

# Microbial Spectrum and Resistance Patterns in Ocular Infections: A 15-Year Review in East China

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**Purpose:** To report antibiotic resistance rates and trends of common ocular isolates collected over a 15-year period.

**Methods:** We collected 3533 isolates from July 1, 2005 to July 31, 2020. Antibiotic sensitivity was determined according to the guidelines of the Clinical and Laboratory Standards Institute. Chi-squared ( $\chi^2$ ) test was used to analyze changes in antibiotic susceptibility over 15 years.

**Results:** Among the 3533 isolates, the predominant pathogens were the staphylococcal species. Methicillin resistance was observed in 381 *Staphylococcus aureus* (*S. aureus*) isolates (46.4%) and 1888 coagulase-negative staphylococci (CoNS) isolates (61.1%), and methicillin-resistant (MR) isolates had a high probability of concurrent resistance to fluoroquinolones and aminoglycosides. The mean percentage of resistance in staphylococcal isolates did not reach statistical significance across patient age groups ( $P = 0.87$ ). Methicillin resistance did not increase in the CoNS ( $P = 0.546$ ) isolates, and resistance to methicillin slightly decreased among *S. aureus* ( $P = 0.04$ ) isolates over 15 years. Additional exploratory analysis revealed a small decrease in resistance to tobramycin ( $P = 0.01$ ) and chloramphenicol ( $P < 0.001$ ) among the CoNS isolates. All staphylococcal isolates were susceptible to vancomycin.

**Conclusion:** Staphylococci were the most common microorganisms responsible for causing ocular infections. Antibiotic resistance was high among staphylococci, with nearly half of these isolates were resistant to methicillin and these had a high probability of concurrent resistance among MR staphylococci to other antibiotics. Overall, ocular resistance did not significantly change during the 15-year study period. We conclude that continued surveillance of antibiotic resistance provides critical data to guide antibiotic selection.

**Keywords:** methicillin, antibiotic resistance, ocular isolate, staphylococci

## Introduction

The emergence of antibiotic resistance among bacterial pathogens poses a serious therapeutic challenge to public health.<sup>1–6</sup> In ophthalmology, antibiotic resistance among pathogens has become a growing concern in the last decades,<sup>7–13</sup> partially because of an increase in the number of contact-lens users and immune-compromised patients.<sup>14–18</sup> According to the Antibiotic Resistance Monitoring in Ocular Microorganisms in the USA (ARMOR), methicillin resistance is prevalent among staphylococcal isolates from ocular infections, with many strains demonstrating multidrug resistance.<sup>19</sup> The emergence of antibiotic resistance among bacterial pathogens can complicate the choice of antibiotic treatment and threaten vision.

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Ocular infections are serious clinical conditions that can lead to vision loss.<sup>20,21</sup> Timely and appropriate treatment is essential to prevent this loss from being irreversible.<sup>22</sup> In the absence of faster treatment approaches with higher accuracy, ophthalmologists continue to treat ocular infections empirically. However, the current recommendation for empirical therapy requires further validation studies. Surveillance of ocular isolates and antibiotic resistance provides valuable information to aid the choice of empirical treatment.

In this study, we aimed to provide data for determining the optimal use of antibiotics and tools for target-oriented infection control measures. Using isolates collected during July 2005–July 2020, we retrospectively analyzed the trends of microbial-spectrum and antibiotic-resistance profiles at a tertiary hospital in Shanghai, China. As no large-scale, retrospective studies have been performed in this region, our results would benefit efforts to identify the most effective treatment regimen.

## Methods

We reported antibiotic resistance among ocular isolates collected from July 1, 2005 to July 31, 2020 at the Eye & ENT Hospital. The study was performed in compliance with the Declaration of Helsinki and was approved by the hospital's ethics committee (No. 2015011). Informed consent from patients was not required as the data were obtained from patient clinical records in the medical database. We confirm that the patient data complied with relevant data protection and privacy regulations.

The ocular sites from which bacteria were isolated were the conjunctiva, cornea, aqueous humor, vitreous humor, and intraocular foreign bodies. Staphylococci were grouped as methicillin-resistant (MR) or methicillin-susceptible (MS). Minimum inhibitory concentrations were interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute.<sup>23</sup> A one-way analysis of variance (ANOVA) was used to evaluate antibiotic resistance rates by the age of the patients. The changes in the resistance rates over time were determined using the  $\chi^2$  test for trends.

Statistical analyses were performed using SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) or Prism version 5.01 (GraphPad Software). Significance was set at  $P < 0.05$ .

## Results

### Source of Isolates

We collected 3533 isolates from the same number of patients, with 2512 (71.1%) males and 935 (26.5%) females; sex was not reported for 86 patients (2.4%). We had data of the originating patient ages of 3447 isolates (345 isolates, <10 years; 170 isolates, 10–19 years; 532 isolates, 20–29 years; 590 isolates, 30–39 years; 645 isolates, 40–49 years; 528 isolates, 50–59 years; 372 isolates, 60–69 years; 265 isolates,  $\geq 70$  years).

Additionally, we had details of the anatomical sources for 3533 isolates, and they were conjunctiva ( $n = 1286$ ), cornea ( $n = 388$ ), aqueous humor ( $n = 210$ ), vitreous humor ( $n = 715$ ), and intraocular foreign body ( $n = 934$ ). Table 1 shows a detailed overview of isolates.

### Microbial Spectrum

Most isolates (82.5%, 2916) were gram-positive, and the remaining (17.5%, 617) were gram-negative. Overall, the predominant pathogens were staphylococcal species (coagulase-negative staphylococci [CoNS] in 1888 cases and *Staphylococcus aureus* [*S. aureus*] in 381). *Pseudomonas aeruginosa* was the most frequently isolated gram-negative bacterium (Table 2).

**Table 1** Demographics and Clinical Characteristics

| Variable         |                          | N (%)       |
|------------------|--------------------------|-------------|
| Age              | <10                      | 345(9.8%)   |
|                  | 10–19                    | 170(4.8%)   |
|                  | 20–29                    | 532(15.1%)  |
|                  | 30–39                    | 590(16.7%)  |
|                  | 40–49                    | 645(18.3%)  |
|                  | 50–59                    | 528(14.9%)  |
|                  | 60–69                    | 372(10.5%)  |
|                  | >70                      | 265(7.5%)   |
|                  | Unknown                  | 86(2.4%)    |
| Gender           | Male                     | 2512(71.1%) |
|                  | Female                   | 935(26.5%)  |
|                  | Unknown                  | 86(2.4%)    |
| Culture Location | Conjunctiva              | 1286(36.4%) |
|                  | Intraocular foreign body | 934(26.4%)  |
|                  | Vitreous humor           | 715(20.2%)  |
|                  | Cornea                   | 388(11.0%)  |
|                  | Aqueous humor            | 210(5.9%)   |

**Notes:** %, percent of total; n, total number of isolates.

**Table 2** Microbiological Spectrum of the Ocular Samples

| Isolates                         | N    | %Total |
|----------------------------------|------|--------|
| <b>Gram positive</b>             | 2916 | 82.5%  |
| Coagulase-negative staphylococci | 1888 | 53.4%  |
| <i>Staphylococcus aureus</i>     | 381  | 10.8%  |
| <i>Streptococcus</i> species     | 236  | 6.7%   |
| <i>Bacillus</i> species          | 229  | 6.5%   |
| <i>Corynebacterium</i> species   | 92   | 2.6%   |
| <i>Streptococcus pneumoniae</i>  | 60   | 1.7%   |
| Other Gram-positive bacteria     | 30   | 0.8%   |
| <b>Gram negative</b>             | 617  | 17.5%  |
| <i>Enterobacteriaceae</i>        | 235  | 6.7%   |
| <i>Pseudomonas aeruginosa</i>    | 126  | 3.6%   |
| Other non-fermentative bacilli   | 205  | 5.8%   |
| <i>Neisseria</i>                 | 34   | 1.0%   |
| Other Gram-negative bacteria     | 17   | 0.5%   |
| <b>Total</b>                     | 3533 | 100%   |

**Notes:** %, percent of total; N, number of isolates.

## Antibiotic Resistance Rates

All staphylococcal species were susceptible to vancomycin (100%) (Table 3). Of the methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, 48.0%, 46.4%, 41.0%, and 57.6% were resistant to ciprofloxacin, ofloxacin, tobramycin, and TMP-SMX, respectively. Resistance to chloramphenicol was 22.6% and that to moxifloxacin was 25.0%. Only 6.5% of methicillin-susceptible *Staphylococcus aureus* (MSSA) was resistant to moxifloxacin. MRSA isolates had higher resistance rates to ciprofloxacin ( $P < 0.0001$ ), ofloxacin ( $P < 0.0001$ ), levofloxacin ( $P = 0.002$ ), and TMP-SMX ( $P < 0.0001$ ) than MSSA isolates (Table 3). The resistance rates to other drugs were not significant. Of the 1888 methicillin-resistant CoNS (MRCoNS) isolates, 48.7%, 42.4%, and 48.8% were resistant to ciprofloxacin, ofloxacin, and TMP-SMX, respectively. Similar to MRSA isolates, MRCoNS isolates had higher resistance rates to these drugs than MSSA isolates ( $P < 0.005$ ; Figure 1).

## Antibiotic Resistance Rates by Patient Age

The mean percentage of resistance in staphylococcal isolates did not reach statistical significance across patient age groups ( $P = 0.87$ ; Figure 2).

## Antibiotic Resistance Trends Over Time

We found a few changes in the resistance rates of the isolated over 15 years (Figure 3). Methicillin resistance

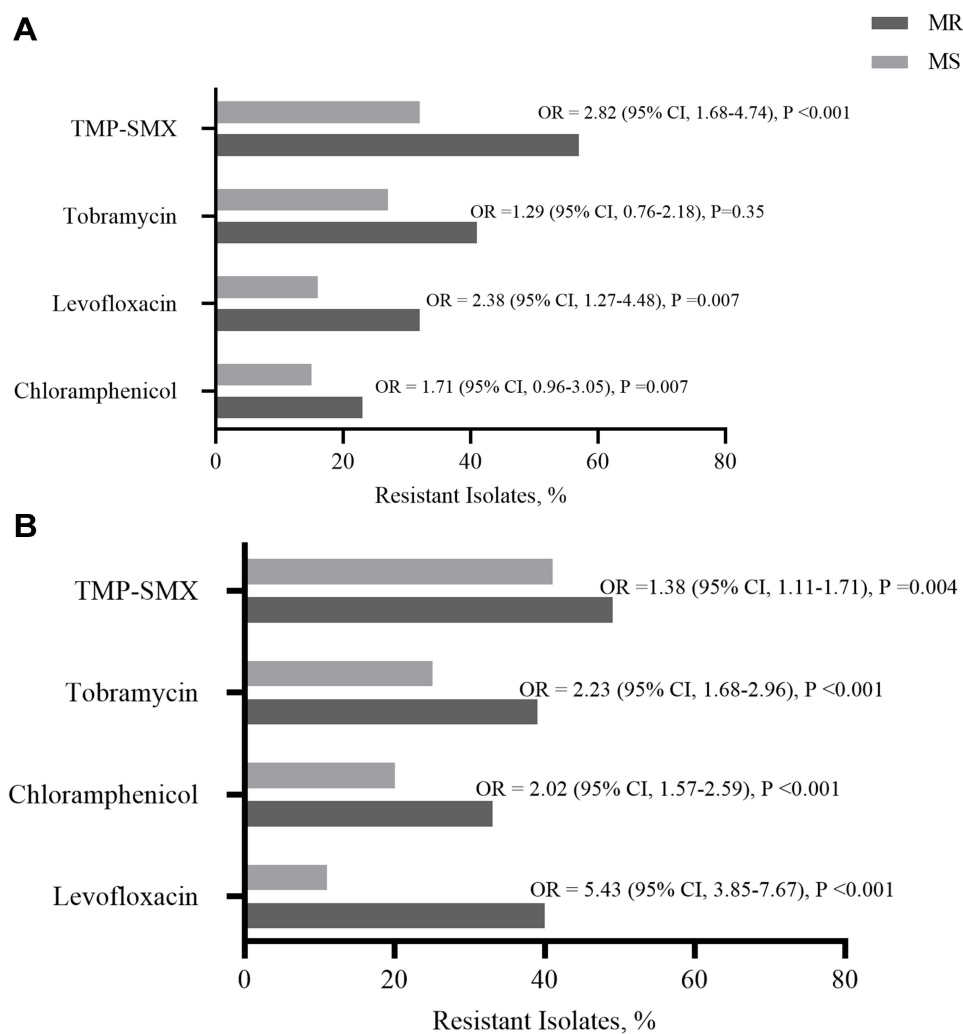
**Table 3** Antibiotic Resistance Profiles for Isolates

| Antibiotic      |        | % (n)      | $\chi^2$ | P             |
|-----------------|--------|------------|----------|---------------|
| Ciprofloxacin   | MSCoNS | 20.8(701)  | 140.4    | $\leq 0.0001$ |
|                 | MRCoNS | 48.7(1125) |          |               |
|                 | MSSA   | 22.0(200)  | 31       | $\leq 0.0001$ |
|                 | MRSA   | 48.0(174)  |          |               |
| Ofloxacin       | MSCoNS | 22.0(480)  | 43.9     | $\leq 0.0001$ |
|                 | MRCoNS | 42.4(603)  |          |               |
|                 | MSSA   | 15.5(142)  | 29.3     | $\leq 0.0001$ |
|                 | MRSA   | 46.4(139)  |          |               |
| Levofloxacin    | MSCoNS | 10.5(418)  | 107.6    | $\leq 0.0001$ |
|                 | MRCoNS | 39.2(769)  |          |               |
|                 | MSSA   | 15.3(144)  | 9.2      | 0.002         |
|                 | MRSA   | 31.4(105)  |          |               |
| Chloramphenicol | MSCoNS | 19.6(577)  | 30.9     | $\leq 0.0001$ |
|                 | MRCoNS | 32.9(875)  |          |               |
|                 | MSSA   | 14.7(163)  | 3.2      | 0.072         |
|                 | MRSA   | 22.6(155)  |          |               |
| Tobramycin      | MSCoNS | 24.8(436)  | 31.1     | $\leq 0.0001$ |
|                 | MRCoNS | 38.9(545)  |          |               |
|                 | MSSA   | 26.3(114)  | 0.9      | 0.351         |
|                 | MRSA   | 41.0(122)  |          |               |
| TMP-SMX         | MSCoNS | 40.3(528)  | 8.7      | 0.003         |
|                 | MRCoNS | 48.8(858)  |          |               |
|                 | MSSA   | 32.3(129)  | 15.6     | $\leq 0.0001$ |
|                 | MRSA   | 57.6(118)  |          |               |
| Moxifloxacin    | MSCoNS | 2.3(132)   | 17.7     | $\leq 0.0001$ |
|                 | MRCoNS | 16.9(236)  |          |               |
|                 | MSSA   | 6.5(31)    | 3.5      | 0.06          |
|                 | MRSA   | 25.0(20)   |          |               |
| Vancomycin      | MSCoNS | 0(701)     | /        | /             |
|                 | MRCoNS | 0(1125)    |          |               |
|                 | MSSA   | 0(200)     | /        | /             |
|                 | MRSA   | 0(174)     |          |               |

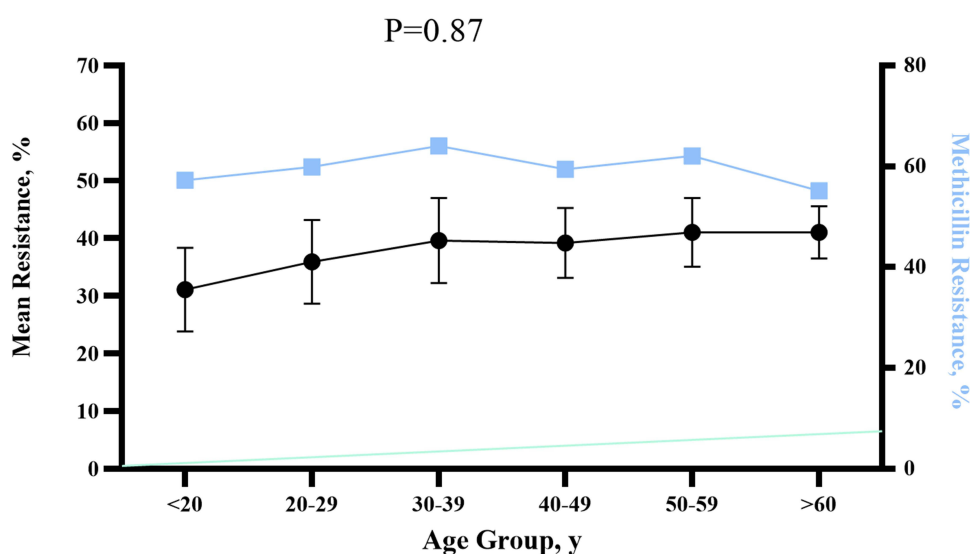
**Notes:** %, percent resistance; n, total number of isolates tested; Underlined values mean statistically significant at  $P < 0.05$ .

**Abbreviations:** MRCoNS, methicillin-resistant coagulase-negative staphylococci; MSCoNS, methicillin-susceptible coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

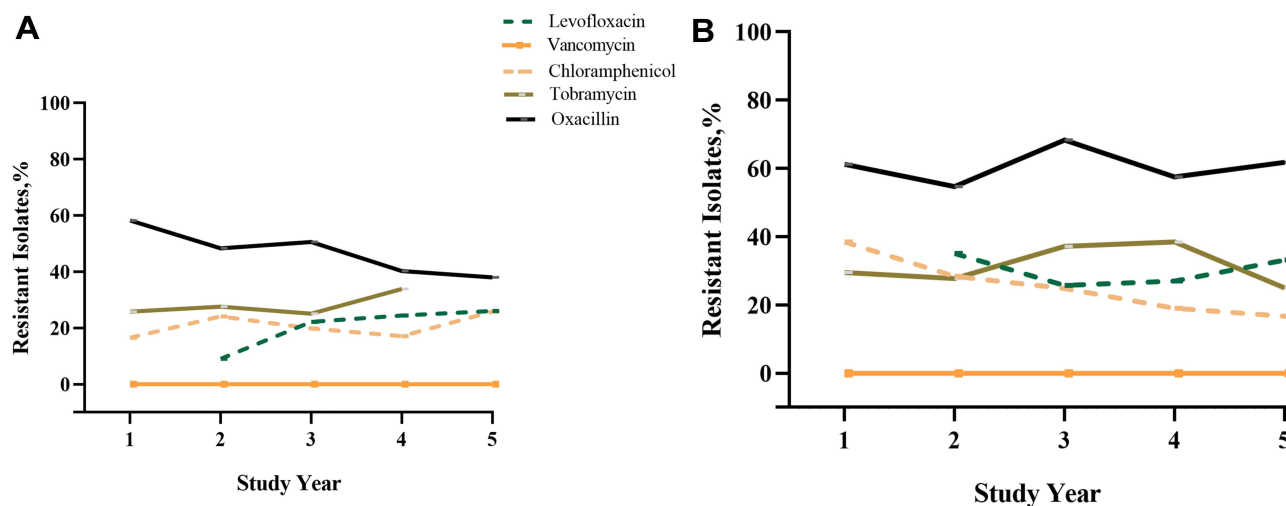
of the CoNS isolates did not increase ( $P = 0.546$ ), rather it slightly decreased among *S. aureus* isolates ( $P = 0.04$ ). Additional exploratory analysis revealed a small decrease in resistance to tobramycin ( $P = 0.01$ ) and chloramphenicol ( $P < 0.001$ ) among the CoNS isolates (Table 4). There were no other changes in the resistance rates.



**Figure 1** Resistance to other antibiotic classes among isolates by methicillin resistance status. **(A)** *Staphylococcus aureus*. **(B)** Coagulase-negative staphylococci. **Abbreviations:** MR, methicillin resistant; MS, methicillin susceptible.



**Figure 2** Resistance among ocular isolates by patient age. Data are expressed as mean (SE) percentage of resistance (black line) and percentage of methicillin resistance (blue line) by decade of life.  $P$  values are calculated using analysis of variance of the mean percentage of resistance.



**Figure 3** Antibiotic resistance trends over time. (A) *Staphylococcus aureus*. (B) Coagulase-negative staphylococci.

## Discussion

Ocular infection is a devastating complication that can have serious anatomical and functional consequences.<sup>24–26</sup> Because the treatment options for ocular infections are limited, antibiotics are commonly used, and increasing resistance to antibiotics is a serious problem that must be overcome. In this study, we investigated the major causative bacteria of ocular infections and antibiotic resistance among ocular pathogens, especially among *S. aureus* and CoNS isolates, to aid the selection of appropriate antibiotics.

Regarding keratitis and conjunctivitis isolates, the most common causative organism of suspected conjunctivitis was staphylococci, similar to that reported previously.<sup>27,28</sup> We also found CoNS to be the most common isolate in conjunctivitis, followed by *S. aureus*. The latter finding differs from that of a previous study.<sup>26</sup> Thus, the high CoNS prevalence we observed may be unique to our public healthcare setting or represent a change in local ocular pathogens. Gram-positive bacteria, and specifically staphylococcal species, remained the predominant

pathogens in patients with endophthalmitis. These patients also had a relatively high incidence of *B. cereus*, a highly virulent bacterium that causes rapid progression to panophthalmitis.<sup>29,30</sup>

The results show that antibiotic resistance continues to be high among staphylococcal species, with nearly half of the isolates resistant to methicillin and a high probability of concurrent resistance among MR staphylococci to other commonly used antibiotics.<sup>31</sup> These findings are consistent with data from other studies.<sup>19,32,33</sup> The MR isolates were 1–3 times more likely to exhibit ciprofloxacin, chloramphenicol, TMP-SMX, levofloxacin, or tobramycin resistance than MS strains, and most MR staphylococci isolates were multidrug-resistant. Within this group, MRSA and MRCoNS isolates were 4 and 7 times more likely to be moxifloxacin-resistant, respectively, although they were sensitive to vancomycin. The cumulative rates of methicillin resistance among *S. aureus* and CoNS isolates in our study (46.5% and 61.1%, respectively) were slightly higher than corresponding rates from the ARMOR study for isolates collected during 2009–2015 (42.2% and 49.7%, respectively).<sup>19</sup> Antibiotic resistance patterns vary over time, geographic location, ethnic groups, and climatic factors.<sup>34–39</sup> In this study, *S. aureus* exhibited a decrease in resistance to oxacillin ( $P = 0.04$ ). Other staphylococcal species exhibited a decrease in resistance to chloramphenicol ( $P < 0.001$ ) and an increase resistance to tobramycin ( $P = 0.01$ ).

## Conclusions

In conclusion, our data demonstrate that staphylococci are the most common microorganisms responsible for causing

**Table 4** Antibiotic Susceptibility Trends for Staphylococci from 2005 to 2020

| P value*        | <i>S. aureus</i> | CoNS          |
|-----------------|------------------|---------------|
| Levofloxacin    | 0.408            | 0.26          |
| Chloramphenicol | 0.553            | <u>≤0.001</u> |
| Tobramycin      | 0.317            | <u>0.01</u>   |
| Methicillin     | <u>0.04</u>      | 0.546         |

**Notes:** \* $\chi^2$  test for trend. Underlined values mean statistically significant at  $P < 0.05$ .

**Abbreviations:** *S. aureus*, *Staphylococcus aureus*; CoNS, coagulase-negative staphylococci.

ocular infections. Antibiotic resistance profiles have not changed significantly between 2015 and 2020, indicating that antibiotic resistance remains a challenge. Therefore, continued surveillance of antibiotic resistance is recommended to guide therapy choices. Clinicians should consider these data when establishing empirical treatment strategies for ocular infections.

## Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

## Acknowledgments

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## Disclosure

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