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Ocular pathogens and antibiotic resistance in microbial keratitis over three years in Harbin, Northeast China

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ABSTRACT.
Purpose: **Understanding the spectrum of ocular pathogens in a given geographic region is important for devising appropriate practical treatment. Therefore, we examined the pathogen spectrum and antibiotic resistance of microbial keratitis in northeast China.**
Methods: **In this retrospective observational study, we reviewed the microbiology laboratory records of patients diagnosed with microbial keratitis in a tertiary eye hospital in Harbin, northeast China, between 2017 and 2019, and analysed the pathogen spectrum and antibiotic susceptibility.**
Results: **We collected 462 specimens, of which 282 exhibited positive cultures. Among these cultures, 257 were bacterial and 25 were fungal. Of the 257 bacterial isolates, 214 (83.27%) were gram positive whereas 43 (16.73%) were gram negative. The most prevalent gram-positive pathogen was coagulase-negative staphylococcus (CoNS; 58.37%), followed by *Staphylococcus aureus* (*S. aureus*; 20.62%) and *Streptococcus pneumoniae* (2.33%). Of the gram-negative bacterial isolates, 10 were *Pseudomonas aeruginosa* (3.89%). The most frequently detected ocular pathogens from fungal isolates were *Fusarium* species (40%). We also found more culture-positive cases of bacterial keratitis in summer. Overall, 16.98% *S. aureus* and 64.00% CoNS isolates were methicillin resistant. These methicillin-resistant bacteria were also more likely to be resistant to other antibiotics, with multidrug resistance found in 77.78% methicillin-resistant *S. aureus* and 90.63% methicillin-resistant CoNS. However, all gram-positive isolates were sensitive to vancomycin and linezolid.**
Conclusion: **Coagulase-negative staphylococcus are the most common ocular pathogens in northeast China. We also show the prevalence of methicillin resistance and concurrent multidrug resistance among staphylococcal isolates. Monitoring the patterns of antimicrobial resistance could help in the management selection.**

Key words: antibiotic resistance – microbial keratitis – ocular pathogen – surveillance

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Introduction

Microbial keratitis (MK) is a sight-threatening, ophthalmologic condition that is prevalent worldwide (Shalchi et al. 2011). This infection may lead to corneal perforation, infectious endophthalmitis, and even loss of vision (Jin et al. 2017). Accurate and rapid diagnosis, timely therapy, and appropriate follow-ups are crucial for preventing nonreversible loss of vision. For treating infectious diseases, the causative pathogen and its antibiotic resistance profiles should be determined before initiating antimicrobial treatment. However, corneal culturing is often not possible in nontertiary hospitals, and bacterial or fungal growth on culture plates takes several days. Hence, clinicians treat ocular infections based on the clinical manifestations of the corneal lesions and history of antibiotic usage in the patients to avoid delay in treatment (Goff et al. 2012). Since the clinical features of corneal microbes are variable, surveillance of the pathogen spectrum is crucial for the treatment of corneal infections.

According to multiple reports, the microbial spectrum of corneal infections varies with weather and geography (Lin et al. 2019a). A retrospective study on the distribution of bacterial keratitis in north China revealed that *Staphylococcus epidermidis* was the most common isolate among 1133 mono-bacterial positive cultures (isolated from 6220 cases), accounting for 26.90% of all bacteria isolated, followed by *Pseudomonas aeruginosa,* which accounted for 11.0% of all isolates (Zhang et al. 2017). However, the two most common bacterial isolates from MK in Taiwan, China, are *P. aeruginosa* (35.2%) and *Staphylococcus* species (13.2%; Liu et al. 2019). These differences in results may be attributed to regional, environmental, and/or seasonal variation. Moreover, the susceptibility of these ocular pathogens to antibiotics could be influenced by the local usage of antibiotics, and therefore, susceptibility profiles must be considered while selecting the appropriate empirical treatment.

Surveillance of local epidemiology of ocular pathogens and their antibiotic resistance can identify the risk factors and susceptibility of these pathogens, which can aid in the choice of empirical treatment. The Antibiotic Resistance Monitoring in Ocular Microorganisms study has been annually publishing the susceptibility data for ocular pathogens in the United States to guide clinical treatment of ocular diseases (Asbell et al. 2020). However, few such surveillance studies have been carried out in China despite the huge burden of corneal ulcers in China (Sun et al. 2002; Zhang et al. 2017; Lin et al. 2019a), especially in the northeastern areas. In this study, we examined the frequency, microbiological characteristics, and antibiotic susceptibility of pathogens isolated from corneal scrapes of patients clinically diagnosed with infectious keratitis in a tertiary eye hospital in northeast China.

Materials and Methods

This was a retrospective, laboratory-based microbiological study of patients who were clinically diagnosed with MK and underwent corneal scraping and culturing at the Eye Hospital of the First Affiliated Hospital of Harbin Medical University between January 1, 2017, and December 31, 2019. Data on patient demographics, predisposing factors, date of specimen submission, isolated pathogens, and subsequent antibiotic sensitivity tests were collected. This research was carried out in accordance with the World Medical Association Declaration of Helsinki and was approved by the Harbin Medical University Research Ethics Committee. All patients provided informed consent prior to participating in the research.

Species identification and antibiotic susceptibility testing

MK was clinically diagnosed based on ocular features such as corneal ulceration, epithelial defect, and/or stromal infiltrate, supplemented by microbiological investigations such as corneal scraping for culturing and testing of antimicrobial sensitivity (Ung et al. 2019; Lin et al. 2019b). Corneal scrapes were taken from patients diagnosed with keratitis who met the inclusion criteria of moderate-sized corneal ulcers (>2 mm diameter) or deep stromal infiltration in accordance with established clinical protocols. Specimens were aerobically inoculated on blood agar, chocolate agar, Sabouraud dextrose agar, and an enrichment liquid medium thioglycolate broth. For suspected cases of *Acanthamoeba keratitis*, non-nutrient *E. coli*-enriched agar plate was used for inoculation. All cultures were incubated for at least 2 weeks while those with suspected acanthamoeba keratitis were incubated for up to 3 weeks. Positive cultures were defined by laboratory staff as growth along the line of inoculation on at least one medium (Shalchi et al. 2011).

All bacterial colonies were subjected to species identification and determination of the antibiotic resistance profile using the VITEK 2 compact automated system (BioMérieux, Marcy l'Etoile, France). The fungi were identified by experienced laboratory staff, based on the colony characteristics and microscopic characteristics of their hyphae and spores. *Staphylococci* isolates were classified as methicillin resistant (MR) or methicillin susceptible (MS) based on their susceptibility to oxacillin. Multidrug resistance (MDR) was defined as resistance to at least three classes of antibiotics.

Patients were given a course of intensive topical antibiotics upon diagnosis after corneal scraping. These treatment protocols included combined therapy (cephalosporin and aminoglycoside), fluoroquinolone monotherapy, or natamycin treatment. The treatment was revised later, based on the results of the culturing and antibiotic sensitivity tests.

Statistical analysis

Continuous data are presented as mean ± SD, whereas categorical data are represented as frequency (%). The Chi-squared test was used to test for differences in proportions of isolates between different seasons. Logistic regression models for concurrent resistance to each antibiotic were used to obtain odds ratios (ORs) based on MR among staphylococci isolates. Statistical analysis were performed using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA). $p < 0.05$ was taken as the criterion for statistical significance.

Results

Demographics and predisposing factors

During the 3-year period from 2017 to 2019, 462 corneal scrapes were performed on 440 patients with clinical keratitis at the Eye Hospital of the First Affiliated Hospital of Harbin Medical University. Repeated corneal scrapings were performed in the same eye in 22 cases because treatment was ineffective in these cases. The mean age of patients was 54.10 ± 21.03 years and 53.68% of patients were women. Cultures were positive in 282 samples (61.04%) in terms of microbial growth. Among these 282 samples, 257 (91.13%) were positive for bacterial species while 25 (8.87%) were positive for fungal species. We did not find any cases with multiple bacteria or both fungi and bacteria in the same culture. We also did not find any *Acanthamoeba* species in these microbial cultures.

The risk factors associated with microbial keratitis are shown in Table 1. Predisposing factors were identified in the eyes of 370 patients in this study (370 of 440 eyes, 84.09%), whereas no obvious documented predisposing factors were recorded in 70 patients (15.91%). Trauma was the most common risk factor (63.64%) with injury due to vegetative materials responsible for nearly half the cases of trauma. Other factors included ocular surgeries (18.18%), ocular diseases (10.91%), and the use of contact lenses (4.55%).

Microbiological profile of ocular samples

Overall, coagulase-negative *Staphylococcus* (CoNS) was the most commonly

Table 1. Predisposing factors of patients with microbial keratitis (*n* = 440).

Predisposing factors	Amount of cases (%)
Trauma	280 (63.64)
Vegetative materials	152 (34.55)
Chemical or thermal injuries	30 (6.82)
Scrap iron	18 (4.09)
Sands	12 (2.73)
Dusts	12 (2.73)
Local irradiation	13 (2.95)
Unidentified foreign bodies	43 (9.77)
Ocular surgeries (cataract surgery, penetrating keratoplasty, pterygium excision)	80 (18.18)
Ocular diseases	48 (10.91)
Contact lens wear	20 (4.55)
Unknown	70 (15.91)

cultured bacterial organism (150 isolates; 58.37% of all bacterial cultures) and hence, the most common gram-positive bacteria, accounting for 70.09% of the gram-positive isolates. The most common CoNS was *S. epidermidis* (142 isolates; 94.67% of all CoNS cultures), followed by *Staphylococcus aureus* (53 isolates; 20.62% of all bacterial cultures) and *Streptococcus pneumonia*e (6 isolates; 2.23% of all bacterial growths).

Of the gram-negative bacteria, the most common isolate was *P. aeruginosa* (10 isolates; 3.89% of all bacterial isolates), accounting for 23.26% of the gram-negative cultures. Other isolates included *Klebsiella* spp. (7 isolates; 2.72% of all bacterial cultures), *Escherichia coli* (6 isolates; 2.23% of all bacterial cultures), and *Acinetobacter* spp. (6 isolates; 2.23% of all bacterial cultures).

The most frequently detected fungal organism was *Fusarium* species (10 isolates, 40% of all fungal cultures), followed by *Aspergillus* species (6 isolates, 24% of all fungal cultures) and *Alternaria* species (4 isolates, 16% of all fungal cultures). A detailed overview of keratitis isolates is provided in Tables 2 and 3.

Table 2. Genus distribution of bacteria isolated from 2017 to 2019 in Harbin Ophthalmic Center.

Genus	Specimens, *n* (%)
Gram-positive organisms	214 (83.27%)
Coagulase-negative staphylococci (CNS)	150 (58.37%)
Staphylococcus epidermidis	142 (55.25%)
Other CNS	8 (3.12%)
Staphylococcus aureus	53 (20.62%)
Streptococcus pneumoniae	6 (2.33%)
Streptococcus mitis/oralis group	3 (1.17%)
Other: *Streptococcus agalactiae*, *Streptococcus pyogenes*	2 (0.78%)
Gram-negative organisms	43 (16.73%)
Pseudomonas aeruginosa	10 (3.89%)
Klebsiella spp.	7 (2.72%)
Escherichia coli	6 (2.33%)
Acinetobacter spp.	6 (2.33%)
Enteric bacilli spp.	5 (1.96%)
Other *Pseudomonas* spp.	2 (0.78%)
Other: *Proteus rettgeri*, *Proteus mirabilis*, *Burkholderia cepacia*, *Citrobacter braakii*, *Citrobacter freundii*, *Aeromonas hydrophila*, *Achromobacter* sp.	7 (2.72%)
Total	257 (100.00%)

Table 3. Genus distribution of fungi isolated from 2017 to 2019 in Harbin Ophthalmic Center.

Genus	Specimens, *n* (%)
Filamentous fungi	23 (92.00%)
Fusarium sp.	10 (40.00%)
Aspergillus sp.	6 (24.00%)
Alternaria sp.	4 (16.00%)
Penicillium	1 (4.00%)
Curvularia	1 (4.00%)
Cladosporium	1 (4.00%)
Nonfilamentous fungi	2 (8.00%)
Candida albicans	1 (4.00%)
Candida parapsilosis	1 (4.00%)
Total	25 (100.00%)

Effects of season on bacterial distribution

According to the internationally recognized astronomical seasons (Ting et al. 2020), spring was defined as the period from March 21 to June 21, summer as June 21 to September 22, fall as September 22 to December 21, and winter as December 22 to March 20 of the following year. Across the cohorts, the highest number of culture-positive cases were recorded in summer (75/257, 29.18%), followed by autumn (73/257, 28.40%), winter (56/257, 21.80%), and spring (53/257, 20.62%). Although the number of culture-positive cases in summer was higher than in other seasons overall seasonal variation in culture-positive cases did not reach statistical significance. We also compared the proportions of gram-positive and gram-negative isolates in different seasons. While the two kinds of isolates occurred more frequently in summer and autumn than in spring and winter, this difference was not statistically significant (χ^2 = 2.2086, p = 0.53).

Sensitivity of gram-positive microorganisms to antibiotics

We found that the cumulative *in vitro* sensitivity of *S. aureus* isolates to linezolid, tetracycline, oxacillin, and gentamicin was 100.00%, 88.68%, 83.02%, and 81.13%, respectively. The sensitivity of *S. aureus* isolates was 81.13% to all fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin). The sensitivity of *S. aureus* to clindamycin (28.30%) and erythromycin (24.53%) was low. All *S. aureus* isolates were susceptible to vancomycin (100.00%). The sensitivity of CoNS to linezolid, gentamicin, and tetracycline was 100.00%, 89.33%, and 70.67% respectively, while only 36.00% of CoNS isolates were sensitive to oxacillin. The sensitivity of CoNS to fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) was 44.67%, 46.00%, and 46.67%, respectively. A lower sensitivity profile was found with clindamycin (38.00%) and erythromycin (16.67%) in CoNS. All CoNS isolates were sensitive to vancomycin. Methicillin/oxacillin-resistant *S. aureus* (MRSA) represented only 16.98% of the isolates whereas methicillin/oxacillin-resistant CoNS (MRCoNS) represented 64.00% of the cultured isolates.

In vitro resistance among *S. pneumoniae* isolates was low for the majority of antibiotics tested, with all isolates being susceptible to vancomycin, linezolid, levofloxacin, moxifloxacin, and cefepime. However, the sensitivity of *S. pneumoniae* isolates to penicillin (83.33%) and tetracycline (33.33%) was lower than that to other antibiotics. The antibiotic sensitive profiles for gram-positive isolates are presented in Table 4.

Concurrent antibiotic resistance and MDR

We further analysed the sensitivity of MRSA and MRCoNS isolates to other antibiotics. While we found 100% sensitivity among these isolates to vancomycin and linezolid, resistance to

Table 4. Overall susceptibility rate of isolated Gram-positive bacteria to different antibiotics in Harbin Ophthalmic Center.

	Staphylococcus aureus	Coagulase-negative staphylococci	*Streptococcus pneumoniae*
Oxacillin	83.02% (44/53)	36% (54/150)	/
Penicillin	/	/	83.33% (5/6)
Cefepime	/	/	100.00% (6.6)
Gentamicin	81.13% (43/53)	89.33% (134/150)	/
Ciprofloxacin	81.13% (43/53)	44.67% (67/150)	/
Levofloxacin	81.13% (43/53)	46.00% (69/150)	100.00% (6/6)
Moxifloxacin	81.13% (43/53)	46.67% (70/150)	100.00% (6/6)
Erythromycin	24.53% (13/53)	16.67% (25/150)	0.00% (0/6)
Tetracycline	88.68% (47/53)	70.67% (106/150)	33.33% (2/6)
Clindamycin	28.30% (15/53)	38.00% (57/150)	0.00% (0/6)
Vancomycin	100.00% (53/53)	100.00% (150/150)	100.00% (6/6)
Linezolid	100.00% (53/53)	100.00% (150/150)	100.00% (6/6)

other antibiotics was variable. Although MRSA isolates were more likely than their methicillin-susceptible counterparts to be concurrently resistant to other antibacterial drug classes, these differences were not statistically significant: tetracycline (22.22 versus 9.09; OR = 2.86 [95% CI, 0.44–18.68]; $p > 0.05$), erythromycin (77.78 versus 75.00; OR = 1.17 [0.21–6.47]; $p > 0.05$), moxifloxacin (33.33 versus 15.91; OR = 2.64 [0.53–13.14]; $p > 0.05$), levofloxacin (33.33 vs 15.91; OR = 2.64 [0.53–13.14]; $p > 0.05$), ciprofloxacin (33.33 vs 15.91; OR = 2.64 [0.53–13.14]; $p > 0.05$), and gentamicin (33.33 vs 15.91; OR = 2.64 [0.53–13.14]; $p > 0.05$). However, methicillin-susceptible isolates were more likely to be concurrently resistant to clindamycin than MRSA isolates (75.00 versus 55.56; OR = 0.42 [0.10–1.83]; $p > 0.05$; Fig. 1A).

Similarly, MRCoNS isolates were significantly more likely to be concurrently resistant to other antibacterial drug classes compared to methicillin-susceptible CoNS isolates: clindamycin (72.92 versus 42.59; OR = 3.63 [1.80–7.33]; $p < 0.05$), tetracycline (35.42 vs 18.52; OR = 2.41 [1.08–5.39]; $p < 0.05$), erythromycin (93.75 versus 64.81; OR = 8.14 [3.00–22.08]; $p < 0.05$), moxifloxacin (67.71 versus 27.78; OR = 5.45 [2.62–11.35]; $p < 0.05$), levofloxacin (67.71 vs 29.63; OR = 4.98 [2.41–10.27]; $p < 0.05$), ciprofloxacin (69.79 vs 29.63; OR = 5.49 [2.65–11.37]; $p < 0.05$). Although this trend was consistent in gentamycin as well, the difference was not statistically significant (12.50 vs 7.41; OR = 1.78 [0.55–5.83]; $p > 0.05$; Fig. 1B).

Multidrug *in vitro* resistance (resistance to ≥ 3 classes of antibiotics) was found in 30.19% of *S. aureus* (Fig. 2A) and 66.00% of CoNS isolates (Fig. 2C). These percentages increased to 77.78% and 90.63% when examining only MRSA (Fig. 2B) and MRCoNS (Fig. 2D), respectively. In contrast, methicillin-susceptible staphylococcal isolates were less likely to be multidrug resistant (20.45% of *S. aureus* and 22.22% of CoNS isolates).

Sensitivity of gram-negative microorganisms to antibiotics

P. aeruginosa isolates demonstrated high rates of *in vitro* susceptibility to all tested antibiotics with only 10.00% resistance against ciprofloxacin. Other gram-negative isolates, such as *Klebsiella* spp., *E. coli*, *Acinetobacter* spp. and *Enteric bacilli* spp., showed higher susceptibility to cefepime and imipenem than they did towards other antibiotics. The antibiotic sensitive profiles for collected isolates are presented according to species or combinations of antibiotics tested in Table 5.

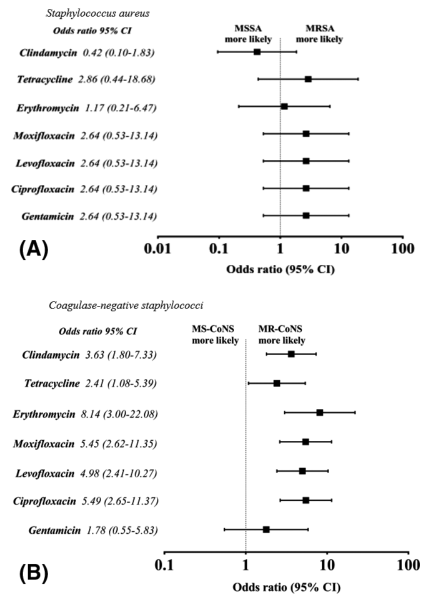

Fig. 1. Concurrent resistance to other antibiotic classes among ocular isolates by methicillin resistance phenotype. Isolates were tested concurrent resistance against clindamycin, tetracycline, erythromycin, moxifloxacin, levofloxacin, ciprofloxacin and gentamicin. MR = methicillin resistant; MS = methicillin susceptible.

Discussion

New technologies, such as *in vivo* confocal microscopy and polymerase chain reaction, have been used in the clinical diagnosis of microbial infections. However, the traditional culturing method remains indispensable for nontertiary hospitals and is the only method to determine antibiotic sensitivity. Regional variation in species distributions and antibiotic resistance profiles of microbes is crucial for the successful management of microbial keratitis. To our knowledge, this study is the first to report the microbial spectrum and the antibiotic susceptibility of microbial isolates from patients with MK in northeast China and provides useful information for clinical treatment decisions. The overall culture-positivity rate of the 462 corneal scrapes from 440 patients at a tertiary eye hospital in Harbin, China from January 1, 2017 to December 31, 2019 was 61.04%.

The culture-positivity rate of 61.04% in this study was within the range of previously reported rates between 32.60% and 79.20% (Amatya et al. 2012; Tan et al. 2017), but slightly higher than average. We used both liquid and solid media in this study to increase the probability of isolating the organism since Sahu et al. previously reported an isolation rate of 13.63% in liquid media and zero in solid media in cases of bacterial keratitis (Bhadange et al. 2013). Our results showed that bacterial isolates accounted for the majority of positive cultures (91.13%) while fungal isolates accounted for only 8.87% of the isolated organisms.

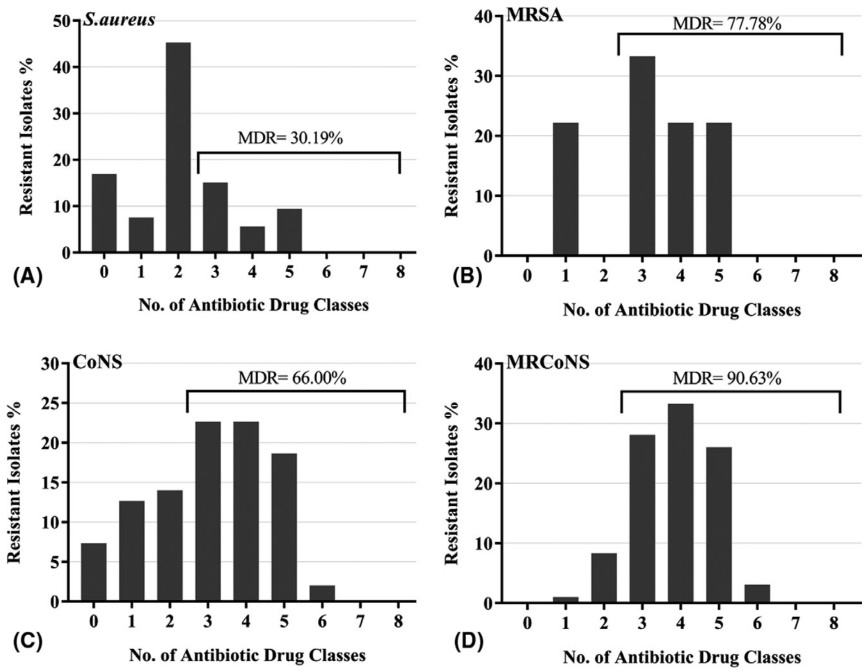

Fig. 2. Multidrug resistance among staphylococcal isolates. Isolates were tested against oxacillin, gentamicin, ciprofloxacin, erythromycin, tetracycline, clindamycin, vancomycin and linezolid. The percentage of resistance includes intermediate resistance. MR = methicillin resistant; MS = methicillin susceptible.

Table 5. Overall susceptibility rate of isolated Gram-negative bacteria to different antibiotics in Harbin Ophthalmic Center.

	Pseudomonas aeruginosa	*Klebsiella* spp.	*Escherichia coli*	*Acinetobacter* spp.	*Enteric bacilli* spp.
Cefepime	100.00% (10/10)	100.00% (7/7)	83.33% (5/6)	33.33% (2/6)	100.00% (5/5)
Imipenem	100.00% (10/10)	100.00% (7/7)	100.00% (6/6)	50.00% (3/6)	100.00% (5/5)
Gentamicin	100.00% (10/10)	57.14% (4/7)	50.00% (3/6)	33.33% (2/6)	60.00% (3/5)
Tobramycin	100.00% (10/10)	57.14% (4/7)	50.00% (3/6)	50.00% (3/6)	60.00% (3/5)
Ciprofloxacin	90.00% (9/10)	57.14% (4/7)	50.00% (3/6)	16.67% (1/6)	80.00% (4/5)
Levofloxacin	100.00% (10/10)	85.71% (6/7)	83.33% (5/6)	50.00% (3/6)	80.00% (4/5)

The distribution of bacterial isolates in our study was consistent with those reported in studies of West Anatolia and England (Yilmaz et al. 2007; Tan et al. 2017; Ting et al. 2018), where CoNS, *S. aureus,* and *Streptococcus* species are the most common gram-positive isolates, with *P. aeruginosa* being the most common gram-negative isolates among the ocular pathogens. Based on similarities in the study designs and average age of patients, this could be explained by the occurrence of similar predisposing factors of patients in both the studies. For instance, the most common risk factor for microbial keratitis in our study was ocular trauma (63.64%) followed by ocular surgeries (18.18%), which was similar to the aforementioned two studies. Furthermore, most of the 620 patients in the study in West Anatolia were agricultural workers, similar to those in our study. CoNS are opportunistic pathogens that commonly colonize the healthy ocular surface and usually cause keratitis under conditions of trauma, while patients with chronic illnesses or immunocompromised states are more prone to infectious diseases caused by *Staphylococcus*. Notably, the frequency distribution of predisposing factors in our data was similar to the China subgroup data reported in the Asia Cornea Society Infectious Keratitis Study (ACSIKS; Khor et al. 2018), suggesting that ocular trauma and ocular surgeries were the most common risk factors for keratitis. This could explain the correlation between risk factors and ocular pathogens, and the high frequency of CoNS in our study. The most common gram-negative bacterial isolate in our study was *P. aeruginosa*, accounting for 23.26% of the gram-negative isolates, similar to reports from other studies conducted in China (Lin et al. 2019a). *Pseudomonas* spp. is commonly found in soil, water, and vegetative material, and its growth is favoured under warm and humid conditions. For instance, cultured isolates from tropical cities exhibit higher rates of *P. aeruginosa*, with 71.00% seen in Brisbane, Australia (Green et al. 2008), and 68.40% seen in Miri, Sarawak, Borneo (Khor et al. 2018). However, the frequency of *P. aeruginosa* in our study was not extremely high, possibly because northeast China has a continental monsoon climate.

The epidemiological features of fungal keratitis also vary across different geographic regions and climatic conditions. For example, filamentous fungi, such as *Fusarium* spp. and *Aspergillus* spp., mostly occur and thrive in tropical conditions and during harvest seasons (Xie et al. 2006). Moreover, yeast-associated keratitis, including cases caused by *Candida* spp., are more common in patients with preexisting ocular surface conditions and/or predisposing systemic diseases (Galarreta et al. 2007). In our study, *Fusarium* spp. (40.00%) predominated among the isolated fungal pathogens, followed by *Aspergillus* spp. (24.00%). This was consistent with the findings of a retrospective study in Qingdao, China, in which *Fusarium* spp. and *Aspergillus* spp. were the two most common fungi (Xie et al. 2006). Similar findings were seen in south China and south India, where *Fusarium* species was the most frequent cause of fungal keratitis (Wang et al. 2000; Gopinathan et al. 2002). Although we found several suspected cases, we did not observe Acanthamoeba growth in the cultured plates. Considering the low frequency of Acanthamoeba keratitis, we may need to combine the examination of clinical features with culturing and *in vivo* confocal microscopy to diagnose this condition. Interestingly, although we cultured bacteria and fungi simultaneously in all cases, we did not find polymicrobial infections in this study. Thus, the treatment of our patients may be less complicated than the treatment of those exhibiting polymicrobial growth.

The species distribution and antimicrobial resistance profiles of causative agents of MK should inform the management of this disease and reaffirm the current treatment protocols. Our initial treatment, either with fluoroquinolone monotherapy or combined therapy (cephalosporin and aminoglycoside), provided good antibiotic coverage for 61.48% (n = 158/257) and 86.45% (n = 217/251) of the cases, respectively. High proportions of resistant microbes seen in our study in the northeast region of China suggest that treatment methods should be chosen carefully. Accordingly, we now use a combination of vancomycin and tobramycin as our initial empirical therapy for all severe suspected cases of bacterial keratitis, and we closely monitor the subsequent clinical response.

Since the introduction of fluoroquinolones in the early 1990s, they have been used widely as prophylactics against bacterial conjunctivitis and keratitis because of their broad spectrum of activity and low toxicity. Ciprofloxacin 0.3%, ofloxacin 0.3%, and levofloxacin 1.5% have been approved by the US Food and Drug Administration (FDA) for the treatment of bacterial keratitis (Lin et al. 2019b). However, several studies have reported an increase in fluoroquinolone-resistant bacteria alongside the increase in topical use of these drugs. In a 2017 study in south India, coagulase-negative *Staphylococcus* species and methicillin-sensitive *Staphylococcus* were reported to exhibit as low as 61.20% and 53.10% susceptibility to moxifloxacin, respectively (Lalitha et al. 2017). In our study, the proportion of CoNS resistant to ciprofloxacin, levofloxacin, and moxifloxacin was extremely high (55.36%), whereas the resistance of *S. aureus* to fluoroquinolones was about 18.87%. The WHO proposed a threshold of 5% resistance for changing the empirical therapy for neonatal sepsis (Muller-Pebody et al. 2011), but no such threshold has been recommended for bacterial keratitis. Our results showed that gram-positive cocci were all sensitive to vancomycin and linezolid, though a previous study reported that vancomycin resistance was increasingly common. For instance, Lixia et al. found around 70% sensitivity of gram-positive cocci to vancomycin, which was similar to the overall sensitivity to other antibiotics (Lin et al. 2019a). Although linezolid, a synthetic oxazolidinone antibiotic approved by the FDA in 2000, has good corneal penetration according to pharmacokinetic studies on animal models, its toxicity has not been reported (Egrilmez & Yildirim-Theveny 2020). Therefore, if resistance to vancomycin becomes more frequent, linezolid should be explored as it shows promise as an antibacterial.

Imipenem is a broad-spectrum antibiotic from the carbapenem group. It is effective against both gram-positive and gram-negative bacteria, including extended-spectrum beta-lactamase-producing bacteria, enterobacteria, and multidrug-resistant *P. aeruginosa* (Egrilmez & Yildirim-Theveny 2020). Gentile et al. (2014) investigated the trends of antibiotic susceptibility in infectious endophthalmitis over the past 25 years and found 93.80% susceptibility of gram-negative isolates for imipenem, which increased over time. Similarly, in our study, we found that 100.00% of *P. aeruginosa* and most other gram-negative bacteria were sensitive to imipenem. Hence, imipenem may be an excellent antibiotic for treating gram-negative infected ocular infections.

In recent years, the prevalence of methicillin/oxacillin-resistant *Staphylococcus* species has become an important issue in ophthalmology (Saïd-Salim et al. 2003; Egrilmez & Yildirim-Theveny 2020). The Antibiotic Resistance Monitoring in Ocular Microorganisms study in April 2020 revealed high rates of methicillin resistance and MDR among *S. aureus* and CoNS isolates, though oxacillin/methicillin resistance was slightly reduced among *S. aureus* isolates and unchanged among CoNS isolates (Asbell et al. 2020). Our findings were consistent with previous reports that MRSA or MRCoNS could also be resistant to multiple drugs. In our study, one in six *S. aureus* isolates and two in three CoNS isolates were resistant to methicillin and approximately three in four MRSA isolates were MDR. Concurrent antibiotic resistance was higher among MRSA compared to methicillin-susceptible *Staphylococcal* isolates, with MRSA isolates being twice more likely to be resistant to other commonly used antibiotics. Similarly, methicillin-resistant CoNS isolates were 1.5 to 2.5 times more likely to be resistant to other antibiotics. However, vancomycin and linezolid are effective against these methicillin-resistant isolates.

Our study highlights antibiotic resistance in microbial keratitis, but suffers from a few limitations. First, since it is a referral hospital-based study, the results may be biased due to the sampling of referred patients. For instance, pretreatment with antibiotics of patients being referred to our hospital could affect the culture-positivity rate and the proportions of pathogen species. In addition, all patients with a corneal infiltrate of >2 mm were subjected to corneal scraping according to national protocol. Hence, patients with mild MK were not included in this study. Therefore, the reported incidence of MK is probably underestimated in our study and some other previous studies. Although our culture-positivity rate (61.04%) was comparable to that reported in previous studies, the use of new technologies such as *in vivo* confocal microscopy and next-generation sequencing could further assist in the diagnosis of MK. Moreover, the three-year data used in our study may be insufficient to analyse the trends of antibiotic resistance of the local ocular pathogens and guide treatment of MK. Hence the length of the study period will be increased in future studies.

In summary, the most common pathogens among infectious keratitis patients in northeast China were CoNS, *S. aureus,* and *P. aeruginosa*. While 17.98% *S. aureus* and 64.00% CoNS were methicillin resistant, 77.78% of MRSA and 90.63% MRCoNS were additionally multidrug resistant. Therefore, fluoroquinolones should be used with caution as monotherapy in the treatment of gram-positive keratitis. Since all gram-positive organisms were sensitive to vancomycin and linezolid, these may be promising candidates for the treatment of staphylococcal ocular infections. Moreover, imipenem may be used to treat gram-negative isolates such as *P. aeruginosa*. Thus, our description of the species distribution and antimicrobial profiles of MK causing microbes could help in devising appropriate protocols for treating MK in northeast China.

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