An Evaluation of Staphylococci from Ocular Surface Infections Treated Empirically with Topical Besifloxacin: Antibiotic Resistance, Molecular Characteristics, and Clinical Outcomes

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ABSTRACT

Introduction: Understanding antibiotic resistance and toxin profiles among staphylococcal isolates in ocular infections can aid in therapeutic management and infection prevention strategies. We evaluated in vitro antibiotic resistance patterns and molecular traits of staphylococci isolated from patients with ocular surface infections. We also report on clinical outcomes for these patients following empirical treatment with topical besifloxacin ophthalmic suspension 0.6%.

Methods: This was a small observational study. Participating investigators from three clinical sites collected an initial ocular culture from the affected eye of patients presenting with ocular surface infections with presumed staphylococcal etiology. Clinical outcome data for patients with confirmed staphylococcal infections were collated later through retrospective review of patient medical records. Staphylococcal species identification in ocular cultures, in vitro antibiotic susceptibility testing, and PCR-based determination of methicillin resistance cassettes and toxin genotypes were conducted at a central laboratory. Isolates were categorized as susceptible or resistant based on systemic breakpoints, where available.

Results: Cultures were collected from 43 patients, and staphylococcal infections were confirmed in 25 patients. Two isolates of Staphylococcus aureus and 27 isolates of Staphylococcus epidermidis were identified. Both S. aureus isolates were methicillin-susceptible, lacked the gene encoding Panton-Valentine leukocidin, and carried few enterotoxin genes. Eight (30%) S. epidermidis were methicillin-resistant (MRSE), and 10 (37%) were ciprofloxacin-resistant. All but two MRSE isolates demonstrated multidrug resistance (MDR), and the staphylococcal cassette chromosome mec (SCCmec) type IVa was detected in five of the eight MRSE isolates. Clinical resolution of the ocular surface infection was reported in all 25 patients following treatment with besifloxacin.

Conclusions: In this study, S. aureus contained few toxins, while SCCmec IVa and MDR was predominant among MRSE from ocular surface infections. Despite significant in vitro fluoroquinolone resistance, there were no cases of...
treatment failure with topical besifloxacin ophthalmic suspension 0.6%.

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**Keywords:** Antibiotic resistance; Besifloxacin; Molecular characteristics; Ocular surface infections; Staphylococci

### Key Summary Points

#### Why carry out this study?

Few studies have examined antibiotic resistance profiles and genotypic characteristics of staphylococci from ocular infections in association with clinical outcome data, and, to our knowledge, none have reported on how molecular or resistance features of ocular staphylococci might correlate with the clinical efficacy of a specific antibiotic treatment.

This study evaluated in vitro antibiotic resistance patterns and molecular traits of staphylococci isolated from patients with ocular surface infections and evaluated corresponding clinical outcomes following treatment with besifloxacin ophthalmic suspension 0.6%.

#### What was learned from the study?

We found few toxins among *Staphylococcus aureus* isolates and a predominance of SCCmec IVa and multidrug resistance among methicillin-resistant *Staphylococcus epidermidis* isolates from these ocular surface infections, and, despite significant in vitro fluoroquinolone resistance, treatment with topical besifloxacin resulted in clinical resolution in all cases.

Multidrug resistance and SCCmec types IV/V were prevalent among community-acquired ocular methicillin-resistant *Staphylococcus epidermidis* isolates; however, a clear association between clinical efficacy and in vitro activity of besifloxacin could not be established in this small study.

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### INTRODUCTION

Staphylococci are important causative pathogens of ocular surface infections, including conjunctivitis and keratitis [1]. The prevalence of antibiotic resistance among staphylococci, especially to methicillin, is of clinical concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were first reported in 1961 [2] and subsequently spread from hospital environments to the community [3]. Given the rapid development of resistance to multiple additional drug classes among MRSA, several studies have focused on microbiologic characterization of the staphylococcal population with respect to phenotypic and genotypic traits that may contribute to pathogenicity [4–6]. Molecular typing research, in particular, has proven useful in the understanding of staphylococcal strain epidemiology, virulence, and clonal evolution and could ultimately help design strategies for successful treatment and infection prevention in hospital and community settings [7, 8]. Among isolates of *S. aureus* and coagulase-negative staphylococci (CoNS, including *Staphylococcus epidermidis*), one such research method involves characterization of the *mecA* gene, which confers resistance to beta-lactam antibiotics including methicillin and is harbored within the staphylococcal cassette chromosome *mec* (SCCmec) element [9–11].

Historically, hospital-acquired MRSA (HA-MRSA) pathogens have been characterized as having high rates of multidrug resistance (MDR), producing few toxins, and carrying SCCmec variants I–III [12, 13]. In contrast to HA-MRSA, community-acquired MRSA (CA-MRSA) pathogens are typically not MDR, but produce high toxin levels [14] and tend to carry SCCmec variants IV–V [12, 15, 16]. Cytotoxins such as Panton-Valentine leukocidin (PVL) enhance pathogenicity [12, 15, 17], and MRSA isolates carrying SCCmec IV are known to also harbor the PVL gene [18]. Similar studies have begun to evaluate the resistance traits of methicillin-resistant *S. epidermidis* (MRSE) isolates from ocular infections [19, 20].

Hesje et al. previously reported on traits of 38 ocular MRSA isolates collected between 2006
and 2008 across 14 states. Of these, 22 (58%) carried SCC\textit{mec} II, while the remaining 16 (42%) carried SCC\textit{mec} IV [16]. Consistent with previous reports for non-ocular isolates, all SCC\textit{mec} type II isolates were MDR and lacked PVL genes, traits typical of HA-MRSA, whereas the SCC\textit{mec} type IV isolates demonstrated greater MDR than expected, and 25% lacked the genes encoding PVL, suggesting the criteria for classifying a MRSA isolate as either CA- or HA-MRSA may be blurring [16]. If confirmed, this trend for CA-MRSA should inform treatment choice in MRSA infections. Further data are thus needed, particularly among staphylococci from ocular surface infections where cultures are not typically collected, to gain insight into the microbiologic and molecular characteristics that contribute to the pathogenesis of these bacteria.

The current study evaluated in vitro antibiotic resistance patterns and molecular traits of staphylococci isolated from patients presenting with ocular surface infections. We also report on the corresponding clinical outcomes in these patients following empirical treatment with topical BESIVANCE® (besifloxacin ophthalmic suspension) 0.6% (Bausch + Lomb; Bridgewater, NJ, USA).

**METHODS**

This was an observational, retrospective review of longitudinal data gathered during routine treatment of patients with staphylococcal eye infections at three investigational sites, including two community-based ophthalmology practices (Dr. Sheppard [Virginia] and Dr. Schechter [Florida]) and one hospital-based outpatient clinic (Dr. Asbell [New York]). Patients had to be 18 years of age or older and had to have a topical ocular infection with presumed staphylococcal etiology (for example, based on clinician’s observation of purulent discharge) for which besifloxacin was prescribed. Patients with a history of hypersensitivity to besifloxacin or other quinolone antibiotics, patients in an immunocompromised state at the time of initial diagnosis, and those for whom the investigator intended to treat with topical or systemic antimicrobials other than or in addition to besifloxacin were not eligible to participate. The protocol was approved by an institutional review board (Biomedical Research Alliance of New York [BRANY IRB], Lake Success, NY, USA), and the study was conducted in compliance with the Declaration of Helsinki and all of its amendments. All patients provided written informed consent.

Investigators obtained an initial ocular swab (rayon) from the affected eye of patients and submitted the swabs immediately to a central laboratory (International Health Management Associates, Inc.; Schaumburg, IL, USA) for culturing and microbiologic and molecular testing. In cases of bilateral ocular infection, the investigator designated the more severely infected eye as the study eye. If both eyes were of equal severity, the right eye was the study eye.

Immediately upon receipt by the central laboratory, swab samples were cultured on blood agar and chocolate agar plates, and semiquantitative growth ratings (1+ to 4+) were obtained by determining the number of plate quadrants with bacterial growth [21]. Bacterial isolates were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Biotyper, Bruker Daltonics, MA, USA). Susceptibility testing was performed on staphylococcal isolates, and minimum inhibitory concentrations (MICs) were determined by broth microdilution [22] for nine classes of antibiotics: fluoroquinolones (besifloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, gatifloxacin, ciprofloxacin, levofloxacin, and ofloxacin), macrolides (azithromycin), aminoglycosides (tobramycin), lincosamides (clindamycin), penicillins (oxacillin), dihydrofolate reductase inhibitors (trimethoprim), amphenicols (chloramphenicol), tetracyclines (tetracycline), and glycopeptides (vancomycin). Isolates were categorized as susceptible or resistant (intermediate plus full resistance) based on systemic breakpoints, where available [23]; oxacillin was used as a surrogate for methicillin. Multidrug resistance (MDR) was categorized as resistance to ≥3 antibiotic classes. Isolates of \textit{S. aureus} and \textit{S. epidermidis} underwent DNA extraction
methicillin-resistant strains were examined by PCR for \textit{mecA} and \textit{SCCmec} subtype as described previously [24] using \textit{S. aureus} specific primers. Isolates of \textit{S. aureus} were also examined for PVL genes [25] as well as the toxic shock syndrome toxin (TSST) gene, 6 staphylococcal enterotoxins (SEs) genes, and 15 SE-like toxin genes [26] as described in the referenced PCR methods.

Demographic and clinical outcome data were obtained retrospectively through review of medical records for those patients with laboratory-confirmed staphylococcal infections. Data collected included demographic data (patient age, gender, initial diagnosis, relevant medical/ocular history), dosage and duration of treatment with besifloxacin, ocular signs and symptoms, visual acuity at baseline and follow-up visits, as well as any adverse events (AEs) during treatment. Clinical resolution of the baseline infection was based on investigator judgment. Before and after ocular photographs were obtained at clinic visits when permitted by patients.

Descriptive statistics were used to summarize demographic variables. Microbiologic results were presented for individual subjects.

**RESULTS**

Ocular cultures were obtained from 43 patients at three investigational sites. Culturing of ocular samples from eight of these patients either produced no growth or were negative for staphylococci. Of 35 patients with suspected staphylococcal infections, 10 were excluded for various reasons including treatment noncompliance \((n = 1)\), no documentation of besifloxacin treatment \((n = 1)\), lack of follow-up \((n = 1)\), or having an infection other than at the ocular surface (i.e., blepharitis, \(n = 7)\).

A total of 25 patients (13 men, 12 women) had staphylococci isolated from ocular surface infections, were treated with topical besifloxacin, and subsequently had their medical records reviewed, including 5 patients with conjunctivitis and 20 patients with blepharoconjunctivitis; all 25 were treated at community-based practices. The mean (SD) age of these patients was 80.5 (11.0) years, with ages ranging from 45 to 92 years; all but two patients were between the ages of 72–92 years. Eight patients had relevant comorbid conditions, including diabetes \((n = 4)\), glaucoma \((n = 2)\), glaucoma with hypertension \((n = 1)\), and lymphoma \((n = 1)\), and 19 had previous cataract surgical procedures. At baseline, 24 of the 25 patients had mild-to-moderate bulbar erythema, while severe discharge was noted in eight patients.

Culturing and analysis of ocular swabs from the 25 included patients resulted in the identification of 73 bacterial isolates, 40 of which were unique staphylococci including \textit{S. aureus} \((n = 2)\), \textit{S. epidermidis} \((n = 27)\), \textit{S. hominis} \((n = 1)\), \textit{S. warneri} \((n = 2)\), \textit{S. lugdunensis} \((n = 2)\), \textit{S. haemolyticus} \((n = 4)\), \textit{S. caprae} \((n = 1)\), and \textit{S. schleiferi} \((n = 1)\). Table 1 presents the comparative MICs of fluoroquinolones for each isolate by patient. Newer fluoroquinolones (besifloxacin, moxifloxacin, and gatifloxacin) generally had lower MICs compared with older fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin). The MIC that inhibited 90% of isolates, or MIC\textsubscript{90}, was 0.5 \(\mu\)g/ml for besifloxacin, 1 \(\mu\)g/ml for moxifloxacin, 2 \(\mu\)g/ml for gatifloxacin, 4 \(\mu\)g/ml for levofloxacin, and 16 \(\mu\)g/ml for both ciprofloxacin and ofloxacin. For the majority of isolates, besifloxacin had the lowest in vitro MICs among the tested fluoroquinolones, either equal to or often below that of moxifloxacin. With few exceptions, besifloxacin MICs were 2- to 16-fold lower than those for moxifloxacin and up to 128-fold lower for other fluoroquinolones when isolates exhibited resistance to ciprofloxacin (MIC \(\geq 2 \mu\)g/ml).

Overall, 2 isolates of \textit{S. aureus} and 27 isolates of \textit{S. epidermidis} were identified from 24 patients. Both isolates of \textit{S. aureus} were methicillin-susceptible \textit{Staphylococcus aureus} (MSSA) and susceptible to all antibiotic classes tested (Table 2). The 2 MSSA isolates lacked the PVL gene and carried at maximum only 2 of the 22 tested enterotoxin genes. Of the 27 \textit{S. epidermidis} isolates, 10 (37%), 13 (48%), and 8 (30%) were resistant to ciprofloxacin, azithromycin, and oxacillin/methicillin, respectively; resistance to trimethoprim and tobramycin was also noted (19% for each). All isolates were
| Swab ID | Pt age | Diagnosis       | Staphylococcal organisms present | Growth rating | MIC (µg/ml) |          |          |          |          | Additional organisms present |
|---------|--------|-----------------|---------------------------------|---------------|------------|----------|----------|----------|----------|--------------------------------|
|         |        |                 |                                 |               | BES MXF GAT CIP LVX OFL |          |          |          |          |                                |
| 15196   | 74     | Conjunctivitis  | *S. epidermidis*                | 1+            | 4          | 64       | 64       | 64       | 256      | 256 None                        |
|         |        |                 | *S. hominis*                    |               | 0.03       | 0.015    | 0.03     | 0.06     | 0.06     | 0.25                            |
| 15199   | 81     | Conjunctivitis  | *S. epidermidis* (1)            | 1+            | 0.25       | 1        | 2        | 8        | 4        | 8 *Corynebacterium bovis*       |
|         |        |                 | *S. epidermidis* (2)            |               | 0.03       | 0.06     | 0.12     | 1        | 0.25     | 0.5                             |
| 15200   | 78     | Blepharoconjunctivitis | *S. epidermidis*            | 1+            | 0.03       | 0.03     | 0.06     | 0.5      | 0.25     | 0.5 *Actinomyces turicensis*    |
|         |        |                 |                                  |               |            |          |          |          |          | *Klebsiella oxytoca*            |
|         |        |                 |                                  |               |            |          |          |          |          | *Proteus mirabilis*             |
|         |        |                 |                                  |               |            |          |          |          |          | *Trueperella bernardiae*        |
| 15202   | 78     | Acute conjunctivitis | *S. epidermidis*            | 1+            | 0.03       | 0.008    | 0.06     | 0.25     | 0.12     | 0.25 *Corynebacterium macginleyi* |
|         |        |                 |                                  |               |            |          |          |          |          | *Corynebacterium striatum*      |
|         |        |                 |                                  |               |            |          |          |          |          | *Streptococcus oralis*          |
| 15203   | 45     | Blepharoconjunctivitis | *S. epidermidis*            | 1+            | 0.03       | 0.03     | 0.12     | 0.5      | 0.25     | 0.5 *Cutibacterium acnes*       |
|         |        |                 | *S. warneri*                    |               | 0.03       | 0.03     | 0.06     | 0.25     | 0.25     | 0.25                            |
| 15204   | 75     | Blepharoconjunctivitis | *S. epidermidis*            | 1+            | 2          | 32       | 32       | 64       | 128      | 256 *Corynebacterium macginleyi* |
|         |        |                 |                                  |               |            |          |          |          |          | *Pantoea septica*               |
| 15206   | 75     | Blepharoconjunctivitis | *S. epidermidis*            | 2+            | 0.03       | 0.06     | 0.12     | 1        | 0.25     | 0.5 *Corynebacterium accolans*  |
|         |        |                 | *S. lugdunensis*                |               | 0.03       | 0.06     | 0.12     | 0.5      | 0.25     | 0.5                            |
| 15207   | 91     | Blepharoconjunctivitis | *S. epidermidis*            | 1+            | 0.03       | 0.03     | 0.06     | 0.25     | 0.25     | 0.25 None                        |
|         |        |                 | *S. haemolyticus*               |               | 1          | 4        | 8        | 128      | 32       | 64                             |
| Swab ID | Pt age | Diagnosis                    | Staphylococcal organisms present | Growth rating | MIC (µg/ml) | Additional organisms present                                      |
|---------|--------|------------------------------|----------------------------------|---------------|-------------|---------------------------------------------------------------|
|         |        |                              |                                  |               | BES MXF GAT CIP LVX OFL |                                                              |
| 15208   | 91     | Blepharoconjunctivitis       | *S. epidermidis*                 | 1+            | 0.03 0.06 0.12 0.5 0.25 0.5 | *Bacillus cereus*                                           |
|         |        |                              |                                  |               |             | *Bacillus thuringensis*                                       |
|         |        |                              |                                  |               |             | *Corynebacterium macginleyi*                                   |
|         |        |                              |                                  |               |             | *Corynebacterium pseudodiphtheriticum*                       |
|         |        |                              |                                  |               |             | *Moraxella catarrhalis*                                       |
| 15211   | 81     | Blepharoconjunctivitis       | *S. epidermidis* (1)             | 2+            | 0.03 0.03 0.06 0.25 0.25 0.25 | None                                                        |
|         |        |                              |                                  |               |             |                                                               |
| 15212   | 83     | Blepharoconjunctivitis       | *S. haemolyticus*                | 1+            | 0.03 0.008 0.06 0.25 0.12 0.25 | *Corynebacterium macginleyi*                                |
| 15596   | 86     | Conjunctivitis               | *S. warneri*                     | 1+            | 0.06 0.06 0.12 0.25 0.25 0.5 | None                                                        |
|         |        |                              |                                  |               |             |                                                               |
| 19313   | 56     | Blepharoconjunctivitis       | *S. epidermidis*                 | 2+            | 0.06 0.12 0.25 2 1 1 | None                                                        |
| 19314   | 78     | Blepharoconjunctivitis       | *S. epidermidis*                 | 1+            | 0.03 0.008 0.06 0.25 0.12 0.25 | *Acinetobacter pittii*                                      |
|         |        |                              |                                  |               |             | *Chryseobacterium gleum*                                      |
| 19315   | 91     | Conjunctivitis               | *S. epidermidis*                 | 1+            | 0.03 0.06 0.12 0.5 0.25 0.5 | *Rothia* (non-speciated)                                    |
| 19316   | 78     | Blepharoconjunctivitis       | *S. epidermidis* (1)             | 2+            | 0.25 0.5 2 4 4 8 | None                                                        |
|         |        |                              |                                  |               |             |                                                               |
| 19317   | 85     | Blepharoconjunctivitis       | *S. epidermidis*                 | 1+            | 0.03 0.03 0.12 0.25 0.25 0.25 | *Bacillus* (non-speciated)                                  |
|         |        |                              |                                  |               |             | *Rothia mucilaginosa*                                         |
|         |        |                              |                                  |               |             | *Streptococcus* (alpha-hemolytic)                            |
| 19318   | 82     | Blepharoconjunctivitis       | *S. haemolyticus*                | 1+            | 0.03 0.008 0.06 0.25 0.12 0.25 | *Corynebacterium macginleyi*                                |
|         |        |                              |                                  |               |             | *Corynebacterium pseudodiphtheriticum*                       |
|         |        |                              | *S. epidermidis*                 | 2+            | 0.03 0.06 0.06 0.25 0.12 0.25 |                                                              |
| Swab ID | Pt age | Diagnosis                  | Staphylococcal organisms present | Growth rating | MIC (µg/ml) | Additional organisms present        |
|---------|--------|----------------------------|---------------------------------|---------------|-------------|-------------------------------------|
|         |        |                            |                                 |               | BES MXF GAT CIP LVX OFL              |                                     |
| 19319   | 92     | Blepharoconjunctivitis     | S. epidermidis                  | 2+            | 0.5 1 2 16 8 16              | Corynebacterium bovis               |
|         |        |                            | S. haemolyticus                 | 1+            | 0.03 0.008 0.06 0.25 0.12 0.25 |                                      |
| 19320   | 90     | Blepharoconjunctivitis     | S. aureus                       | 1+            | 0.015 0.008 0.06 0.25 0.25 0.25 | None                                |
|         |        |                            | S. lugdunensis                  | 1+            | 0.06 0.06 0.12 0.25 0.25 0.5   |                                      |
|         |        |                            | S. epidermidis                  | 1+            | 0.25 0.25 2 8 4 8             |                                      |
| 19321   | 72     | Blepharoconjunctivitis     | S. epidermidis                  | 1+            | 0.03 0.03 0.12 0.25 0.25 0.5   | Corynebacterium pseudodiphtheriticum |
|         |        |                            | S. caprae                       | 1+            | 0.5 0.06 2 16 0.25 16         |                                      |
| 19322   | 89     | Blepharoconjunctivitis     | S. epidermidis                  | 1+            | 0.25 1 2 8 4 8               | Corynebacterium amycolatum          |
|         |        |                            | S. schleiferi                   | 1+            | 0.06 0.06 0.12 0.25 0.25 0.5   |                                      |
| 20032   | 86     | Blepharoconjunctivitis     | S. aureus                       | 1+            | 0.015 0.008 0.06 0.25 0.25 0.25 | Corynebacterium macginleyi         |
| 20033   | 91     | Blepharoconjunctivitis     | S. epidermidis                  | 1+            | 0.25 1 2 4 4 8               | Bacillus cereus                     |
| 20034   | 85     | Blepharoconjunctivitis     | S. epidermidis (1)              | 1+            | 0.015 0.008 0.06 0.25 0.12 0.25 | Coriobacterium (non-speciated)      |
|         |        |                            | S. epidermidis (2)              | 1+            | 0.03 0.06 0.12 0.25 0.25 0.5   | Streptococcus (alpha-hemolytic)     |

*BES* besifloxacin, *MXF* moxifloxacin, *GAT* gatifloxacin, *CIP* ciprofloxacin, *LVX* levofloxacin, *OFL* ofloxacin
susceptible to vancomycin, with MICs of either 1 \( \mu \text{g/ml} \) or 2 \( \mu \text{g/ml} \). Of the eight MRSE, five carried SCC\textit{mec} type IVa, one carried SCC\textit{mec} type V, and two isolates contained un-typeable SCC\textit{mec} variants. Multidrug resistance was observed in eight \textit{S. epidermidis} isolates (30%), whereas six of eight (75%) MRSE demonstrated MDR.

Daily dosing with topical besifloxacin ranged from 2 to 4 doses per day (1 drop per dose), while besifloxacin treatment duration ranged from 7 to 14 days. The follow-up clinic visit occurred 6–21 days (mean of 11) after initiation of besifloxacin therapy. Clinical resolution of the ocular surface infections was reported for all 25 patients at follow-up. All signs/symptoms were absent at follow-up with few exceptions (mild discharge in one patient; superficial punctate keratitis in another). Visual acuity findings were unremarkable at either baseline or follow-up, and there were no AEs reported for any patient during besifloxacin treatment. Notably, eight patients reported relief of ocular signs/symptoms as early as 1–2 days and 14 as early as 3–4 days, following treatment initiation. Representative photographs of patient eyes prior to and following treatment with besifloxacin are shown in Fig. 1.

DISCUSSION

The current study was undertaken to evaluate in vitro antibiotic resistance patterns and molecular traits of staphylococci isolated from patients presenting with ocular surface infections and to report on clinical outcomes following treatment with besifloxacin ophthalmic suspension 0.6%. Pending results, a secondary objective was to begin to formulate an ocular breakpoint for this drug. Besifloxacin is a fluoroquinolone with structural modifications intended to increase its inhibition of bacterial DNA gyrase and topoisomerase IV [32] and has been reported to be highly bactericidal with broad-spectrum activity against a range of bacterial pathogens, including drug-resistant pathogens [33–36]. The clinical outcomes in this study attest to the efficacy of this chlorinated fluoroquinolone necessary for empiric use and confirm findings from prospective studies specific to bacterial conjunctivitis [37–39]. Importantly, this is the first report of besifloxacin efficacy in blepharoconjunctivitis, although randomized, vehicle-controlled, clinical trials are needed to confirm these observations. More than half of patients were infected with two or more species or

Of the 40 staphylococci collected at baseline from 25 patients with either conjunctivitis or blepharoconjunctivitis, only 2 were identified as \textit{S. aureus}. The low number of \textit{S. aureus} isolates was surprising but probably a consequence of the small sample size. Neither of the isolates was methicillin-resistant, and both produced few toxins, which is encouraging. In contrast, approximately one-third of \textit{S. epidermidis} isolates were MRSE, and all but two MRSE were also MDR. This finding is consistent with data obtained in the Antibiotic Resistance Monitoring in Ocular micRoorganisms (ARMOR) study, an ongoing surveillance program specific to ocular bacterial pathogens, which reported that approximately three-quarters of MRSA and methicillin-resistant CoNS (MRCoNS) isolates were MDR whether considering all ocular isolates regardless of anatomical source [30] or conjunctival isolates [31]. Similarly, in vitro fluoroquinolone (ciprofloxacin) resistance rates observed among \textit{S. epidermidis} isolates in the current study (37%) are also consistent with those reported in ARMOR (~ 30%), with newer fluoroquinolones having lower MICs within the class [30, 31].

Despite evidence of in vitro fluoroquinolone resistance, treatment of patients with topical besifloxacin resulted in clinical resolution of the baseline infection in all 25 patients by the follow-up visit. While these results were welcomed, they, however, precluded the possibility of defining an ocular breakpoint for this drug. Besifloxacin is a fluoroquinolone with structural modifications intended to increase its inhibition of bacterial DNA gyrase and topoisomerase IV [32] and has been reported to be highly bactericidal with broad-spectrum activity against a range of bacterial pathogens, including drug-resistant pathogens [33–36]. The clinical outcomes in this study attest to the efficacy of this chlorinated fluoroquinolone necessary for empiric use and confirm findings from prospective studies specific to bacterial conjunctivitis [37–39]. Importantly, this is the first report of besifloxacin efficacy in blepharoconjunctivitis, although randomized, vehicle-controlled, clinical trials are needed to confirm these observations. More than half of patients were infected with two or more species or
| Swab ID | Resistance profile | Molecular characteristics |
|---------|--------------------|--------------------------|
|         | CIP    | AZI    | CHL    | CLI    | TET    | TOB    | TMP    | VAN    | OXA    | MDR | meCA | SCCmec type | PVL | Toxins |
| **S. aureus** | | | | | | | | | | | | | | |
| 19320   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   | Neg  | Neg       | SE-like L |
| 20032   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   | Neg  | Neg       | SEA, SE-like X |
| **S. epidermidis** | | | | | | | | | | | | | | |
| 15196   | R      | R      | S      | R      | S      | R      | S      | R      | Yes   | Pos  | IVa  | | | |
| 15199   | R      | S      | S      | I      | S      | S      | S      | S      | R      | Yes  | Pos  | IVa  | | |
| 15199   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 15200   | S      | S      | S      | S      | S      | R      | S      | S      | S      | No   |   |   | |
| 15202   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 15203   | S      | R      | S      | S      | I      | S      | S      | S      | S      | No   |   |   | |
| 15204   | R      | R      | S      | R      | S      | S      | S      | S      | S      | Yes  |   |   | |
| 15206   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 15207   | S      | R      | S      | S      | S      | R      | S      | R      | Yes   | Pos  | Un-typeable | | |
| 15208   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 15211   | S      | S      | S      | I      | S      | S      | R      | S      | S      | No   |   |   | |
| 15211   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 15596   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19313   | I      | R      | S      | S      | R      | R      | S      | S      | R      | Yes  | Pos  | IVa  | | |
| 19314   | S      | S      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19315   | S      | R      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19316   | R      | S      | S      | S      | S      | S      | S      | S      | R      | No   | Pos  | IVa  | | |
| 19316   | R      | R      | S      | S      | R      | S      | S      | R      | Yes   | Pos  | IVa  | | |
| 19317   | S      | R      | S      | I      | S      | S      | S      | S      | S      | No   |   |   | |
| 19318   | S      | R      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19319   | R      | R      | S      | S      | S      | R      | S      | S      | Yes   |   |   |   | |
| 19320   | R      | R      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19321   | S      | R      | S      | S      | S      | S      | S      | S      | S      | No   |   |   | |
| 19322   | R      | S      | S      | S      | R      | R      | R      | S      | R      | Yes  | Pos  | V | | |
strains of staphylococcal species in this study, and other bacterial species in addition to staphylococcal species were recovered from nearly three quarters of patients (18/25). Thus, in this study besifloxacin also demonstrated efficacy in mixed pathogen or polybacterial infections.

Recent publications suggest that resistance and virulence may be converging and that SCCmeC types associated with community-acquired staphylococci are now exhibiting increased antibiotic resistance [16, 19, 20, 28, 40–42]. Despite the predominance of SCCmeC types IV/V among MRSE in the current study (n = 6), nearly all (83%) showed MDR. These findings are consistent with those from an analysis of 30 MRSE isolates from ocular infections in Sao Paulo, Brazil, which found that of 17 isolates containing SCCmeC IV/V, at least 70% were MDR [19]. Similarly, Jena et al. examined the molecular traits of 52 ocular S. epidermidis isolates (23 from infections and 29 from asymptomatic healthy conjunctiva) in India and determined that all isolates containing SCCmeC IV/V, at least 70% were MDR [19]. Consistent with results reported from health-care settings, an analysis of 643 staphylococci isolated from environmental samples in a community in the UK found that of 46 CoNS isolates for which SCCmeC types were determined, 18 were type IV/V, and 16 of these demonstrated resistance to 3 or more antibiotics [40].

While findings for MRSE do not inform on convergence of virulence and resistance in MRSA, there is an increasing recognition that MRCoNS may play a role in the pathogenesis of community-acquired infections [40, 43] since it is thought that CoNS may be an important reservoir of resistance genes for S. aureus [10, 42, 44]. This hypothesis is based in part on the greater prevalence of methicillin resistance among S. epidermidis relative to S. aureus isolates [30, 42, 44] and the reporting of in vivo transfer of SCCmeC from S. epidermidis to S. aureus [45], notwithstanding that CoNS and S. aureus co-colonize and/or commonly coinfect the ocular surface [46–48]. The transfer of antimicrobial resistance genes across staphylococcal species [11, 44] represents one potential mechanism underlying the rapid spread of antimicrobial resistance into the community and may be a factor contributing to the high proportion of MDR observed among SCCmeC type IV MRCoNS in the current study. To what degree polymicrobial infections contribute to or result from this phenomenon is another interesting area of research.

Our study is limited by the small sample size and the very few S. aureus isolates obtained, thereby limiting any inferences as to whether

| Swab ID | Resistance profile | Molecular characteristics |
|---------|-------------------|---------------------------|
|         | CIP | AZI | CHL | CLI | TET | TOB | TMP | VAN | OXA | MDR | meC | SCCmeC type | PVL | Toxins |
| 20033   | R   | S   | S   | S   | S   | S   | S   | S   | R   | No  | Pos | Un-typeable |
| 20034   | S   | S   | S   | S   | S   | S   | S   | S   | S   | No  |     |               |
| 20034   | S   | R   | S   | R   | S   | S   | S   | S   | S   | No  |     |               |

CIP ciprofloxacin, AZI azithromycin, CHL chloramphenicol, CLI clindamycin, TET tetracycline, TOB tobramycin, TMP trimethoprim, VAN vancomycin, OXA oxacillin, MDR multidrug resistance (to ≥ 3 antibiotic classes), S susceptible, I intermediate, R resistant, Pos positive, Neg negative
resistance and virulence may be converging among ocular MRSA. While there was some geographic diversity among the three study sites, all were in the eastern part of the US, and only two of the sites had patients with confirmed staphylococcal ocular surface infections. Furthermore, since almost all patients were 72 years of age or older, the results may simply reflect real-world pathology of ocular surface infections in this age group. Systemic breakpoints were used to interpret in vitro susceptibility/resistance of antibiotics other than besifloxacin, which is of limited value for determining clinical antibiotic resistance given the expected achievable drug concentrations in the eye. Finally, there were no cases of treatment failure with besifloxacin precluding the possibility of beginning to formulate an ocular breakpoint for this drug.

CONCLUSIONS

The findings of this small observational study found few toxins among S. aureus isolates and a predominance of SCCmec IVa and MDR among
MRSE isolates from ocular surface infections obtained at community-based practices. Future studies with larger numbers of S. aureus and MRSA isolates from a more diverse patient population, including from patients with hospital-acquired infections, could further our knowledge of the comparative molecular traits of MRSA and MRCoNS from ocular surface infections and inform on any potential convergence of resistance and virulence among MRSA. Finally, besifloxacin appeared effective in this study of staphylococcal infections with no cases of treatment failure and no AEs.

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Compliance with Ethics Guidelines. The protocol was approved by an institutional review board (Biomedical Research Alliance of New York [BRANY IRB], Lake Success, NY), and the study was conducted in compliance with the Declaration of Helsinki and all of its amendments. All patients provided written informed consent.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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