In Vitro Antibiotic Resistance among Bacteria from the Cornea in the Antibiotic Resistance Monitoring in Ocular MicRoorganisms Surveillance Study

Randall K. Thomas, OD, MPH, FAAO,[1]* Ron Melton, OD, FAAO,[2] Patrick M. Vollmer, OD, FAAO,[3] and Penny A. Asbell, MD[4]

PURPOSE: This study aimed to report on *in vitro* susceptibility patterns among corneal isolates collected in the Antibiotic Resistance Monitoring in Ocular micRoorganisms (ARMOR) study.

METHODS: Each year, from 2009 to 2019, *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* isolates cultured from patients with ocular infections at participating ARMOR sites were submitted to a central laboratory for species confirmation and antibiotic susceptibility testing. In this analysis of corneal isolates, odds ratios for concurrent resistance were based on sample proportions, one-way ANOVA was used to evaluate resistance by patient age, and Cochran-Armitage tests were used to examine changes in antibiotic resistance over time.

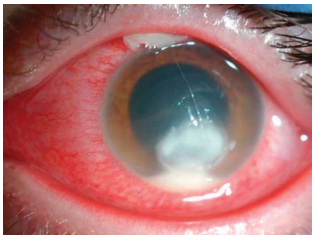
RESULTS: A total of 1499 corneal isolates were collected from 61 sites over the 11-year period. Overall, 34.5% (148 of 429) of *S. aureus* and 41.9% (220 of 525) of CoNS isolates were methicillin resistant and had higher odds ratios for concurrent resistance to azithromycin (17.44 and 5.67), ciprofloxacin (39.63 and 12.81), and tobramycin (19.56 and 19.95), respectively, relative to methicillin-susceptible isolates ($P < .001$, all); also, a high proportion of methicillin-resistant *S. aureus* (85.1%) and methicillin-resistant CoNS (81.8%) were multidrug resistant (at least three classes of antibiotics). Resistance among *S. pneumoniae* isolates was highest for azithromycin (33.1%), whereas *P. aeruginosa* and *H. influenzae* isolates demonstrated low resistance overall. Among staphylococci, antibiotic resistance differed by patient age (*S. aureus*: $F = 6.46$, $P < .001$; CoNS: $F = 4.82$, $P < .001$), and few small changes in resistance (≤3.60% per year), mostly decreases, were observed over time.

CONCLUSIONS: Although rates of *in vitro* antibiotic resistance among presumed keratitis isolates obtained in ARMOR seemed stable between 2009 and 2019, resistance among staphylococci and pneumococci remains high (and should be considered when treating keratitis).

Optom Vis Sci 2021;98:1113–1121. doi:10.1097/OPX.0000000000001768

Supplemental Digital Content: *Direct URL links are provided within the text.*

OPEN SDC



Author Affiliations:
[1]Educators in Primary Eye Care, LLC, Concord, North Carolina
[2]Charlotte Eye, Ear, Nose and Throat Associates, Charlotte, North Carolina
[3]Vita Eye Clinic, Shelby, North Carolina
[4]University of Tennessee Health Science Center, Knoxville, Tennessee
*1randallthomas@gmail.com

Microbial keratitis is a sight-threatening infection of the cornea with clinical findings of a corneal epithelial defect with underlying stromal infiltrate and inflammation.[1,2] Common risk factors for microbial keratitis include contact lens wear, ocular trauma, ocular surface disease, diabetes, and ocular surgery.[1–7] Globally, the incidence of microbial keratitis ranges from 3.6 to 799 per 100,000 persons,[8] whereas incidence in the United States is estimated to be 27.6 per 100,000 person-years overall versus 130.4 per 100,000 person-years among contact lens wearers.[1]

Causative pathogens of microbial keratitis in the United States are most commonly bacteria (up to 95%), followed by fungi,[1,2,6,7,9,10] although it is not uncommon for keratitis to be polymicrobial (i.e., polybacterial or fungal and bacterial).[7,9,11–16] Common bacterial pathogens associated with keratitis include staphylococci (especially *Staphylococcus aureus* and coagulase-negative staphylococci, with *Staphylococcus epidermidis* being predominant among coagulase-negative staphylococci), *Streptococcus pneumoniae*, and gram-negative rods (*Pseudomonas* species),[2,3,5–7,9,10,17–19] whereas *Serratia* and *Moraxella* species are also often implicated.[1,7,18] The epidemiology of bacterial keratitis differs across studies, possibly because of differences in climate, rural versus urban area, and keratitis etiology. For example, although data from several studies have shown gram-positive to be more common than gram-negative isolates,[2,9,10,17] gram-negative organisms were found to be more prevalent in the southern versus the northern United States.[20] Among contact lens wearers, the most common pathogens are coagulase-negative staphylococci and *Pseudomonas aeruginosa*.[3,7]

Prompt diagnosis and appropriate treatment are critical for achieving good clinical outcomes and minimizing visual loss.[2,19,21] Because cultures may take hours to days to process, initial treatment is typically empirical,[19,22] with cases treated as bacterial until proven otherwise. Although keratitis guidelines suggest smears or cultures be taken of severe, chronic, treatment-unresponsive, or atypical infections,[19] some have recommended corneal culture and susceptibility testing for all corneal ulcers, given concerns about antibiotic resistance among bacterial keratitis pathogens.[21] Indeed, a number of studies have reported on keratitis treatment failures due to antibiotic-resistant bacteria.[23–25] In this context, selecting initial

antibiotic treatment may be aided by surveillance data and then modified depending on the clinical course and culture results.

The ongoing Antibiotic Resistance Monitoring in Ocular micRoorganisms (ARMOR) study invites centers across the United States to submit clinically relevant isolates of *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* cultured from ocular infections for *in vitro* antibiotic susceptibility testing.[26,27] Here, we present antibiotic resistance data for isolates specifically obtained from the cornea collected in the ARMOR study to date, with the aim of helping guide antibiotic selection for patients with bacterial keratitis due to these common species and ultimately improving treatment outcomes.

METHODS

ARMOR Study Design

The design and methods of the ARMOR surveillance study have been described.[26,27] Briefly, community hospitals, university hospitals, specialty or ocular centers, and reference laboratories across the United States are asked to provide clinically relevant *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* isolates from patients with ocular infections (i.e., isolates meeting each laboratory's criteria of "significant pathogen") to a central laboratory for confirmation of bacterial species and susceptibility testing. There were no human participants involved, or specimens or tissue samples actively collected as part of the ARMOR study. Because this was a laboratory study and no patient identifying information was provided with isolates, institutional review board approval was not required per Title 45 of the Code of Federal Regulations part 46.101(b); however, the ARMOR study protocol deferred the final need for institutional review board review to individual participating sites based on their discretion. The current analysis reports antibiotic resistance data among ocular isolates collected from the cornea in the ARMOR study from 2009 to 2019.

Antibiotic Susceptibility Testing

Each year of the ARMOR study collection, bacterial isolates were sent to an independent central laboratory (Eurofins Medinet [2009 to 2013]; International Health Management Associates Inc. [2014 to 2019]) for species confirmation and *in vitro* susceptibility testing by broth microdilution methodology with frozen antibiotic microtiter panels.[28] The lowest drug concentrations that inhibited growth of 90% of isolates (minimum inhibitory concentration [$MIC]_{90}$) were recorded for each species-antibiotic combination. Representative antibiotics from 10 different classes were tested as appropriate based on bacterial species, including azithromycin (macrolide); clindamycin (lincosamide); besifloxacin, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin (fluoroquinolones); chloramphenicol (amphenicol); oxacillin and penicillin (β-lactams); polymyxin B (polypeptide); tetracycline (tetracycline); tobramycin (aminoglycoside); trimethoprim (dihydrofolate reductase inhibitor); and vancomycin (glycopeptide).

Clinical and Laboratory Standards Institute interpretive criteria, also known as break points, were used when available to determine whether an isolate was susceptible, intermediate, or resistant to each antibiotic based on the MIC; because besifloxacin was developed for topical ophthalmic use only, no break points are available for interpretation of besifloxacin MICs.[29] Staphylococci were categorized as methicillin susceptible or methicillin resistant based on oxacillin susceptibility, and the break point for oral penicillin was used to determine *Streptococcus pneumoniae* susceptibility to penicillin. Unless otherwise indicated, break points for ciprofloxacin were used to interpret resistance to the fluoroquinolone class. Calculations for the percentage of antibiotic resistance included isolates of both intermediate and full resistance. Multidrug resistance was defined as resistance to at least three antibiotic classes.[30]

Statistical Analysis

Odds ratios, confidence intervals, and *P* values for resistance of methicillin-susceptible and methicillin-resistant staphylococci to each antibiotic were based on sample proportions computed directly from the data, with *P* values calculated using the lognormal distribution. Mean overall antibiotic resistance of staphylococcal isolates by age was evaluated using one-way ANOVA, with ages categorized by decade of life. Because not all antibiotic classes were assessed each year, ANOVA used means of the percentage of drug classes to which each isolate was resistant based on the number of antibiotic classes tested. Means were considered not equal if $P \leq .05$; subsequently, the Tukey honest significant difference test using the $P = .05$ criterion for statistical significance was applied to compare all possible pairs of means (i.e., detect pairwise differences).[31] Additional differences among staphylococci by methicillin resistance were evaluated using χ^2 tests, followed by a multiple-comparison test for proportions. Trends in antibiotic resistance over time were evaluated using a Cochran-Armitage test for linear trends in a proportion, with two-tailed $P < .05$ values reported; magnitude of any change (i.e., slope) was estimated with weighted least squares regression analysis. All statistical analyses were performed using Statistix 10 (Analytical Software, Tallahassee, FL).

RESULTS

Demographics/Species Breakdown

Overall, 1499 keratitis isolates were collected from 61 sites (27 community hospitals, 24 university hospitals, 7 specialty or ocular centers, and 3 reference laboratories) across 30 states. These isolates included *Staphylococcus aureus* (n = 429), coagulase-negative staphylococci (n = 525), *Haemophilus influenzae* (n = 33), *Pseudomonas aeruginosa* (n = 385), and *Streptococcus pneumoniae* (n = 127). Of the 1499 patients from whom isolates were obtained, 677 (45.2%) were female and 632 (42.2%) were male; sex was not reported for 190 patients (12.7%). A total of 1203 isolates were obtained from patients with specified ages (n = 36, <10 years; n = 59, 10 to 19 years; n = 114, 20 to 29 years; n = 106, 30 to 39 years; n = 167, 40 to 49 years; n = 179, 50 to 59 years; n = 195, 60 to 69 years; n = 163, 70 to 79 years; n = 124, 80 to 89 years; n = 60, ≥90 years).

In Vitro Antibiotic Resistance Profiles

Cumulative MIC_{90}s and antibiotic susceptibility/resistance profiles of keratitis isolates are presented by species-antibiotic combination in Appendix Table A1, available at http://links.lww.com/OPX/A518. Of 429 *Staphylococcus aureus* isolates, 148 (34.5%) were methicillin/oxacillin resistant. Among *Staphylococcus aureus* isolates, 52.5% were resistant to azithromycin, and approximately one-third were resistant to fluoroquinolones such as ciprofloxacin (34.7%); none were resistant to vancomycin. Compared with methicillin-susceptible *Staphylococcus aureus* isolates, antibiotic resistance was more prevalent among methicillin-resistant *Staphylococcus aureus*

isolates, with resistance greater than 70.0% for fluoroquinolones (not applicable for besifloxacin) and 89.9% for azithromycin. Overall MIC_{90}s were lower for the later-generation fluoroquinolones (besifloxacin, gatifloxacin, and moxifloxacin) compared with earlier-generation fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin). Among fluoroquinolones, besifloxacin had the lowest MIC_{90}s of the fluoroquinolones, and ciprofloxacin had the highest.

Antibiotic resistance profiles among 525 coagulase-negative staphylococci isolates were similar to those observed for *Staphylococcus aureus* isolates, although the rate of oxacillin/methicillin resistance was slightly higher (n = 220; 41.9%). As with *Staphylococcus aureus* isolates, methicillin-resistant coagulase-negative staphylococci demonstrated higher rates of resistance to various antibiotics than did methicillin-susceptible coagulase-negative staphylococci, and later-generation fluoroquinolones had lower overall MIC_{90}s against coagulase-negative staphylococci isolates than did older-generation fluoroquinolones. Besifloxacin had the lowest MIC_{90}s of the fluoroquinolones, and levofloxacin had the highest.

Resistance rates against *Streptococcus pneumoniae* isolates were less than 10% for all antibiotics tested except for azithromycin (33.1%) and penicillin (29.9%). Rates of resistance among *Pseudomonas aeruginosa* and *Haemophilus influenzae* were low for all antibiotics tested. Against *Pseudomonas aeruginosa* isolates, the lowest MIC_{90}s were observed with ciprofloxacin, gatifloxacin, levofloxacin, and tobramycin, and the highest

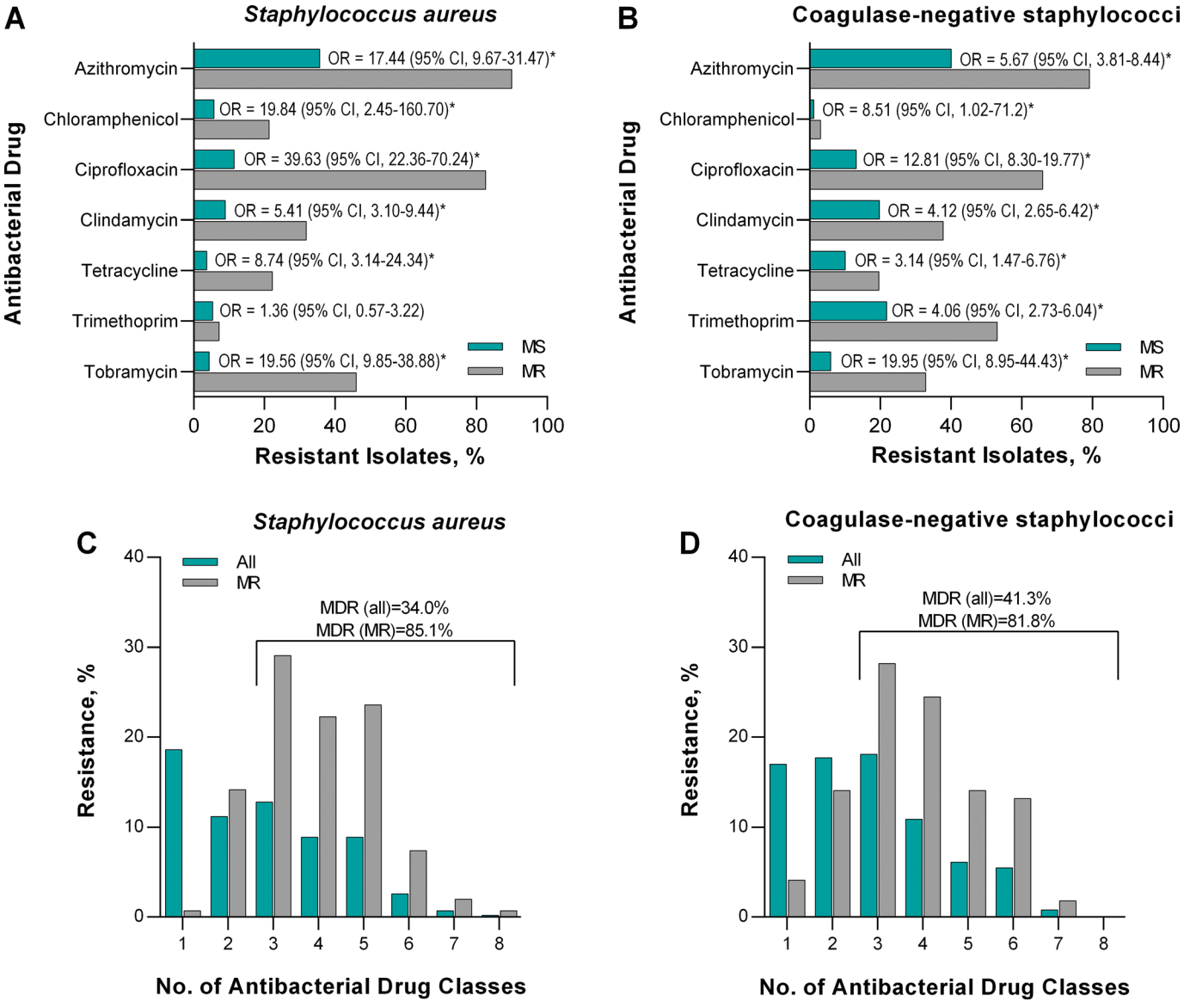


FIGURE 1. Methicillin-resistant staphylococci exhibited high levels of concurrent resistance to other antibiotics and multidrug resistance. The OR and 95% CI for concurrent resistance to antibiotics among MS and MR isolates of *Staphylococcus aureus* (A) and coagulase-negative staphylococci (B) were computed directly from the data, with *P* values calculated using the lognormal distribution (*$P < .05$). Multidrug resistance percentages for all and for MR isolates of *Staphylococcus aureus* (C) and coagulase-negative staphylococci (D) were computed directly from the data; isolates were tested against ciprofloxacin, azithromycin, chloramphenicol, clindamycin, oxacillin, tetracycline, tobramycin, trimethoprim, and vancomycin (representing nine drug classes). CI = confidence interval; MDR = multidrug resistance; MR = methicillin-resistant; MS = methicillin-susceptible; OR = odds ratio.

MIC_{90}s with azithromycin, whereas against *Haemophilus influenzae* isolates, gatifloxacin and ciprofloxacin demonstrated the lowest MIC_{90}s, and azithromycin had the highest.

Concurrent Antibiotic Resistance and Multidrug Resistance

With the exception of trimethoprim (and vancomycin to which there was no concurrent resistance), methicillin-resistant *Staphylococcus aureus* isolates were significantly more likely to be concurrently resistant to antibiotics representative of another drug class than methicillin-susceptible *Staphylococcus aureus* isolates, with $P < .001$ for resistance to azithromycin (odds ratio, 17.44), chloramphenicol (odds ratio, 19.84), ciprofloxacin (odds ratio, 39.63), clindamycin (odds ratio, 5.41), tetracycline (odds ratio, 8.74), and tobramycin (odds ratio, 19.56; Fig. 1A). For all drugs tested, methicillin-resistant coagulase-negative staphylococci were significantly more likely than methicillin-susceptible coagulase-negative staphylococci to be concurrently resistant to all antibiotics tested, with $P \leq .001$ for resistance to azithromycin (odds ratio, 5.67), ciprofloxacin (odds ratio, 12.81), clindamycin (odds ratio, 4.12), trimethoprim (odds ratio, 4.06), and tobramycin (odds ratio, 19.95); with $P = .003$ for resistance to tetracycline (odds ratio, 3.14); and with $P = .05$ for resistance to chloramphenicol (odds ratio, 8.51; Fig. 1B). Figs. 1C and D summarize the percentage of multidrug resistance among staphylococcal isolates. The multidrug resistance rate among all *Staphylococcus aureus* isolates was 34.0%, and that among all coagulase-negative staphylococci isolates was 41.3%, whereas the rates among methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible coagulase-negative staphylococci were 85.1 and 81.8%, respectively.

Mean Percent Resistance and Methicillin Resistance by Age

ANOVA of the mean percentage of resistance by patient age (categorized by decade of life) demonstrated differences among *Staphylococcus aureus* ($F = 6.46$, $P < .001$; Fig. 2A) and coagulase-negative staphylococci ($F = 4.82$, $P < .001$; Fig. 2B), with the lowest resistance among patients in the 10- to 19- and 20- to 29-year categories and increasing by decade of life thereafter. Among *Staphylococcus aureus* isolates, significant pairwise differences were found between isolates both from patients 20 to 29 years of age and from patients 40 to 49 years of age compared with patients 60 years and older in all age groups, as well as between isolates from patients 30 to 39 years of age compared with isolates from patients 80 to 89 years of age. Similarly, among coagulase-negative staphylococci isolates, significant pairwise differences were found between isolates from patients 20 to 29 years of age compared with patients in the age groups 60 to 69, 70 to 79, and 80 to 89 years; between isolates from patients 30 to 39 years of age compared with those from patients 80 to 89 years of age; and between isolates from patients 50 to 59 years of age compared with patients in the age groups 60 to 69 and 80 to 89 years. Oxacillin/methicillin resistance for coagulase-negative staphylococci isolates also differed by patient age ($P = .001$), with isolates from patients 20 to 29 years of age showing significantly lower rates of methicillin resistance compared with isolates from patients in the age groups 60 to 69, 70 to 79, and 80 to 89 years in pairwise comparisons; although, overall, there was a significant difference in oxacillin/methicillin resistance between age groups for *Staphylococcus aureus* ($P = .01$), no significant pairwise differences between isolates from specific age groups were found.

Mean resistance rates among *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* isolates did not differ by age group ($P = .14$ and $P = .93$, respectively).

Trends Over Time

Fig. 3 presents antibiotic resistance rates over time from 2009 to 2019. Oxacillin/methicillin resistance did not change significantly among *Staphylococcus aureus* or coagulase-negative staphylococci isolates. Small but significant decreases in resistance over time were observed to tobramycin among *Staphylococcus aureus* isolates and to ciprofloxacin among coagulase-negative staphylococci isolates; mean changes per year in percent of antibiotic resistance were –1.65% ($P = .001$) for tobramycin among *Staphylococcus aureus* isolates and –0.95% ($P = .03$) for ciprofloxacin among coagulase-negative staphylococci isolates. Among methicillin-resistant *Staphylococcus aureus* isolates, there was a significant decrease over time in resistance to azithromycin (mean change, –0.84%; $P = .003$), ciprofloxacin (mean change, –1.14%; $P = .01$), and tobramycin (mean change, –3.60%; $P = .001$), whereas methicillin-resistant coagulase-negative staphylococci showed an increase in resistance to tobramycin (mean change, +3.43%; $P = .001$). No changes over time in antibiotic resistance were observed among *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* isolates.

DISCUSSION

Since 2009, the ARMOR surveillance study has provided information on the *in vitro* antibiotic susceptibility of common ocular bacterial pathogens collected nationwide in the United States. This is the first ARMOR study report specifically focused on the subset of pathogens presumed causative in bacterial keratitis, comprising nearly 1500 isolates obtained from the cornea over an 11-year span. Overall findings from the current analysis demonstrate high levels of *in vitro* resistance to commonly used antibiotics among staphylococci and pneumococci sourced from the cornea. Given the frequent isolation of these organisms from bacterial keratitis infections and the negative impact that antibiotic resistance may have on successful treatment, these data warrant consideration when selecting appropriate therapies.

In vitro antimicrobial resistance patterns obtained in this analysis were generally similar to those reported in recent regional/single-center U.S. keratitis studies.[4,6,9,10,17,18,32] The rates of methicillin resistance among *Staphylococcus aureus* (34.5%) and coagulase-negative staphylococci (41.9%) corneal isolates in the ARMOR study were comparable with those from other studies (16 to 53 and 25 to 51%, respectively), and similarly, none of the staphylococcal isolates, including methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci, seemed resistant to vancomycin.[2,4,6,9,10,17,18,32] As in the current analysis, the majority of other keratitis studies also found increased resistance to fluoroquinolones among staphylococci, particularly in strains demonstrating methicillin resistance (~35 to 90% resistance to second- and/or fourth-generation agents),[6,9,17,18,32] with little resistance observed among *Pseudomonas aeruginosa*.[4,6,9,10,18] Similarly, around 30% of *Streptococcus pneumoniae* isolates from other keratitis studies exhibited resistance to macrolides (erythromycin),[4,6,18]

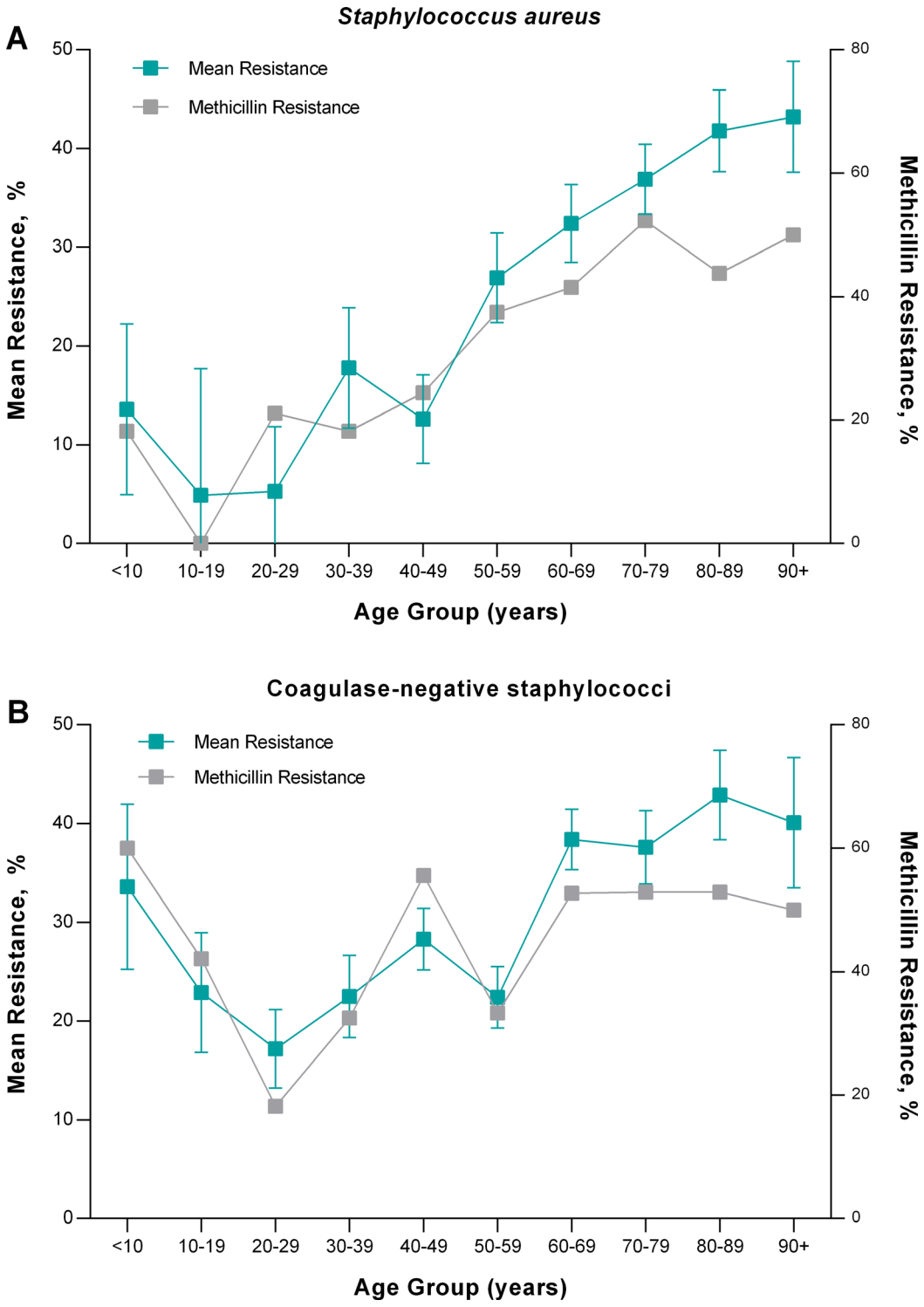


FIGURE 2. Mean percentage of antibiotic resistance (bars denote standard error) and methicillin resistance among isolates of *Staphylococcus aureus* (A) and coagulase-negative staphylococci (B) differed by patient age group (characterized by decade of life). *P* values were calculated using ANOVA for mean percentage of resistance and the χ^2 test for oxacillin/methicillin resistance.

analogous to the 33.1% that were azithromycin resistant herein. One may speculate that any variances observed in cumulative antibiotic resistance profiles for corneal isolates from the ARMOR study and those for keratitis isolates from single/regional institutions are likely due to differences in sample sizes and the time frame and/or geographic location of isolate collection.

In the present analysis, concurrent antibiotic resistance was higher among methicillin-resistant versus methicillin-susceptible

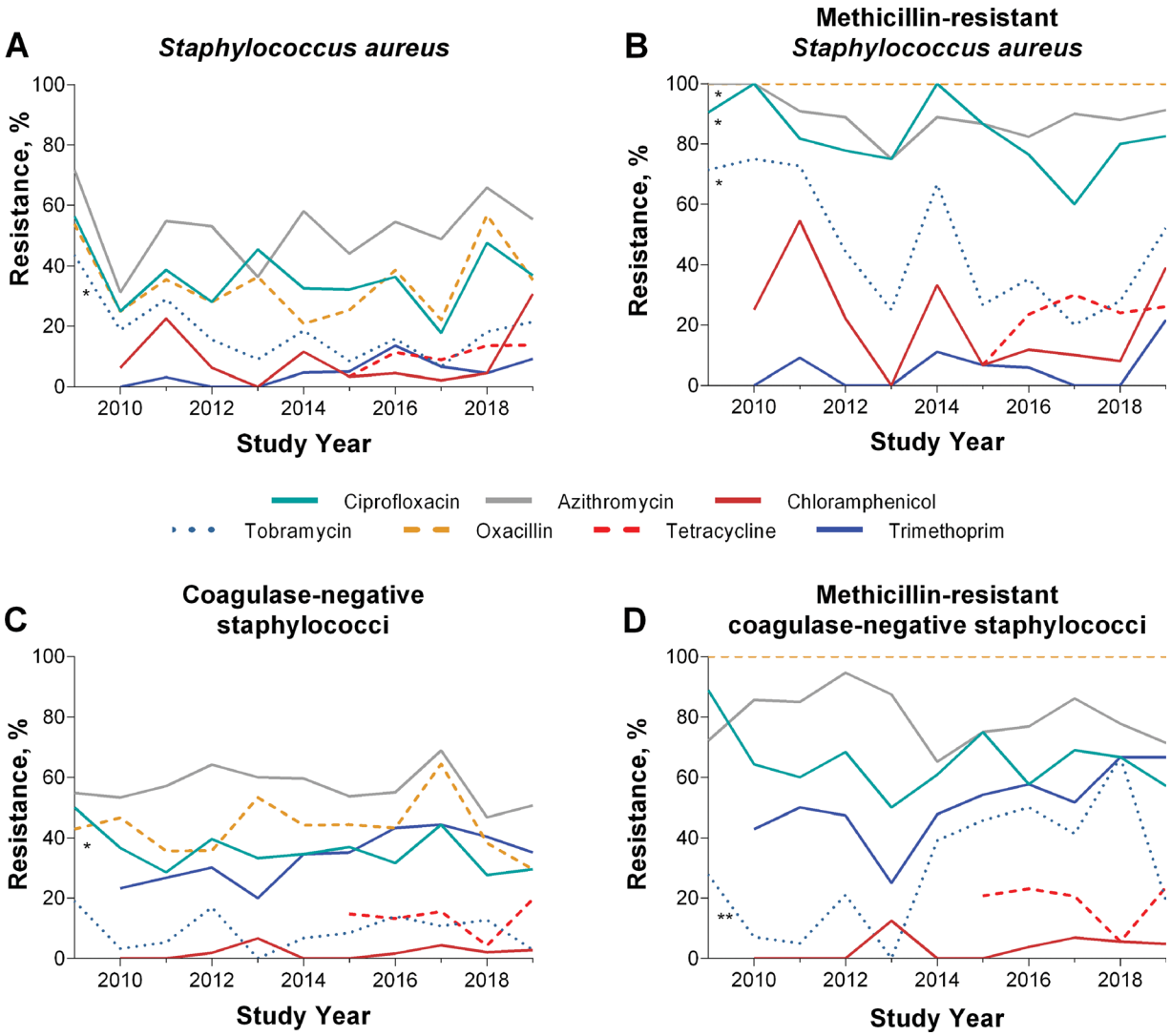


FIGURE 3. Few changes in antibiotic resistance over time were observed among isolates of *Staphylococcus aureus* (A), methicillin-resistant *Staphylococcus aureus* (B), coagulase-negative staphylococci (C), and methicillin-resistant coagulase-negative staphylococci (D). Cochran-Armitage tests were used to identify significant decreasing (*) and increasing (**) trends in antibiotic resistance over the 11-year period.

staphylococcal isolates from the cornea, with methicillin-resistant *Staphylococcus aureus* isolates being 5 to 40 times more likely than methicillin-susceptible *Staphylococcus aureus* to be resistant to other antibiotics tested, with the exception of trimethoprim, and with methicillin-resistant coagulase-negative staphylococci isolates being 3 to 20 times more likely to be resistant to other antibiotics based on calculated odds ratios. Previous ARMOR study results, inclusive of all ocular isolates and not limited to those obtained from the cornea,[27] reflected a similar trend in odds ratios for concurrent antibiotic resistance among methicillin-resistant versus methicillin-susceptible staphylococcal isolates; however, slightly higher odds ratios were observed among the subset of presumed keratitis staphylococcal pathogens. The reason for this difference may be due to the fact that the 10-year ARMOR study results also encompassed isolates from potentially milder and less resistant infections (e.g., conjunctivitis). Nonetheless, similar patterns in the broader ARMOR study data set compared with those specifically from corneal pathogens suggest that antibiotic resistance may not differ much by etiology, although additional study is needed. Trimethoprim was equally active against methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* corneal isolates, a finding consistent with results of the broader ARMOR study data set and with those from Ocular Tracking Resistance in US Today (Ocular TRUST),[27] an older prospective surveillance study of bacterial isolates from ocular infections collected between October 2005 and June 2006.[33] Furthermore, overall rates of multidrug resistance (at least three drug classes) among corneal *Staphylococcus aureus* (34.0%) and coagulase-negative staphylococci (41.3%) were comparable with the proportions of isolates exhibiting oxacillin/methicillin resistance (34.5 and 41.9%, respectively), and rates of multidrug resistance were greater than 80% among methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci. Taken together, these findings are consistent with methicillin resistance often serving as a hallmark for increased resistance to other antibiotics.[17,33]

As was previously reported among all ocular isolates in the ARMOR study,[27] comparisons of resistance rates between patient

age groupings (decade of life) among *Staphylococcus aureus* and coagulase-negative staphylococci keratitis isolates reflected increases in antibiotic resistance with patient age. This association is likely a result of older people having a higher risk of exposure to antibiotic-resistant bacteria than younger patients because of frequent time spent in health care facilities. In addition, a lack of quality tear film/drier eyes in older individuals[34] may contribute to an increased risk of infection and thus a greater probability that such an infection may be caused by a pathogen with antibiotic resistance; indeed, more than 700 of the ~1200 isolates from patients with known ages in the current study were obtained from those 50 years or older.

No significant changes were observed from 2009 to 2019 in oxacillin/methicillin resistance among staphylococcal isolates from the cornea. Rates of resistance to other antibiotics remained relatively stable over time, with no change or generally small decreases in resistance (–1 to –2%) observed among staphylococcal isolates; small but significant decreases in antibiotic resistance to azithromycin, ciprofloxacin, and tobramycin in methicillin-resistant *Staphylococcus aureus*; and a modest increase in resistance to tobramycin in methicillin-resistant coagulase-negative staphylococci. Given the small magnitudes of these changes, further studies are needed to determine whether these trends persist and the potential impact of yearly fluctuations. There were no significant changes over time in antibiotic resistance among *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* isolates. In contrast, previous studies have reported an increase in resistance to moxifloxacin and gatifloxacin among methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* between 1993 and 2012 as well as an increase in resistance to moxifloxacin over time (2006 to 2014) among streptococcal and staphylococcal isolates.[17,18] Although the current ARMOR study findings are encouraging in terms of resistance not generally increasing, resistance nonetheless remains an issue.

Despite increased resistance observed among corneal pathogens, topical antibacterial eye drops remain the preferred method for treatment in most bacterial keratitis cases, as they are expected to achieve high concentrations in conjunctival and corneal tissues.[19] The fluoroquinolone class of antibiotics in particular is considered the *de facto* standard therapy for the management of bacterial corneal ulcers (small peripheral infiltrates and or peripheral infiltrates approaching 2 mm).[35] To date, only the early-generation fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin) are approved by the U.S. Food and Drug Administration for the treatment of corneal ulcers, although later-generation fluoroquinolones (besifloxacin, moxifloxacin, and gatifloxacin) are widely used for this purpose.[19,35] Examination of MIC_{90}s in the current ARMOR study analysis revealed notable differences within the fluoroquinolone class of agents: among all staphylococcal isolates, MIC_{90}s were lower for the later-generation fluoroquinolones compared with earlier-generation fluoroquinolones, and besifloxacin had the lowest MIC_{90}s.

Studies in bacterial keratitis have shown a correlation between low fluoroquinolone MICs and improved treatment outcomes,[36–38] suggesting that the differences observed in MIC_{90}s from the current ARMOR analysis may have clinical relevance. For instance, a 43% reduction in improvement and a 29% reduction in cure were found among ciprofloxacin-treated bacterial keratitis infections having a ciprofloxacin MIC of >1 μg/mL compared with those in patients with more sensitive isolates.[36] Significant associations between MIC and clinical outcomes were also observed among patients treated with fluoroquinolone monotherapy whose corneal ulcers healed without surgical intervention[37] and in the Steroids for Corneal Ulcers Trial, where higher moxifloxacin MICs were associated with decreased visual acuity, larger infiltrate/scar size, and slower time to reepithelialization.[38]

In studies including randomized controlled trials, both the newer-generation fluoroquinolones moxifloxacin and gatifloxacin have performed at least as well as older fluoroquinolones, compounded fortified cefazolin/tobramycin combination therapy, and potentially better than ciprofloxacin in the treatment of keratitis.[39–43] Besifloxacin has also shown clinical utility in the management of bacterial keratitis in a prospective, randomized trial[44]; in a retrospective safety surveillance study[45]; and in case reports.[46–48] In addition, *in vitro*[49] and *in vivo* animal studies have provided further potential for this indication.[50–52] Besifloxacin ophthalmic suspension (0.6%) is unique among the fluroquinolones in that the formulation contains the DuraSite delivery system designed to increase ocular surface residence time.[53] This formulation attribute, together with low MIC_{90}s/high potency against corneal isolates, may confer the potential for greater efficacy against the common bacterial pathogens of keratitis. However, comparative trials with besifloxacin are needed to evaluate whether MIC differences are indeed meaningful in the clinical setting.

Several limitations are inherent to the current study. Although the data analysis was limited to isolates characterized as originating from the cornea and that were presumed to represent keratitis infections, most participating laboratories lacked confirmatory diagnostic information. Other limitations associated with the ARMOR study data in general included sampling bias associated with infrequent culturing of ocular organisms during routine clinical practice and sites' selection of isolates for submission, inconsistencies in documenting patient age and sex, selection of antibiotics tested, and the use of systemic break points to interpret MIC data. The validity of using systemic break points to interpret MIC data for ocular isolates has not been established and may potentially result in overreporting of resistance because higher antibiotic concentrations are achievable on the ocular surface after topical instillation.[54] In the case of besifloxacin, systemic break points were not available to interpret MIC data because this medication has only been developed as an ophthalmic formulation. Nonetheless, in the absence of topical ophthalmic break points, the application of systemic interpretive criteria remains a valuable tool for the determination and comparison of antibiotic resistance profiles among ocular bacteria.

CONCLUSIONS

Data from the ARMOR surveillance study indicate that levels of antibiotic resistance among presumed keratitis isolates were relatively stable from 2009 to 2019, although resistance levels among staphylococci and pneumococci remain high. Methicillin resistance and multidrug resistance are common among staphylococcal isolates, with methicillin-resistant strains specifically demonstrating an increased likelihood for concurrent resistance to other drug classes; these findings should be considered when treating keratitis, especially in older patients. Small decreases in antibiotic resistance among methicillin-resistant *Staphylococcus aureus* are encouraging but require further monitoring.

ARTICLE INFORMATION

Supplemental Digital Content: Appendix Table A1, available at http://links.lww.com/OPX/A518. Minimum inhibitory concentrations and antibiotic resistance profiles for *S. aureus,* coagulase-negative staphylococci, *S. pneumoniae, P. aeruginosa,* and *H. influenzae* keratitis isolates from ARMOR 2009–2019.

Submitted: January 18, 2021

Accepted: April 25, 2021

Funding/Support: Bausch + Lomb (a division of Bausch Health US, LLC).

Conflict of Interest Disclosure: RKT is a consultant to Bausch + Lomb and ICARE. RM is a consultant to, on the speakers bureau of, on the advisory committee of, or involved in research for the following companies: ICARE and Bausch + Lomb. PMV has no conflicts to disclose. PAA reports serving as a consultant and receiving personal fees from Alcon, Kao, Medscape, Perrigo, Santen, ScientiaCME, Senju, and Shire and serving on advisory boards for Allakos, Allergan, Bausch + Lomb, Dompé, Kala, Novaliq, Novartis, Regeneron Pharmaceuticals, and Sun Pharmaceuticals, outside the submitted work. The Antibiotic Resistance Monitoring in Ocular micRoorganisms study was funded by Bausch + Lomb. Medical writing assistance was provided by Churchill Communications (Maplewood, NJ) and funded by Bausch + Lomb.

Author Contributions: Conceptualization: PAA; Data Curation: RKT, RM, PMV, PAA; Formal Analysis: RKT, RM, PMV, PAA; Methodology: PAA; Writing – Review & Editing: RKT, RM, PMV, PAA.

REFERENCES

1. Jeng BH, Gritz DC, Kumar AB, et al. Epidemiology of Ulcerative Keratitis in Northern California. Arch Ophthalmol 2010;128:1022–8.

2. Truong DT, Bui MT, Cavanagh HD. Epidemiology and Outcome of Microbial Keratitis: Private University versus Urban Public Hospital Care. Eye Contact Lens 2018;44: S82–6.

3. Bourcier T, Thomas F, Borderie V, et al. Bacterial Keratitis: Predisposing Factors, Clinical and Microbiological Review of 300 Cases. Br J Ophthalmol 2003;87:834–8.

4. Jin H, Parker WT, Law NW, et al. Evolving Risk Factors and Antibiotic Sensitivity Patterns for Microbial Keratitis at a Large County Hospital. Br J Ophthalmol 2017; 101:1483–7.

5. Rossetto JD, Cavuoto KM, Osigian CJ, et al. Paediatric Infectious Keratitis: A Case Series of 107 Children Presenting to a Tertiary Referral Centre. Br J Ophthalmol 2017;101:1488–92.

6. Hsu HY, Ernst B, Schmidt EJ, et al. Laboratory Results, Epidemiologic Features, and Outcome Analyses of Microbial Keratitis: A 15-year Review from St. Louis. Am J Ophthalmol 2019;198:54–62.

7. Puig M, Weiss M, Salinas R, et al. Etiology and Risk Factors for Infectious Keratitis in South Texas. J Ophthalmic Vis Res 2020;15:128–37.

8. Ung L, Bispo PJM, Shanbhag SS, et al. The Persistent Dilemma of Microbial Keratitis: Global Burden, Diagnosis, and Antimicrobial Resistance. Surv Ophthalmol 2019;64:255–71.

9. Sand D, She R, Shulman IA, et al. Microbial Keratitis in Los Angeles: The Doheny Eye Institute and the Los Angeles County Hospital Experience. Ophthalmology 2015;122:918–24.

10. Truong DT, Bui MT, Memon P, et al. Microbial Keratitis at an Urban Public Hospital: A 10-year Update. J Clin Exp Ophthalmol 2015;6:498.

11. Pate JC, Jones DB, Wilhelmus KR. Prevalence and Spectrum of Bacterial Co-infection during Fungal Keratitis. Br J Ophthalmol 2006;90:289–92.

12. Stefan C, Nenciu A. Post-traumatic Bacterial Keratitis—A Microbiological Prospective Clinical Study. Oftalmologia 2006;50:118–22.

13. Preechawat P, Ratananikom U, Lerdvitayasakul R, et al. Contact Lens–related Microbial Keratitis. J Med Assoc Thai 2007;90:737–43.

14. Lim NC, Lim DK, Ray M. Polymicrobial versus Monomicrobial Keratitis: A Retrospective Comparative Study. Eye Contact Lens 2013;39:348–54.

15. Pakzad-Vaezi K, Levasseur SD, Schendel S, et al. The Corneal Ulcer One-touch Study: A Simplified Microbiological Specimen Collection Method. Am J Ophthalmol 2015;159:37–43.e1.

16. Termote K, Joe AW, Butler AL, et al. Epidemiology of Bacterial Corneal Ulcers at Tertiary Centres in Vancouver, B.C. Can J Ophthalmol 2018;53:330–6.

17. Chang VS, Dhaliwal DK, Raju L, et al. Antibiotic Resistance in the Treatment of *Staphylococcus aureus* Keratitis: A 20-year Review. Cornea 2015;34:698–703.

18. Peng MY, Cevallos V, McLeod SD, et al. Bacterial Keratitis: Isolated Organisms and Antibiotic Resistance Patterns in San Francisco. Cornea 2018;37:84–7.

19. Lin A, Rhee MK, Akpek EK, et al. Bacterial Keratitis Preferred Practice Pattern®. Ophthalmology 2019;126:P1–55.

20. Estopinal CB, Ewald MD. Geographic Disparities in the Etiology of Bacterial and Fungal Keratitis in the United States of America. Semin Ophthalmol 2016;31:345–52.

21. Austin A, Lietman T, Rose-Nussbaumer J. Update on the Management of Infectious Keratitis. Ophthalmology 2017;124:1678–89.

22. Austin A, Schallhorn J, Geske M, et al. Empirical Treatment of Bacterial Keratitis: An International Survey of Corneal Specialists. BMJ Open Ophthalmol 2017; 2:e000047.

23. Chatterjee S, Agrawal D. Multi-drug Resistant *Pseudomonas aeruginosa* Keratitis and Its Effective Treatment with Topical Colistimethate. Indian J Ophthalmol 2016;64:153–7.

24. Garg P, Sharma S, Rao GN. Ciprofloxacin-resistant *Pseudomonas* Keratitis. Ophthalmology 1999;106:1319–23.

25. Moshirfar M, Mirzaian G, Feiz V, et al. Fourth-generation Fluoroquinolone-resistant Bacterial Keratitis After Refractive Surgery. J Cataract Refract Surg 2006; 32:515–8.

26. Haas W, Pillar CM, Torres M, et al. Monitoring Antibiotic Resistance in Ocular Microorganisms: Results from the Antibiotic Resistance Monitoring in Ocular micRorganisms (ARMOR) 2009 Surveillance Study. Am J Ophthalmol 2011;152:567–74.e3.

27. Asbell PA, Sanfilippo CM, Sahm DF, et al. Trends in Antibiotic Resistance among Ocular Microorganisms in the United States from 2009 to 2018. JAMA Ophthalmol 2020;138:439–50.

28. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. Approved Standard. CLSI Document M07-A11.

29. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 27th Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2019. CLSI Document M100-S29.

30. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, Extensively Drug-resistant and Pandrug-resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. Clin Microbiol Infect 2012;18:268–81.

31. Tukey JW. Comparing Individual Means in the Analysis of Variance. Biometrics 1949;5:99–114.

32. Elsahn AF, Yildiz EH, Jungkind DL, et al. *In Vitro* Susceptibility Patterns of Methicillin-resistant *Staphylococcus aureus* and Coagulase-negative *Staphylococcus* Corneal Isolates to Antibiotics. Cornea 2010;29:1131–5.

33. Asbell PA, Colby KA, Deng S, et al. Ocular TRUST: Nationwide Antimicrobial Susceptibility Patterns in Ocular Isolates. Am J Ophthalmol 2008;145:951–8.

34. Wang MT, Muntz A, Lim J, et al. Ageing and the Natural History of Dry Eye Disease: A Prospective Registry-based Cross-sectional Study. Ocul Surf 2020;18: 736–41.

35. Miller D. Pharmacological Treatment for Infectious Corneal Ulcers. Expert Opin Pharmacother 2013;14: 543–60.

36. Wilhelmus KR, Abshire RL, Schlech BA. Influence of Fluoroquinolone Susceptibility on the Therapeutic Response of Fluoroquinolone-treated Bacterial Keratitis. Arch Ophthalmol 2003;121:1229–33.

37. Kaye S, Tuft S, Neal T, et al. Bacterial Susceptibility to Topical Antimicrobials and Clinical Outcome in Bacterial Keratitis. Invest Ophthalmol Vis Sci 2010;51: 362–8.

38. Lalitha P, Srinivasan M, Manikandan P, et al. Relationship of *In Vitro* Susceptibility to Moxifloxacin and *In Vivo* Clinical Outcome in Bacterial Keratitis. Clin Infect Dis 2012;54:1381–7.

39. Constantinou M, Daniell M, Snibson GR, et al. Clinical Efficacy of Moxifloxacin in the Treatment of Bacterial Keratitis: A Randomized Clinical Trial. Ophthalmology 2007;114:1622–9.

40. Sharma N, Arora T, Jain V, et al. Gatifloxacin 0.3% versus Fortified Tobramycin-cefazolin in Treating Nonperforated Bacterial Corneal Ulcers: Randomized, Controlled Trial. Cornea 2016;35:56–61.

41. Sharma N, Goel M, Bansal S, et al. Evaluation of Moxifloxacin 0.5% in Treatment of Nonperforated Bacterial Corneal Ulcers: A Randomized Controlled Trial. Ophthalmology 2013;120:1173–8.

42. Parmar P, Salman A, Kalavathy CM, et al. Comparison of Topical Gatifloxacin 0.3% and Ciprofloxacin 0.3% for the Treatment of Bacterial Keratitis. Am J Ophthalmol 2006;141:282–6.

43. Shah VM, Tandon R, Satpathy G, et al. Randomized Clinical Study for Comparative Evaluation of Fourth-generation Fluoroquinolones with the Combination of Fortified Antibiotics in the Treatment of Bacterial Corneal Ulcers. Cornea 2010;29:751–7.

44. Zaguia F, Ross M, Darvish M, et al. Besifloxacin Ophthalmic Suspension in Patients with Bacterial Keratitis: A Prospective, Randomized Clinical Study. Invest Ophthalmol Vis Sci 2018;59:3659.

45. Schechter BA, Parekh JG, Trattler W. Besifloxacin Ophthalmic Suspension 0.6% in the Treatment of Bacterial Keratitis: A Retrospective Safety Surveillance Study. J Ocul Pharmacol Ther 2015;31:114–21.

46. Michaud L. Efficacy of Besifloxacin in the Treatment of Corneal Ulcer. Clin Refract Optom 2011;5:90–3.

47. Pandit RT. Brevundimonas Diminuta Keratitis. Eye Contact Lens 2012;38:63–5.

48. Nguyen AT, Hong AR, Baqai J, et al. Use of Topical Besifloxacin in the Treatment of *Mycobacterium chelonae* Ocular Surface Infections. Cornea 2015;34:967–71.

49. Miller D, Chang JS, Flynn HW, et al. Comparative *In Vitro* Susceptibility of Besifloxacin and Seven Comparators against Ciprofloxacin- and Methicillin-susceptible/Nonsusceptible Staphylococci. J Ocul Pharmacol Ther 2013;29:339–44.

50. Sanders ME, Norcross EW, Moore QC, 3rd, et al. Efficacy of Besifloxacin in a Rabbit Model of Methicillin-resistant *Staphylococcus aureus* Keratitis. Cornea 2009;28:1055–60.

51. Sanders ME, Moore QC, 3rd, Norcross EW, et al. Efficacy of Besifloxacin in an Early Treatment Model of Methicillin-resistant *Staphylococcus aureus* Keratitis. J Ocul Pharmacol Ther 2010;26:193–8.

52. Sanders ME, Moore QC, 3rd, Norcross EW, et al. Comparison of Besifloxacin, Gatifloxacin, and Moxifloxacin against Strains of *Pseudomonas aeruginosa* with Different Quinolone Susceptibility Patterns in a Rabbit Model of Keratitis. Cornea 2011;30:83–90.

53. Besivance® (Besifloxacin Ophthalmic Suspension) 0.6%. Prescribing Information. Bausch + Lomb; 2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/022308s013lbl.pdf. Accessed July 16, 2021.

54. Kowalski RP. Is Antibiotic Resistance a Problem in the Treatment of Ophthalmic Infections? Expert Rev Ophthalmol 2013;8:119–26.