Title

Emerging Moxifloxacin Resistance in Pseudomonas aeruginosa Keratitis Isolates in South India

Journal

Ophthalmic Epidemiology, 20(3)

ISSN

0928-6586

Authors

Oldenburg, Catherine E
Lalitha, Prajna
Srinivasan, Muthiah
et al.

Publication Date

2013-06-01

DOI

10.3109/09286586.2013.790978

Peer reviewed

Published in final edited form as:
Ophthalmic Epidemiol. 2013 June ; 20(3): 155–158. doi:10.3109/09286586.2013.790978.

Emerging moxifloxacin resistance in *Pseudomonas aeruginosa* keratitis isolates in South India

Catherine E. Oldenburg[1], **Prajna Lalitha**[2], **Muthiah Srinivasan**[2], **Revathi Rajaraman**[3], **Meenakshi Ravindran**[4], **Jeena Mascarenhas**[2], **Durga S. Borkar**[1], **Kathryn J Ray**[1], **Michael E. Zegans**[5], **Stephen D. McLeod**[1,6], **Travis C. Porco**[1,7], **Thomas M. Lietman**[1,6,7], and **Nisha R. Acharya**[1,6]

[1]Francis I. Proctor Foundation, University of California, San Francisco

[2]Aravind Eye Care System, Madurai, Tamil Nadu, India

[3]Aravind Eye Care System, Coimbatore, Tamil Nadu, India

[4]Aravind Eye Care System, Tirunelveli, Tamil Nadui, India

[5]Dartmouth Medical School, Hanover, NH

[6]Department of Ophthalmology, University of California, San Francisco

[7]Department of Epidemiology and Biostatistics, University of California, San Francisco

Abstract

Purpose—To describe temporal trends in *Pseudomonas aeruginosa* resistance to moxifloxacin in keratitis isolates from South India.

Methods—The Steroids for Corneal Ulcers Trial (SCUT) was a randomized, double-masked, placebo-controlled trial assessing outcomes in patients with culture positive bacterial corneal ulcers randomized to receive prednisolone phosphate or placebo. All patients received moxifloxacin, and susceptibility to moxifloxacin was measured at baseline using Etest. We investigated trends in moxifloxacin susceptibility of *P. aeruginosa* during 2007, 2008, and 2009 isolated in SCUT in South India.

Results—There were 89 *P. aeruginosa* isolates during 2007, 2008, and 2009 in SCUT that were eligible for this study. There was an increase in the proportion of resistant isolates from 19% in 2007 to 52% in 2009 (P=0.02, Chi-square test for trend). Logistic regression showed that there was a 2-fold increase in odds of resistance per one year increase during the study period (OR 2.16, 95% CI 1.09 to 4.26, P=0.027).

Conclusions—We found a sharp increase in the proportion of isolates that were resistant to moxifloxacin from 2007 to 2009. Further work needs to be done to characterize the nature of this increase.

Corresponding Author: Nisha R. Acharya, MD, MS, 513 Parnassus Ave, Box 0412, San Francisco, CA 94143, Phone: 415-476-8131, Fax: 415-476-0527, nisha.acharya@ucsf.edu.

Conflicts of Interest: None of the authors have any conflicts of interest to declare.

Declaration of Interest
None of the authors have any conflicts of interest to declare.

Introduction

Bacterial keratitis is a sight-threatening infection of the cornea that can lead to poor visual outcomes in the absence of appropriate therapy. While the true incidence of bacterial keratitis is unknown, there are thought to be at least 1.5-2 million cases of infectious keratitis annually.[1] While there are geographic variations in the distribution of causative organisms, a large proportion of infectious keratitis is due to bacteria, with *Pseudomonas aeruginosa* being one of the most common isolates.[2] Fourth-generation fluoroquinolones were introduced within the last decade. They are one of the newest classes of antibiotics used to treat ocular infections, and are thought to be particularly efficacious in the treatment of bacterial keratitis.[3]

Previous reports have documented an increase in second- and third-generation fluoroquinolone-resistant *P. aeruginosa* from ocular infections.[4, 5] Case reports have described *P. aeruginosa* resistance to the newer fourth-generation fluoroquinolones, such as moxifloxacin, in ocular isolates.[6] Here, we analyze moxifloxacin resistance trends in *P.aeruginosa* isolated from bacterial corneal ulcers in South India as part of a randomized controlled trial over a three-year period.

Methods

The Steroids for Corneal Ulcers Trial was a National Institutes of Health-sponsored (www.clinicaltrials.gov NCT# 00324168) randomized placebo-controlled double-masked clinical trial comparing outcomes in patients randomized to receive 1% prednisolone phosphate or placebo as adjunctive therapy in the treatment of bacterial keratitis. All patients received topical 0.5% moxifloxacin (Vigamox®, Alcon, Fort Worth, TX). Specific methods for the trial have been described previously.[7] In brief, patients were enrolled from September 2006 to February 2010 at the Aravind Eye Care System (Madurai, Tirunelveli, Coimbatore), the Francis I. Proctor Foundation, University of California San Francisco, and Dartmouth-Hitchcock Medical Center. All patients with a culture-positive bacterial corneal ulcer seen during the enrollment period were considered for the trial. Exclusion criteria included evidence of fungus, acanthamoeba, or herpetic keratitis, age less than 16 years at the time of the presenting corneal ulcer, impending corneal perforation, and vision worse than 20/200 in their fellow eye. Ethical approval was obtained from the University of California Committee on Human Research, the Aravind Eye Hospital Institutional Review Board, and the Dartmouth Committee for the Protection of Human Subjects.

At presentation, corneal scraping was performed. Two scrapings were smeared directly on two separate slides for Gram stain and KOH wet mount. Three further scrapings were inoculated on sheep's blood agar, chocolate agar, and potato dextrose agar or Sabouraud's agar for bacterial and fungal cultures. The criterion for positivity for bacteria was growth of the organism on one solid medium at the site of inoculation. For *Staphylococcus epidermis* and diphtheroids, cultures were considered positive only if moderate growth was seen on at least two solid media or on one solid medium plus identification of the organism on the corneal smear (in order to rule out possible contamination of normal flora from the lids).[8] All patients were checked for fungal elements on smear and culture. Antibiotic susceptibility testing was performed using the Etest method (AB BIODISK, Solna, Sweden). Quality control was performed according to the Clinical and Laboratory Standards Institute (CLSI) performance standards (CLSI M100-S21).

Since there are currently no standards published by the CLSI for *P. aeruginosa* sensitivity to moxifloxacin, we defined "resistant" as a minimum inhibitory concentration (MIC) greater than or equal to 4 μg/mL, the threshold for resistance to ciprofloxacin. This cutoff has also

been published by the British Society for Antimicrobial Therapy (BSAC) for *P. aeruginosa* resistance to moxifloxacin.[9] The number of resistant isolates per year, using this definition, was tabulated. A Chi-square test for trend in proportions was used to assess for a trend in the proportion of resistant isolates for each complete year for which there was enrollment in the trial: 2007, 2008, and 2009. The analysis was restricted to isolates from South India, and isolates with more than one causative organism were excluded. The $\log_2$-tranformed MIC was used for statistical analyses. Univariate logistic regression was used to assess the odds per year of having a resistant isolate and odds per year of having already received topical fluoroquinolone treatment prior to enrollment. Multivariable logistic regression was used to assess possible confounding of the temporal trend of resistance by prior treatment with a fluoroquinolone. While we assumed that the samples were independent in our primary analyses, there could be temporal correlation because the lab personnel's technique could change over time (autocorrelation). As a sensitivity analysis, bootstrap resampling of temporally adjacent pairs of results was performed to account for autocorrelation. Statistical analyses were performed in Stata 10.0 (StataCorp, College Station, TX).

Results

There were 111 *P. aeruginosa* isolates identified in the trial. Of these, 106 were from South India. MIC results were not available for two isolates, and one isolate was excluded due to mixed infection. Of these, 14 were not enrolled during the analysis periods (2007-2009), and were therefore excluded. Therefore, 89 isolates were analyzed in this study. The MIC_{50} of *P. aeruginosa* against moxifloxacin was 3.0 μg/mL (interquartile range 2.0 to 4.0). Of the 89 isolates, 31 (34%) were identified as having an MIC greater than 4 μg/mL. The proportion of resistant organisms rose from 19% (95% CI 5.4% to 41.9%) in 2007 to 52% (95% CI 29.8% to 74.3%) in 2009 (P=0.024, Table 1). In an unadjusted univariate logistic regression model, each 1-year increase was associated with a two-fold increase in odds of having a resistant isolate (Odds Ratio (OR) 2.16, 95% CI 1.09 to 4.26, P=0.027). Adjusting for autocorrelation as a sensitivity analysis did not change these results (OR 2.2, 95% CI 1.02 to 4.6, P=0.04). The odds of pre-treatment with fluoroquinolone increased 2-fold per 1-year increase, however this association was not statistically significant (OR 1.95, 95% CI 0.85 to 4.46, P=0.11).. In a multivariate logistic regression model including pre-treatment with a fluoroquinolone as a covariate, adjusting for autocorrelation, each 1-year increase was associated with a twofold increase in odds of having a resistant isolate, similar to the model that did not adjust for pre-treatment with a fluoroquinolone (OR 2.0, 95% CI 0.92 to 4.33, P=0.07).

Discussion

Fluoroquinolones are commonly used as empiric therapy for ocular infections such as bacterial keratitis.[10] Since they target both DNA gyrase and topoisomerase IV, two mutations rather than one are required to result in resistance to fourth-generation fluoroquinolones, thus effectively lowering their mutation prevention concentration.[11] Consequently, spontaneous resistance may be less likely to occur.[3] At the time of the introduction of fourth-generation fluoroquinolones in ophthalmology, Mather et al suggested that this requirement of two mutations would substantially slow the development of resistance among pathogens.[3]

There are few studies documenting temporal trends in fourth-generation fluoroquinolone resistance in ocular isolates. In this study, the MIC_{50} of moxifloxacin against *P. aeruginosa* was considerably higher than previous reports in the literature.[12] In addition, we found a dramatic increase in the proportion of moxifloxacin-resistant organisms isolated over a three-year period. It is not clear what is responsible for this increase in moxifloxacin

resistance. Prior studies have shown that pre-treatment with a fluoroquinolone is a risk factor for fluoroquinolone resistance in ocular bacterial isolates.[13, 14] In this study, the odds of pre-treatment with fluoroquinolone increased by approximately 2-fold during the study period, suggesting that there may be an increasing trend of fluoroquinolone use, although this was not statistically significant. However, increased resistance with time did not change appreciably when pre-treatment with a fluoroquinolone was taken into account. The increase in resistance could be explained by an increase in community oral fluoroquinolone use, or an increase in topical fluoroquinolone use resulting in *de novo* resistance. A community survey of fluoroquinolone use would be useful to characterize consumption of these antibiotics in this region to try to explain the reason for such an increase in resistance. The sample size in this study was small, and we only had data for 3 complete years. A larger study that covered more years would more definitively characterize temporal trends in *P. aeruginosa* resistance to moxifloxacin, and would allow for adjustment for autocorrelation by year. Resistance to newer antibiotics should continue to be monitored.

This report demonstrates the need for vigilance with fourth-generation fluoroquinolones, and awareness of the potential for resistant *P. aeruginosa* keratitis isolates. The rise of resistance in these ocular isolates is worrisome given that these isolates are from serious infections, and a higher MIC to moxifloxacin has been shown to be associated with worse clinical outcomes in bacterial keratitis.[15] There is a need for further analysis of fourth-generation fluoroquinolone-resistant strains of *P. aeruginosa*, to determine if particular strains or virulence factors are associated with increasing resistance patterns, and to characterize the cause of this increase in resistance.

Acknowledgments

We are grateful for the invaluable guidance and advice of the SCUT data and safety monitoring board: Marian Fisher, Ph.D. (chair), Anthony Aldave, M.D., Donald Everett, M.A., Jacqueline Glover, Ph.D., K. Ananda Kannan, M.D., Steven Kymes, Ph.D., G.V.S. Murthy, M.D., and Ivan Schwab, M.D.

Funding

Funding for the Steroids for Corneal Ulcers Trial was from the National Eye Institute, U10 EY015114. Ms. Oldenburg is supported by a National Institute of Allergy and Infectious Disease grant, T32AI007535. Dr. Acharya is supported by a National Eye Institute K23EY017897 grant and a Research to Prevent Blindness Award. The Department of Ophthalmology at UCSF is supported by a core grant from the National Eye Institute, EY02162, an unrestricted grant from Research to Prevent Blindness, New York, NY, and That Man May See, Inc, San Francisco, CA. Alcon provided moxifloxacin (Vigamox) for the trial. The sponsors did not have a role in the design and conduct of the study; collection, management, analysis and interpretation of the data; and preparation, review or approval of the manuscript.

Financial Support: Funding for the Steroids for Corneal Ulcers Trial was from the National Eye Institute, U10 EY015114. Ms. Oldenburg is supported by a National Institute of Allergy and Infectious Disease grant, T32AI007535. Dr. Acharya is supported by a National Eye Institute K23EY017897 grant and a Research to Prevent Blindness Award. The Department of Ophthalmology at UCSF is supported by a core grant from the National Eye Institute, EY02162, an unrestricted grant from Research to Prevent Blindness, New York, NY, and That Man May See, Inc, San Francisco, CA. Alcon provided moxifloxacin (Vigamox) for the trial. The sponsors did not have a role in the design and conduct of the study; collection, management, analysis and interpretation of the data; and preparation, review or approval of the manuscript.

References

1. Whitcher J, et al. Corneal blindness: a global perspective. Bulletin of the World Health Organization. 2001; 79:214–221. [PubMed: 11285665]
2. Shah A, et al. Geographic variations in microbial keratitis: an analysis of the peer-reviewed literature. British Journal of Ophthalmology. 2011; 95:762–767. [PubMed: 21478201]
3. Mather R, et al. Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. American Journal of Ophthalmology. 2002; 133:463–466. [PubMed: 11931779]

4. Chaudhary N, et al. Emerging ciprofloxacin-resistant *Pseudomonas aeruginosa*. American Journal of Ophthalmology. 1999; 128:509–510. [PubMed: 10577596]
5. Kunimoto D, et al. In vitro susceptibility of bacterial keratitis pathogens to cirpofloxacin: emerging resistance. Ophthalmology. 1999; 106:80–85. [PubMed: 9917785]
6. Moshirfar M, et al. Fourth-generation fluoroquinolone-resistant bacterial keratitis after refractive surgery. Journal of Cataract and Refractive Surgery. 2006; 32:515–518. [PubMed: 16631067]
7. Srinivasan M, et al. The steroids for corneal ulcers trial: study design and baseline characteristics. Archives of Ophthalmology. 2012; 130:143–150. [PubMed: 21987582]
8. Wilhelmus, K., et al. Laboratory diagnosis of ocular infection. In: Specter, S., editor. Cumitech: cumulative techniques and procedures in clinical microbiology. American Society for Microbiology Press; 1994.
9. Andrews J. for the BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 8). Journal of Antimicrobial Therapy. 2009; 64:454–489.
10. Hsu H, et al. Community opinions in the management of corneal ulcers and ophthalmic antibiotics: a survey of 4 states. Eye & Contact Lens. 2010; 36:195–200. [PubMed: 20626115]
11. Drlica K, Zhao X. Mutant selection window hypothesis updated. Clinical Infectious Diseases. 2007; 44:681–688. [PubMed: 17278059]
12. Epstein S, Bottone D, Asbell P. Susceptibility testing of clinical isolates of *Pseudomonas aeruginosa* to levofloxacin, moxifloxacin, and gatifloxacin as a guide to treating *Pseudomonas* ocular infections. Eye & Contact Lens. 2006; 32:240–244. [PubMed: 16974157]
13. Fintelmann R, et al. Topical fluoroquinolone use as a risk factor for in vitro resistance in ocular cultures. Archives of Ophthalmology. 2011; 129:399–402. [PubMed: 21482865]
14. Milder E, et al. Changes in antibiotic resistance patterns of conjunctival flora due to repeated use of topical antibiotics after intravitreal injection. Ophthalmology. 2012; 119:1420–1424. [PubMed: 22420958]
15. Lalitha P, et al. Relationship of in vitro susceptibility to moxifloxacin and in vivo clinical outcome in bacterial keratitis. Clinical Infectious Diseases. 2012; 54:1381–1387. [PubMed: 22447793]

Table 1

Proportion of moxifloxacin-resistant isolates of *Pseudomonas aeruginosa*, 2007-2009

Year	Moxifloxacin-resistant isolates (N, %, 95% CI *)	*P*
2007 (N=21)	4 (19%, 95% CI 5.4 to 41.9%)	0.024 **
2008 (N=47)	16 (34%. 95% CI 20.9 to 49.3%)	
2009 (N=21)	11 (52%, 95% CI 29.8 to 74.3%)	
Total (N=89)	31 (35%, 95% CI 25.0 to 45.7%)	

* Exact binomial 95% confidence interval

** Chi-square test for trend in proportions