Is Resistance to Gatifloxacin and Moxifloxacin Here Already?

New evidence suggests that the incidence of in vitro resistance to these drugs is on the rise. How do we counter this trend?

BY DAVID G. HWANG, MD

In the 4 years since their approval by the FDA, gatifloxacin 0.3% (Zymar; Allergan, Inc., Irvine, CA) and moxifloxacin 0.5% (Vigamox; Alcon Laboratories, Inc., Fort Worth, TX) have quickly emerged as the preferred antimicrobials of many ophthalmologists for the prophylaxis and treatment of serious ocular infections.[1] The chief advantages of these agents (sometimes referred to as *fourth-generation fluoroquinolones* but more precisely and hereinafter termed *8-methoxyfluoroquinolones*) compared with older ophthalmic fluoroquinolones such as ciprofloxacin (Ciloxan; Alcon Laboratories, Inc.) and ofloxacin (Ocuflox; Allergan, Inc.) are their enhanced in vitro gram-positive antibacterial activity and favorable pharmacokinetics.[2,3]

Another factor that has encouraged the use of gatifloxacin and moxifloxacin is the well-documented rise in resistance to ciprofloxacin and ofloxacin among ocular isolates.[2,4] Both gatifloxacin and moxifloxacin show better activity against gram-positive isolates compared with ciprofloxacin and ofloxacin.[3] Furthermore, on in vitro testing, the 8-methoxyfluoroquinolones have demonstrated less susceptibility to the development of *de novo* gram-positive–related resistance in wild-type *Staphylococcus aureus* due to their activity against both bacterial topoisomerase II and IV.[5] On this basis, the first-line use of these agents for prophylaxis and treatment has been suggested as a potential means of forestalling the development of gram-positive resistance.

It thus is perhaps surprising that, in the short time since the introduction of gatifloxacin and moxifloxacin for ophthalmic use, significant rates of in vitro resistance to these fluoroquinolones have already been observed by at least one research group. A recently published study by Miller and colleagues confirmed that resistance to both drugs is prevalent among the ocular isolates responsible for endophthalmitis.[6] These investigators reported that the proportion of coagulase-negative staphylococcal endophthalmitis isolates with in vitro resistance to gatifloxacin and moxifloxacin increased from 3% among historical isolates banked from

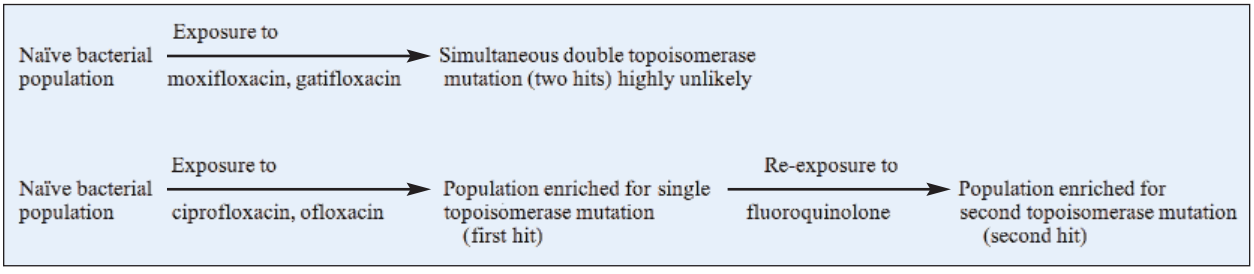

Figure 1. A serial-hit hypothesis may explain resistance to newer fluoroquinolones. Topoisomerase-based resistance in *Staphylococcus* to 8-methoxyfluoroquinolones requires mutations to two different topoisomerase genes.

1990 to 1994 to 35% among isolates recovered in 2000 to 2004. These data suggest that the rise in resistance to the drugs may have predated their introduction for clinical use. The investigators also reported that the prevalence of cross-resistance to fluoroquinolones was high (ie, isolates that were resistant to ciprofloxacin and ofloxacin were likely to be resistant to gatifloxacin and moxifloxacin).

The findings of the study by Miller and colleagues raise a number of important questions. First, how could resistance have arisen so rapidly, especially given the intrinsic resistance-fighting properties of the 8-methoxyfluoroquinolones? Second, what are the potential clinical implications of this increase in resistance? Finally, what can be done to forestall the further development of resistance to fluoroquinolones?

PRIOR EXPOSURE TO FLUOROQUINOLONES GENERATES LOW-LEVEL RESISTANCE

Resistance to older fluoroquinolones in ocular isolates has been on the rise for some time, dating to before the introduction of gatifloxacin and moxifloxacin.[3] Likely explanations for this trend include not just increasing human systemic use of fluoroquinolones but also the widespread use of these agents in US poultry farming in the recent past. Another potential contributing factor is indiscriminate and incorrect ophthalmic usage of fluoroquinolones.[2]

The increased use of fluoroquinolones generates selection pressure that, over time, enriches an exposed bacterial population for one or more mutations that confer resistance to fluoroquinolones. Depending on the mechanism of such resistance and the bacterial species, these mutations may confer either absolute resistance to gatifloxacin and moxifloxacin or a greatly reduced threshold for the subsequent development of resistance to these agents.

Against gram-negative bacteria, the 8-methoxyfluoroquinolones have no advantage in activity over ciprofloxacin or ofloxacin[3]; accordingly, a *Pseudomonas aeruginosa* strain that is resistant to ciprofloxacin will be equally resistant to gatifloxacin or moxifloxacin.[7] Clinical and in vitro resistance to gatifloxacin and moxifloxacin has recently been reported in a case of *P. aeruginosa* keratitis after PRK,[8] and research suggests that the prevalence of gram-negative resistance to fluoroquinolones appears to be rising in systemic isolates.[9]

A SECOND MUTATION CONFERS RESISTANCE TO 8-METHOXYFLUOROQUINOLONES

For gram-positive bacteria, one of the principal (but not exclusive) mechanisms of fluoroquinolone resistance is a mutation to bacterial topoisomerase II (DNA gyrase) and/or topoisomerase IV. Each topoisomerase is made up of separate subunits (A and B), and mutations in either subunit may confer resistance. Whereas ciprofloxacin and ofloxacin primarily target topoisomerase IV, the 8-methoxyfluoroquinolones require mutations in both DNA gyrase (encoded by *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by *grlA* and *grlB* genes) to be rendered inactive. In a naïve population of wild-type gram-positive bacteria, the likelihood of two such mutations' occurring simultaneously in the same bacterium is extremely low. The situation is quite different, however, in a bacterial population that has had prior exposure to ciprofloxacin and ofloxacin and through such selection pressure has undergone enrichment for mutations conferring fluoroquinolone resistance. In *S. aureus* isolates that have been selected for resistance to older fluoroquinolones, the acquisition of mutations to the topoisomerase IV A subunit gene (*grlA*) is commonly observed.[10] In this population, resistance to 8-methoxyfluoroquinolones would occur with the acquisition of just one additional mutation in either the *gyrA* or *gyrB* gene.

This serial-hit hypothesis to explain the development of 8-methoxyfluoroquinolone resistance is illustrated in Figure 1.

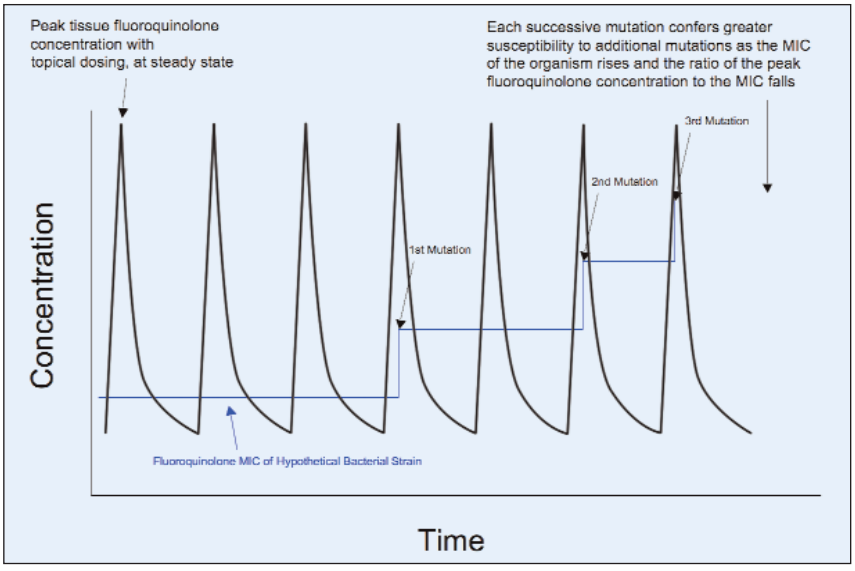

Figure 2. Each subsequent fluoroquinolone mutation is acquired more readily, leading to superresistant bacteria. This theoretical graph shows the development of mutational resistance in a hypothetical bacterial strain exposed to repeated dosing of a topical fluoroquinolone. Each successive mutation confers greater susceptibility to additional mutations as the minimum inhibitory concentration (MIC) of the organism rises and the ratio of the peak fluoroquinolone concentration to the MIC falls.

SUCCESSIVE MUTATIONS OCCUR WITH GREATER PROBABILITY

An additional mechanism must be invoked to explain the rapid acquisition of serial mutations in a given bacterial strain, which results in the development of high-level resistance to fluoroquinolones, including 8-methoxyfluoroquinolones. Under optimal conditions, if a given bacterial strain were continuously exposed to a fluoroquinolone concentration in excess of roughly four times the MIC of that strain, the probability of a mutational resistance event would be less than 10^{-9}. At this frequency, the likelihood of even a single mutational resistance event would therefore be expected to be low in the ophthalmic setting, given the relatively low organism burden present in the normal ocular flora and even in active ocular infections.

The threshold antibiotic concentration associated with a spontaneous mutational resistance probability of less than 10^{-9} is termed the *mutant prevention concentration* (MPC).[11]

When the tissue concentration of a fluoroquinolone drops to a range below the MPC but above the MIC (this range is termed the *mutant selection window*), mutations can occur. For fluoroquinolones in particular, the author has demonstrated that the mutational resistance rate increases exponentially, by several orders of magnitude, as the ratio between the antibiotic concentration decreases linearly within the mutant selection window.[2] In the course of normal topical ophthalmic dosing, organisms may be exposed for prolonged durations to concentrations only slightly in excess of the MIC or even to concentrations below the MIC. It is during these intervals that the selection for resistance-conferring mutations can occur with a relatively high probability. Additionally, as each subsequent mutation raises the MIC of the strain further, the ratio between the antibiotic's concentration in tissue and the MIC falls proportionally, and the mutational frequency increases exponentially yet further. Thus, the probability of acquiring each successive mutation increases (Figure 2).

Furthermore, under conditions of antibiotic selection pressure, those isolates with more than one resistance mutation enjoy a strong survival advantage relative to other isolates within a bacterial population. As the number of resistant bacteria rises, so does the likelihood that, within this already resistant subpopulation, there will arise new chance mutations that confer yet greater degrees of resistance to fluoroquinolones.

SUPERBUGS RESULT FROM MULTIPLE MUTATION ACQUISITION

One prediction of the proposed hypothesis is that multidrug resistance may occur as different types of drug-resistant genes are acquired in the process of sequential selection. Because resistance to fluoroquinolones can occur by mechanisms other than an alteration in the topoisomerase target, such as altered permeability or efflux, some of these acquired resistance mechanisms may confer cross-resistance to other antimicrobials. It is therefore interesting to note that exposure to fluoroquinolones has been shown to increase the likelihood of selecting methicillin-resistant strains in a heterogeneous population of *S. aureus*,[12] although a causal mechanistic explanation for this association has yet to be fully elucidated. Conversely, methicillin-resistant staphylococci are also likely to carry multiple fluoroquinolone-resistance genes. A recent study demonstrated that, among methicillin-resistant staphylococcal strains isolated from ocular surface flora, approximately 70% were concomitantly resistant to fluoroquinolones, including the 8-methoxyfluoroquinolones.[13]

A further prediction of the preceding hypothesis is that the acquisition of successive mutations conferring resistance would occur in a runaway fashion, as the probability of acquiring additional mutations rises and the gap in survival advantage between more and less resistant strains within a given bacterial population widens. This process of acquiring further mutations would be expected to stop once the MIC exceeded the peak concentration of antibiotic in tissue, at which point there would be no additional survival advantage to the acquisition of further mutations. This model would therefore predict that (1) resistant isolates obtained after fluoroquinolone selection pressure exerted in vivo would be likely to manifest multiple fluoroquinolone-resistance mutations, rather than just one or two, and that (2) in such strains, high-level resistance to fluoroquinolones would occur, as well as possibly multidrug resistance.

These predictions have been borne out by at least two studies in the ophthalmic literature. I conducted a study of patients given a prolonged postoperative topical dosing regimen of either ofloxacin or ciprofloxacin tapered from q.i.d. to b.i.d. over 4 weeks.[2] At the end of the treatment period, cultures of lid and ocular surface flora were performed. The rates of recovery of fluoroquinolone-resistant ocular flora increased from under 10% prior to exposure to fluoroquinolones to over 30% after exposure. The MIC_{50} to ciprofloxacin of recovered isolates was in excess of 64 µg/mL, and the MIC_{50} to ofloxacin was 16 µg/mL, a finding implying that the sequential acquisition of multiple fluoroquinolone-related mutations had occurred. The majority of these resistant isolates also manifested resistance to multiple other antibiotics, including methicillin. In a recently published study by Iihara and colleagues,[14] a high proportion of methicillin-resistant ocular isolates were also found to have high-level resistance to fluoroquinolones, including 8-methoxyfluoroquinolones. Upon genomic analysis of these isolates, more than 60% of the isolates had four or five topoisomerase mutations, 32% had two topoisomerase mutations, and just 5% had only one topoisomerase mutation.

GUIDELINES FOR THE PROPER DOSING OF FLUOROQUINOLONES

- Dose no less frequently than q.i.d.
- Dose only for the required period of time:
  Bacterial conjunctivitis: 1 week
  Bacterial keratitis: 2 to 3 weeks
  Post-LASIK: 3 to 7 days
  Postcataract surgery: 1 week
- Curtail treatment by abrupt discontinuation rather than by tapering
- Hit hard, get in, and get out

WHAT IS THE CLINICAL SIGNIFICANCE OF IN VITRO RESISTANCE?

In vitro antimicrobial resistance does not always translate into in vivo resistance, particularly if the resistance is low level and the organism burden is modest.[15] In such cases, high tissue levels achieved by adequate ophthalmic dosing may be sufficient to eradicate the relatively modest numbers of low-level resistant organisms. In cases in which high-level resistance is present and/or the organism burden is high, however, the likelihood that the fluoroquinolone will successfully eradicate such resistant organisms in vivo decreases.

Dajcs and colleagues demonstrated in a rabbit model of ofloxacin-resistant staphylococcal keratitis that the initiation of topical moxifloxacin therapy within 9 hours after inoculation successfully treated the infection, but a delay of just 6 hours resulted in reduced efficacy in the treatment of the keratitis.[16] Wilhelmus and colleagues reported that the treatment response to ciprofloxacin experimental keratitis correlated in proportion to the strain's susceptibility to the drug.[17] In another study, Wilhelmus et al described a retrospective analysis of data from clinical trials that found a positive correlation between clinical therapeutic response rates and the quantitative level of ciprofloxacin susceptibility of each of the causative isolates.[18]

Although a confirmation of the finding of rising fluoroquinolone resistance is needed, and further substantiation of the earlier hypothesis will be required, a delay in implementing measures to counter this threat risks disastrous consequences—a rapid rise in fluoroquinolone resistance to the point where the effectiveness of this class of antimicro-

bials is substantially diminished. Two measures need to be taken by prescribers: (1) limiting their use of fluoroquinolones (perhaps the hardest pill to swallow) and (2) applying optimal strategies for fluoroquinolone dosing and antimicrobial selection, based on pharmacodynamic principles.

THE USE OF AGENTS OTHER THAN FLUOROQUINOLONES

The 8-methoxyfluoroquinolones are considered by many ophthalmologists in the US to be first-line antimicrobials for the treatment and prophylaxis of serious infections such as bacterial keratitis, post-LASIK keratitis, and postoperative endophthalmitis. Nonetheless, there is a surprising lack of clinical data to support the notion that these antimicrobials are clinically superior to older fluoroquinolones or to other antimicrobial agents of a different structural class. In fact, in a recently completed head-to-head comparison of topical levofloxacin and intracameral cefuroxime for surgical prophylaxis in cataract surgery, intracameral cefuroxime proved superior for first-line antimicrobial prophylaxis in intraocular surgery.[19]

Although the merits of topical fluoroquinolones versus intracameral cephalosporins may be the subject of ongoing debate, it is at least clear that not every infectious indication requires the use of fluoroquinolone antibiotics. Particularly for minor, self-limited, or chronic infections such as blepharitis or uncomplicated conjunctivitis, agents other than fluoroquinolones should be considered. Older antibiotics with a narrower spectrum of activity can generally treat less serious ocular infections effectively. Examples of excellent antimicrobials for these indications include bacitracin for staphylococcal blepharitis and polymyxin B-trimethoprim for uncomplicated bacterial conjunctivitis. Not only are these particular antimicrobials less likely to exert a strong degree of selection pressure that can promote resistance, but they also have the advantage of being considerably less expensive than the newer fluoroquinolones.

For most routine, uncomplicated cases of bacterial conjunctivitis, older agents such as polymyxin B-trimethoprim are comparable to the newer fluoroquinolones in effecting a clinical cure. Using one of the newer fluoroquinolones may be appropriate, however, in the exceptional instances in which laboratory resistance to first-line agents has been documented or when clinical unresponsiveness or relapse has been encountered after treatment with a first-line antimicrobial. Furthermore, because fluoroquinolones with better antimicrobial activity may in some cases effect a more rapid microbiological cure than older fluoroquinolones,[20] it may be preferable to use a newer fluoroquinolone as first-line therapy for cases of bacterial conjunctivitis in which rapid bacterial eradication is essential. Examples include patients at risk for secondary infectious complications from bacterial conjunctivitis (eg, those with bullous keratopathy or an avascular filtering bleb) or individuals for whom there is a need to limit horizontal transmission to their contacts at home, school, or workplace.

It is essential to recognize that the hypothesis discussed previously predicts that a strategy of using older fluoroquinolones in preference to the 8-methoxyfluoroquinolones will not result in reduced fluoroquinolone selection pressure but, in fact, may actually increase the likelihood of resistance to 8-methoxyfluoroquinolones. The reason, as previously discussed, is that first-step mutational resistance is much more likely to occur with ciprofloxacin or ofloxacin than with gatifloxacin or moxifloxacin. Thus, if a fluoroquinolone is needed, an extrapolation from in vitro data would predict that an 8-methoxyfluoroquinolone would have a lower propensity to select for resistant isolates. Further clinical testing, however, is needed to substantiate this prediction.

CORRECT DOSING

Some investigators have suggested that the ophthalmic use of fluoroquinolones should rarely lead to the development of antibiotic resistance, due to the relatively small quantity of ophthalmic fluoroquinolones used compared with the amounts needed for systemic and veterinary use. Incorrect ophthalmic dosing, however, can lead to the rapid development of high-level fluoroquinolone resistance. Insufficiently frequent daily dosing leads to the prolonged exposure of the lid flora to subinhibitory concentrations of a fluoroquinolone, a situation that rapidly promotes the development of drug-resistant strains. The consequences of inadequate dosing include not only a suboptimal therapeutic effect but also an increased propensity for the development of antibiotic resistance.

Examples of regimens that are likely to be less than optimal include a dosing frequency of less than q.i.d., a length of treatment or prophylaxis that extends well beyond the susceptible period for infection, and tapering rather than simply abruptly discontinuing the antibiotic. For example, a 4-week course of topical ofloxacin or ciprofloxacin (q.i.d. for 2 weeks, followed by b.i.d. for 2 weeks) leads to frequent recovery of resistant ocular isolates,[2] whereas a 3-day course of ofloxacin given q.i.d. does not result in an increased recovery of resistant isolates.[21]

The fact that the newer fluoroquinolones generally show better activity than their older counterparts does not mean that they need not be adequately dosed. In the absence of available scientific studies needed to create evidence-based guidelines for the proper dosing of ophthalmic fluoroquinolones, the author offers some guidelines for the dosing of these drugs (see *Guidelines for the Proper Dosing of*

Fluoroquinolones). A general principle in the use of ophthalmic fluoroquinolones is to prescribe a sufficiently high dose for the shortest required duration. In other words, hit hard, get in, and get out.

The precise determination of the optimal clinical dosing regimens for 8-methoxyfluoroquinolones will require pharmacodynamic studies relevant to ocular pharmacotherapeutics. Pharmacodynamics is a discipline that attempts to describe how pharmacology, pharmacokinetics, pharmacogenetics, and other factors work together to govern the biological activity of a given drug over time in a living organism. Although the application of pharmacodynamics to ophthalmology (and ocular infectious disease in particular) is still in its infancy, this discipline promises to help clinicians develop antimicrobial dosing guidelines that should improve therapeutic efficacy and even reduce the propensity for the development of resistance.

As an example, for fluoroquinolones, there is a dose-response relationship between the tissue concentration of the antibiotic and biological response. In general, the higher the achieved concentration of fluoroquinolone in the target tissue or fluid, the better. A higher tissue concentration of fluoroquinolone (relative to the MIC) translates into better bacterial killing, an improved clinical response to infection, and a lower propensity for the development of resistance.[2] In contrast, for cephalosporin antibiotics, bacterial killing is dependent on time rather than concentration. Raising the concentration of cephalosporin would therefore have little benefit, but extending the duration of treatment with this agent would be advantageous.

Recent pharmacodynamic evidence from the literature on systemic infectious disease suggests that the optimal target concentration of an antibiotic is not the MIC but a concentration significantly higher than that value. For empiric treatment and prophylaxis, the minimum target value should be the MIC_{90}, not the more commonly reported MIC_{50}. The MIC_{90} designates the concentration of antibiotic at which 90% of tested bacterial strains of a given species are inhibited, and it is generally several times higher than the more commonly reported MIC_{50}, which represents the antibiotic concentration at which 50% of tested bacterial strains of a given species are inhibited. The minimum bacteriocidal concentration is slightly higher yet and designates the antibiotic concentration needed to kill 90% of tested bacterial strains of a given species. Finally, the MPC designates the concentration at which the mutational resistance occurs at a frequency of less than 10^{-9} or, alternatively, the concentration that is sufficient to prevent the growth of a first-step resistant mutant.[11]

According to the MPC hypothesis, keeping the antibiotic concentration at or above the MIC can prevent the development of mutational resistance. The MPC must be determined experimentally for each strain, species, and antibiotic. In general, for many fluoroquinolones, it is three- to four-fold higher than the corresponding MIC. Because a much larger population of bacteria is exposed during treatment than during prophylaxis, the MPC may be a more important goal when dosing for treatment than when dosing for prophylaxis.

It can be argued, therefore, that the optimal target concentration for a fluoroquinolone for prophylaxis is the MIC_{90}, whereas for treatment it is the MPC_{90}. These respective values may be two- to 10-fold higher than the MIC_{50}. When using older fluoroquinolones such as ofloxacin and ciprofloxacin, even very frequent dosing (eg, five times in 1 hour) may only produce aqueous humor levels that approximate the MIC_{50}.[22] The MIC_{90} of the 8-methoxyfluoroquinolones for nonfluoroquinolone-resistant gram-positive species is relatively low, especially in comparison to ciprofloxacin or ofloxacin.[3] This factor, combined with the favorable corneal and aqueous penetration characteristics of gatifloxacin and moxifloxacin, should theoretically result in the ability of the 8-methoxyfluoroquinolones—assuming proper and frequent dosing—to more readily achieve target tissue concentrations that yield the optimally desired antibacterial effect while reducing the propensity for developing resistance.

CONCLUSION

Ophthalmologists have grown increasingly reliant on the newer fluoroquinolones, gatifloxacin and moxifloxacin in particular, as first-line agents in the prophylaxis and treatment of serious ophthalmic infections. The recently reported rise in resistance to these agents, occurring so soon after their introduction, is worrisome. If confirmed, it could require substantial modifications to current antibiotic prescribing patterns. An explanation for these findings may lie in the fact that selection pressure from years of ciprofloxacin and ofloxacin use has resulted in an increasing prevalence of low-level fluoroquinolone resistance. Against this background, the development of multistep mutations that confer 8-methoxyfluoroquinolone resistance is more likely to occur. In the setting of suboptimal dosing of fluoroquinolones, runaway resistance can rapidly develop, leading to high-level fluoroquinolone resistance that is likely to be clinically significant.

Further experimental and clinical work is needed to develop rational strategies for limiting the development of fluoroquinolone resistance. Until such data become available, the recommendations presented in this article may serve as a roadmap for how the judicious, restrained, and reduced use of fluoroquinolones, coupled with appropriate dosing and antimicrobial selection, can maximize the therapeutic effect of the newer-generation fluoroquinolones. Perhaps

more importantly, such a disciplined approach may extend the useful lifetime of this important group of ophthalmic antimicrobials. ■

David G. Hwang, MD, is Professor of Ophthalmology and Director of the Cornea Service at the University of California, San Francisco. He is also on the faculty of the Francis I. Proctor Foundation for Research in Ophthalmology. He acknowledged no financial interest in the products or companies mentioned herein. Dr. Hwang may be reached at (415) 476-7977; david.hwang@ucsf.edu.

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