


# Epidemiology of Microbial Keratitis at a Tertiary Care Hospital in Southern Thailand

Ratchapol Ngarmsom, Orasa Horatanaruang 

Department of Ophthalmology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Correspondence: Orasa Horatanaruang, Department of Ophthalmology, Faculty of Medicine, Prince of Songkla University, 15 Karnjanavanich Road, Hat Yai, Songkhla, 90110, Thailand, Tel +66 74 451380, Fax +66 74 451381, Email orasaho@gmail.com

**Purpose:** This study aimed to ascertain the microbial profiles and predisposing risk factors for microbial keratitis and to analyze the trend of mixed microbial infection cases over 8 years.

**Patients and Methods:** In this retrospective analysis, we reviewed the electronic medical records of inpatients diagnosed with microbial keratitis between January 2012 and December 2019. Data on demographics, risk factors, and causative pathogens were analyzed. Multivariate logistic regression models were utilized to identify risk factors associated with pathogens.

**Results:** This study included 640 eyes of 638 patients. Trauma was the most common predisposing risk factor (57.2%), followed by a combination of factors (14.4%). Among diagnostic test results, bacteria, fungi, and mixed pathogens were identified in 46.72%, 46.56%, and 21.41% of cases, respectively. Positive culture results were obtained in 324 eyes (53.6%), with *Pseudomonas aeruginosa* (25.1%) and *Fusarium* spp. (17.4%) being the most prevalent pathogens. In the multivariate logistic regression model, contact lens use, presence of diabetes mellitus, and HIV infection were statistically significant risk factors for *Pseudomonas aeruginosa* infection ( $p = 0.001$ ,  $p = 0.046$ , and  $p = 0.04$ , respectively). Trauma was associated with *Fusarium* spp. infection ( $p = 0.001$ ). An increase in the percentage of mixed microbial infection cases was observed when comparing the periods of 2016–2019 with 2012–2015 ( $p = 0.023$ ).

**Conclusion:** Bacteria and fungi are equally common causes of microbial keratitis, with *Pseudomonas aeruginosa* and *Fusarium* spp. being the predominant pathogens causing bacterial and fungal infections. Trauma is the primary predisposing risk factor for microbial keratitis. There was a notable increase in mixed infection cases over the study period.

**Keywords:** microbial keratitis, infectious keratitis, corneal ulcer, risk factors

## Introduction

Microbial keratitis stands as a significant contributor to ocular morbidity and blindness globally.<sup>1–3</sup> The causative agents of microbial keratitis encompass bacteria, fungi, viruses, or parasites. Numerous predisposing risk factors have been documented, including corneal trauma, contact lens usage, ocular surface disease, prior ocular surgery, systemic illness, and a history of herpes simplex keratitis. Initiation of appropriate empirical treatment before culture results has become imperative to mitigate ocular morbidity, relying on epidemiological insights such as the spectrum of causative agents and predisposing risk factors. Nonetheless, these epidemiological data may exhibit variations contingent on geographic location, climate, socioeconomic status, and time.<sup>4,5</sup>

Several studies have delineated the prevalence of microbial keratitis solely through positive culture results;<sup>6–8</sup> however, other diagnostic methodologies exist to identify the pathogen. It is imperative to ascertain the epidemiological landscape of a specific region to formulate efficacious strategies for the diagnosis, treatment, and prevention of microbial keratitis.

There is a shortage of contemporary local epidemiological surveys on microbial keratitis in southern Thailand. Consequently, this study pursued two objectives. First, to delineate the microbial profiles and predisposing risk factors of patients afflicted with microbial keratitis at Songklanagarind Hospital, a tertiary care facility in southern Thailand. Second, to examine the trend of mixed microbial infection cases over 8 years.

## Materials and Methods

This retrospective study included all patients diagnosed with microbial keratitis and admitted to Songklanagarind Hospital between January 1, 2012, and December 31, 2019. Patients were identified using the H160 (corneal ulcers) code from an International Classification of Diseases, Tenth Revision, search. Exclusion criteria encompassed patients diagnosed with peripheral ulcerative keratitis, Mooren's ulcers, marginal keratitis, interstitial keratitis, sterile ulcers, keratitis-associated autoimmune conditions, and cases with incomplete data. The study adhered to the principles outlined in the Declaration of Helsinki. The Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand approved this study (no. 62-467-2-4). The requirement for written informed consent from patients was waived, as this research posed a miniscule risk to patients, and their rights and welfare would not be adversely affected. Patient data were kept confidential.

Data were retrieved from the electronic medical record system of Songklanagarind Hospital, encompassing demographic data; visit dates; predisposing risk factors; clinical features; corneal scraping results including smears, culture, and polymerase-chain reaction (PCR); histopathological results; and confocal microscopy results.

Regarding the clinical features of microbial keratitis, ulcer locations were classified as central, paracentral, marginal, or total, based on the extent of infiltration. The central zone was defined as a 3 mm diameter area centered on the fixation point. The marginal zone was the infiltration area within 3 mm of the limbus periphery. The paracentral zone was defined as the area between the central and marginal zones. A total lesion was defined as the involvement of >80% of the cornea. The greatest infiltration diameter was utilized to categorize ulcer sizes into small (<2 mm), medium (2–6 mm), and large (>6 mm).

Corneal scraping was performed guided by an operating microscope after the instillation of tetracaine hydrochloride 0.5%. Tissue samples from the edge and base of the ulcer were obtained using a separate crescent knife for each culture media (blood agar, chocolate agar, thioglycolate broth, and Sabouraud's dextrose agar), Gram stain, 10% potassium hydroxide smear, and/or PCR. Topical antimicrobial agents were withheld for 24 hours prior to scraping.

Descriptive statistics were employed, including mean, standard deviation, median, and percentage. Categorical variables were analyzed using Pearson's chi-square and Fisher's exact tests. Univariate analysis, Pearson's chi-square test, and Fisher's exact test were utilized to determine the predisposing risk factors associated with the pathogens. Risk factors ( $p$ -value <0.2 in univariate analysis) were simultaneously analyzed using multivariate analysis. Statistical significance was set at  $p < 0.05$ . All statistical analyses were performed using the R program version 4.1.0 (The R Group, Vienna, Austria) with EpiCalc software.

## Results

### Demographic and Clinical Characteristics

This study included 640 eyes of 638 patients diagnosed with microbial keratitis. Among them, 436 (68.3%) were male and 202 (31.7%) were female (Table 1). The mean age was  $50.27 \pm 19.82$  years (range, 1–96 years). The majority of patients (60.6%) were laborers and farmers. Ophthalmologists previously treated 543 eyes (84.8%) before referral to Songklanagarind Hospital. The duration of symptoms preceding presentation to ophthalmologists was within 7 days in 75.5% of cases and more than 1 month in 2.5% of cases.

Most patients (75.5%) presented with visual acuity worse than 20/200. The infiltration was located centrally in 336 (52.5%), paracentrally in 101 (31.6%), marginally in 41 (6.4%), and totally in 61 (9.5%) eyes. The size of infiltration was small in 65 eyes (10.2%), medium in 381 (59.5%), and large in 194 (30.3%).

### Predisposing Risk Factors

Predisposing risk factors for microbial keratitis were identified in 493 (77%) eyes. The most prevalent risk factor was trauma (57.2%), followed by multiple factors (14.4%), ocular surface disease (9.7%), systemic illness (7.3%), contact lens use (5.9%), history of herpes simplex keratitis (3.7%), and previous ocular surgery (1.8%) (Table 2).

### Microbial Analysis

Corneal scrapings were performed on 604 (94.4%) of the 640 eyes. Gram staining results were positive in 124 eyes (20.5%), and potassium hydroxide smears were positive in 7.6% of eyes (Table 3). The positive culture rate was 53.6%. Among the 395

**Table 1** Demographic and Clinical Characteristics of Patients with Microbial Keratitis

Variable	n (%)
Age, mean $\pm$ SD (year)	50.27 $\pm$ 19.82
Sex	
Male	436 (68.3)
Female	202 (31.7)
Laterality	
Right	327 (51.1)
Left	313 (48.9)
Occupation	
Laborers	192 (30.0)
Farmers	196 (30.6)
Senior citizens	87 (13.6)
Children & students	43 (6.7)
Others	122 (19.1)
Prior treatment	
Ophthalmologists	543 (84.8)
General practitioners	20 (3.1)
Pharmacists	7 (1.1)
None	70 (10.9)
Duration of symptom (day)	
$\leq$ 7	483 (75.5)
8–30	141 (22.0)
> 30	16 (2.5)
Initial VA	
> 20/70	92 (14.4)
20/70–20/200	57 (8.8)
< 20/200	483 (75.5)
Unknown	8 (1.3)
Location	
Central	336 (52.5)
Paracentral	202 (31.6)
Marginal	41 (6.4)
Total	61 (9.5)
Size, mean $\pm$ SD (mm)	5.0 $\pm$ 2.55
Small	65 (10.2)
Medium	381 (59.5)
Large	194 (30.3)

**Abbreviations:** VA, visual acuity; SD, standard deviation.

isolates, 256 (64.8%) were bacteria, 127 (32.2%) were fungi, and 12 (3.0%) were special pathogens (including four *Mycobacterial* spp., one *Nocardia asteroides*, and seven *Pythium insidiosum*) (Figure 1). Mixed microbial organisms were isolated from 63 (19.4%) eyes. The most commonly isolated organism was *Pseudomonas aeruginosa* (25.1%), followed by *Fusarium* spp. (17.5%), *Staphylococcus epidermidis* (7.6%), and *Propionibacterium* spp. (7.3%) (Table 4). Additionally, PCR results from corneal scrapings were positive for *Pythium* in seven eyes, *Mycobacteria* in two eyes, and human herpesvirus in one eye.

**Table 2** Predisposing Risk Factors in Patients with Microbial Keratitis

Predisposing Risk Factors	n (%)
Trauma	282 (57.2)
Vegetative	107 (21.7)
Soil	47 (9.5)
Contaminated water	16 (3.2)
Others	112 (22.7)
Multiple factors	71 (14.4)
Ocular surface disease	48 (9.7)
Systemic illness	36 (7.3)
Diabetes mellitus	27 (5.5)
HIV infection	4 (0.8)
Autoimmune disease	5 (1.0)
Contact lens wear	29 (5.9)
History of herpes simplex keratitis	18 (3.7)
Previous ocular surgery	9 (1.8)

**Table 3** Results from Corneal Scraping

Variable	n (%)
Gram stain	
Positive results	124 (20.5)
Gram-positive	23 (3.8)
Gram-negative	33 (5.5)
Filamentous fungi	40 (6.6)
Yeast	16 (2.6)
Mixed organism	12 (2.0)
Potassium hydroxide	
Positive results	46 (7.6)
Culture	
Positive results	324 (53.6)
Monomicrobial infection	261 (80.6)
Mixed-microbial infection	63 (19.4)

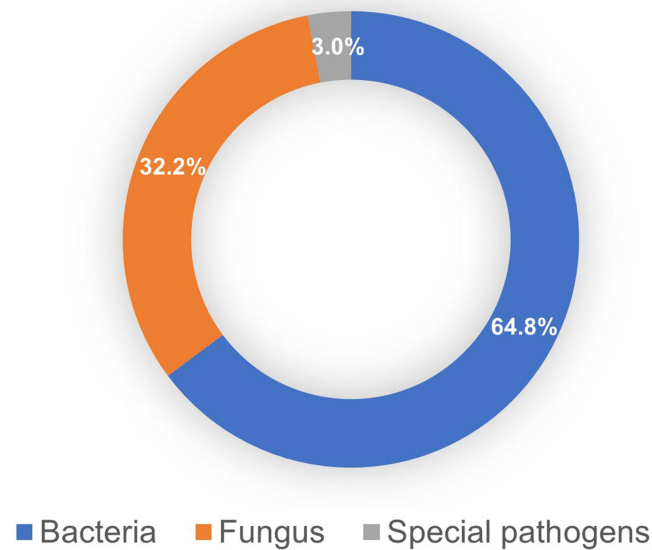
### Risk Factors Related to Organisms in Microbial Keratitis

Multivariate analysis assessed the relationship between risk factors and organisms in microbial keratitis. Contact lens use, presence of diabetes mellitus, and HIV infection were significantly associated with *Pseudomonas aeruginosa* infection ( $p = 0.001$ ,  $p = 0.046$ , and  $p = 0.040$ , respectively) (Table 5). Trauma was associated with infections caused by *Fusarium* spp. and *Propionibacterium* spp. ( $p = 0.047$  for both). Furthermore, diabetes mellitus was correlated with *Staphylococcus epidermidis* infection ( $p = 0.005$ ).

### Other Diagnostic Test Results

In addition to corneal scraping, other diagnostic procedures were performed to identify organisms in select patients, such as confocal microscopy and histopathological staining of tissues from corneal biopsies, corneal transplantation, evisceration, or enucleation. Confocal microscopy was conducted on 385 eyes (60.2%), yielding positive results in 212 eyes (55%). Among these positive results, hyperreflective lines in 195 eyes, cysts in 13 eyes, and both hyperreflective lines

## Isolated organisms



**Figure 1** Percentage of isolated organisms from culture.

and cysts in 4 eyes. Histopathological staining revealed the presence of 12 organisms, including 3 *Pythium* spp., 2 *Microsporidia*, 5 *Mycobacterium* spp., and 2 fungi.

Regarding all diagnostic test results, patients with microbial keratitis were categorized into monomicrobial organism infection (50.5%), mixed microbial organism infection (21.4%), and unknown organism infection (28.1%). Bacteria and fungi were identified as the causative agents of microbial keratitis in 46.7% and 46.6% of patients, respectively (Figure 2).

**Table 4** Isolated Organisms from Cases of Microbial Keratitis

Organism	n (%)
Gram-positive	
<i>Staphylococcus epidermidis</i>	30 (7.6)
<i>Propionibacterium</i> spp.	29 (7.3)
Other coagulase-negative <i>Staphylococcus</i>	15 (3.8)
Other <i>Streptococcus</i> spp.	11 (2.8)
<i>Streptococcus pneumoniae</i>	10 (2.5)
<i>Corynebacterium</i> spp.	8 (2.0)
<i>Staphylococcus aureus</i>	8 (2.0)
<i>Bacillus</i> spp.	6 (1.5)
Others	7 (1.8)
Gram-negative	
<i>Pseudomonas aeruginosa</i>	99 (25.1)
Other <i>Pseudomonas</i> spp.	5 (1.3)
<i>Citrobacter</i> spp.	3 (0.8)
<i>Enterobacter</i> spp.	3 (0.8)
<i>Proteus mirabilis</i>	3 (0.8)
<i>Serratia marcescens</i>	3 (0.8)
<i>Acinetobacter baumannii</i>	2 (0.5)
<i>Moraxella</i> spp.	2 (0.5)
Others	12 (3.0)

(Continued)

**Table 4** (Continued).

Organism	n (%)
<b>Fungi</b>	
<i>Fusarium</i> spp.	69 (17.4)
<i>Aspergillus</i> spp.	16 (4.1)
Unidentified fungus	10 (2.5)
<i>Candida</i> spp.	8 (2.0)
<i>Penicillium</i> spp.	7 (1.8)
<i>Cladosporium</i> spp.	5 (1.3)
<i>Acremonium</i> spp.	4 (1.0)
<i>Curvularia</i> spp.	2 (0.5)
Others	6 (1.5)
<b>Special pathogens</b>	
<i>Pythium insidiosum</i>	7 (1.8)
<i>Mycobacterium</i> spp.	4 (1.0)
<i>Nocardia asteroides</i>	1 (0.3)

**Table 5** Risk Factors Related to Organisms in Microbial Keratitis

Organism	Risk Factor	aOR (95% CI)	p-value
<i>Pseudomonas aeruginosa</i>	Contact lens wear	5.50 (1.93, 15.67)	0.001
	Diabetes mellitus	3.15 (1.02, 9.74)	0.046
	HIV infection	9.00 (1.11, 72.88)	0.040
<i>Fusarium</i> spp.	Trauma	2.47 (1.09, 6.26)	0.047
<i>Staphylococcus epidermidis</i>	Diabetes mellitus	7.37 (1.85, 29.41)	0.005
<i>Propionibacterium</i> spp.	Trauma	7.54 (1.04, 60.22)	0.047

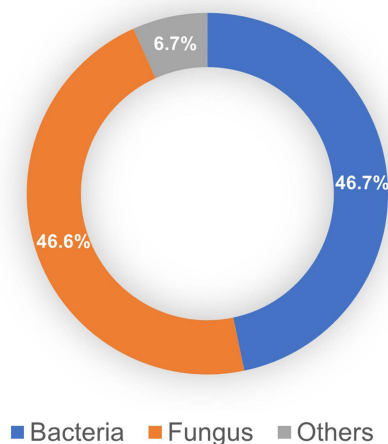
**Notes:** Multivariate logistic regression.

**Abbreviations:** aOR, Adjusted Odds ratio; CI, Confidential interval.

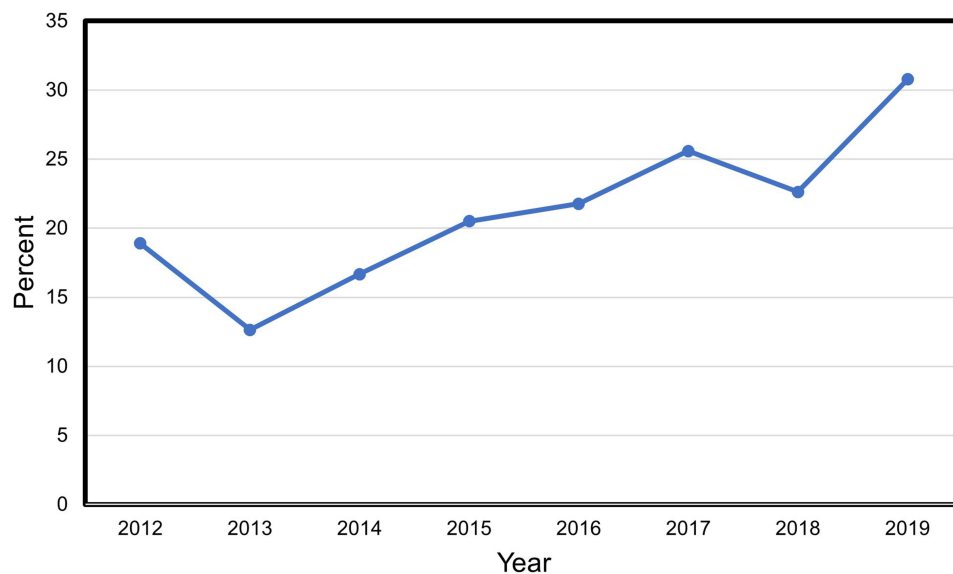
### Trend of Mixed Microbial Infection Cases

Over the 8 years, there was a significant increase in the percentage of mixed microbial infection cases when comparing 2016–2019 with 2012–2015 ( $p = 0.023$ ). Figure 3 illustrates the percentage of mixed microbial infections in each study year.

**Organisms from all diagnostic tests**



**Figure 2** Percentage of organisms from all diagnostic tests.



**Figure 3** Trend of mixed microbial infection cases in 8 years.

## Discussion

Microbial keratitis stands as a primary cause of corneal blindness in Thailand.<sup>9</sup> Our investigation revealed a predominant demographic profile of male, laborers and farmers, a trend congruent with studies conducted in Thailand and India.<sup>7,10,11</sup>

Seventy-seven percent of patients diagnosed with microbial keratitis exhibited at least one associated risk factor, a proportion slightly lower than that reported in comparable studies.<sup>6,10</sup> Trauma emerged as the most prevalent risk factor, consistent with previous observations from developing nations.<sup>12,13</sup> In contrast, contact lens use, prevalent in developed countries, assumed prominence as the leading risk factor for microbial keratitis.<sup>6,14,15</sup>

The percentage of positive smears was quite low, at 20.5% for Gram stain and 7.6% for potassium hydroxide smear. This could be because our center is a referral hospital and the majority of patients (84.8%) had already been treated with antimicrobial agents by other ophthalmologists.

The positive culture rate varied among reports, ranging from 25% to 75%.<sup>6,8,11–14</sup> In our study, the positive culture rate was 53.6%, which aligns with findings from other referral centers in Thailand, such as King Chulalongkorn Memorial Hospital (47.3%),<sup>13</sup> Thammasat University Hospital (52%),<sup>12</sup> and Chiang Mai University Hospital (25.6%).<sup>11</sup> Among the positive culture results, bacteria accounted for 64.8%, followed by fungi at 32.2%. This distribution contrasts with studies conducted in tropical countries, where bacteria and fungi were found to be equally prevalent.<sup>7,11</sup> *Pseudomonas aeruginosa* emerged as the most frequently isolated bacterial pathogen. It exhibited significant associations with contact lens wearers and those with diabetes mellitus and HIV infection, a departure from previous findings, where contact lens use was commonly associated with this organism.<sup>15,16</sup> Our study identified *Fusarium* spp. as the most commonly isolated fungus, which is consistent with several studies conducted in tropical areas.<sup>7,10,11</sup> Trauma was also found to be related to *Fusarium* infection.

However, based on the culture results, 46.4% of the causes of microbial keratitis remain unidentified. Consequently, additional diagnostic tests were conducted to ascertain the remaining pathogens. The proportion of unidentified cases has decreased to 28.1%. All diagnostic test results indicate an almost equal prevalence of bacteria and fungi at 46.7% and 46.6%, respectively, a discrepancy from findings solely reliant on culture-positive results. This study underscores the inadequacy of culture results alone in delineating the epidemiology of microbial keratitis.

In our current clinical practice, we have observed a rise in cases of mixed microbial infection associated with microbial keratitis. Consequently, another objective of this study was to examine the trends in such cases. Among positive culture results, the prevalence of mixed microbial infection cases stood at 19.4%, surpassing figures reported in studies from China<sup>17</sup> and the UK,<sup>18</sup> which reported rates of 10.3% and 11.9%, respectively. However, we identified an

increasing prevalence of mixed microbial infections (21.4%) when all diagnostic tests were considered. These findings underscore the importance of initiating treatment with empiric antimicrobial agents, subsequently adjusting based on clinical response and the results of smears, cultures, and other pertinent tests. Furthermore, there was a significant increase in the percentage of mixed microbial infections when comparing the periods 2016–2019 and 2012–2015 ( $p = 0.023$ ). This may be attributed to shifts in physician experience, technological advancements, alteration in pathogens, and environmental factors.

A limitation of this study is its retrospective design, which inherently incorporates selection bias, loss, and incomplete data.

## Conclusion

When comprehensive diagnostic tests were performed, bacteria and fungi emerged as equally prevalent causes of microbial keratitis. Notably, *Pseudomonas aeruginosa* was the predominant bacterial pathogen, whereas *Fusarium spp.* accounted for the majority of fungal infections. Trauma emerged as the primary predisposing risk factor for microbial keratitis. An upward trend was observed in the number of mixed infections.

## Abbreviation

PCR, Polymerase Chain Reaction.

## Data Sharing Statement

The data supporting the findings of this study are accessible upon request from the author RN (Ratchapol.top@gmail.com). These data have not been made publicly available owing to privacy considerations regarding the research participants.

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## Disclosure

The authors report no conflicts of interest in this work.

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