

Antimicrobial Resistance Profiles of Bacterial Conjunctivitis Isolates from a Secondary Hospital in Shanghai: A 5-Year Retrospective Study (2020–2024)

Weihong Xu¹, YiTing Yao², Yifei Jia², LiLun Jiang¹, Lei Li¹, Yunqi Pan², Yanan Lai²

¹Department of Clinical Laboratory, Shibe Hospital, Shanghai, People's Republic of China; ²Department of Clinical Laboratory, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

Correspondence: Weihong Xu, Department of Clinical Laboratory, Shibe Hospital, No. 4500 Gonghexin Road, Jing'an District, Shanghai, 200435, People's Republic of China, Email xu_weihong@163.com

Purpose: To analyze the pathogen distribution, epidemiological characteristics, and antimicrobial resistance patterns of bacterial conjunctivitis in a Shanghai secondary hospital from 2020 to 2024, providing evidence for clinical treatment optimization.

Patients and Methods: Conjunctival swab specimens from patients clinically diagnosed with bacterial conjunctivitis were collected between January 2020 and December 2024. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system and Kirby-Bauer disk diffusion method, respectively. Statistical analyses were conducted using WHONET 5.6 and SPSS 26.0.

Results: Among the 611 specimens, 58 bacterial isolates were identified (9.5% positivity rate). Gram-positive cocci predominated (70.7%, 41/58), primarily *Staphylococcus epidermidis* (21 strains) and *Staphylococcus aureus* (10 strains). Gram-negative bacilli accounted for 15.5% (9/58), including *Pseudomonas aeruginosa* and multidrug-resistant *Acinetobacter baumannii*. A significant seasonal variation was observed, with higher incidence in summer-autumn (72.4%) than in winter (12.1%, $P=0.005$). Cases in 2023–2024 nearly doubled those in 2020–2022 (65.5% vs 34.5%). The detection rate of *S. aureus* increased significantly annually ($P=0.043$). High resistance rates were observed among Gram-positive cocci to penicillin (89.5%), oxacillin (60.5%), and erythromycin (55.3%). All Gram-positive isolates remained susceptible to vancomycin, linezolid, and tigecycline. Gram-negative isolates exhibited 100% resistance to ampicillin, with *A. baumannii* demonstrating pandrug-resistance.

Conclusion: Gram-positive cocci, particularly *Staphylococcus* spp. were the predominant pathogens in bacterial conjunctivitis, with an increasing trend of *S. aureus* and multidrug-resistant *A. baumannii*. The significant seasonal pattern and high resistance to first-line antibiotics emphasize the necessity for culture-guided therapy and enhanced antimicrobial stewardship in ocular infections.

Plain Language Summary:

Why we did this study

Bacterial conjunctivitis (pink eye) is a common eye infection. We noticed that standard antibiotics sometimes fail to clear these infections in Shanghai. This study aimed to identify the bacteria causing conjunctivitis and track their changing responses to antibiotics over time.

What we did

We examined 611 eye swabs from patients with bacterial conjunctivitis between 2020 and 2024. Laboratory tests helped us identify the bacteria and determine which antibiotics effectively treated them.

What we found

Staphylococcal infections were common: Most cases (70.7%) involved staphylococcus bacteria, especially *Staphylococcus epidermidis*. Over half of these bacteria resisted common antibiotics like penicillin.

Seasonal patterns emerged: Infections occurred more often in warm months (June–November) than in winter. Cases nearly doubled in 2023–2024 compared to earlier years.

Effective treatments exist. While resistance to first-line antibiotics was high, all tested bacteria remained susceptible to certain reserve antibiotics like vancomycin and linezolid.

Why this matters

Our findings suggest that doctors can improve treatment by considering local resistance patterns when prescribing eye drops. Patients can support recovery by completing prescribed treatments and maintaining good hand hygiene, especially in summer. This research underscores the need for continuous monitoring of antibiotic resistance in eye infections to guide effective treatment strategies.

Keywords: microbial sensitivity, drug resistance, eye infections, seasonal variation, bacterial pathogens

Introduction

The ocular surface microbiota represents a dynamic equilibrium between bacterial communities and host ocular tissues,¹ playing a pivotal role in maintaining ocular surface homeostasis and defending against pathogenic invasion.² Under physiological conditions, the eye's defense mechanisms – including mechanical barriers, antimicrobial peptides (eg, defensins, cathelicidins), and immune regulation – effectively suppress excessive microbial proliferation. However, when local or systemic factors disrupt this delicate balance, opportunistic pathogens can breach these defenses, leading to infectious conjunctivitis.

As the most prevalent ocular infection, bacterial conjunctivitis requires prompt and effective intervention. Left untreated, it may progress to serious complications including keratitis, endophthalmitis, and even irreversible vision loss.^{3,4} Recent years have witnessed a concerning rise in antimicrobial resistance among ocular pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant Gram-negative bacilli. This trend has significantly increased the risk of empirical treatment failure.

In this context, systematic analysis of regional pathogen profiles and resistance patterns becomes crucial for developing personalized treatment regimens and optimizing antimicrobial stewardship programs. This retrospective study analyzes microbiological surveillance data from 2020 to 2024 at an ophthalmic center in Shanghai, aiming to provide contemporary evidence for clinical decision-making.

Patients and Methods

Bacterial Isolates and Inclusion Criteria

This retrospective study was conducted at Shibe Hospital, a large regional general hospital in Shanghai's Jing'an District. The hospital provides comprehensive medical services to a population of nearly 400,000 residents.

The study examined conjunctival swab specimens obtained from patients diagnosed with bacterial conjunctivitis at the hospital's Ophthalmology Center between January 2020 and December 2024. Laboratory procedures (bacterial culture and identification) were performed at the time of specimen collection as part of routine clinical care. The data for this study were retrospectively analyzed from these existing laboratory records. Eligible specimens met the following criteria:^{5,6} (1) clinical diagnosis of bacterial conjunctivitis (2) no antibiotic use within 48 hours before specimen collection, and (3) only the first isolate from each patient was included to avoid duplication. Specimens were excluded for insufficient volume, transport delays exceeding 2 hours, or contamination (eg, mixed growth of multiple Gram-negative bacilli). Out of the 611 qualified specimens, 58 bacterial isolates were identified, yielding a positivity rate of 9.5%. This rate may reflect factors such as prior antibiotic use, viral etiology, or sampling timing relative to disease onset.

Microbiological Procedures

Conjunctival swab specimens were processed and cultured according to the standardized methodologies outlined in the National Guide to Clinical Laboratory Procedures (National Health Commission of the People's Republic of China, 2015).⁷ Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system (bioMérieux, Marcy-l'Étoile, France). Gram-positive and Gram-negative microorganisms were differentiated using specific identification cards (GP and GN, respectively), with incubation maintained at 35±2°C in a 5% CO₂ atmosphere for 18–24 hours. Antimicrobial susceptibility testing followed the latest Clinical and Laboratory Standards

Institute (CLSI) guidelines supplemented by the disk diffusion method (OXOID, UK) for specific antimicrobials.⁸ Our testing panel included antibiotics commonly used for conjunctivitis treatment in our region: fluoroquinolones, aminoglycosides, macrolides, and β -lactams. Quality control strains included *E. coli* ATCC 25922 and ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 25923 and ATCC 29213, *S. pneumoniae* ATCC 49619, ensuring standardized testing procedures throughout the study. Bacterial species names are italicized throughout the text (*Staphylococcus aureus*).

Statistical Analysis

Statistical analyses were performed using WHONET 5.6 (WHO Collaborating Centre for Surveillance of Antimicrobial Resistance) for data management and initial resistance pattern analysis, along with SPSS 26.0 (IBM Corp.) for advanced statistical computations. We employed descriptive statistics to characterize pathogen distributions and χ^2 -tests with Yates' correction to evaluate seasonal variations in infection rates. Temporal trends in antimicrobial resistance patterns were assessed using the Cochran-Armitage trend test. A two-sided p-value of <0.05 was considered statistically significant.

Results

Bacterial Distribution

A total of 58 bacterial isolates were cultured from conjunctival specimens, with Gram-positive cocci predominating (70.7%, 41/58). Among these, coagulase-negative staphylococci (CoNS) were most prevalent (68.3%, 28/41), primarily represented by *Staphylococcus epidermidis* (36.2%, 21/58). Other CoNS species included *S. simulans* (3 isolates), *S. xylosum*, *S. hominis*, *S. warneri*, and *S. haemolyticus* (1 isolate each). *Staphylococcus aureus* accounted for 17.2% (10/58) of total isolates. Other Gram-positive cocci included *Streptococcus pneumoniae* and *S. parasanguinis* (3.4% and 1.7%, respectively) (Table 1 and Figure 1).

Gram-negative bacilli constituted 15.5% (9/58) of isolates, featuring clinically relevant species such as *Pseudomonas aeruginosa* (5.2%, 3/58) and multidrug-resistant *Acinetobacter baumannii* (3.4%, 2/58). The remaining Gram-negative isolates included *Escherichia coli* (2 cases), *Morganella morganii*, and *Citrobacter koseri* (1 case each). Other isolates (13.8%, 8/58) included *Corynebacterium* spp., *Bacillus cereus*, and unclassified Gram-positive bacilli.

Table 1 Distribution of Pathogenic Bacteria in Bacterial Conjunctivitis (2020–2024, n=58)

Organism	No. of Strains	Percentage (%)
Gram-positive cocci	41	70.7
<i>Staphylococcus epidermidis</i>	21	36.2
<i>Staphylococcus aureus</i>	10	17.2
<i>Staphylococcus simulans</i>	3	5.2
<i>Streptococcus pneumoniae</i>	2	3.4
<i>Streptococcus parasanguinis</i>	1	1.7
Other CoNS*	4	6.9
Gram-negative bacilli	9	15.5
<i>Pseudomonas aeruginosa</i>	3	5.2
<i>Escherichia coli</i>	2	3.4
<i>Acinetobacter baumannii</i>	2	3.4
Other Gram-negatives**	2	3.4
Other isolates***	8	13.8
Total	58	100.0

Notes: Bold values represent category totals. *Other CoNS: *Staphylococcus xylosum*, *S. hominis*, *S. warneri*, *S. haemolyticus* (1 each). **Other Gram-negative: *Morganella morganii*, *Citrobacter koseri* (1 each). ***Other isolates: *Corynebacterium* spp. (2), *Bacillus cereus* (1), unclassified Gram-positive bacilli (5).

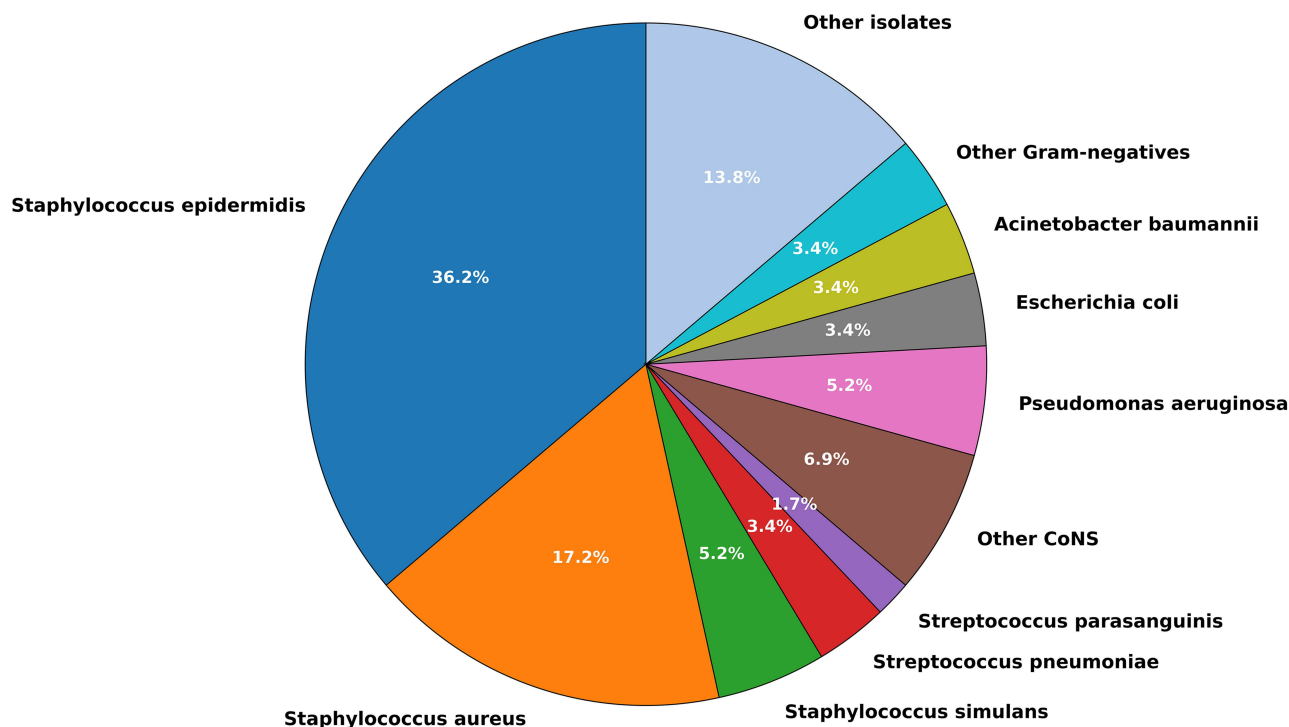


Figure 1 Distribution of Pathogenic Bacteria in Bacterial Conjunctivitis (2020–2024, N=58).

Notes: The pie chart illustrates the proportional distribution of bacterial isolates from conjunctivitis cases, showing the predominance of Gram-positive cocci (70.7%) over Gram-negative bacilli (15.5%) and other isolates (13.8%).

Epidemiological Characteristics

Our analysis revealed significant temporal and seasonal patterns in bacterial conjunctivitis cases. The annual case burden demonstrated a progressive increase from 4 cases (6.9%) in 2020 to 21 cases (36.2%) in 2024, with the 2023–2024 period accounting for 65.5% of total cases – nearly double the cumulative incidence of the preceding three years (34.5%). Trend analysis identified a statistically significant annual increase in *Staphylococcus aureus* isolation rates (0% in 2020 to 28.6% in 2024; $P=0.043$), while the proportion of *S. epidermidis* isolates remained stable (25.0–44.4%; $P=0.812$) (Table 2 and Figure 2).

A pronounced seasonal distribution was observed, with 72.4% of cases (42/58) occurring during summer–autumn months compared to only 12.1% (7/58) in winter ($\chi^2=8.04$, $df=1$, $P=0.005$). Summer months (June–August) accounted for 41.4% of cases ($n=24$), predominantly caused by *S. aureus* ($n=8$) and *S. epidermidis* ($n=10$). The autumn period (September–November) represented 31.0% of cases ($n=18$), featuring notable isolates including all *Pseudomonas aeruginosa* cases ($n=3$) and *Acinetobacter baumannii* ($n=2$) (Table 3 and Figure 3).

Antimicrobial Resistance Patterns

The antimicrobial resistance profiles revealed concerning patterns among ocular isolates. Among Gram-positive cocci ($n=38$), we observed alarmingly high resistance rates to first-line agents: penicillin (89.5%), oxacillin (60.5%), and erythromycin (55.3%) (Table 4 and Figure 4). Notably, *Staphylococcus aureus* isolates demonstrated 60.0% resistance to tetracycline.

Table 2 Temporal Distribution of Major Pathogen Isolation (2020–2024, n=58)

Organism	2020 (n=4)	2021 (n=7)	2022 (n=9)	2023 (n=17)	2024 (n=21)	P (trend)
<i>Staphylococcus epidermidis</i>	1 (25.0%)	3 (42.9%)	4 (44.4%)	6 (35.3%)	8 (38.1%)	0.812
<i>Staphylococcus aureus</i>	0 (0.0%)	1 (14.3%)	1 (11.1%)	4 (23.5%)	6 (28.6%)	0.043*

Notes: P-values calculated using Cochran-Armitage trend test (two-sided). *Statistical significance ($P<0.05$).

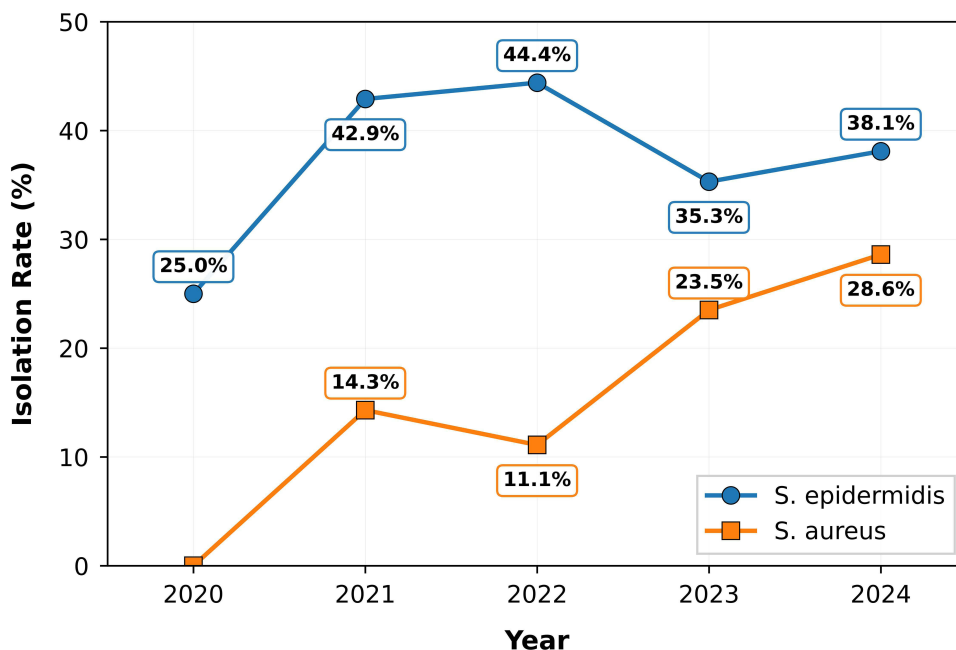


Figure 2 Temporal Trends in Isolation Rates of Major Ocular Pathogens (2020–2024).

Notes: Line graph demonstrating the annual variation in isolation rates of *Staphylococcus epidermidis* and *Staphylococcus aureus* over the five-year study period. A statistically significant increasing trend was observed for *S. aureus* ($P=0.043$).

Methicillin-resistant strains were prevalent, with 66.7% of *S. epidermidis* (14/21) and 60.0% of *S. aureus* (6/10) isolates exhibiting resistance. However, all Gram-positive isolates remained fully susceptible to vancomycin, linezolid, and tigecycline.

Gram-negative bacilli ($n=9$) showed universal resistance to ampicillin (100%). Of particular concern, *Acinetobacter baumannii* isolates displayed pan-resistance to all tested β -lactams, aminoglycosides, and carbapenems. While *Pseudomonas aeruginosa* demonstrated complete resistance to ciprofloxacin (3/3), susceptibility to other agents was maintained. Enterobacteriaceae isolates preserved carbapenem susceptibility, suggesting that this class remains a viable treatment option for these pathogens.

Discussion

Our five-year retrospective analysis of bacterial conjunctivitis cases reveals several clinically significant findings that warrant careful consideration in ophthalmic practice.

Microbiological Profile and Antimicrobial Resistance Trends

The microbiological profile of bacterial conjunctivitis isolates revealed a predominance of Gram-positive cocci (70.7%), among which coagulase-negative staphylococci (CoNS) accounted for 68.3% of all isolates—a distribution pattern consistent with national surveillance data.⁹ Among these, *Staphylococcus epidermidis* represented the most prevalent pathogen (21 strains, 75.0% of CoNS isolates), a finding of particular clinical significance given this microorganism’s

Table 3 Seasonal Distribution of Bacterial Conjunctivitis Cases and Predominant Pathogens

Season	Months	Cases (n=58)	Percentage (%)	Predominant Pathogens
Spring	Mar-May	9	15.5	<i>Staphylococcus epidermidis</i> (6)
Summer	Jun-Aug	24	41.4	<i>Staphylococcus aureus</i> (8), <i>Staphylococcus epidermidis</i> (10)
Autumn	Sep-Nov	18	31.0	<i>Staphylococcus epidermidis</i> (4), <i>Pseudomonas aeruginosa</i> (3)
Winter	Dec-Feb	7	12.1	<i>Streptococcus pneumoniae</i> (2)

Notes: Summer-autumn incidence (72.4%, 42/58) was significantly higher than winter (12.1%, 7/58) ($\chi^2=8.04$, $df=1$, $P=0.005$).

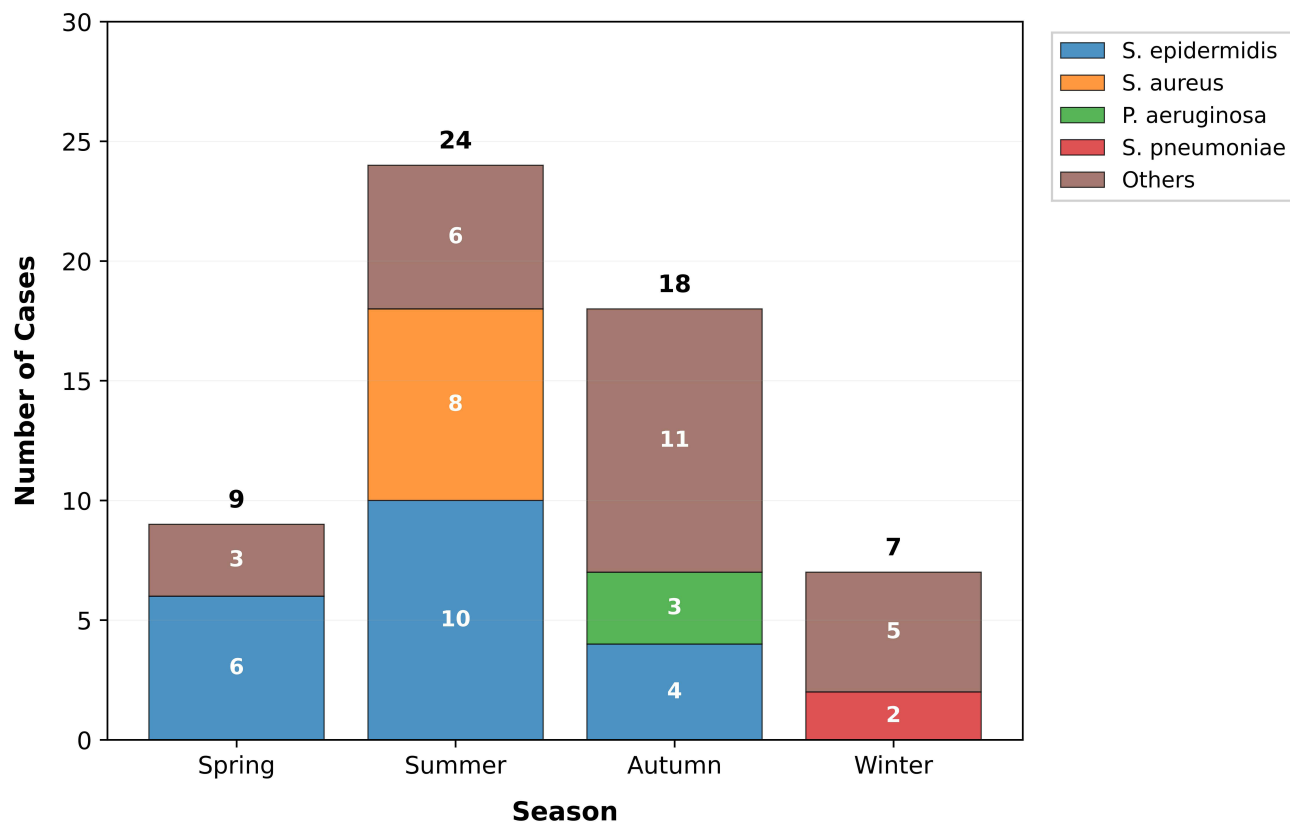


Figure 3 Seasonal Distribution of Bacterial Conjunctivitis Cases (n=58).

Notes: Bar chart showing the seasonal variation in conjunctivitis cases, with significantly higher incidence in summer-autumn months (72.4%) compared to winter (12.1%, P=0.005).

well-established capacity for biofilm formation and associated antimicrobial resistance phenotypes.¹⁰ These microbial characteristics may warrant the consideration of additional therapeutic strategies aimed at biofilm disruption.

Our longitudinal resistance analysis revealed a statistically significant increase in methicillin-resistant *S. aureus* (MRSA) prevalence (Cochran–Armitage test, P=0.043), with an annual growth rate surpassing the 15.3% reported for general hospital isolates in Eastern China.¹¹ This disproportionate increase suggests the presence of ophthalmology-specific selective pressures, potentially attributable to intensive exposure to topical ophthalmic antibiotics, particularly fluoroquinolones. Supporting this hypothesis, antimicrobial utilization data from Shanghai documented a 37.2% increase in fluoroquinolone ophthalmic prescriptions between 2020 and 2022,¹² reflecting broader national prescription trends.¹³

Table 4 Antimicrobial Resistance Patterns of Gram-Positive Cocci

Antimicrobial Agent	<i>S. epidermidis</i> (n=21)	<i>S. aureus</i> (n=10)	Other Staphylococci (n=7)	Total (n=38)
Penicillin	19 (90.5%)	10 (100.0%)	5 (71.4%)	34 (89.5%)
Oxacillin	14 (66.7%)	6 (60.0%)	3 (42.9%)	23 (60.5%)
Erythromycin	12 (57.1%)	6 (60.0%)	3 (42.9%)	21 (55.3%)
Tetracycline	7 (33.3%)	6 (60.0%)	2 (28.6%)	15 (39.5%)
Levofloxacin	5 (23.8%)	2 (20.0%)	1 (14.3%)	8 (21.1%)
Clindamycin	4 (19.0%)	2 (20.0%)	1 (14.3%)	7 (18.4%)
Trimethoprim-Sulfamethoxazole	4 (19.0%)	0 (0.0%)	1 (14.3%)	5 (13.2%)
Moxifloxacin	2 (9.5%)	1 (10.0%)	1 (14.3%)	4 (10.5%)
Vancomycin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Linezolid	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tigecycline	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

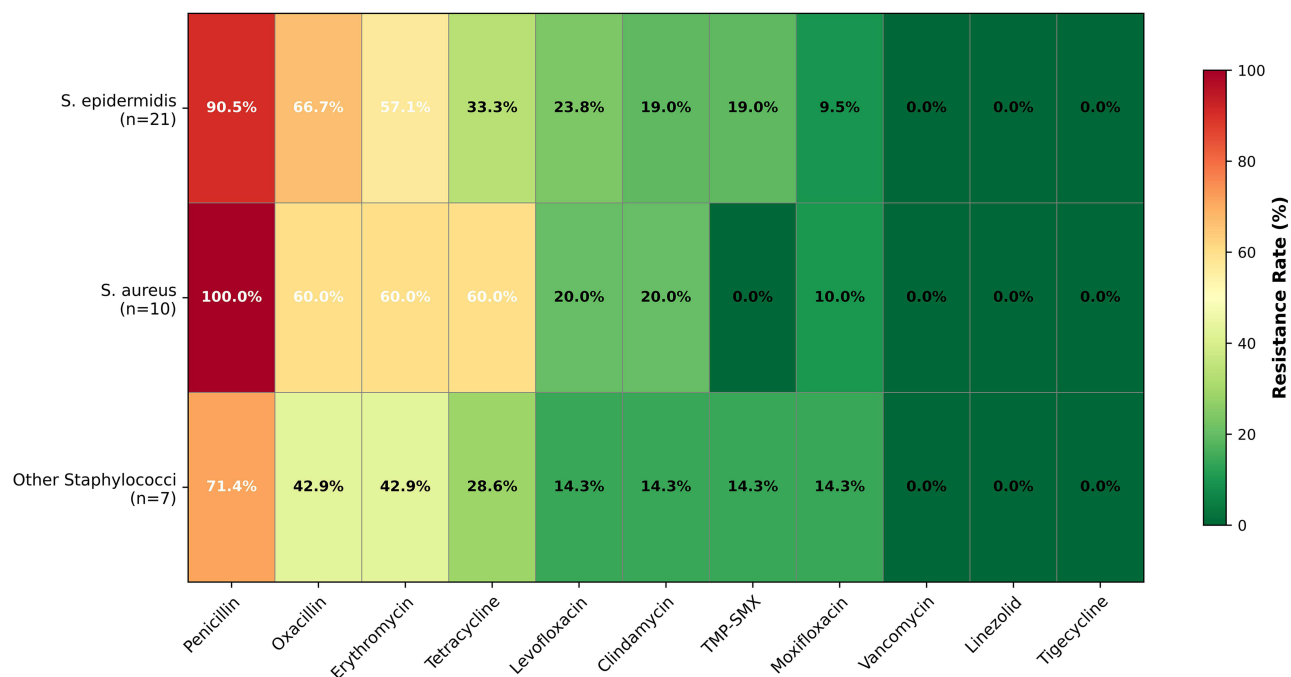


Figure 4 Antimicrobial Resistance Profiles of Gram-positive Cocci.

Notes: Bar graph displaying the resistance rates of Gram-positive cocci to various antimicrobial agents. High resistance was observed to penicillin (89.5%), oxacillin (60.5%), and erythromycin (55.3%), while all isolates remained susceptible to vancomycin, linezolid, and tigecycline.

Epidemiological Characteristics and Temporal Trends

Our epidemiological analysis identified several key patterns in bacterial conjunctivitis distribution. The disease exhibited marked seasonality, with 72.4% of cases occurring in summer–autumn months (June–November) versus only 12.1% in winter ($\chi^2=8.04$, $P=0.005$). This seasonal predilection may be mediated through multiple mechanisms: (1) enhanced bacterial viability in high temperature/humidity conditions (mean summer relative humidity >80%);¹⁴ (2) increased recreational water exposure; and (3) temperature-modulated virulence factor expression.¹⁵ Pathogen-specific seasonal patterns were particularly noteworthy: while *Staphylococcus* spp. demonstrated year-round prevalence with summer amplification ($P<0.05$), *Pseudomonas aeruginosa* exhibited exclusive summer occurrence, and *Streptococcus* spp. showed winter predominance – possibly indicating respiratory-ocular transmission pathways.

The post-pandemic period (2023–2024) witnessed a dramatic epidemiological shift, with case numbers nearly doubling pre-2023 levels (65.6% vs 34.4%). This surge may reflect the confluence of multiple factors: reduced microbial exposure during pandemic restrictions leading to altered immune responses, and increased outpatient visits post-pandemic.

Antimicrobial Resistance Patterns and Therapeutic Implications

The antimicrobial resistance patterns observed in this study present significant therapeutic challenges for clinical management of bacterial conjunctivitis. Gram-negative isolates exhibited universal resistance to ampicillin (100%), with *Acinetobacter baumannii* strains showing pan-drug resistance to all tested antimicrobial classes. These resistance profiles necessitate careful reconsideration of current treatment paradigms.

Among Gram-positive isolates, we documented alarmingly high resistance rates to first-line antibiotics, with 89.5% of strains resistant to penicillin and 60.5% to oxacillin – findings that are consistent with China’s national antimicrobial resistance surveillance data.⁹ Of particular concern, MRSA isolates demonstrated tetracycline resistance rates (60.0%) that exceeded the national average (30%),¹⁴ indicating potentially unique resistance selection pressures in ocular infections (Table 4 and Figure 4).

Based on our findings, we recommend: (1) avoiding empirical use of penicillin and erythromycin for Gram-positive infections due to their diminished efficacy; (2) reserving fluoroquinolones for culture-confirmed susceptible cases to prevent

further resistance development; and (3) considering vancomycin only for confirmed MRSA infections with resistance to other antimicrobials, given its maintained 100% susceptibility in our study. These recommendations align with antimicrobial stewardship principles while addressing the specific resistance challenges identified in ocular pathogens.

Study Limitations

This study has several limitations. As a single-center retrospective study, our findings require validation through multicenter prospective studies. The relatively small sample size ($n=58$) may limit generalizability, though our epidemiological trends show strong statistical significance. The 9.5% positivity rate may reflect factors such as prior antibiotic use, viral etiology, or sampling timing. Despite these limitations, this investigation provides critical baseline data for monitoring ocular antimicrobial resistance in urban China, particularly highlighting the emerging threat of ocular MRSA.

Although this single-center study was conducted in a secondary hospital setting, which may limit the direct generalizability of the incidence rates to tertiary care centers, the identified antimicrobial resistance profiles and temporal trends of key pathogens like MRSA are consistent with broader regional surveillance data.¹⁶ This suggests that the challenges of antimicrobial resistance in ocular infections are widespread across different levels of the healthcare system.

Conclusion

This five-year study (2020–2024) in Shanghai found that Gram-positive cocci dominated bacterial conjunctivitis, with a significant increase in MRSA ($P=0.043$) and the emergence of multidrug-resistant *A. baumannii*. Infections peaked significantly in summer–autumn (72.4%, $P=0.005$). High resistance to first-line antibiotics contrasted with 100% susceptibility to vancomycin and linezolid. These findings support the adoption of culture-guided therapy, restriction of fluoroquinolone use, and enhanced resistance surveillance, particularly for emerging resistant strains in ocular infections.

Data Sharing Statement

All data used to analyze and generate the results of this study are included in this article.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shabei Hospital, Jing'an District, Shanghai (Approval Reference No.: YL-20250909-33). The need for informed consent was waived by the committee due to the retrospective nature of the study, which involved minimal risk to participants and used anonymized clinical data. All patient data were de-identified and processed confidentially to ensure privacy protection.

Acknowledgments

The authors would like to express their sincere gratitude to the colleagues at the Ophthalmology Center of Shabei Hospital, Shanghai, for their invaluable assistance in specimen collection and clinical data coordination.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research received no external funding.

Disclosure

The authors report no conflicts of interest in this work.

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