· Investigation ·

Five-year analysis of isolated pathogens and antibiotic resistance of ocular infections from two large tertiary comprehensive hospitals in east China

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Abstract
● **AIM:** To analyze the spectrum of isolated pathogens and antibiotic resistance for ocular infections within 5y at two tertiary hospitals in east China.
● **METHODS:** Ocular specimen data were collected from January 2019 to October 2023. The pathogen spectrum and positive culture rate for different infection location, such as keratitis, endophthalmitis, and periocular infections, along with antibiotic resistance were analyzed.
● **RESULTS:** We included 2727 specimens, including 827 (30.33%) positive cultures. A total of 871 strains were isolated, 530 (60.85%) bacterial and 341 (39.15%) fungal strains were isolated. Gram-positive cocci (GPC) were the most common ocular pathogens. The most common bacterial isolates were *Staphylococcus epidermidis* (25.03%), *Staphylococcus aureus* (7.46%), *Streptococcus pneumoniae* (4.59%), *Corynebacterium macginleyi* (3.44%), and *Pseudomonas aeruginosa* (3.33%). The most common fungal genera were *Fusarium* spp. (12.74%), *Aspergillus* spp. (6.54%), and *Scedosporium* spp. (5.74%). *Staphylococcus epidermidis* strains showed more than 50% resistance to fluoroquinolones. *Streptococcus pneumoniae* and *Corynebacterium macginleyi* showed more than 90% resistance to erythromycin. The percentage of bacteria showing multidrug resistance (MDR) significantly decreased (χ^2=17.44, *P*=0.002).
● **CONCLUSION:** GPC are the most common ocular pathogens. *Corynebacterium macginleyi*, as the fourth common bacterium, may currently be the local microbiological feature of east China. *Fusarium* spp. is the most common fungus. More than 50% of the GPC are resistant to fluoroquinolones, penicillins, and macrolides. However, the proportion of MDR strains has been reduced over time.
● **KEYWORDS:** ocular infections; bacteria; fungus; antibiotic resistance; multidrug resistance
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INTRODUCTION
Ocular infections may occur in the anatomical structures surrounding the eye (conjunctivitis, blepharitis, canaliculitis, dacryocystitis, orbital and periorbital cellulitis), on the surface of the eye (keratitis), or within the globe of the eye (endophthalmitis and uveitis/retinitis); these conditions are common in ophthalmology and vary from self-limiting to sight-threatening[1-2]. Secondary infections caused by the destruction of normal structures of the eye after ocular trauma have a significant impact on the patient's vision[3]. Timely administration of preventive antibiotics can reduce the occurrence of complications. Pathogenic microorganism examination is the gold standard for the diagnosis of ocular infections. Once a specific microorganism is isolated, it is possible to adjust the treatment based on the culture and antibiogram results[1]. Empiric antibiotic treatment is often initiated before the identification of the causative organism. The widespread use of antibiotics for the treatment of ocular infections and prophylaxis in ophthalmic procedures has led to the emergence of antibiotic-resistant bacterial isolates.

There have been many studies on pathogen distribution and antibiotic susceptibility, most of which were limited to ocular infectious diseases such as keratitis or endophthalmitis, and the sensitivity rate was conducted according to the classification of gram-positive or gram-negative bacteria. The spectrum of pathogens in each area is influenced by geographical location, the occupation of residents, predisposing factors and antibiotics used[4]. In addition, the profiles of antibiotic sensitivity vary significantly by region and evolve over time[5], so it is necessary to regularly summarize these profiles. In light of this, we simultaneously included different kinds of ocular infectious diseases, analyzed common causative agents and their antibiotic susceptibility, and further analyzed the resistance of bacteria classified according to gram-positive cocci (GPC), gram-negative bacilli (GNB), gram-positive bacilli (GPB) and gram-negative cocci (GNC) to antibiotics in two tertiary eye hospitals in East China.

SUBJECTS AND METHODS

Ethical Approval The study was performed in accordance with the ethical principles of the Declaration of Helsinki and approved by the hospital's ethics committee (2021KT273; 2019-K068). Informed consent was not needed to obtain the clinical records of the participants. Patient records were anonymized and deidentified prior to analysis.

Subjects This retrospective study included patients who underwent ocular causative organism identification and antimicrobial susceptibility testing (AST), no matter whether patients were receiving antibiotic treatment previously or at the time of culture collection, and excluded those patients who were immunocompromised or they have systemic immune diseases from January 2019 to October 2023 at the Department of Ophthalmology, Affiliated Hospital of Nantong University and Second Affiliated Hospital of Nantong University, Jiangsu Province, China.

Causative Organism Identification Clinical specimens were obtained from the conjunctiva (a sterile dry cotton swab was used to wipe the lower conjunctival sac from the nasal to the temporal side and backwards while rotating the swab), cornea (a sterile scalpel blade was used under the visualization of slit lamp biomicroscope to obtain corneal samples), aqueous humor (0.1 mL of aqueous humor was removed from the anterior chamber puncture), vitreous (undiluted vitreous samples 0.2 to 0.5 mL were collected by pars plana vitrectomy or vitreous tap), eyelid margin (two sterile cotton swabs were used to squeeze the lower meibomian glands from the bottom to top both inside and outside of the lower eyelid until the meibum was visible at the openings, and the lower eyelid margin including the squeezed meibum was wiped with another sterile swab from the nasal to temporal side and backward), lacrimal passage (a sterile cotton swab was pressed on the lacrimal sac and pushed along the lacrimal canaliculi towards the lacrimal dot opening to extract the middle pus secretions or soya residue spills), and other ocular sites of the patients in the inpatient wards or outpatient clinics. The specimens were inoculated in four culture media (blood agar, chocolate agar, Sabouraud agar, and potato glucose agar). All cultured positive causative organisms were isolated, purified through passage and subsequently identified using a fully automated mass spectrometry organism identification instrument (Autof ms1000, Autof, China).

Antimicrobial Susceptibility Testing The technique used for AST was an automated photometric system (VITEK-2 Compact, bioMerieux, France), which uses the minimum inhibitory concentration (MIC) breakpoint to rapidly identify antibiotic susceptibility. Not every antibiotic was tested for identical quantities of specimens. The antibiotic susceptibilities were determined according to the criteria of the Clinical and Laboratory Standards Institute (CLSI)[6]. Multidrug resistance (MDR) was defined as resistance to at least three classes of antibiotics[7]. The quality control strains used were *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212 and *Streptococcus pneumoniae* ATCC49619. No susceptibility tests were conducted for the fungal cultures.

Statistical Analysis The statistical analysis was performed using SPSS for Windows software (version 22; SPSS, Inc.). Continuous data are presented as the mean±standard deviation (SD), whereas categorical data are represented as frequencies (%). The Chi-square test was used to determine statistical significance, and the level of statistical significance was always $P<0.05$.

RESULTS

A total of 2727 ocular specimens were obtained from 2727 patients (918 females, 1809 males) with suspected ocular infections, and these patients were subjected to microbiological identification. The average age of these patients was 56.41±15.80y (range 10d to 95y). Among the 827 (30.33%) positive culture specimens, 324 had keratitis, 30 had conjunctivitis, 74 had endophthalmitis, 120 had lacrimal passage infections, 21 had other periocular infections, and 258 had emergency ocular traumas. The positive culture rates of the isolated microorganisms from 2019 to 2023 were 29.58%, 33.94%, 28.01%, 38.26% and 23.65%, respectively (χ^2=30.88, $P<0.001$). General information on the cultivation of each ocular infection was shown in Table 1.

Among these 827 specimens (14 specimens coinfected with a bacterium and a fungus, 15 specimens infected with two bacteria, and 15 specimens infected with two fungi), a total of 871 strains were isolated. We did not find any patients

Table 1 General information on ocular infection cultivation

Parameters	Specimens source	Positive specimens (*n*)	Specimens (*n*)	Positive rate (%)
Keratitis	Cornea	324	922	35.14
Endophthalmitis	Aqueous humor or vitreous	74	175	42.29
Periocular infections				
Conjunctivitis	Conjunctiva	30	105	28.57
Lacrimal passage infections	Lacrimal passage secretion	120	273	43.96
Other periocular infections	Conjunctival sac, eyelid margin, or orbit	21	50	42.00
Emergency ocular traumas	Secretion around the wound	258	1202	21.46
Total	-	827	2727	30.33

with multiple microorganisms associated with conjunctivitis. The distributions of the microorganisms associated with keratitis, endophthalmitis, lacrimal passage infections, other periocular infections and emergency ocular traumas are shown in Figure 1.

Spectrum of Causative Agents Among these 871 strains, 530 (60.85%) bacterial strains (32 genera and 73 species) and 341 (39.15%) fungal strains (17 genera and 37 species) were isolated. The most common bacterial genera were *Staphylococcus* spp. (35.02%), *Streptococcus* spp. (6.02%), *Corynebacterium* spp. (4.36%), *Pseudomonas* spp. (3.67%), *Bacillus* spp. (2.07%), and *Klebsiella* spp. (1.61%), which accounted for 78.99% (447/530) of all bacterial cultures. The most common fungal genera were *Fusarium* spp. (12.74%), *Aspergillus* spp. (6.54%), *Scedosporium* spp. (5.74%), *Alternaria* spp. (5.63%) and *Penicillium* spp. (4.82%), which accounted for 90.62% (309/341) of all fungal cultures. The distributions of bacterial and fungal genera isolated from ocular specimens with suspected microbial infections from 2019 to 2023 were presented in Table 2.

The most common bacterial isolates were *Staphylococcus epidermidis* (25.03%), *Staphylococcus aureus* (7.46%), *Streptococcus pneumoniae* (4.59%), *Corynebacterium macginleyi* (3.44%), and *Pseudomonas aeruginosa* (3.33%). *Staphylococcus epidermidis* and *Streptococcus pneumoniae* were the most common bacterial isolates from keratitis, and *Staphylococcus epidermidis* was also the most common bacterial isolate from conjunctivitis, endophthalmitis, lacrimal passage infections and emergency ocular traumas. *Staphylococcus aureus* was the most common bacterial isolate from the other periocular infections. *Fusarium* spp. were the most common fungal isolates from keratitis, followed by *Scedosporium apiospermum* and *Alternaria alternans*. *Penicillium* spp. were the most common fungal isolates from lacrimal passage infections and emergency ocular traumas. Table 3 shows the detailed distribution of microorganisms isolated from each ocular infectious disease (several fungal species were distributed discretely in different kinds of ocular infections and counted by genus).

Table 2 Distribution of bacterial and fungal genera isolated from ocular specimens with microbial infections

Parameters	Strains (*n*=871)	Proportion (%)
Gram-positive cocci	373	42.82
Staphylococcus spp.	305	35.02
Streptococcus spp.	54	6.20
Enterococcus spp.	8	0.92
Kocuria spp.	3	0.34
Others	2	0.23
Gram-negative bacilli	90	10.33
Pseudomonas spp.	32	3.67
Klebsiella spp.	14	1.61
Acinetobacter spp.	8	0.92
Aeromonas spp.	5	0.57
Enterobacter spp.	4	0.46
Proteus spp.	3	0.34
Sphingomonas spp.	3	0.34
Stenophagomonas spp.	3	0.34
Others	18	2.07
Gram-positive bacilli	62	7.12
Corynebacterium spp.	38	4.36
Bacillus spp.	18	2.07
Nocardia spp.	3	0.34
Others	3	0.34
Gram-negative cocci	5	0.57
Moraxella spp.	3	0.34
Neisseria spp.	2	0.23
Fungi	341	40.09
Fusarium spp.	111	12.74
Aspergillus spp.	57	6.54
Scedosporium spp.	50	5.74
Alternaria spp.	49	5.63
Penicillium spp.	42	4.82
Candida spp.	8	0.92
Cladosporium spp.	6	0.69
Paecilomyces spp.	3	0.34
Chaetomium spp.	3	0.34
Others	12	1.38

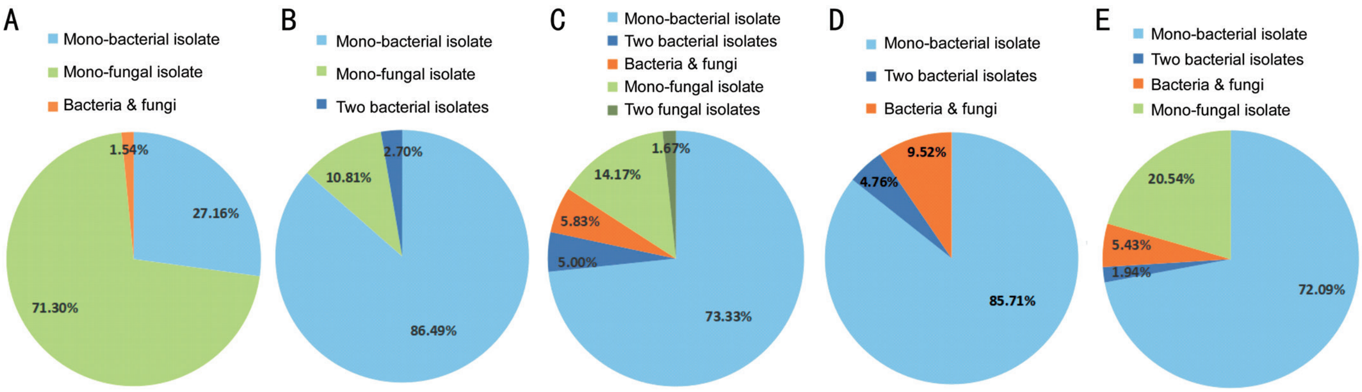

Figure 1 Distribution of the microorganisms associated with each ocular infections A: From 324 specimens with keratitis, 88 (27.16%) specimens infected with a bacterium, 231 (71.30%) specimens infected with a fungus, 5 (1.54%) specimens coinfected with a bacteria and a fungi; B: From 74 specimens with endophthalmitis, 64 (86.49%) specimens infected with a bacterium, 8 (10.81%) specimens infected with a fungus, 2 (2.70%) specimens infected with two bacteria; C: From 120 specimens with lacrimal passage infections, 88 (73.33%) specimens infected with a bacterium, 17 (14.17%) specimens infected with a fungus, 7 (5.83%) specimens coinfected with a bacterium and a fungus, 6 (5.00%) specimens infected with two bacteria, 2 (1.67%) specimens infected with two fungi; D: From 21 specimens with other periocular infections, 18 (85.71%) specimens infected with a bacterium, 1 (4.76%) patient infected with two bacteria, 2 (9.52%) specimens coinfected with a bacterium and a fungus; E: From 258 specimens with emergency ocular traumas, 186 (72.09%) specimens infected with a bacterium, 53 (20.54%) specimens infected with a fungus, 14 (5.43%) specimens coinfected with a bacterium and a fungus, 5 (1.94%) specimens infected with two bacteria.

Table 3 Frequency and composition of microorganisms isolated from each ocular infectious disease *n* (%)

Parameters	All ocular infectious diseases	Keratitis	Endophthalmitis	Conjunctivitis	Acrimal passage infections	Other periocular infections	Emergency ocular traumas
Bacteria	530 (60.85)	93 (23.87)	68 (89.47)	30 (100.00)	107 (79.26)	22 (91.67)	210 (75.81)
Staphylococcus epidermidis	218 (25.03)	21 (6.38)	26 (34.21)	16 (53.33)	28 (20.74)	4 (16.67)	123 (44.40)
Staphylococcus aureus	67 (7.69)	8 (2.43)	4 (5.26)	3 (10.00)	21 (15.56)	12 (50.00)	19 (6.86)
Streptococcus pneumoniae	40 (4.59)	21 (6.38)	3 (3.95)	1 (3.33)	13 (9.63)	-	2 (0.72)
Corynebacterium macginleyi	30 (3.44)	6 (1.82)	-	3 (10.00)	4 (2.96)	1 (4.17)	16 (5.78)
Pseudomonas aeruginosa	29 (3.33)	15 (4.56)	5 (6.58)	-	4 (2.96)	3 (12.50)	2 (0.72)
Klebsiella pneumoniae	10 (1.15)	-	7 (9.21)	-	1 (0.74)	-	2 (0.72)
Bacillus subtilis	10 (1.15)	2 (0.61)	3 (3.95)	-	1 (0.74)	-	4 (1.44)
Bacillus cereus	7 (0.80)	1 (0.30)	3 (3.95)	-	1 (0.74)	-	2 (0.72)
Others	119 (22.85)	19 (5.78)	17 (22.37)	7 (22.33)	34 (25.19)	2 (8.33)	40 (14.44)
Fungi	341 (39.15)	236 (71.73)	8 (10.53)	-	28 (20.74)	2 (8.33)	67 (24.19)
Fusarium spp.	111 (12.74)	101 (30.70)	-	-	2 (1.48)	1 (4.17)	7 (2.53)
Aspergillus spp.	57 (6.54)	33 (10.03)	4 (5.26)	-	4 (2.96)	-	16 (5.78)
Scedosporium apiospermum	50 (5.74)	35 (10.64)	1 (1.32)	-	5 (3.70)	1 (4.17)	8 (2.89)
Alternaria alternans	49 (5.63)	40 (12.16)	1 (1.32)	-	2 (1.48)	-	6 (2.17)
Penicillium spp.	42 (4.82)	11 (3.34)	1 (1.32)	-	9 (6.67)	-	21 (7.58)
Candida spp.	8 (0.92)	6 (1.82)	1 (1.32)	-	1 (0.74)	-	-
Others	24 (2.76)	10 (3.04)	-	-	5 (25.19)	-	9 (3.25)
Total	871 (100.00)	329 (100.00)	76 (100.00)	30 (100.00)	135 (100.00)	24 (100.00)	277 (100.00)

Among the 530 bacterial strains, 70.38% (373/530) of the isolates were GPC, and 16.98% (90/530) were GNB. The proportions of GPC, GNB, GPB and GNC in patients with ocular infections per year during the 5y were shown in Figure 2. Similarly, the proportions of annual components of GPC and GPB fluctuated slightly, but not significantly (P>0.05).

Sensitivity of Microorganisms to Antibiotics A total of 506 bacterial strains underwent AST. ASTs of the 5 most common bacteria were shown in Figure 3. We found that 95.41% of *Staphylococcus epidermidis* strains were resistant to penicillin G; 66.97%, 64.68%, 55.96%, 55.50% and 50.91% of the strains were resistant to erythromycin, oxacillin and levofloxacin, moxifloxacin and ciprofloxacin, respectively. *Staphylococcus aureus* showed 95.45% resistance to penicillin G and 56.72% and 50.75% resistance to erythromycin and clindamycin, respectively. *Staphylococcus aureus* strains exhibited 91.67% and 87.18% resistance to erythromycin and tetracycline, respectively, and 48.72% resistance to cotrimoxazol. *Corynebacterium macginleyi* was 92.59% resistant to clindamycin and erythromycin, and *Pseudomonas aeruginosa* was more than 85% sensitive to different antibiotics.

The resistance of the GPC, GNB, and GPB isolates to the antibiotics available in our study was shown in Table 4. Several common used new generation of antibiotics (such as moxifloxacin and oxacillin) presented a high level of resistance compare to the old ones (gentamicin, chloramphenicol). Only 5 GNC isolates underwent AST; thus, this pathogen group was not included in this resistance analysis.

For levofloxacin, 45.08%, 15.66%, and 25.00% of GPC, GNB, and GPB were resistant, respectively. For gentamicin, 14.29%, 8.86%, and 9.09% of GPC, GNB, and GPB were resistant, respectively. For ceftriaxone, 10.87%, 17.78%, and 0 of GPC, GNB, and GPB were resistant, respectively. For oxacillin, 54.75% of GPC was resistant. For meropenem and imipenem, 0 and 3.90% of GNB was resistant respectively. For linezolid, 0.54% and 0 of GPC and GNB, respectively, were resistant. For vancomycin, 0 of GPC and GPB were resistant.

Among the MDR bacteria (133 *Staphylococcus epidermidis*, 20 *Streptococcus pneumoniae*, 18 *Staphylococcus aureus*, and 44 others), 42.49% (215/506) were MDR in our study. The percentages of MDR strains in patients with ocular infections per year during the 5y were 53.90%, 60.00%, 38.71%, 35.14% and 33.33%, respectively (χ^2=17.44, P=0.002), as shown in Figure 4.

DISCUSSION

In this retrospective study, we report the spectrum of pathogens and the sensitivity of microorganisms to antibiotics in patients with ocular infections in two tertiary comprehensive hospitals in East China. The culture-positivity rate of 30.33% in this study was within the range of previously reported rates between 18.6% and 62.4%[5,8-10]. The reason for the low proportion and positive rate of conjunctivitis is that the causative agent of conjunctivitis is not routinely cultured in these two hospitals; patients with conjunctivitis included in this study planned to receive intraocular surgery before they were diagnosed with conjunctivitis, and all of these patients were prophylactically treated with antibiotics, which would significantly reduce the rate of positive microbial cultures.

Consistent with the findings of other studies, GPC was the predominant pathogen[2,11]. The difference in the proportion of annual composition according to GPC was not statistically significant in our study. However, another study reported that the proportion of GPC significantly decreased in southern China from 2010 to 2018[12].

The five most common bacterial isolates, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus*

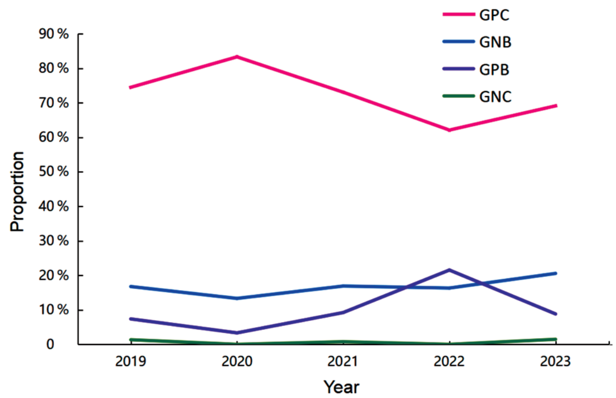

Figure 2 Proportion of GPC, GNB, GPB and GNC in patients with ocular infections per year during the 5y GPC: Gram-positive cocci; GNB: Gram-negative bacilli; GPB: Gram-positive bacilli; GNC: Gram-negative cocci.

Table 4 Resistance of the GPC, GNB, and GPB isolates to the antibiotics available

Parameters	GPC	GNB	GPB
Fluoroquinolones			
Ciprofloxacin	51.59 (162/314)	21.95 (18/82)	15.15 (5/33)
Levofloxacin	45.08 (165/366)	15.66 (13/83)	25.00 (1/4)
Moxifloxacin	43.73 (150/343)	-	-
Penicillins			
Penicillin G	83.89 (302/360)	-	18.92 (7/37)
Oxacillin	54.75 (138/305)	-	-
Piperacillin	-	8.45 (6/71)	0
Piperacillin/tazobactam		0	0
Aminoglycosides			
Tobramycin	-	9.59 (7/73)	0
Gentamicin	14.29 (45/315)	8.86 (7/79)	9.09 (3/33)
Amikacin	-	6.09 (5/82)	0
Carbapenems			
Meropenem	-	0	5.41 (2/37)
Imipenem	-	3.90 (3/77)	0
Tetracyclines			
Tetracycline	24.80 (91/367)	-	8.11 (3/37)
Tigecycline	0	-	-
Cephalosporins			
Cefotaxime	13.73 (7/51)	-	
Ceftriaxone	10.87 (5/46)	17.78 (8/45)	0
Ceftazidime	-	5.19 (4/77)	0
Cefepime	0	4.94 (4/81)	0
Macrolides			
Erythrocin	68.66 (252/367)	-	94.59 (34/37)
Clindamycin	42.59 (138/324)	-	88.89 (32/36)
Others			
Cotrimoxazol	31.88 (110/345)	19.30 (11/57)	11.76 (4/34)
Aztreonam	-	17.39 (12/69)	-
Chloramphenicol	7.84 (4/51)	0	0
Quinuputin/dafuputin	1.06 (2/188)	-	-
Rifampicin	0.65 (2/306)	50.00 (1/2)	16.67 (2/12)
Linezolid	0.54 (2/367)	-	0
Vancomycin	0	-	0

GPC: Gram-positive cocci; GNB: Gram-negative bacilli; GPB: Gram-positive bacilli.

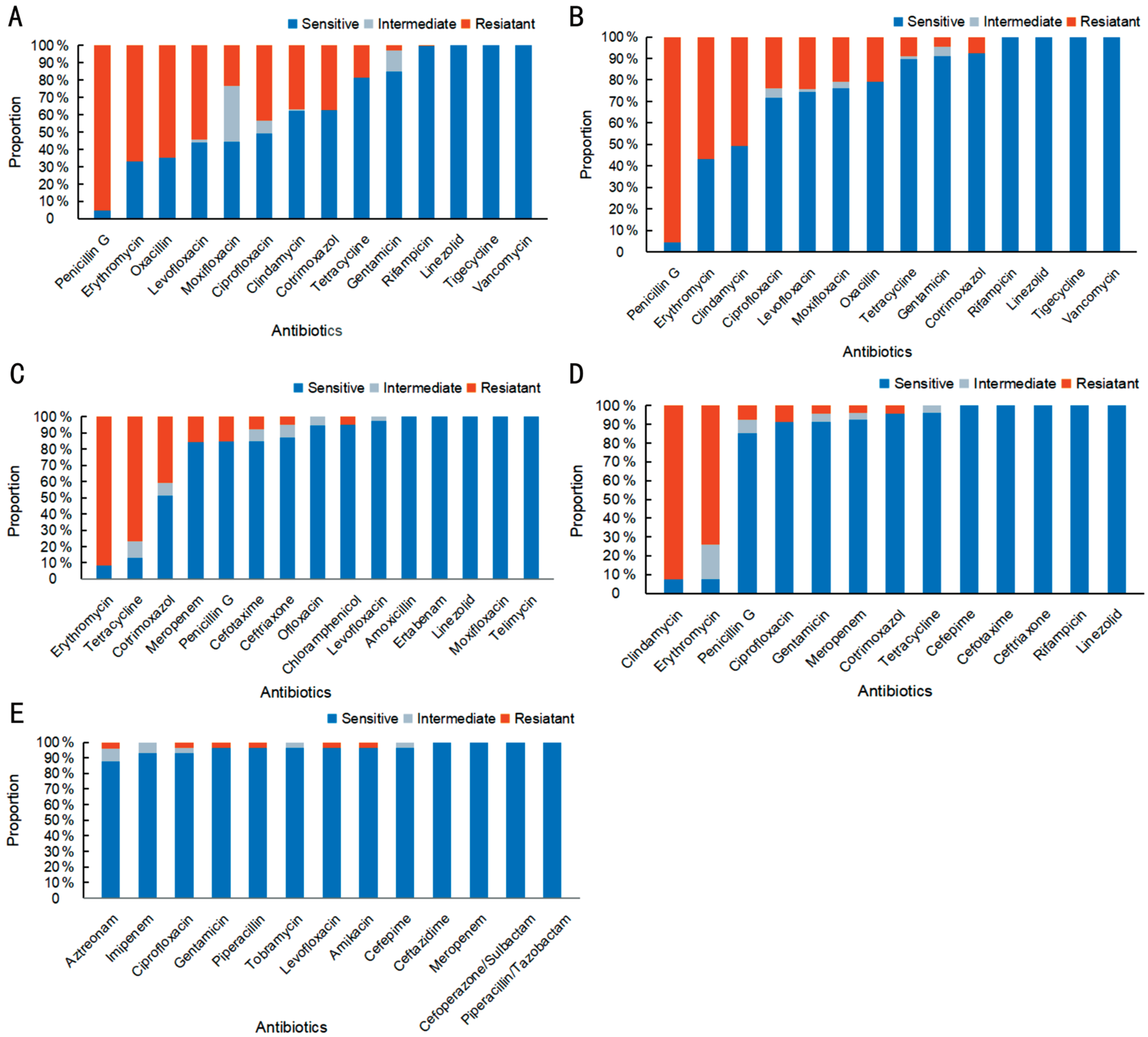

Figure 3 ASTs of the five most common bacteria A: *Staphylococcus epidermidis* showed 95.41% resistance to penicillin G, 66.97%, 64.68%, 55.96%, 55.50%, and 50.91% resistance to erythromycin, oxacillin and levofloxacin, moxifloxacin and ciprofloxacin, respectively, 37.78% and 37.33% resistance to clindamycin and cotrimoxazol, more than 80% sensitivity to other antibiotics; B: *Staphylococcus aureus* showed 95.45% resistance to penicillin G, 56.72% and 50.75% resistance to erythromycin and clindamycin, respectively, 28.35%, 25.76%, 23.88%, and 26.42% resistance to ciprofloxacin, levofloxacin, moxifloxacin and oxacillin and more than 80% sensitivity to other antibiotics; C: *Streptococcus pneumoniae* showed 91.67% and 87.18% resistance to erythromycin, and tetracycline, 48.72% resistance to cotrimoxazol, and more than 80% sensitivity to other antibiotics; D: *Corynebacterium macginleyi* showed 92.59% resistance to clindamycin and erythromycin and more than 85% sensitivity to other antibiotics; E: *Pseudomonas aeruginosa* showed more than 85% sensitivity to different kinds of antibiotics. AST: Antimicrobial susceptibility testing.

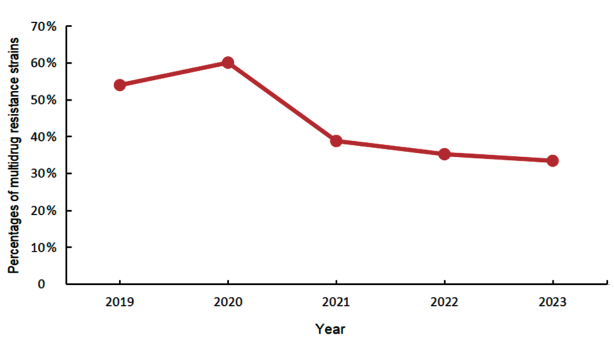

Figure 4 Percentages of multidrug resistance strains in patients with ocular infections per year during the 5y.

pneumoniae, *Corynebacterium macginleyi*, and *Pseudomonas aeruginosa*, accounted for 72.45% (384/530) of the bacterial cultures in our study. Consistent with the findings of other studies, *Staphylococcus* spp. were the predominant pathogens, and *Staphylococcus epidermidis*, which is the main pathogen causing bacterial keratitis[4]. and endophthalmitis[9], remained the most common infectious bacteria. We also found that *Staphylococcus epidermidis* was the main pathogen causing lacrimal passage infections and emergency ocular traumas. A recent study of the ocular surface microbiome provided relevant evidence via sequencing of the samples harvested from the conjunctiva and reported that *Staphylococcus epidermidis* was recovered from 73% of the healthy subjects[13]. Therefore, positive culture results with *Staphylococcus epidermidis* maybe do not necessarily determine the etiology of ocular infections, and a preponderance of *Staphylococcus epidermidis* should be interpreted with caution. In central China, a study reported that *Streptococcus pneumoniae* was the most common isolate in both adults (11, 14.86%) and pediatric patients (30, 24.79%) with dacryocystitis[14]. Coagulase-negative *Staphylococci* (CNS), *Corynebacterium* spp., and *Staphylococcus aureus* constitute the majority of the isolates involved in canaliculitis in India[15]. Due to the special anatomical structure of the lacrimal sac and the lacrimal passage (connecting the conjunctival sac to the nasal cavity), the type of specimen and sampling method may have strongly influenced the results of the study. *Corynebacterium macginleyi,* a slow-growing, lipid-requiring GPB, was first described in 1995[16]. It was the fourth most common isolated agent and caused conjunctivitis, keratitis, lacrimal passage infections, other periocular infections and emergency ocular trauma in our study. However, *Corynebacterium macginleyi* rarely appeared on the list of common bacteria in most of the related literature, except for two reports on the microbiological profile of keratitis in Portugal. *Corynebacterium macginleyi* was the most common or second most common isolated agent: 18.41% (88/478) and 20% (13/65)[17-18]. *Pseudomonas aeruginosa* is the most common gram-negative microorganism; in the case of multidrug-resistant isolates, both the functional and anatomical prognoses are very poor[19]. In the literature, some studies have shown *Pseudomonas aeruginosa* to be the most common or the second agent in bacterial keratitis[12,20-21]. In our study, *Pseudomonas aeruginosa* was the third most common bacterium involved in keratitis.

Bacillus cereus, which is not commonly found in clinical practice, is known to be highly virulent and to cause rapid progression to panophthalmitis. We found 3 *Bacillus cereus* strains isolated from patients with endophthalmitis. *Bacillus cereus* endophthalmitis is a devastating intraocular infection that might be associated with open-globe injuries caused by intraocular foreign bodies, particularly metal objects[9]. Most *Bacillus cereus* endophthalmitis cases result in substantial vision loss within 12-48h[22]. Therefore, the window of therapeutic intervention for this disease is quite narrow compared to that for other diseases.

Ocular fungal infections continue to be an important cause of ocular morbidity and loss of vision, particularly in the developing world, and the fungal genera isolated from different sites of the eye are not consistent[23-24]. *Fusarium* spp. (42.80%, 101/236), *Alternaria alternans* (16.95%, 40/236), *Scedosporium apiospermum* (14.83%, 35/236) and *Aspergillus* spp. (13.98%, 33/236) were found to be the most common fungal isolates from fungal keratitis (FK), which was consistent with previous studies in China[24] and India[25]. One Australian study revealed that *Fusarium* spp. (27%, 15/55) and *Candida parapsilosis* (18%, 10/55) were the most common isolates in FK[26].

Our findings on fungal endophthalmitis (FE) were similar to previous study, which found *Aspergillus* spp. (66.67%, 4/6) were the most common fungi[22]. Another study from Australia showed that the most common organism causing FE was *Candida albicans* (46.43%, 39/84), which was more commonly associated with endogenous endophthalmitis[27].

Fungal conjunctivitis is a rare disorder in ophthalmic care because of its low incidence and nonspecific clinical findings[23]. We did not find any fungi involved in this disease. However, there was a study reported that *Sporothrix* spp. may cause fungal conjunctivitis in Brazial[28].

A previous study reported that lacrimal system fungal infections include *Aspergillus* spp., *Candida* spp., and *Sporothrix* spp[23]. In our study, *Penicillium* spp. (32.14%, 9/28), *Scedosporium apiospermum* (17.86%, 5/28) and *Aspergillus* spp. (14.29%, 4/28) were the most common causative agents of fungal lacrimal passage infections. *Penicillium* spp. (31.34%, 21/67) was also the most common causative agent of trauma coinfected with fungus, followed by *Aspergillus* spp. (23.88, 16/67). We did not find another study that reported the fungal spectrum in trauma patients without signs of keratitis or endophthalmitis. Previous study isolated *Penicillium* spp. and *Aspergillus* spp. from normal ocular surface samples in Chinese[29]. Whether these fungi are merely contaminants or are true pathogens requires further study.

Recently, the continual evolution of bacterial resistance has represented a worldwide challenge in the management of clinical infections, and microorganisms can gradually develop resistance following exposure to antibiotics, thereby decreasing the success rate of empiric antimicrobial treatment[12]. In this retrospective study, we found that several common used new generation of antibiotics, have presented a high level of resistance compare to the old ones.

Fluoroquinolones have been widely used against gram-positive, gram-negative, anaerobic and atypical microorganisms and used prophylactically before eye surgery because of their broad spectrum of activity and low toxicity. Several studies have reported an increase in fluoroquinolone-resistant bacteria alongside the increase in topical use of these drugs[2,12]. Levofloxacin, a third-generation fluoroquinolone, has been used by hospitals and community sectors as a "first-line" antibiotic applied topically to the eye for several years. Our study showed that the resistance rate of the GPC was approximately 50%, which is consistent with the findings of

Gao *et al*[2], who suggested that levofloxacin may no longer be suitable for prophylactic use before eye surgery in China.

Aminoglycosides are particularly useful for treating infections caused by GNB, which include *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp[30]. We found that GNB had less than 10% resistance to aminoglycosides. *Pseudomonas aeruginosa* was only 3.7% resistant to tobramybine, which is a commonly used antibiotic in ophthalmology.

Carbapenems are highly effective against gram-negative and gram-positive drug-resistant infections[31]. Imipenem is effective against extended-spectrum β-lactamase-producing bacteria, enterobacteria, and multidrug-resistant *Pseudomonas aeruginosa*[19]. In our study, GNB was 3.9% (3/77) resistant, and *Pseudomonas aeruginosa* was 7.14% sensitive to imipenem, which was according to previous study that 100% of *Pseudomonas aeruginosa* and most other GNB were sensitive to imipenem[4]. We also found that 100% of GNB were sensitive to meropenem. Hence, imipenem and meropenem are still excellent antibiotics for treating GNB-related ocular infections.

Tetracyclines demonstrate a broad spectrum of activity against a wide range of gram-positive, gram-negative, and atypical pathogens, which is why these drugs have been extensively used in the clinic since they were discovered[32]. The utility of these antibiotics has declined over time through the emergence of antibiotic resistance. Tigecycline is a third-generation of tetracyclines that was reported to be used for treating bacterial keratitis that is resistant to current antimicrobial agents[12]. We also found that 100% of GPC were sensitive to tigecycline, and tigecycline is available only in intravenous formulations.

Cephalosporins encompass five generations of β-lactam antimicrobial agents used to treat gram-positive and gram-negative bacteria in different infections. GPC showed lower resistance rates to cephalosporins (less than 15%). Vancomycin is considered the last effective antibiotic for treating GPB infections[12]. The current recommendation for empirical therapy includes the use of intravitreal vancomycin and ceftazidime for suspected bacterial endophthalmitis[33]. Our results showed that all GPB strains were sensitive to vancomycin and that 5.19% of the GNB strains were resistant to ceftazidime, which verified the recommendation. However, a previous study in southern China reported approximately 30% resistance of GPC to vancomycin and 50% resistance of GNB and GNC to ceftazidime, which was disagree with the recommendation[12].

Linezolid is effective against many resistant gram-positive bacteria, including vancomycin-resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA)[19] and has good corneal penetration according to pharmacokinetic studies on animal models[34]. However, linezolid can cause reversible, duration-dependent optic neuropathy[35].

The emergence of MDR strains of bacteria is quite challenging and has become a global concern. Encouragingly, we found that the proportion of MDR strains decreased over time, which suggests that increased attention has been given to antibiotic stewardship in recent years.

Nonetheless, our study has several limitations: it included only *in vitro* resistance, and these standards are based on susceptibility to systemic antibiotics, which may be different from topical antibiotics and underestimate the clinical efficacy of those antibiotics. Moreover, as two large public tertiary comprehensive hospitals, most patients were referred from community hospitals and had received antibiotic treatment previously

In conclusion, our results indicated that *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Corynebacterium macginleyi*, and *Pseudomonas aeruginosa* are the most common bacteria causing ocular infection, and *Corynebacterium macginleyi* may currently be the local microbiological feature of our region. *Fusarium* spp., *Aspergillus* spp., and *Scedosporium* spp. were the most common fungi involved in ocular infection and these fungi accounted for more than 90% of all fungal cultures. The occurrence of antibiotic resistance in East China was not encouraging, as more than 50% of the GPCs were resistant to fluoroquinolones, penicillins, and macrolides. However, our finding that the proportion of MDR strains has been reduced over time indicates that the recent focus on antibiotic stewardship has been effective.

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