



DOI: 10.14744/eer.2025.27928
Eur Eye Res 2025;5(2):138–144

EUROPEAN
EYE
RESEARCH

ORIGINAL ARTICLE

Microbiological profile and antibiotic susceptibility results in corneal samples: Sharing 4-year data

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Abstract

Purpose: The purpose of the study is to evaluate the microbiological profile, antibiotic susceptibility patterns, and clinical outcomes of infectious keratitis cases managed at a tertiary referral center over 4 years.

Methods: This retrospective study included 451 patients with suspected infectious keratitis, whose corneal scrapings were subjected to microbiological culture and antibiotic susceptibility testing between June 2020 and October 2024. Patients with non-infectious keratitis or insufficient follow-up were excluded. Microbial identification was performed using matrix-assisted laser desorption ionization time of flight mass spectrometry, and antibiotic susceptibility was assessed per EUCAST 2024 standards. Demographic data, clinical risk factors, culture positivity rates, and treatment outcomes were analyzed statistically.

Results: Culture positivity was observed in 39.4% of cases (n=178). Among these, 61.3% were gram-positive bacteria, 35.4% gram-negative bacteria, and 3.3% fungi. The most frequently isolated pathogen was *Staphylococcus spp.* (42.1%), primarily *Staphylococcus epidermidis* (18%), followed by *Pseudomonas aeruginosa* (13.4%) and *Streptococcus spp.* (9.5%). Methicillin susceptibility of *S. epidermidis* was 58%, while susceptibility rates to moxifloxacin, gentamicin, and fusidic acid were 88.9%, 83.3%, and 83.3%, respectively.

Conclusion: This study highlights the microbiological profile and regional antibiotic susceptibility patterns of infectious keratitis in a tertiary care setting. The predominance of *Staphylococcus spp.* and variability in antibiotic resistance emphasize the importance of prompt microbiological testing and evidence-based empirical therapy. These findings provide valuable data to optimize treatment strategies and improve clinical outcomes in infectious keratitis management.

Keywords: Antibiotic susceptibility; Bacteria; Culture; Fungus; Keratitis.



Cite this article as: Ceylan A, Aydin FO, Akbaş YB, Oncel B, Akgul BA, Yildiz BK, et al. Microbiological profile and antibiotic susceptibility results in corneal samples: Sharing 4-year data. Eur Eye Res 2025;5(2):138–144.

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Submitted Date: 14.02.2025 **Revised Date:** 15.04.2025 **Accepted Date:** 27.04.2025 **Available Online Date:** 26.08.2025

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Infectious keratitis, a relatively common and rapidly progressing condition, remains a major cause of corneal blindness worldwide due to its significant impact on vision.^[1-4] A wide variety of pathogens, including bacteria, viruses, fungi, and parasites, have been implicated in its etiology.^[5] The prevalence of distinct microbial populations varies across different geographical regions, reflecting the influence of various risk factors, climate, and geographical differences on the patient population affected.^[6] The most prevalent risk factors include the use of contact lenses, organic trauma, ocular surface disease, a history of previous ocular surgery, the presence of systemic diseases, and the administration of immunosuppressive agents.^[2,7-9]

The choice of an initial antibacterial therapy at the optimal time is of great consequence for the achievement of favourable treatment outcomes. In the absence of laboratory confirmation of microbial infection, clinicians must rely on clinical findings and regional antimicrobial susceptibility patterns to guide the choice of an empiric antibiotic regimen. Among the most valuable tests for diagnosis are corneal scrapings and culture, anterior segment optical coherence tomography, and confocal microscopy findings.^[10-12]

The objective of the study was to evaluate epidemiological data, microbiological results, and clinical outcomes of cases of infectious keratitis attending a tertiary referral clinic.

Materials and Methods

This retrospective study was conducted in accordance with the Declaration of Helsinki and with the permission of the Ethics Committee of Başakşehir City Hospital (Research protocol code: 2024-98/Date:16.07.2024). The study included eye specimens from which corneal scrapings were obtained and sent to the microbiology laboratory of Basaksehir Cam and Sakura Sehir Hospital for infective keratitis between June 2020 and October 2024.

Cases with corneal opacity, viral keratitis, or sterile, neurotropic or autoimmune keratitis were excluded from the study. However, patients with a history of trauma leading to infectious keratitis were included, as this was a predisposing factor for infection.

Patients with <1 month of follow-up or no microbiological intervention were also excluded.

The data set comprised demographic information, predisposing factors, isolated pathogens, antibiotic resistance, and clinical course.

Corneal scrapings were initially inoculated directly at the bedside in the clinic using a disposable number 15 scalpel

blade or disposable needles. Subsequently, the samples were applied directly onto a slide for examination and staining. Following this, the samples were sent to the clinical microbiology laboratory in our hospital for analysis. In most cases, empirical antibiotic therapy was initiated shortly after corneal sampling, typically within a few hours. Bacterial culture results were generally available within 48–72 h, whereas fungal cultures required longer incubation periods, often yielding growth between 10 and 14 days.

Samples were inoculated on sheep's blood agar, chocolate agar and Sabouraud's agar and incubated under conditions optimal for the growth of the inoculated bacteria. A positive culture was defined as the growth of any bacterial or fungal organism at the inoculation line of any culture medium. Bacterial identification was conducted using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry Bruker, Germany. Where necessary, additional confirmatory tests were conducted. Antibiotic susceptibility testing was conducted using the Phoenix M50 system (Bruker Daltonics, Germany). The results of the antibiotic susceptibility tests were evaluated in accordance with the EUCAST 2024 clinical cut-off values table.

Statistical Analysis

The statistical analysis of the data was conducted using Statistical Package for the Social Sciences (SPSS) for Windows, version 25.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics summarized the socio-demographic, clinical characteristics, pathology, and treatment for this case series. Wilcoxon signed-rank test was used when appropriate. Statistical significance was defined as $p < 0.05$.

Results

Corneal scrapings from 451 patients were included in the study. Culture growth was detected in 178 (39.4%) of these 451 scrapings. Among the patients with corneal scrapings, 82 (46.0%) were female and 96 (53.9%) were male. The mean age of the patients was 49.89 ± 23.98 years. Diabetes was observed in 33 (18.6%) of these patients, while immunosuppression was observed in 5 (2.8%) patients. The comorbidity status of the patients is summarized in Table 1.

The ocular predisposing factors for keratitis included the use of contact lenses in 30 (16.9%) patients, antiglaucomatous medication for glaucoma in 22 (12.3%) patients, a history of ocular surgery in 36 (20.2%) patients, and a history of ocular trauma in 45 (25.4%) patients. Trauma-related keratitis was more frequently observed in male patients ($n=29$, 64.4% of trauma cases), whereas contact lens-related keratitis was more common among female patients ($n=18$, 60%

Table 1. Comorbidities of patients

Comorbidity	n	%
Ocular trauma	45	25.4
Previous ocular surgery	35	19.7
DM	33	18.6
Contact lens use	30	16.9
Use of anti-glaucomatous drugs	21	11.9
Others	19	10.7
HT	17	9.6
Immune deficiency	5	2.8

DM: Diabetes mellitus; HT: Hypertension.

of contact lens cases), indicating possible gender-related exposure differences. Of the entire patient group, 164 patients (36.3%) had received some form of topical antibiotic therapy before admission. However, due to the absence of standardized referral documentation, specific details regarding the antibiotic used or the duration of prior treatment were not always available.

Among these patients with corneal scrapings, 89 (50.0%) were inpatient and 35 (19.8%) were treated with intravenous antibiotic therapy. Of these patients, 23 (13.0%) developed corneal melting, 4 (2.2%) developed corneal perforation, and 12 (6.8%) developed endophthalmitis. 38 (21.3%) patients underwent amniotic membrane transplantation, 12 (6.8%) patients underwent tectonic penetrating keratoplasty, 2 (1.1%) patients underwent evisceration, 2 (1.1%) patients underwent pars plana vitrectomy, and 10 (5.6%) patients with endophthalmitis were followed up with intravitreal antibiotic injection. Microorganisms in patients who developed endophthalmitis or underwent tectonic penetrating keratoplasty are shown in Table 2.

The distribution of the microorganisms grown is shown in Table 3. The results of the culture growth demonstrated that 109 (61.3%) corneal scrape samples exhibited the growth of gram-positive bacteria, 63 (35.4%) scrape samples showed the growth of Gram-negative bacteria, and 6 (3.3%) scrape samples demonstrated the growth of fungi. The most frequently isolated microorganism was *Staphylococcus spp.* (42.1%), followed by *Pseudomonas aeruginosa* (13.4%) and *Streptococcus spp.* (9.5%) (Table 3). Polymicrobial infections were identified in 10 (5.6%) of the culture-positive patients, with the most common combinations being *Staphylococcus epidermidis* with *P. aeruginosa* (2.2%) and *Staphylococcus aureus* with *Streptococcus pneumoniae* (1.7%).

The most frequently isolated species among the *Staphylococcus* genus was *S. epidermidis*, which constituted 18% of all microorganisms grown in culture. While methicillin

Table 2. Microorganisms in keratoplasty patients and endophthalmitis cases after keratitis

Microorganism name	Keratoplasty (n)	Endophthalmitis (n)
<i>Pseudomonas aeruginosa</i>	3	1
<i>Staphylococcus epidermidis</i>	2	0
<i>Staphylococcus haemolyticus</i>	0	1
<i>Fusarium species</i>	1	2
<i>Moraxella nonliquefaciens</i>	1	3
<i>Streptococcus pneumoniae</i>	0	2
<i>Streptococcus dysgalactiae</i>	0	1
<i>Micrococcus luteus</i>	1	0
<i>Klebsiella pneumoniae</i>	0	1
<i>Enterococcus faecalis</i>	1	0
<i>Haemophilus influenzae</i>	1	0
<i>Corynebacterium striatum</i>	1	0
<i>Aspergillus flavus</i>	1	0
<i>Acinetobacter spp.</i>	0	1
Total	12	12

Table 3. Microorganisms in Keratoplasty Patients and Endophthalmitis Cases After Keratitis

Microorganism Name	Keratoplasty (n)	Endophthalmitis (n)
<i>Pseudomonas aeruginosa</i>	3	1
<i>Staphylococcus epidermidis</i>	2	0
<i>Staphylococcus haemolyticus</i>	0	1
<i>Fusarium species</i>	1	2
<i>Moraxella nonliquefaciens</i>	1	3
<i>Streptococcus pneumoniae</i>	0	2
<i>Streptococcus dysgalactiae</i>	0	1
<i>Micrococcus luteus</i>	1	0
<i>Klebsiella pneumoniae</i>	0	1
<i>Enterococcus faecalis</i>	1	0
<i>Haemophilus influenzae</i>	1	0
<i>Corynebacterium striatum</i>	1	0
<i>Aspergillus flavus</i>	1	0
<i>Acinetobacter spp.</i>	0	1
Total	12	12

sensitivity was observed in 58% of *S. epidermidis* isolates, antibiotic susceptibility rates for moxifloxacin, gentamicin, and fusidic acid, which can be administered topically in eye infections, were found to be 88.9%, 83.3% and 83.3%, respectively. The results of the antibiotic susceptibility tests for other antibiotics and microorganisms are presented in Figure 1. The distribution of antibiotic resistance status of *Staphylococcus spp.* isolates and *P. aeruginosa* isolates, which are the most frequently detected microorganisms, according to years, are shown in Figures 2 and 3.

	Methicillin	AMP	PTZ	CAZ	CRO	CPM	GEN	AMK	CIP	MXF	LEV	MEM	IPM	TET	LNZ	FA	VA	TEC	ERY	CLI	SXT	DAP
Staphylococcus aureus	S 9 (%64.2)	0					13 (%93)	12 (%85.7)	0	12 (%85.7)	4 (%28.5)			10 (%71.4)	10 (%100)	14 (%100)	14 (%100)	14 (%100)	11 (%78.5)	12 (%85.7)	13 (%93)	14 (%100)
	I 0	0					0	0	11 (%78.5)	0	7 (%50)			0	0	0	0	0	0	0	1 (%7)	0
	R 5 (%35.8)	12 (%100)					1 (%7)	2 (%14.3)	3 (%21.5)	2 (%14.3)	3 (%21.5)			4 (%28.6)	0	0	0	0	3 (%21.5)	2 (%14.3)	0	0
Staphylococcus epidermidis	S 11 (%58)	0					15 (%83.3)	18 (%100)	0	16 (%88.9)	0			8 (%42.1)	17 (%94.4)	15 (%83.3)	17 (%94.4)	17 (%100)	6 (%31.5)	16 (%88.9)	19 (%100)	17 (%100)
	I 0	0					0	0	15 (%79)	0	16 (%88.9)			1 (%5.3)	0	0	0	0	0	0	0	0
	R 8 (%42)	13 (%100)					3 (%16.7)	0	4 (%21)	2 (%11.1)	2 (%11.1)			10 (%52.6)	1 (%5.6)	3 (%16.7)	1 (%5.6)	0	13 (%68.5)	2 (%11.1)	0	0
Other Staphylococcus	S 15 (%71.4)	0					18 (%85.7)	20 (%95.2)	0	17 (%81)	0			13 (%62)	20 (%95.2)	16 (%76)	20 (%100)	17 (%85)	11 (%52.4)	17 (%81)	19 (%90.6)	20 (%95.2)
	I 0	0					0	0	15 (%75)	0	17 (%81)			1 (%4.7)	0	0	0	0	0	0	1 (%4.7)	0
	R 6 (%28.6)	6 (%100)					3 (%14.3)	1 (%4.8)	5 (%25)	4 (%19)	4 (%19)			7 (%33.3)	1 (%4.8)	5 (%24)	0	3 (%15)	10 (%47.6)	4 (%19)	1 (%4.7)	1 (%4.8)
Pseudomonas aeruginosa	S		0	0		0	21 (%91.3)	0		0	19 (%82.6)	0										
	I		20 (%87)	20 (%87)		20 (%87)	0	17 (%77.3)		16 (%72.7)	0	17 (%74)										
	R		3 (%13)	3 (%13)		3 (%13)	2 (%18.7)	5 (%22.7)		6 (%27.3)	4 (%17.4)	6 (%26)										
Enteric Gram Negatives	S	2 (%18)	12 (%92.3)	10 (%77)	10 (%77)	10 (%77)	11 (%82)	12 (%92.3)	9 (%70)	10 (%77)	11 (%82)	9 (%75)									9 (%70)	
	I	0	0	0	0	0	0	0	3 (%23)	2 (%15.3)	0	0									0	
	R	11 (%82)	1 (%7.7)	3 (%23)	3 (%23)	3 (%23)	2 (%18)	1 (%7.7)	1 (%7)	1 (%7.7)	2 (%18)	3 (%25)									4 (%30)	
Other Gram Negatives	S	3 (%75)		8 (%100)				6 (%50)		6 (%75)	9 (%90)										9 (%69.2)	
	I	0		0				3 (%25)		0	0										2 (%15.4)	
	R	1 (%25)		0				3 (%25)		2 (%25)	1 (%10)										2 (%15.4)	
Streptococcus spp	S								5 (%100)	0	8 (%100)	4 (%80)	5 (%100)	10 (%100)	10 (%100)	3 (%60)					3 (%100)	
	I								0	5 (%100)	0			0	0	0	0	0	0		0	
	R								0	0	0			1 (%20)	0	0	0	2 (%40)		0		

Fig. 1. Antibiotic susceptibility test results of microorganisms grown in eye culture samples. AMP: Ampicillin, PTZ: Piperacillin-Tazobactam, CAZ: Ceftazidime, CRO: Ceftriaxone, CPM: Cefepime, GEN: Gentamicin, AMK: Amikacin, CIP: Ciprofloxacin, MXF: Moxifloxacin, LEV: Levofloxacin, MEM: Meropenem, IPM: Imipenem, TET: Tetracycline, LNZ: Linezolid, FA: Fusidic Acid, VA: Vancomycin, TEC: Teicoplanin, ERY: Erythromycin, CLI: Clindamycin, SXT: Trimethoprim-Sulfamethoxazole, DAP: Daptomycin, CL: Colistin. S: Susceptible, I: Susceptible with increased exposure, R: Resistant.

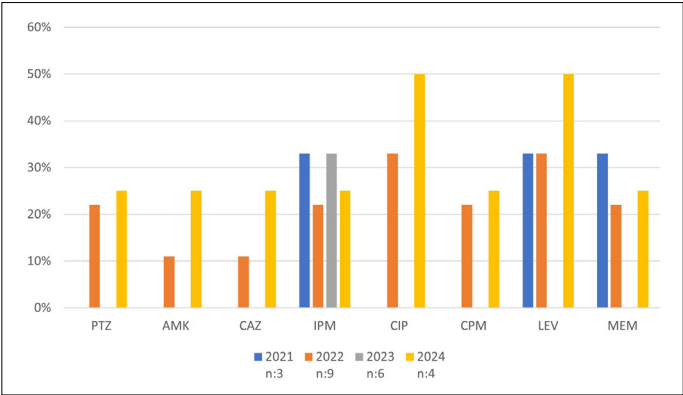


Fig. 2. Distribution of antibiotic resistance status of *Pseudomonas aeruginosa* isolates from eye specimens according to years. Antibiotic susceptibility test results were considered resistant except those in the susceptible and susceptible with increased exposure categories. PTZ: Piperacillin-Tazobactam, AMK: Amikacin, CAZ: Seftazidim, IPM: Imipenem, CIP: Ciprofloxacin, CPM: Cefepim, LEV: Levofloxacin, MEM: Meropenem.

Discussion

Microbial keratitis is a suppurative inflammation of the cornea that has the potential to become a significant public health concern. The prevalence of corneal blindness caused by microbial keratitis is estimated to range from 1.5 to 2 million cases per year on a global scale.^[4] Similar trends in corneal blindness are observed in Turkey due to

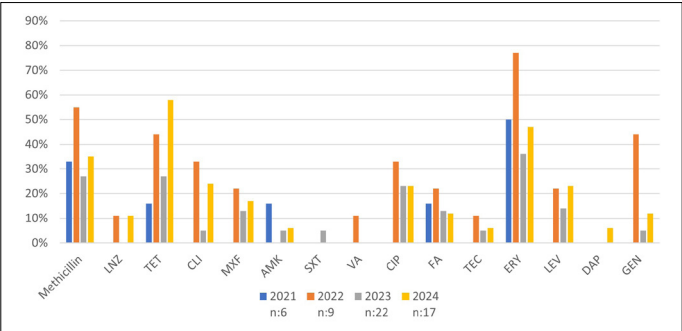


Fig. 3. Distribution of antibiotic resistance status of *Staphylococcus* spp. isolates from eye specimens according to years. Antibiotic susceptibility test results were considered resistant except those in the susceptible and susceptible with increased exposure categories. LNZ: Linezolid, TET: Tetracycline, CLI: Clindamycin, MXF: Moxifloxacin, AMK: Amikacin, SXT: Trimethoprim-Sulfamethoxazole, VA: Vancomycin, CIP: Ciprofloxacin, FA: Fusidic Acid, TEC: Teicoplanin, ERY: Erythromycin, LEV: Levofloxacin, DAP: Daptomycin, GEN: Gentamicin

the aggressive nature of the disease, the late presentation of patients, and the lack of appropriate diagnostic and management protocols. Microbiological patterns and clinical outcomes in epidemiological patterns may vary from country to country and even between different regions within a country. Therefore, it is essential to know the etiological agents that can cause infectious keratitis and the susceptibility pattern

in the geographical area to make an evidence-based decision on the appropriate empirical treatment.^[13]

The mean age of the subjects in our study was 49.89 ± 23.98 years, with a male predominance (53.9%). This may be attributed to the fact that men in this age group are more likely to engage in outdoor work than women. This finding aligns with those reported in studies by Mohod et al.^[14] and Parmar et al.^[15]

Although some studies have previously documented contact lens wear as the predominant ocular predisposing factor for keratitis,^[16,17] our investigation revealed that the most common history of ocular trauma (45 cases, 25.4%) was observed, followed by the second most prevalent history of ocular surgery (36 cases, 20.2%) and contact lens wear (30 cases, 16.9%).

The present study revealed that culture positivity was detected in 178/451 (39.4%) patients. It is notable that cultural positivity has been found to be highly variable in a number of different regions across the world.^[18] Indeed, even studies conducted in the same region have yielded markedly disparate results. A study from South India documented a culture positivity rate of 20.1%, while another study from the same region reported 51.9%.^[19] Similarly, one study from the same region reported 42.08%, while another study reported 26.5%.^[20,21] These discrepancies may be attributed to geographical characteristics, climate, socio-cultural life, and even sample collection and laboratory conditions.

In accordance with other studies,^[22-24] gram-positive bacteria represented the most frequently isolated microorganisms (61.3%). The most frequently isolated microorganism was *Staphylococcus spp.* (42.1%), comprising primarily *S. epidermidis* (18%) and followed by *P. aeruginosa* (13.4%) and *Streptococcus spp.* (9.5%). Similar results to our study have been shown in other studies.^[25,26] However, in another study conducted in our country, *P. aeruginosa* was the most frequently isolated microorganism.^[27] The most frequently isolated pathogens may vary in different regions. For example, in some regions, the most common pathogen may be a fungus^[15] (as observed in India), while in another region it may be a different genus of bacteria.^[28] The low detection rate of fungal keratitis in our study (3.3%) may be due to regional climatic conditions less favorable to fungal growth or potential limitations in the sensitivity of laboratory culture. Future studies using molecular diagnostic methods may improve the accuracy of detection.

The global health problem of antibiotic resistance has a significant impact on ophthalmology and numerous other

medical specialties. In daily ophthalmological practice, antibiotic resistance presents considerable challenges. The increasing prevalence of antibiotic-resistant strains jeopardises the effectiveness of standard treatment regimens, leading to prolonged and more severe infections, as well as an increased risk of complications and sight-threatening outcomes.^[29] Therefore, this should be taken into an account when selecting antibiotics for ocular infections, and the common causes of infection and antibiotic susceptibility in that region should be well understood.

In the present study, the most frequently isolated *Staphylococcus* species was *S. epidermidis*, with a methicillin susceptibility rate of 58%. The topical moxifloxacin, gentamicin, and fusidic acid susceptibility rates for *S. epidermidis* were 88.9%, 83.3% and 83.3%, respectively. In the literature reveals that susceptibility rates of *Staph. epidermis* to fluoroquinolones (44–93.0%), macrolides (34.3–52%) and methicillin/oxacillin (45–70.4%) have been demonstrated.^[30,31] In contrast to the findings of our study, the ARMOR study^[32] demonstrated a low susceptibility to fluoroquinolones (63.9%) in keratitis. However, the susceptibility to aminoglycosides (72–95.3%) and vancomycin (99–100%) was high, which is consistent with our study.

A small number of ophthalmological studies have been conducted in Turkey to evaluate the etiological cause and antibiotic susceptibility;^[13,16,33-35] however, the retrospective nature of our study, the limited scope of our hospital and patient population, and the discrepancy between clinical response and in vitro antibiotic susceptibility should be considered as potential limitations. Therefore, caution should be exercised when extrapolating the results of this study to different geographical areas or specific populations. Another limitation of this study is that the follow up time of the eyes is not long enough to fully evaluate long-term visual outcomes, recurrence rates, or permanent complications such as corneal scarring. Future prospective studies with longer follow-up are warranted to assess treatment efficacy and visual prognosis fully.

Conclusion

This study provides insight into the microbiological landscape and antibiotic susceptibility patterns of infectious keratitis in a tertiary care centre over a 4-year period. The prevalence of *Staphylococcus spp.*, including *S. epidermidis*, and the observed variations in antibiotic resistance patterns highlight the necessity of region-specific empirical

treatment strategies. Furthermore, our findings emphasise the significance of prompt microbiological testing and targeted therapy to achieve favourable clinical outcomes. The objective of this study is to provide updated data on the etiology and resistance trends in infectious keratitis, thus supporting clinicians in making informed treatment decisions and improving patient care.

Ethics Committee Approval: The Başakşehir City Hospital Ethics Committee granted approval for this study (date: 16.07.2024, number: 2024-98).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: A.C., F.O.A.; Design: A.C., Y.B.A., B.K.Y., Y.Y.; Supervision: A.C., B.K.Y., Y.Y.; Resource: A.C.; Materials: A.C.; Data Collection and/or Processing: A.C., F.O.A., B.O., B.A.; Analysis and/or Interpretation: A.C., F.O.A., Y.B.A., Y.Y.; Literature Search: A.C., F.O.A., Y.B.A., B.O., B.A., B.K.Y.; Writing: A.C., F.O.A., Y.B.A., B.O., B.A., B.K.Y.; Critical Reviews: A.C., B.K.Y., Y.Y.

Conflict of Interest: None declared.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: The authors declared that this study has received no financial support.

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