

Emerging 8-Methoxyfluoroquinolone Resistance among Methicillin-Susceptible *Staphylococcus epidermidis* Isolates Recovered from Patients with Endophthalmitis

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Fluoroquinolones remain the most commonly used antimicrobials for the prevention and management of bacterial endophthalmitis. Coagulase-negative staphylococci are the most frequently recovered pathogens. Increasing resistance among this group has paralleled the presence of methicillin resistance. From 2005 to 2010, we recovered 38 methicillin-susceptible *Staphylococcus epidermidis* (MSSE) isolates from endophthalmitis patients at our institute, including 15 (39.5%) isolates resistant to gatifloxacin and moxifloxacin, members of the C-8-methoxyfluoroquinolones family. Mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* were determined and correlated with fluoroquinolone MICs based on Etests of these 15 MSSE isolates. High-level resistance (MIC, >32 µg/ml) to gatifloxacin and moxifloxacin was documented for 46.7% of the MSSE isolates, and low-level resistance (MIC, 2 to 4 µg/ml) was determined for 53.3%. The MICs for ciprofloxacin, levofloxacin, and ofloxacin were >32 µg/ml for all isolates. The amino acid substitution Ser84Phe in *gyrA* was found among all isolates. A second mutation in *gyrA* (Glu88Lys) resulted in high-level resistance to moxifloxacin and gatifloxacin. Almost all (92.8%) isolates presented double point mutations in the *parC* gene at codons 80 and 84 with different combinations. Eighty-seven percent of the patients had prior exposure to topical 8-methoxyfluoroquinolones. Prior exposure to the 8-methoxyfluoroquinolones may contribute to the selection of MSSE strains containing multiple mutations in the QRDRs of *gyrA* and *parC* that results in low- and high-level resistance to these agents.

Bacterial endophthalmitis is a rare but severe inflammation of the intraocular fluids and tissues resulting from the presence and growth of microorganisms. Microorganisms gain access to the intraocular chambers following intraocular surgery, intravitreal injections (postoperative), open globe injury (posttraumatic), or through hematological spread (endogenous). Postoperative endophthalmitis is the most common category and is caused predominantly by Gram-positive organisms originating from the normal conjunctiva and eyelid microflora (1–3). Coagulase-negative staphylococci (CoNS), including the most common species, *Staphylococcus epidermidis*, are the most frequently isolated organisms from culture-proven endophthalmitis cases (1, 4, 5). Topical fluoroquinolone agents (ciprofloxacin, ofloxacin, and levofloxacin) have been widely used as the first-line option for both prophylaxis and management of endophthalmitis and other ocular infections (6, 7). However, recovery of ocular fluoroquinolone-resistant pathogens emerged soon after the introduction and widespread use of these agents in the 1990s and has significantly increased in the last 2 decades (7, 8).

The 8-methoxyfluoroquinolones, gatifloxacin (Zymar; Allergan) and moxifloxacin (Vigamox; Alcon), are the most frequent and widely used agents in ophthalmology due to their increased potency against Gram-positive pathogens and reduced rates of resistance compared with the older fluoroquinolones (6, 7). Despite its withdrawal for systemic use, gatifloxacin is still available for topical ophthalmic use in well-tolerated eye drop formulations of 0.3% and 0.5%. Both gatifloxacin and moxifloxacin inactivate simultaneously the topoisomerases II (DNA gyrase) and IV, which are necessary for DNA replication, while older fluoroquinolones, including levofloxacin, preferentially target either topoisomerase II or IV. Dual-acting fluoroquinolones not only demonstrate increased potency but also are thought to minimize

selection of resistant strains because of the double point mutations in both DNA gyrase and topoisomerase IV that are necessary for an organism to become resistant to these newer agents (7). Although older fluoroquinolones are associated with higher resistance rates than the newer compounds, evolving resistance to gatifloxacin and moxifloxacin among CoNS isolates from endophthalmitis patients has been documented (9, 10). Fluoroquinolone resistance among ocular staphylococci is particularly more common for methicillin-resistant isolates, which are also more likely to exhibit high-level resistance (11–13). National surveillance studies designed to monitor the antimicrobial resistance profile of ocular pathogens have reported a significantly higher percentage of resistance to ciprofloxacin and moxifloxacin among methicillin-resistant *Staphylococcus aureus* (MRSA) and also methicillin-resistant CoNS (MRCoNS) in comparison to methicillin-susceptible *S. aureus* (MSSA) and CoNS (14, 15). However, this difference in fluoroquinolone resistance between these two groups is probably narrowing. Some studies have reported increasing resistance rates to fluoroquinolones as well as lower *in vitro* poten-

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cies against MSSA and methicillin-susceptible CoNS ocular isolates (13–16). Several studies have confirmed the development of multiple mutation profiles in the quinolone resistance-determining region (QRDR) of the topoisomerase subunits GyrA (DNA gyrase) and ParC (topoisomerase IV) and also its association with the level of fluoroquinolone resistance in *S. epidermidis* recovered from the normal conjunctival microbiota as well as from infected eyes (17–19). In this study, we report the emergence of low- and high-level resistance to 8-methoxyfluoroquinolones associated with multiple mutations in the QRDRs of the *gyrA* and *parC* genes among methicillin-susceptible *S. epidermidis* (MSSE) isolates recovered from endophthalmitis patients.

MATERIALS AND METHODS

Study design. Microbiological laboratory records of consecutive culture-proven *S. epidermidis* endophthalmitis cases seen at the Department of Ophthalmology, Bascom Palmer Eye Institute, between 1 January 2005 and 31 December 2010 were retrospectively reviewed with Institutional Review Board approval. Susceptibility results determined by using the Vitek 2 automated system (bioMérieux, Durham, NC) and Etest for oxacillin, gatifloxacin, and/or moxifloxacin were computed for all *S. epidermidis* (methicillin-susceptible and -resistant) isolates during the period. *S. epidermidis* isolates resistant to 8-methoxyfluoroquinolone but susceptible to methicillin (oxacillin) were selected for further characterization. Clinical data, including age, gender, endophthalmitis category (postoperative, posttraumatic, endogenous, or miscellaneous), surgery, preoperative antibiotic usage, and outcome were recovered from the medical records.

Bacterial isolates and DNA extraction. Species identification was routinely performed using the Vitek 2 system. Selected *S. epidermidis* isolates resistant to 8-methoxyfluoroquinolone but susceptible to methicillin were recovered from vials of frozen skim milk, thawed, and inoculated onto 5% sheep blood agar plates. Plates were incubated at 35°C for 18 to 24 h. Two isolates did not grow, and the DNA was extracted from the skim milk tube for molecular testing. One isolate was not available. Total DNA of the remaining *S. epidermidis* isolates was extracted using the QIAamp DNA minikit (Qiagen) according to the manufacturer's recommendations.

Etest MIC determinations. MICs of ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, ofloxacin, oxacillin, linezolid, teicoplanin, and vancomycin were determined by Etest (bioMérieux, Durham, NC). The test inoculum for each isolate and quality control strain (*S. aureus* ATCC 29213) was prepared at 0.5 McFarland standard and then swabbed onto a Mueller-Hinton agar plate. Etest strips were dropped after 15 min, and the plates were incubated for 18 to 24 h at 35°C in an aerobic atmosphere.

Amplification and sequencing of QRDRs. Amplification of QRDRs of the *gyrA* and *parC* genes was performed as previously described (19) using the following primer pairs: *gyrA* forward, 5'-ATGCGTGAATCAT TCTTAGACTATGC-3', and reverse, 5'-GAGCCAAAGTTACCTTGAC C-3', designed to generate an amplification product of 284 bp, and *parC* forward, 5'-TCGCAATGTATTCAAGTGGG-3', and reverse, 5'-ATCGT TATCGATACTACCATT-3', designed to amplify a region of 197 bp in length. PCR was set up using 10 µl HotStarTaq master mix (Qiagen; final concentrations of 1.25 U HotStarTaq DNA polymerase, 1.5 mM MgCl₂, and 200 µM each deoxynucleoside triphosphate), 0.5 µM each primer, and 1 µl of DNA template. The PCR conditions were as follows: initial denaturation at 95°C for 10 min and 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s. The amplified products were then purified (QIAquick PCR purification kit; Qiagen), and both strands were sequenced. Sequences were edited using the software SeqMan (Lasergene Software package) and then aligned against the reference *S. epidermidis* RP62A sequence from GenBank using the blastx program with automatically adjusted parameters.

TABLE 1 Frequency of ciprofloxacin and 8-methoxyfluoroquinolone resistance rates for MSSE, MRSE, and all *S. epidermidis* isolates from 2005 to 2010 at BPEI^a

Yr	No. of isolates per yr		Resistance rate (%) among:					
			MSSE (<i>n</i> = 38)				MRSE (<i>n</i> = 55)	
	MSSE	MRSE	CIP-R	8-FQ-R	CIP-R	8-FQ-R	CIP-R	8-FQ-R
2005	8	4	37.5	28.6	100	100	58.3	54.5
2006	9	16	33.3	25.0	68.8	60.0	56.0	45.8
2007	6	7	66.7	66.7	100	85.7	84.6	76.9
2008	4	9	50.0	50.0	55.6	55.6	53.8	53.8
2009	10	7	80.0	62.5	71.4	57.1	76.5	60.0
2010	1	12	0 ^b	0 ^b	83.3	75.0	76.9	69.2
All yrs	38	55	52.6	39.5	76.4	60.0	66.7	58.4

^a Abbreviations: BPEI, Bascom Palmer Eye Institute; 8-FQ-R, resistant to both moxifloxacin and gatifloxacin; CIP-R, resistant to ciprofloxacin.

^b Only one MSSE isolate was recovered in 2010.

RESULTS

During the study period (2005 to 2010), a total of 146 *Staphylococcus* spp. isolates were recovered from endophthalmitis patients at our institution. The most frequent species was *S. epidermidis*, which accounted for 63.7% (93) of the isolates. Among the *S. epidermidis* isolates, 59.1% (55) were methicillin resistant and 40.9% (38) were methicillin susceptible. Methicillin resistance ranged from 33.3% in 2005 to 92.3% in 2010. Consequently, the percentage of MSSE declined from 66.7% in 2005 to 7.7% in 2010. Resistance increased for all fluoroquinolone antibiotics during the period of study. The ciprofloxacin resistance rate was 66.7% and ranged from 58% in 2005 to 77% in 2010. The frequency of isolates resistant to 8-methoxyfluoroquinolones was 58.4% and ranged from 55% in 2005 to 69% in 2010 (Table 1).

No significant increase in resistance to ciprofloxacin or the 8-methoxyfluoroquinolones was observed for MRSE isolates during the study period. However, ciprofloxacin and 8-methoxyfluoroquinolone resistance rates among MSSE isolates were more than double in 2007 compared to 2005 and 2006 and remained high in the following 2 years, 2008 and 2009. In 2010, only one MSSE isolate was recovered (also fluoroquinolone susceptible). The ciprofloxacin resistance rate among MSSE was 52.6% (20 out of 38) and ranged from 37.5% in 2005 to 80% in 2009. The resistance rate to 8-methoxyfluoroquinolones was 39.5% (15 out of 38) and ranged from 25% in 2005 to 50% in 2009 (Table 1). These selected MSSE isolates (*n* = 15) resistant to 8-methoxyfluoroquinolones were further analyzed.

Clinical features, ocular history, and prophylactic antibiotic usage among the 15 patients diagnosed with endophthalmitis caused by MSSE isolates resistant to 8-methoxyfluoroquinolones are summarized in Table 2. The mean age was 73 years (range, 56 to 92 years). Most of the patients were female (53.3%). At the time of clinical diagnosis, 13 patients (86.6%) were using prophylactic topical antibiotics. Information on the prophylaxis regimen for 2 patients was not available. Four patients had received gatifloxacin, 7 received moxifloxacin, 1 received gatifloxacin and ofloxacin, and 1 received moxifloxacin and vancomycin. Most cases were postoperative (6 acute and 1 delayed). Four patients experienced an infection after intravitreal injection of vascular epithelial

TABLE 2 Clinical features, ocular history, prophylactic antibiotic usage, and outcomes for patients with endophthalmitis from whom MSSE isolates resistant to 8-methoxyfluoroquinolones were recovered^a

Patient	Age		Yr isolate collected	Specimen	Eye	Prophylactic antibiotic(s)		Category	History	Initial	
	(yrs)	Gender								VA	Final VA ^c
1	72	M	2005	Vitreous	OS	Moxifloxacin	Acute PO	Phacoemulsification 4 days prior ^b	HM	HM	
2	NA	NA	2005	Vitreous	OS	Gatifloxacin	NA	NA	NA	NA	
3	92	F	2006	Vitreous	OD	Gatifloxacin	After IV injection	Chronic macular edema; underwent multiple IV injections of avastin and macugen; last injection 1 day prior	LP	8/200	
4	NA	NA	2006	AC	OS	NA	NA	NA	NA	NA	
5	NA	NA	2007	Vitreous	OD	Moxifloxacin	NA	NA	NA	NA	
6	56	M	2007	AC	OD	Moxifloxacin and vancomycin	Fungal keratitis	Underwent intracameral wash with voriconazole and PK for fungal keratitis	NA	NA	
7	62	M	2007	Vitreous	OD	Moxifloxacin	Acute PO	Phacoemulsification 5 days prior	HM	NA	
8	62	M	2008	Vitreous	OD	NA	Acute PO	Phacoemulsification 13 days prior	HM	20/60	
9	62	F	2008	AC	OS	Gatifloxacin and ofloxacin	Delayed PO	Phacoemulsification 4 yrs prior and PK 18 mos prior; graft corneal suture lost 1 week prior	HM	NA	
10	76	M	2008	Vitreous	OD	Moxifloxacin	Acute PO	Phacoemulsification 9 days prior	LP	20/200	
11	88	F	2009	Vitreous	OD	Moxifloxacin	After IV injection	Patient with AMD; underwent multiple IV injections of lucentis and avastin; last injection 3 days prior	CF	20/40	
12	78	F	2009	Vitreous	OD	Gatifloxacin	After IV injection	Patient with AMD; underwent multiple IV injections of avastin and macugen; last injection 4 days prior	HM	20/200 ^d	
13	82	F	2009	Vitreous	OD	Moxifloxacin	After IV injection	Underwent IV injection of lucentis 2 days prior	HM	20/40 ^d	
14	74	F	2009	Vitreous	OS	Gatifloxacin	Acute PO	Phacoemulsification 10 days prior	HM	20/80	
15	68	F	2009	Vitreous	OS	Moxifloxacin	Acute PO	Phacoemulsification 4 days prior	HM	20/30 ^d	

^a Abbreviations: NA, not available; M, male; F, female; AC, anterior chamber; OS, left eye; OD, right eye; PO, postoperative; IV, intravitreal injection; VA, visual acuity; PK, penetrating keratoplasty; AMD, age-related macular degeneration; HM, hand motion; LP, light perception; CF, counting fingers.

^b The time interval between the procedure and patient presentation at our eye institute.

^c Visual acuity at last follow-up.

^d Visual acuity with glasses.

growth factor (VEGF) antagonists, and 1 patient's infection occurred after severe fungal keratitis. Initial visual acuity at presentation ranged from counting finger to light perception, and final visual acuity ranged from 20/30 to hand motion.

Microbiology and molecular characterizations of the 15 MSSE

isolates resistant to 8-methoxyfluoroquinolones are summarized in Table 3. The MIC₉₀ for oxacillin among these isolates was 0.25 μg/ml; 6 (40%) of isolates had an oxacillin MIC of 0.19 μg/ml, and for the remaining 9 (60%) it was 0.25 μg/ml. High-level resistance to 8-methoxyfluoroquinolones (MIC, >32 μg/ml) was docu-

TABLE 3 Mutations in the QRDRs of *gyrA* and *parC* and MICs for fluoroquinolones and comparator agents

Patient no.	QRDR mutation(s) in:		Etest MIC (μg/ml)							
	<i>gyrA</i>	<i>parC</i>	OXA	GAT	MOX	LEV	CIP	OFX	LZD	VAN
1	Ser84Phe	Ser80Phe, Asp84Asn	0.25	3	2	ND	ND	ND	1	1.5
2	Ser84Phe, Glu88Lys	Ser80Tyr, Asp84Tyr	0.25	>32	>32	>32	>32	>32	4	2
3	Ser84Phe, Glu88Lys	Ser80Tyr, Asp84Tyr	0.19	>32	>32	>32	>32	>32	1	2
4	Ser84Phe, Glu88Lys	Ser80Phe, Asp84Asn	0.25	>32	>32	>32	>32	>32	1	2
5	ND	ND	0.19	2	3	ND	ND	ND	4	2
6	Ser84Phe	Ser80Phe, Asp84Gly	0.25	2	3	ND	ND	ND	1	2
7	Ser84Phe, Glu88Lys	Ser80Tyr, Asp84Tyr	0.19	>32	>32	>32	>32	>32	1	1.5
8	Ser84Phe, Glu88Lys	Ser80Phe, Asp84Gly	0.25	>32	>32	>32	>32	>32	1	3
9	Ser84Phe	Ser80Phe, Asp84Gly	0.25	2	2	>32	>32	>32	1	2
10	Ser84Phe	Ser80Phe, Asp84Val	0.19	2	2	>32	>32	>32	2	2
11	Ser84Phe	Ser80Phe, Asp84Asn	0.19	3	2	>32	>32	>32	1.5	2
12	Ser84Phe	Ser80Phe, Asp84Asn	0.19	3	3	>32	>32	>32	1.5	2
13	Ser84Phe	Ser80Phe	0.25	2	4	>32	>32	>32	1.5	2
14	Ser84Phe, Glu88Lys	Ser80Tyr, Asp84Tyr	0.25	>32	>32	>32	>32	>32	1	2
15	Ser84Phe, Glu88Lys	Ser80Tyr, Asp84Tyr	0.25	>32	>32	>32	>32	>32	1	2

^a Abbreviations: OXA, oxacillin; GAT, gatifloxacin; MOX, moxifloxacin; LEV, levofloxacin; CIP, ciprofloxacin; OFX, ofloxacin; LZD, linezolid; VAN, vancomycin; ND, not determined.

mented in 46.7% (7 out of 15) of the isolates. Low-level resistance (MIC, 2 to 4 $\mu\text{g/ml}$) was identified for the remaining 8 (53.3%) isolates. All isolates were also resistant to ciprofloxacin, levofloxacin, and ofloxacin, with MICs of >32 $\mu\text{g/ml}$. All isolates were susceptible to linezolid (MICs ranging from 1 to 4 $\mu\text{g/ml}$) and vancomycin (MICs ranging from 1.5 to 3 $\mu\text{g/ml}$).

Mutations in the QRDRs of the *gyrA* and *parC* genes were found for the 14 tested isolates (Table 2). In the *gyrA* gene, the substitution of a serine for a phenylalanine at codon 84 (Ser84Phe) was found for all isolates. In addition, a second point mutation at codon 88 (Glu88Lys) was also identified for 7 out of 14 isolates (50%), which was associated with a higher MIC of 8-methoxyfluoroquinolones. All isolates with a double point mutation in the *gyrA* gene demonstrated high-level resistance to moxifloxacin and gatifloxacin (MICs, >32 $\mu\text{g/ml}$), regardless of the number and combination of point mutations in the QRDR of *parC*.

In the *parC* gene, a single point mutation (Ser80Phe) was found in only one isolate, and all the other (13/14) harbored a double point mutation with four different combinations, including Ser80Tyr and Asp84Tyr in 5 isolates (35.7%), Ser80Phe and Asp84Asn in 4 isolates (28.6%), Ser80Phe and Asp84Gly in 3 isolates (21.4%), and Ser80Phe and Asp84Val in 1 isolate (7.1%).

DISCUSSION

Fluoroquinolone resistance among staphylococci endophthalmitis isolates is a major and increasing concern in ophthalmology. The documentation of high-level moxifloxacin and gatifloxacin resistance in methicillin-susceptible isolates will further limit the use of this drug class in the treatment and prevention of ocular infections. The development of endophthalmitis caused by CoNS isolates resistant to 8-methoxyfluoroquinolones in eyes treated prophylactically with these compounds has been documented (20, 21). Moreover, an important increase in the resistance rates to gatifloxacin and moxifloxacin among CoNS isolates from endophthalmitis cases over the last 2 decades has been reported (9, 10). However, to our knowledge this is the first report showing the emergence of 8-methoxyfluoroquinolone resistance and multiple mutations in the QRDRs of the *gyrA* and *parC* genes among MSSE recovered from endophthalmitis cases.

The rates of resistance to ciprofloxacin and 8-methoxyfluoroquinolones among the MSSE isolates included in this study were 52.6% and 39.5%, respectively. Moreover, there was an increasing trend of fluoroquinolone resistance among MSSE between the years 2005 and 2009. The overall resistance rates to moxifloxacin (26.9%) and ciprofloxacin (38.4%) for all CoNS (methicillin susceptible and resistant) isolated from postoperative endophthalmitis cases in the same institution were approximately half as high between 2000 and 2004 (9).

Fluoroquinolone-resistant CoNS isolates from the normal conjunctiva microbiota have been recovered from patients undergoing intraocular surgery and intravitreal injection (4, 5, 22–24). In addition, two studies found mutations within the QRDR in around 50% to 58% of *S. epidermidis* isolates from in the conjunctival sacs before preoperative instillation of antibiotic from patients undergoing intraocular surgeries (18, 19). Although a double point mutation was found for a few isolates in both studies, 38% to 41.6% of *S. epidermidis* isolates from conjunctival sacs preoperatively harbored at least one point mutation in the *gyrA* gene. Moreover, it has been demonstrated that prophylactic use of topical levofloxacin and moxifloxacin is associated with selection

of fluoroquinolone-resistant *S. epidermidis* and others CoNS from the normal conjunctival and nasopharynx microbiota (4, 18, 25). The MSSE isolates resistant to 8-methoxyfluoroquinolones included in our study were recovered mostly from endophthalmitis cases following intraocular surgery and intravitreal injection. Almost all patients (87%) received prophylactic moxifloxacin or gatifloxacin eye drops. It has been recently reported that topical application of both gatifloxacin and moxifloxacin results in subtherapeutic levels in the aqueous humor against staphylococci isolates from endophthalmitis (26). Achieving therapeutic levels is not only important to maximize the bactericidal effect and bacteriological cure but also to prevent the potential for selection and dissemination of subpopulations of bacterial cells harboring spontaneous mutations in drug target-encoding genes (27). In this scenario, prophylactic use of topical fluoroquinolones is likely to increase the odds for selection of fluoroquinolone-resistant mutants, especially isolates presenting high-level resistance.

Nearly half of the isolates (46.7%) included in our study showed high-level resistance to both gatifloxacin and moxifloxacin. The higher MICs were correlated with the number of point mutations in the *gyrA* gene. Low-level resistance isolates demonstrated only a single point mutation (Ser84Phe) in *gyrA*, whereas isolates carrying a second point mutation (Ser84Phe and Glu88Lys) showed high-level resistance. Previous work has also shown that the additional substitution at codon 88 from a glutamic acid to lysine in the *gyrA* of *S. epidermidis* ocular isolates is related to a higher MIC of 8-methoxyfluoroquinolones (18, 19). Although overexpression of the efflux pump might play a role in the high-level fluoroquinolone resistance, our present and previous data suggest that the level of resistance for 8-methoxyfluoroquinolones increases as additional mutations are included in the QRDR of *gyrA* (28, 29).

In conclusion, our results demonstrated that low- and high-level resistance to 8-methoxyfluoroquinolone was detected and is increasing among MSSE isolates from patients with endophthalmitis following intraocular procedures. Prior exposure to the 8-methoxyfluoroquinolones may contribute to the selection of MSSE strains containing multiple mutations in the QRDRs of *gyrA* and *parC* that result in low- and high-level resistance to these agents. To prevent the growing resistance to the newer fluoroquinolones among staphylococci isolates from endophthalmitis cases, the extensive use of gatifloxacin and moxifloxacin pre- and postoperatively for the prevention of endophthalmitis should be reconsidered.

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We have no conflicts of interest.

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