such high detection rates, particularly in shelter or community cats, may result from increased stress, poor hygiene, or immunosuppression, which promote bacterial colonization and infection (Bierowiec *et al.,* 2016; Bijoy *et al.,* 2025).

Hematological analysis in this study revealed that all cats with conjunctivitis exhibited blood parameters within the physiological reference range (Tilley *et al.,* 2021). Erythrocyte count, hemoglobin level, hematocrit, leukocyte count, and platelet values showed no significant deviations, suggesting the absence of a systemic hematologic response to the ocular infection. This supports the notion that conjunctivitis caused by *S. aureus* in these cases was a localized ocular infection, without involving systemic inflammation or hematopoietic disruption.

Comparable results were reported in Indonesia, where cats with confirmed bacterial conjunctivitis showed normal hematological profiles unless the infection had spread systemically or was complicated by other conditions. That study also noted that significant hematological changes, such as leukocytosis, occurred only in severe ocular infections involving ulceration (Adnyana *et al.,* 2025). In the current study, the normal eosinophil values further suggest that the conjunctivitis was not associated with allergic or parasitic causes. This contrasts with cases of feline eosinophilic conjunctivitis or hypersensitivity reactions, where eosinophilia is typically present (Allgoewer *et al.,* 2001). The hematological findings indicate that the infections were uncomplicated and localized, supporting the use of targeted ocular treatment without systemic therapy. They also highlight the value of hematological testing in differentiating bacterial conjunctivitis from viral, allergic, or systemic infections.

The diagnostic workflow combining phenotypic and molecular identification was essential for achieving accurate and specific results. Phenotypic traits such as Gram staining, catalase, and coagulase tests remain standard, but PCR targeting the 23S rRNA gene provided definitive identification, particularly in distinguishing *S. aureus* from coagulase-negative staphylococci. The presence of a ~1250 bp amplicon in most isolates confirmed the high specificity of the

primers used and supported the reliability of PCR as a diagnostic tool for accurate identification of *S. aureus*. These results are consistent with previous molecular diagnostic studies in veterinary microbiology, reinforcing the role of 23S rRNA-based PCR as a gold standard for species-level confirmation (Koupahi *et al.,* 2016; Prihandani *et al.,* 2024). The genotypic confirmation provided strong evidence supporting the phenotypic findings and minimized the risk of misidentification, especially in samples where biochemical reactions were ambiguous.

Antibiotic susceptibility tests showed 100% sensitivity of *S. aureus* isolates to gentamicin, chloramphenicol, and ciprofloxacin, which is encouraging. However, the intermediate susceptibility to amoxicillin observed in 57.89% of isolates indicates a potential for emerging resistance. This is particularly concerning given the widespread use of beta-lactam antibiotics in veterinary practice (Prescott, 2013; Pandey and Cascella, 2023; Araujo *et al.,* 2024). Further studies incorporating molecular detection of FHV-1, Chlamydia felis, and Mycoplasma spp. are necessary to fully elucidate the multifactorial etiology of feline conjunctivitis and to define the role of *S. aureus* in its pathogenesis.

Zoonotic Implications and One Health Context: The isolation of *S. aureus* from cats, particularly from sites with frequent human contact such as the eye, has important zoonotic implications. *S. aureus* is well recognized as a pathogen capable of crossing species barriers, and pets may serve as reservoirs, vectors, or even victims of human-origin strains. Direct contact during petting, grooming, or treating infected cats may facilitate the transmission of pathogenic or antibiotic-resistant strains between animals and humans, especially in immunocompromised individuals or veterinary personnel. Moreover, the concern extends beyond methicillin-sensitive *S. aureus* (MSSA). The global emergence of methicillin-resistant *S. aureus* (MRSA) in companion animals has heightened awareness of bidirectional transmission between pets and humans. Multiple studies have documented MRSA colonization or infection in cats, dogs, and even their owners, with identical strain genotypes confirmed by molecular typing. Although MRSA detection was beyond the scope of this study, the high rate of intermediate resistance to amoxicillin suggests the need for future screening of resistance genes, such as mecA and blaZ (Bierowiec *et al.,* 2016; Drougka *et al.,* 2016; Locke *et al.,* 2025). Furthermore, considering the limitations of the present study, future research should employ cefoxitin and/or oxacillin to improve the accuracy of phenotypic MRSA determination.

This highlights the relevance of the One Health approach, which emphasizes the interconnectedness of human, animal, and environmental health. Surveillance of antimicrobial resistance in companion animals is critical not only for veterinary treatment planning but also for preventing the zoonotic spread of resistant pathogens within the broader community (Locke *et al.,* 2025). In countries like Indonesia, where companion animals increasingly live in close proximity to humans, stronger integration between veterinary and public health surveillance is warranted.

The findings of this study highlight the presence of *Staphylococcus aureus* as an associated bacterial isolate in feline conjunctivitis, emphasizing its potential clinical and epidemiological importance. Beyond its local veterinary relevance, the possible cross-species transmission of *Staphylococcus* spp., including *S. aureus*, between cats and humans warrants further investigation. Compared with earlier local studies that focused solely on phenotypic identification, the present research provides a more comprehensive overview by incorporating clinical, hematological, microbiological, molecular, and pharmacological dimensions. These findings not only serve as baseline data for future regional surveillance but also promote evidence-based antimicrobial use in veterinary ophthalmology. Given that domestic cats often live in close proximity to humans—sharing living spaces, furniture, and direct physical contact—they may act as both sentinels and sources of zoonotic pathogens such as *S. aureus*. This underscores the critical importance of early diagnosis, responsible antibiotic use, and integrated surveillance strategies to minimize the risk of cross-species transmission in both household and clinical settings.

In conclusion, this study demonstrated that *Staphylococcus aureus* is the predominant bacterial pathogen in feline conjunctivitis cases in Sleman, Yogyakarta, Indonesia. Among 20 conjunctival swab samples collected, 19 (95%) were phenotypically identified as *Staphylococcus* spp., and 15 (75%) were genotypically confirmed as *S. aureus* through PCR targeting the 23S rRNA gene. This highlights the value of combining classical microbiological methods with molecular diagnostics for accurate identification of ocular pathogens.

All *S. aureus* isolates showed complete sensitivity to gentamicin, chloramphenicol, and ciprofloxacin, whereas 57.89% exhibited intermediate susceptibility to amoxicillin, suggesting the early development of resistance. These findings are clinically significant, particularly given the widespread use of beta-lactam antibiotics in veterinary practice.

Considering the close humans and companion animals interaction, the detection of potentially resistant *S. aureus* in cats underscores a zoonotic risk. Routine implementation of antimicrobial susceptibility testing, prudent antibiotic use, and molecular surveillance is essential to guide appropriate therapy and prevent cross-species transmission, in alignment with the One Health approach

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