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BRIEF REPORT

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* (SCC*mec*) 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 SCC*mec* IVa and MDR was predominant among MRSE from ocular surface infections. Despite significant in vitro fluoroquinolone resistance, there were no cases of

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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 SCC*mec* 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 SCC*mec* 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.

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* (SCC*mec*) 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 SCC*mec* 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 SCC*mec* variants IV–V [12, 15, 16]. Cytotoxins such as Panton-Valentine leukocidin (PVL) enhance pathogenicity [12, 15, 17], and MRSA isolates carrying SCC*mec* 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*mec* II, while the remaining 16 (42%) carried SCC*mec* IV [16]. Consistent with previous reports for non-ocular isolates, all SCC*mec* type II isolates were MDR and lacked PVL genes, traits typical of HA-MRSA, whereas the SCC*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, 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 *S. aureus* and *S. epidermidis* underwent DNA extraction

(QIAcube, QIAGEN Inc., CA, USA), and any methicillin-resistant strains were examined by PCR for *mecA* and SCC*mec* subtype as described previously [24] using *S. aureus* specific primers. Isolates of *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 *S. aureus* ($n = 2$), *S. epidermidis* ($n = 27$), *S. hominis* ($n = 1$), *S. warneri* ($n = 2$), *S. lugdunensis* ($n = 2$), *S. haemolyticus* ($n = 4$), *S. caprae* ($n = 1$), and *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_{90}, was 0.5 μg/ml for besifloxacin, 1 μg/ml for moxifloxacin, 2 μg/ml for gatifloxacin, 4 μg/ml for levofloxacin, and 16 μg/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 μg/ml).

Overall, 2 isolates of *S. aureus* and 27 isolates of *S. epidermidis* were identified from 24 patients. Both isolates of *S. aureus* were methicillin-susceptible *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 *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

Table 1 All isolated staphylococcal organisms and in vitro fluoroquinolone susceptibilities

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	1+	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)	1+	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	1+	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	1+	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+	1	4	8	128	32	64	

Table 1 continued

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 thuringiensis
											Corynebacterium macginleyi
											Corynebacterium propinquum
											Moraxella catarrhalis
15211	81	Blepharoconjunctivitis	*S. epidermidis* (1)	2+	0.03	0.03	0.06	0.25	0.25	0.25	None
			S. epidermidis (2)	2+	0.03	0.03	0.06	0.25	0.25	0.25	
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
			S. epidermidis	1+	0.03	0.03	0.06	0.25	0.25	0.25	
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 pitii*
											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
			S. epidermidis (2)	1+	0.25	0.5	2	4	4	8	
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*
			S. epidermidis	2+	0.03	0.06	0.06	0.25	0.12	0.25	*Corynebacterium pseudodiphtheriticum*

Table 1 continued

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	0.5	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	0.5	0.25	0.5	
20032	86	Blepharoconjunctivitis	*S. aureus*	1+	0.015	0.008	0.06	0.5	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	0.5	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 μg/ml or 2 μg/ml. Of the eight MRSE, five carried SCC*mec* type IVa, one carried SCC*mec* type V, and two isolates contained un-typeable SCC*mec* variants. Multidrug resistance was observed in eight *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 fluoroquinolone. To date, few studies have examined antibiotic resistance profiles and genotypic characteristics of staphylococci from ocular infections in association with clinical outcome data [27–29], 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.

Of the 40 staphylococci collected at baseline from 25 patients with either conjunctivitis or blepharoconjunctivitis, only 2 were identified as *S. aureus*. The low number of *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 *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 *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

Table 2 In vitro susceptibility profiles and molecular characteristics of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates

Swab ID	Resistance profile										Molecular characteristics			
	CIP	AZI	CHL	CLI	TET	TOB	TMP	VAN	OXA	MDR	*mecA*	SCC*mec* 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	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	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	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	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		

Table 2 continued

Swab ID	Resistance profile										Molecular characteristics			
	CIP	AZI	CHL	CLI	TET	TOB	TMP	VAN	OXA	MDR	*mecA*	SCC*mec* 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 $\geq$ 3 antibiotic classes), *S* susceptible, *I* intermediate, *R* resistant, *Pos* positive, *Neg* negative

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 SCC*mec* types associated with community-acquired staphylococci are now exhibiting increased antibiotic resistance [16, 19, 20, 28, 40–42]. Despite the predominance of SCC*mec* 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 SCC*mec* 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 SCC*mec* IV/V (10 from infections; 11 from healthy conjunctiva) were MDR [20]. 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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

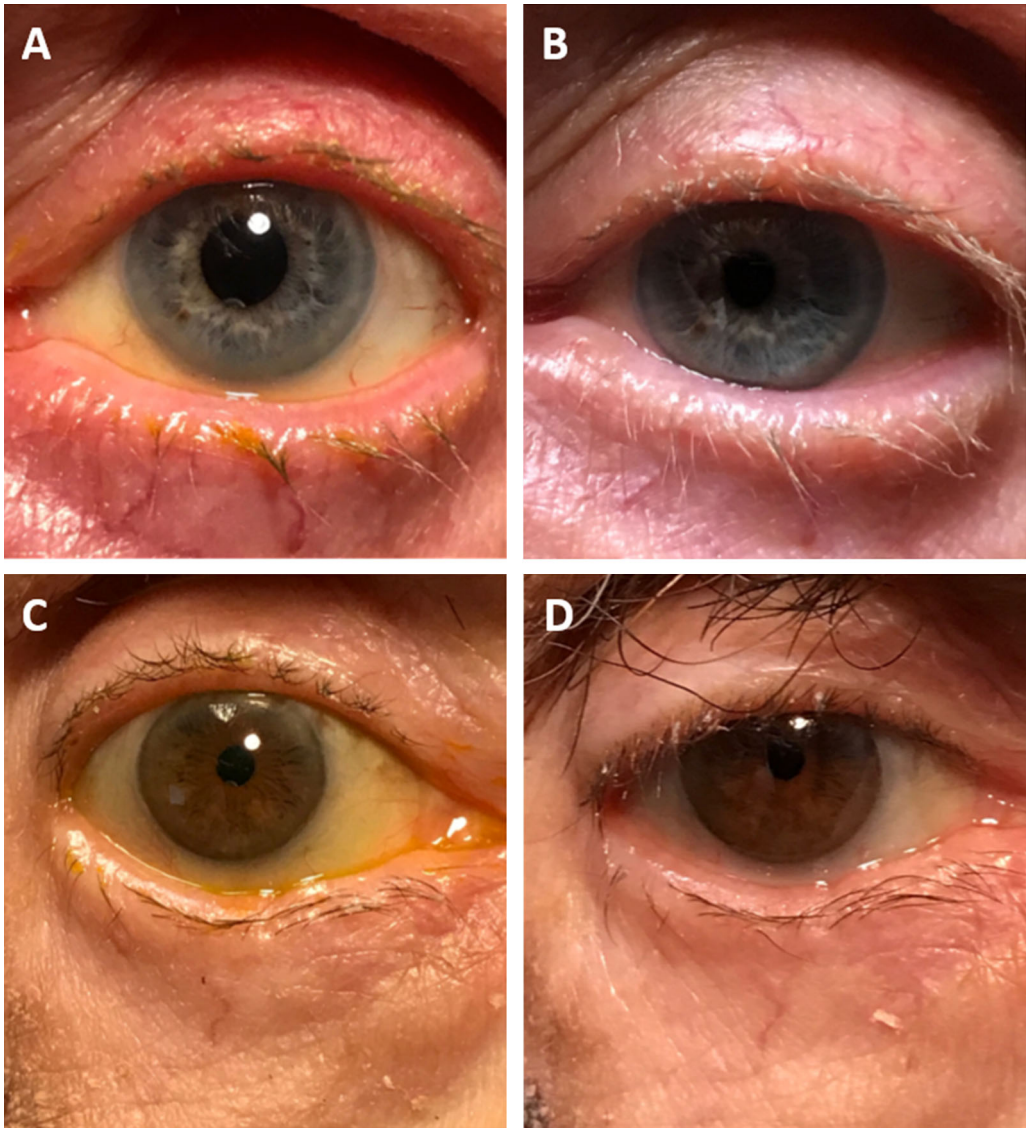

Fig. 1 Photographs from representative eyes with staphylococcal ocular surface infections before (**a**, **c**) and after (**b**, **d**) besifloxacin treatment

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 SCC*mec* 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.

REFERENCES

1. O'Callaghan RJ. The pathogenesis of Staphylococcus aureus eye Infections. Pathogens. 2018;7(1):E9.
2. Jevons MP. "Celbenin"-resistant Staphylococci. Br Med J. 1961;1(5219):124–5.
3. Chambers HF. The changing epidemiology of staphylococcus aureus? Emerg Infect Dis. 2001;7(2):178–82.

4. Harada D, Nakaminami H, Miyajima E, et al. Change in genotype of methicillin-resistant *Staphylococcus aureus* (MRSA) affects the antibiogram of hospital-acquired MRSA. J Infect Chemother. 2018;24(7):563–9.

5. Chen SY, Liao CH, Wang JL, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) staphylococcal cassette chromosome mec genotype effects outcomes of patients with healthcare-associated MRSA bacteremia independently of vancomycin minimum inhibitory concentration. Clin Infect Dis. 2012;55(10):1329–37.

6. Kempker RR, Farley MM, Ladson JL, Satola S, Ray SM. Association of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 genotype with mortality in MRSA bacteremia. J Infect. 2010;61(5):372–81.

7. Goudarzi M, Seyedjavadi SS, Nasiri MJ, et al. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis. Microb Pathog. 2017;104:328–35.

8. Hesari MR, Salehzadeh A, Darsanaki RK. Prevalence and molecular typing of methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin gene. Acta Microbiol Immunol Hung. 2018;65(1):93–106.

9. Petinaki E, Arvaniti A, Dimitracopoulos G, Spiliopoulou I. Detection of *mecA*, mecR1 and mecI genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions. J Antimicrob Chemother. 2001;47(3):297–304.

10. Wielders CL, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mecA* gene is widely disseminated in *Staphylococcus aureus* population. J Clin Microbiol. 2002;40(11):3970–5.

11. Hanssen AM, Kjeldsen G, Sollid JU. Local variants of Staphylococcal cassette chromosome mec in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococci: evidence of horizontal gene transfer? Antimicrob Agents Chemother. 2004;48(1):285–96.

12. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 2003;290(22):2976–84.

13. Deurenberg RH, Stobberingh EE. The molecular evolution of hospital- and community-associated methicillin-resistant *Staphylococcus aureus*. Curr Mol Med. 2009;9(2):100–15.

14. Tsuji BT, Rybak MJ, Cheung CM, Amjad M, Kaatz GW. Community- and health care-associated methicillin-resistant Staphylococcus aureus: a comparison of molecular epidemiology and antimicrobial activities of various agents. Diagn Microbiol Infect Dis. 2007;58(1):41–7.

15. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978–84.

16. Hesje CK, Sanfilippo CM, Haas W, Morris TW. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolated from the eye. Curr Eye Res. 2011;36(2):94–102.

17. Lo WT, Wang CC. Panton-Valentine leukocidin in the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Pediatr Neonatol. 2011;52(2):59–65.

18. Kilic A, Li H, Stratton CW, Tang YW. Antimicrobial susceptibility patterns and staphylococcal cassette chromosome mec types of, as well as Panton-Valentine leukocidin occurrence among, methicillin-resistant Staphylococcus aureus isolates from children and adults in middle Tennessee. J Clin Microbiol. 2006;44(12):4436–40.

19. Bispo PJ, Hofling-Lima AL, Pignatari AC. Characterization of ocular methicillin-resistant *Staphylococcus epidermidis* isolates belonging predominantly to clonal complex 2 subcluster II. J Clin Microbiol. 2014;52(5):1412–7.

20. Jena S, Panda S, Nayak KC, Singh DV. Identification of major sequence types among multidrug-resistant *Staphylococcus epidermidis* strains isolated from infected eyes and healthy conjunctiva. Front Microbiol. 2017;8:1430.

21. Garcia LS. Clinical microbiology procedures handbook. 3rd ed. Washington, DC: ASM Press; American Society for Microbiology; 2014.

22. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. 10th ed. CLSI document M7-A10. Wayne: Clinical and Laboratory Standards Institute; 2015.

23. CLSI. Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne: Clinical and Laboratory Standards Institute; 2017.

24. Zhang K, McClure JA, Conly JM. Enhanced multiplex PCR assay for typing of staphylococcal cassette chromosome *mec* types I to V in methicillin-

resistant *Staphylococcus aureus*. Mol Cell Probes. 2012;26(5):218–21.

25. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of panton-valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 1999;29(5):1128–32.

26. Salgado-Pabón W, Case-Cook LC, Schlievert PM. Molecular analysis of staphylococcal superantigens. In: Ji Y, editor. Methicillin-resistant *Staphylococcus aureus* (MRSA) protocols. Methods in molecular biology (methods and protocols), vol. 1085. Totowa: Humana Press; 2014.

27. Sueke H, Shankar J, Neal T, et al. lukSF-PV in *Staphylococcus aureus* keratitis isolates and association with clinical outcome. Invest Ophthalmol Vis Sci. 2013;54(5):3410–6.

28. Kang YC, Hsiao CH, Yeh LK, et al. Methicillin-resistant staphylococcus aureus ocular infection in Taiwan: clinical features, genotying, and antibiotic susceptibility. Medicine (Baltimore). 2015;94(42):e1620.

29. Hsiao CH, Ong SJ, Chuang CC, Ma DH, Huang YC. A comparison of clinical features between community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* keratitis. J Ophthalmol. 2015;2015:923941.

30. Thomas RK, Melton R, Asbell PA. Antibiotic resistance among ocular pathogens: current trends from the ARMOR surveillance study (2009–2016). Clin Optom (Auckl). 2019;11:12–26.

31. Asbell PA, DeCory HH. Antibiotic resistance among bacterial conjunctival pathogens collected in the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) surveillance study. PLoS One. 2018;13(10):e0205814.

32. Cambau E, Matrat S, Pan XS, et al. Target specificity of the new fluoroquinolone besifloxacin in *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. J Antimicrob Chemother. 2009;63(3):443–50.

33. Haas W, Pillar CM, Zurenko GE, et al. Besifloxacin, a novel fluoroquinolone, has broad-spectrum in vitro activity against aerobic and anaerobic bacteria. Antimicrob Agents Chemother. 2009;53(8):3552–60.

34. Haas W, Pillar CM, Hesje CK, Sanfilippo CM, Morris TW. Bactericidal activity of besifloxacin against staphylococci, *Streptococcus pneumoniae* and Haemophilus influenzae. J Antimicrob Chemother. 2010;65(7):1441–7.

35. Haas W, Gearinger LS, Usner DW, Decory HH, Morris TW. Integrated analysis of three bacterial conjunctivitis trials of besifloxacin ophthalmic suspension, 0.6%: etiology of bacterial conjunctivitis and antibacterial susceptibility profile. Clin Ophthalmol. 2011;5:1369–79.

36. Miller D, Chang JS, Flynn HW, Alfonso EC. Comparative in vitro susceptibility of besifloxacin and seven comparators against ciprofloxacin- and methicillin-susceptible/nonsusceptible staphylococci. J Ocul Pharmacol Ther. 2013;29(3):339–44.

37. Karpecki P, Depaolis M, Hunter JA, et al. Besifloxacin ophthalmic suspension 0.6% in patients with bacterial conjunctivitis: a multicenter, prospective, randomized, double-masked, vehicle-controlled, 5-day efficacy and safety study. Clin Ther. 2009;31(3):514–26.

38. Tepedino ME, Heller WH, Usner DW, et al. Phase III efficacy and safety study of besifloxacin ophthalmic suspension 0.6% in the treatment of bacterial conjunctivitis. Curr Med Res Opin. 2009;25(5):1159–69.

39. McDonald MB, Protzko EE, Brunner LS, et al. Efficacy and safety of besifloxacin ophthalmic suspension 0.6% compared with moxifloxacin ophthalmic solution 0.5% for treating bacterial conjunctivitis. Ophthalmology. 2009;116(9):1615–23.

40. Xu Z, Shah HN, Misra R, et al. The prevalence, antibiotic resistance and *mecA* characterization of coagulase negative staphylococci recovered from non-healthcare settings in London, UK. Antimicrob Resist Infect Control. 2018;7:73.

41. Wurster JI, Bispo PJM, Van Tyne D, et al. Staphylococcus aureus from ocular and otolaryngology infections are frequently resistant to clinically important antibiotics and are associated with lineages of community and hospital origins. PLoS One. 2018;13(12):e0208518.

42. Saber H, Jasni AS, Jamaluddin TZMT, Ibrahim R. A review of staphylococcal cassette chromosome mec (SCCmec) types in coagulase-negative Staphylococci (CoNS) species. Malays J Med Sci. 2017;24(5):7–18.

43. Barbier F, Ruppé E, Hernandez D, et al. Methicillin-resistant coagulase-negative staphylococci in the community: high homology of SCCmec IVa between *Staphylococcus epidermidis* and major clones of methicillin-resistant *Staphylococcus aureus*. J Infect Dis. 2010;202(2):270–81.

44. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46(1):8–20.

45. Wielders CLC, Vriens MR, Brisse S, et al. In-vivo transfer of mecA DNA to *Staphylococcus aureus* [corrected]. Lancet. 2001;357(9269):1674–5.

46. Blondeau JM, Sanfilippo CM, DeCory HH. Incidence of polybacterial infections in three bacterial conjunctivitis studies and outcomes with besifloxacin ophthalmic suspension 0.6%. In: Presented at the annual meeting of the Association for Research in Vision and Ophthalmology. Vancouver, Canada, April 28–May 3, 2019.

47. Willcox MD. Characterization of the normal microbiota of the ocular surface. Exp Eye Res. 2013;117:99–105.

48. Iwalokun BA, Oluwadun A, Akinsinde KA, Niemogha MT, Nwaokorie FO. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria. J Ophthalmic Inflamm Infect. 2011;1(3):95–103.