An Ophthalmologist's Guide to Understanding Antibiotic Susceptibility and Minimum Inhibitory Concentration Data

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Purpose: To address the need to establish appropriate evaluation criteria for analyzing in vitro antibiotic susceptibility based on original data.

Design: In vitro laboratory investigation.

Participants: Bacterial isolates from patients with conjunctivitis.

Main Outcome Measures: Minimum inhibitory concentrations (MICs), descriptive statistics, antibiotic susceptibility, potency, and statistical analysis.

Methods: Minimum inhibitory concentrations were determined for 80 bacterial conjunctivitis isolates to moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin. Using the MIC values, descriptive statistics (median, MIC_{50}, MIC_{90}, mode, range), antibiotic susceptibility, and potency of each antibiotic were calculated for each bacterial group. The data were analyzed statistically using appropriate randomization and nonparametric tests.

Results: The descriptive statistics of gram-positive bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*) followed a consistent trend where the median, MIC_{50}, MIC_{90}, and mode demonstrated the lowest values, in all instances, for moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin. The descriptive statistics for *Haemophilus* species (the predominant gram-negative bacteria implicated in conjunctivitis) did not describe any consistent trend. In contrast, antibiotic susceptibility testing did not demonstrate any advantage among the 5 fluorquinolones tested, except for moxifloxacin in the *S. aureus* fluoroquinolone-resistant group. Potency studies indicated that moxifloxacin and gatifloxacin were the most potent for gram-positive bacteria, whereas gatifloxacin and ciprofloxacin were the most potent for *Haemophilus* species.

Conclusion: In the absence of human clinical trial data to guide care, in vitro susceptibility data should be analyzed with a set of descriptive statistics along with a nonparametric statistical analysis. No single parameter or test should be relied upon in all instances to demonstrate the in vitro superiority of one antibiotic over another. In this study, fourth-generation fluoroquinolones did have some potency advantages over second-generation fluoroquinolones against gram-positive conjunctival bacterial isolates, but not for *Haemophilus* isolates. *Ophthalmology 2005;112:1987–1991 © 2005 by the American Academy of Ophthalmology.*

Ophthalmologists now have available moxifloxacin (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX) and gatifloxacin (Zymar, Allergan, Inc., Irvine, CA) to add to their arsenal of levofloxacin, ciprofloxacin, and ofloxacin. Although clinical efficacy is the best parameter for the comparison of competing antibiotics, clinical studies and complete in vivo data are not entirely available for comparing the 5 fluoroquinolones.[1] In addition, it is highly unlikely that these head-to-head clinical studies will ever be undertaken due to expense, difficulty of patient recruitment, and marketing considerations. Until and unless comparative clinical data are available, in vitro studies will remain by default the principle source of information used to compare

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fluoroquinolone antibiotics. The challenge to the busy ophthalmologist is how best to serve his or her patients by evaluating in vitro data as presented in the literature and by the pharmaceutical industry. Although descriptive statistics for minimum inhibitory concentration (MIC) data (median, antibiotic concentration that would inhibit the growth of 50% of the tested bacterial isolates [MIC_{50}], MIC_{90}, etc.) are frequently used to compare antibiotics and have been used by pharmaceutical companies to present their products in a most favorable light, previous reports have not identified specifically any consistent predictive parameters for determining the most effective antibiotics.[2–4]

Our goals in this in vitro laboratory report were (1) to address the need to establish appropriate evaluation criteria (statistical parameters and tests) for analyzing and comparing in vitro antibiotic susceptibility data and (2) to apply these criteria (descriptive statistics, antibiotic susceptibility, and potency testing) to compare 5 commercially available topical fluoroquinolones against 80 bacterial conjunctival isolates in an original study.

Materials and Methods

The MICs (micrograms per milliliter) of 80 bacterial conjunctivitis isolates were determined for moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin using Etests (AB Biodisk, Piscataway, NJ). In contrast to an epidemiological prevalence study, the current study was designed to compare the relative susceptibilities for the *Staphylococcus aureus* group to different fluoroquinolones by deliberate selection of representative isolates that were both susceptible and resistant to second-generation fluoroquinolones (ciprofloxacin and ofloxacin) (as determined by disk diffusion). The bacterial isolates of this laboratory study included 20 isolates of *S. aureus* that were resistant to ciprofloxacin and ofloxacin, 20 isolates of *S. aureus* that were susceptible to ciprofloxacin and ofloxacin, 20 isolates of *Streptococcus pneumoniae,* and 20 isolates of *Haemophilus* species (the predominant gram-negative bacteria implicated in conjunctivitis). The isolates were collected consecutively from September 1998 to October 2002 at the Charles T. Campbell Ophthalmic Microbiology Laboratory and stored at −76° C (University of Pittsburgh, Pittsburgh, Pennsylvania, Institutional Review Board no.: 000943). Selection was based on the most recently collected and retrieving backwards chronologically. All bacterial isolates were collected from the conjunctiva. *Staphylococcus aureus, S. pneumoniae,* and *Haemophilus* species represented 66% (738/1107) of the bacteria isolated from conjunctivitis at our institution from 1993 to January 1, 2005 (http://eyemicrobiology.upmc.com/conjunctivitis.htm).

The MICs were compared using standard descriptive statistics and appropriate statistical analysis, as the following describe.

Descriptive Statistics

In this study, a descriptive statistic was a numerical value (i.e., median, MIC_{50}, MIC_{90}, etc.) that was used to describe the MIC data. A low descriptive statistic value for an antibiotic in comparison with other antibiotics is advantageous, because this would indicate that less antibiotic is required to inhibit the bacteria.

To start calculating descriptive statistics for a given antibiotic, the MICs for the 20 bacterial isolates must first be ranked from the lowest value to the highest value. A median, MIC_{50}, or MIC_{90} is determined by the position or rank within the 20 MIC values defined by the highest and lowest values.

Types of Descriptive Statistics

1. *Median.* The value in the middle of the rank. For an odd number of values, the middle value is the median. For an even number of values, the middle 2 values are averaged. In the present study, the values of the 10th and 11th positions were averaged for the median.
2. *MIC_{50}.* The antibiotic concentration that would inhibit the growth of *50%* of the tested bacterial isolates. This is not the antibiotic concentration that is required to decrease the amount of a *single* isolate by 50%. Nor is it the average of all the MICs. For example, the MICs of 10 fictitious isolates were determined to be, respectively, 1, 1, 2, 2, 4, 8, 8, 8, 16, and 32 μg/ml. The MIC_{50} would be 4 μg/ml. This is the MIC at the 5 position (50% rank). Similar to median, the value is determined at the 50% position of the values. For an odd number of values, the value would be the rank closest to the 50% position. In the present study, the value at the 10th position was the MIC_{50}.
3. *MIC_{90}.* The antibiotic concentration that would inhibit the growth of *90%* of the tested bacterial isolates. This is not the antibiotic concentration that is required to decrease the amount of a single isolate by 90%. For example, the MICs of the same 10 fictitious isolates were determined to be, respectively, 1, 1, 2, 2, 4, 8, 8, 8, 16, and 32 μg/ml. The MIC_{90} would be 16 μg/ml. This is the MIC at the 9 position (90% rank). The value is determined at the rank of the 90% position of the values. For an odd number of values, the value would be the rank closest to the 90% position. In the present study, the value at the 18th position was the MIC_{90}. The MIC_{90} is more meaningful when a large number of values are included. A small number of values, even at 10 or 20 observations, can be misleading when multiple resistant isolates are part of the data set.
4. *Range (minimum − maximum values).* Rather than completing the actual subtraction, the minimum to maximum values can be substituted. This parameter can indicate outliers that may skew descriptive statistics and statistical analysis.
5. *Mode.* The value amongst all observations that occurs at the greatest frequency. For example, the MIC mode of the 10 fictitious isolates (1, 1, 2, 2, 4, 8, 8, 8, 16, and 32 μg/ml) would be 8 μg/ml. There can be multiple modes for a data set (i.e., bimodal).
6. *Antibiotic susceptibility.* The antibiotic susceptibility of each bacterial isolate was determined by comparing the MIC of each with the National Committee of Clinical Laboratory standards for each fluoroquinolone antibiotic.[5] The standards are based on the safe achievable concentrations of antibiotic in the serum. There are no standards for topical ocular therapy that represent the concentrations of antibiotics in the ocular tissue. For an isolate to be susceptible to ciprofloxacin, the MIC was to be ≤1 μg/ml. For an isolate to be susceptible to ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin, the MIC was to be ≤2 μg/ml. For the cumulative antibiotic susceptibility of each bacterial group, the number of susceptible isolates was divided by the number of susceptible and nonsusceptible isolates.
7. *Potency.* The antibiotic with the lowest MIC values is deemed the most potent. In comparing antibiotics, an antibiotic with a low MIC requires less antibiotic to inhibit the same amount of bacteria than an antibiotic with a higher MIC, which requires more antibiotic.

Statistical Analysis

Susceptibility patterns were analyzed statistically with the Monte-Carlo randomization test (True Epistat, Richardson, TX) using contingency tables (2×5) of all 5 antibiotics for each bacterial group. This analysis was more appropriate because the chi-square test is less accurate when 25% of the cells in the contingency tables contain values that are <5. A P value of ≤0.05 was set as significant. (Note: the Fisher exact test also could have been used for analyzing the susceptibility pattern data with similar results.)

Minimum inhibitory concentration values are discrete data that must be analyzed with nonparametric statistics. This is mandated because MICs, by convention, are preset to fixed known antibiotic concentrations. If MICs were truly unknown random values, then parametric statistical analysis would be appropriate if a normal distribution was assumed. The MICs of the 5 fluoroquinolones against each bacterial group were compared using the Kruskal–Wallis test (True Epistat). The analysis ranked all the MICs from lowest to highest for each antibiotic and compared the antibiotics by analysis of variance (ANOVA) of the ranks (not the actual MICs) using Duncan multiple comparisons at $P = 0.05$ significance. The antibiotic with the lowest mean rank was determined to have the lowest MICs and, therefore, was depicted as the most potent.

Results

Table 1 presents the descriptive statistics of the MICs for bacterial conjunctivitis isolates to 5 fluoroquinolone antibiotics. The medians, MIC_{50}s, MIC_{90}s, and modes for moxifloxacin and gatifloxacin were lower than those for levofloxacin, ciprofloxacin, and ofloxacin for all gram-positive bacteria. The same parameters were lower for moxifloxacin than for gatifloxacin for all gram-positive bacteria.

For *Haemophilus* species, the medians and MIC_{50}s for gatifloxacin, ciprofloxacin, and levofloxain were lower than those for moxifloxacin and ofloxacin. For MIC_{90}s, moxifloxacin was the lowest, followed by gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin. For modes, gatifloxacin was the lowest, followed by ciprofloxacin, levofloxacin, moxifloxacin, and ofloxacin.

Table 2 presents the statistical comparison of in vitro susceptibility of the bacterial conjunctivitis isolates to 5 fluoroquinolones and the relative potency of the antibiotics to each other. The *S. aureus*–fluoroquinolone-resistant group was more susceptible to moxifloxacin than the other 4 fluoroquinolones. Susceptibilities were equivalent for all 5 fluoroquinolones for the other 3 bacterial groups. Moxifloxacin and gatifloxacin were the most potent fluoroquinolones for gram-positive bacteria, and gatifloxacin and ciprofloxacin were the most potent fluoroquinolones for *Haemophilus* species. Levofloxacin was statistically just as potent as moxifloxacin and gatifloxacin for the *S. aureus*–fluoroquinolone-resistant group.

Discussion

The dilemma facing many busy ophthalmologists is how best to serve their patients by correctly evaluating the conflicting data of rival pharmaceutical companies for the lucrative topical antibiotic market for surgical prophylaxis and therapy. Most ophthalmologists have limited experience in microbiological methodology and data analysis and, therefore, must rely on the literature supplied by the phar-

Table 1. Descriptive Statistics of Minimum Inhibitory Concentrations (MICs) (μg/ml) for Bacterial Conjunctivitis Isolates to 5 Fluoroquinolone Antibiotics

	n	Median	MIC_{50}	MIC_{90}	Mode*	Minimum MIC–Maximum MIC Values	Susceptibility
Staphylococcus aureus–fluoroquinolone susceptible							
Moxifloxacin	20	0.047	0.064	0.094	0.064	0.047–0.094	100%
Gatifloxacin	20	0.125	0.125	0.125	0.125	0.094–0.19	100%
Levofloxacin	20	0.25	0.25	0.25	0.25	0.094–0.38	100%
Ciprofloxacin	20	0.38	0.38	0.5	0.38	0.19–0.75	100%
Ofloxacin	20	0.5	0.5	0.75	0.5	0.38–0.75	100%
S. aureus–fluoroquinolone resistant							
Moxifloxacin	20	2.0	2.0	6.0	1.5	1.5–12.0	40%
Gatifloxacin	20	6.0	6.0	64	6.0	3.0–64	0%
Levofloxacin	20	>32	>32	>32	>32	8–>32	0%
Ciprofloxacin	20	>32	>32	>32	>32	>32–>32	0%
Ofloxacin	20	>32	>32	>32	>32	>32–>32	0%
Streptococcus pneumoniae							
Moxifloxacin	20	0.064	0.064	0.094	0.094	0.023–0.19	100%
Gatifloxacin	20	0.125	0.125	0.19	0.125	0.064–0.38	100%
Levofloxacin	20	0.38	0.38	0.75	0.38	0.19–1.0	100%
Ciprofloxacin	20	0.38	0.38	0.75	0.38	0.19–1.5	95%
Ofloxacin	20	1.0	1.0	2.0	0.75, 1.0	0.75–3.0	90%
Haemophilus species							
Moxifloxacin	20	0.047	0.047	0.125	0.047	0.023–0.19	100%
Gatifloxacin	20	0.016	0.016	0.25	0.012	0.012–0.38	100%
Levofloxacin	20	0.023	0.023	0.75	0.023	0.016–1.5	100%
Ciprofloxacin	20	0.016	0.016	1.0	0.016	0.012–1.5	100%
Ofloxacin	20	0.056	0.047	2.0	0.047	0.047–4.0	90%

MIC_{50} = antibiotic concentration that would inhibit the growth of 50% of the tested bacterial isolates; MIC_{90} = antibiotic concentration that would inhibit the growth of 90% of the tested bacterial isolates.
*The value among all observations that occurs at the greatest frequency.

Table 2. Statistical Comparison of In Vitro Susceptibility and Potency of Bacterial Conjunctivitis Isolates to 5 Fluoroquinolones*

	Susceptibility	Potency
Staphyllococcus aureus–fluoroquinolone susceptible	M=G=L=C=O	M=G>L=C=O
S. aureus–fluoroquinolone resistant	M>G=L=C=O	M=G=L>C=O
Streptococcus pneumoniae	M=G=L=C=O	M=G>C=L>=O
Haemophilus species	M=G=L=C=O	G=C>L=M=O

C = ciprofloxacin; G = gatifloxacin; L = levofloxacin; M = moxifloxacin; O = ofloxacin.
The antibiotic susceptibility of each bacterial group was determined by comparing the minimum inhibitory concentrations (MICs) to the National Committee of Clinical Laboratory Standards for each fluoroquinolone antibiotic (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 4th ed. Villanova, PA: National Committee for Clinical Laboratory Standards; 2000. Approved standard M7-A5). For an isolate to be susceptible to ciprofloxacin, the MIC was to be ≤ 1 μg/ml. For an isolate to be susceptible to O, L, G, and M, the MIC was to be ≤ 2 μg/ml. Susceptibility patterns were statistically analyzed with the Monte-Carlo randomization test using contingency tables (2×5) of all 5 antibiotics for each bacterial group. The MICs of the 5 fluoroquinolones against each bacterial group were compared using the Kruskal–Wallis test. The analysis ranked all the MICs from lowest to highest for each antibiotic and compared the antibiotics by analysis of variance of the ranks (not the actual MICs) using the Duncan multiple comparisons at $P = 0.05$ significance. The antibiotic with the lowest mean rank was determined to have the lowest MICs and, therefore, was depicted as the most potent.
*Susceptible or resistant as determined by disk diffusion testing.

maceutical industry for information to guide their choice of topical antibiotic therapy. However, it is necessary to understand the descriptive statistics of in vitro data, assuming there is an in vivo correlation, to determine any apparent advantage of one antibiotic over another. We believe that the ophthalmology community, the pharmaceutical industry, and the ophthalmic literature do not reflect a consensus opinion about the role of descriptive statistics in this process and the most appropriate analysis of antibiotic susceptibility data. The goal of the current study was to consider this situation and offer our suggestions as to how best to approach this important issue.

An appropriate data analysis begins with the recognition of the data type, either discrete or continuous. Minimum inhibitory concentration data are discrete because the values are predetermined to a set of values (32, 24, 16, 12, . . . 0.002). Continuous data are random and not confined to a set of predetermined values (i.e., colony counts). The common descriptive statistics for discrete data are the median and mode, whereas the common descriptive statistics for continuous (parametric) data are the mean and standard deviation. The MIC_{50} and MIC_{90} values can be calculated for both discrete and continuous data because these values are based on rank. Minimum inhibitory concentration data should be analyzed with nonparametric statistical analysis. The Mann–Whitney test compares 2 groups, whereas the Kruskal–Wallis test is used for multiple comparisons. The Kruskal–Wallis is based on the rank of the values and not the actual value, and is less sensitive to large outliers that can skew the data. It is not unusual for MIC data to be handled as parametric (continuous) data in the literature. We suspect that most authors and reviewers are unaware that this is an incorrect analysis, and readers should be wary of the interpretations that are made in such articles.

The descriptive statistics for MICs of gram-positive bacteria to the fluoroquinolones followed a consistent trend, whereas median, $MIC_{50,}$ MIC_{90}, and mode demonstrated the lowest values, in all instances, for moxifloxacin, gatifloxacin, levofloxacin, ciprofloxacin, and ofloxacin. Susceptibility to the serum antibiotic standards did not distinguish among the antibiotics, except for *S. aureus*–fluoroquinolone-resistant that were more susceptible to moxifloxacin. The potency data indicated that the MICs were lowest to moxifloxacin and gatifloxacin, compared with levofloxacin, ciprofloxacin, and ofloxacin.

The descriptive statistics for *Haemophilus* species did not describe any consistent trends. Table 3 (available at http://aaojournal.org) presents the raw MIC data and descriptive statistics of these MIC values for *Haemophilus* species. The medians and MIC_{50}s for gatifloxacin and ciprofloxacin have the lowest values, whereas the MIC_{90} for moxifloxacin is the lowest compared with the other fluoroquinolones. Antibiotic susceptibility, as for gram-positive bacteria, did not distinguish among the fluoroquinolones. The potency data supported the median and MIC_{50} values by indicating that the MICs for gatifloxacin and ciprofloxacin were the lowest compared with the other 3 fluoroquinolones.

An inappropriate parametric analysis (ANOVA) (Minitab, State College, PA) of the data was deliberately included in Table 3 (available at http://aaojournal.org) for important illustrative purposes. Treating the MIC data as continuous, moxifloxacin had the lowest mean value. Perusing the data, the range for moxifloxacin was smaller than ranges for the other fluoroquinolones. This resulted in the data being more sensitive to larger values that increased the mean for some of the fluoroquinolones. An ANOVA, based on the mean of the data, demonstrated a confusing analysis, with little distinction between the antibiotics. This example demonstrates how the application of an inappropriate statistical analysis produces misleading results.

As ocular clinical efficacy is based on several factors, the choice of an appropriate topical antibiotic should be based upon low MICs, along with other equally important variables (e.g., costs, antibiotic tissue concentration, concentration of antibiotic used, dosing regimen, solubility, toxicity, allergenicity, patient compliance). These latter factors are generally established before reaching the market, and some of them (i.e., toxicity, allergenicity, and compliance) undergo a continuous evaluation by the ophthalmologist on a daily personal level during clinical practice.

We believe that in vitro efficacy should be analyzed with a set of descriptive statistics along with a nonparametric analysis of the data. No single descriptive statistic or parameter should be relied upon in all instances to determine the superiority of one antibiotic over another. When reviewing in vitro data, the clinician should remember that proven clinical efficacy remains the ultimate measure of any topical antibiotic. In vitro susceptibility interpretation for treating

eye infections may not correlate with the clinical reality of treating eye infections, because effective therapy is based on systemic parameters that may not represent high antibiotic tissue levels achieved by topical administration. Presently, there are no predetermined ocular standards for susceptibility or resistance. If topical susceptibility standards did exist, these parameters would probably be higher than those for systemic therapy because of the high concentrations of antibiotics that would be achieved in the ocular tissue. It would be nearly impossible to derive susceptibility standards for ocular topical antibiotics. These topical standards would need to be established using a clinical trial where MIC values would correlate with clinical cure. The well-planned trial would need to treat all bacterial infections regardless of whether antibiotic resistance was indicated using conventional in vitro susceptibility testing based on the serum standards. These studies would never be approved by an institutional review board due to the potential danger of knowingly treating a patient's ocular infection with an antibiotic with demonstrated in vitro resistance.

The in vitro analysis of the 5 fluoroquinolones against bacterial conjunctivitis isolates supports our previous endophthalmitis[2] and keratitis[3] findings that the fourth-generation fluoroquinolones have some potency advantages over the second-generation fluoroquinolones for covering gram-positive bacteria. The new data also correlate with the previous studies that there is no distinct advantage of the fourth-generation fluoroquinolones over the second-generation fluoroquinolones for gram-negative bacteria.

In conclusion, we recommend that routine bacterial cultures be obtained in cases of severe conjunctivitis and in those patients in whom *S. aureus* is suspected and antibiotic resistance may exist. Antibiotic susceptibility studies should be undertaken for alternative antibiotics to treat fluoroquinolone-resistant bacteria.

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Table 3. Raw Minimum Inhibitory Concentration (MIC) Data and Descriptive Statistics of Ocular *Haemophilus* Isolates Tested against 5 Fluoroquinolone Antibiotics

Rank	Ciprofloxacin	Ofloxacin	Levofloxacin	Gatifloxacin	Moxifloxacin
1	0.012	0.047	0.016	0.012	0.023
2	0.012	0.047	0.016	0.012	0.023
3	0.016	0.047	0.016	0.012	0.023
4	0.016	0.047	0.016	0.012	0.023
5	0.016	0.047	0.016	0.012	0.023
6	0.016	0.047	0.023	0.012	0.032
7	0.016	0.047	0.023	0.012	0.032
8	0.016	0.047	0.023	0.016	0.032
9	0.016	0.047	0.023	0.016	0.047
10—MIC_{50}	**0.016**	**0.047**	**0.023**	**0.016**	**0.047**
11	0.016	0.064	0.023	0.016	0.047
12	0.023	0.064	0.023	0.016	0.047
13	0.023	0.064	0.023	0.023	0.047
14	0.023	0.064	0.023	0.023	0.047
15	0.023	0.094	0.032	0.023	0.064
16	0.190	0.500	0.190	0.125	0.094
17	0.380	0.750	0.250	0.190	0.125
18—MIC_{90}	**1.000**	**2.000**	**0.750**	**0.250**	**0.125**
19	1.000	3.000	0.750	0.250	0.125
20	1.500	4.000	1.500	0.380	0.190
Median	**0.016**	0.056	0.023	**0.016**	0.047
Mean	0.216	0.553	0.188	0.071	**0.061**

Bolded values denote the antibiotic concentration that would inhibit the growth of 50% of the tested bacterial isolates (MIC_{50}) and MIC_{90} values, and the lowest MIC values for median and mean. The correct statistical analysis for comparing the antibiotics MICs should be the Kruskal–Wallis (nonparametric) test. The data determined that the median MICs from the lowest to highest were gatifloxacin = ciprofloxacin < levofloxacin = moxifloxacin = ofloxacin. Incorrectly comparing the antibiotics MICs with an analysis of variance (parametric) test would result in a confusing analysis with the following results: ofloxacin = ciprofloxacin, gatifloxacin = levofloxacin = moxifloxacin > ofloxacin, ciprofloxacin = gatifloxacin = levofloxacin = moxifloxacin.