Challenges and Progress in the Treatment and Prevention of Ocular Infection

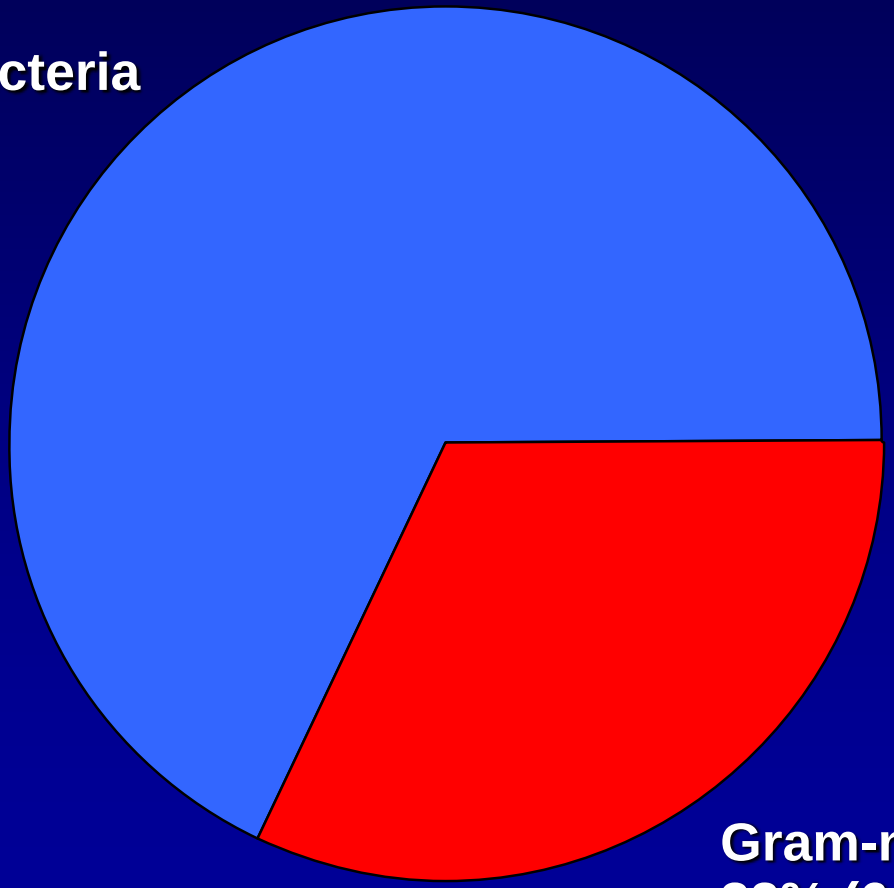
Introduction

- Challenges
  - Pathogens and Rates of Infection
  - Increasing Resistance
  - Effects of Wound Architecture
  - Ability to Define/Measure Properties of Ideal Anti-infectives
  - Need for 4th-Generation Fluoroquinolones
- Progress
  - Introduction of the 4th-Generation of Fluoroquinolones
  - Efficacy of Gatifloxacin, a 4th-Generation Fluoroquinolone
  - Gatifloxacin for the Treatment of Atypical Pathogens Post-LASIK
  - Gatifloxacin Pharmacokinetic, Toxicity, and Safety Profile

Pathogens and Rates of Postsurgical Infection

Distribution of Bacteria From Ocular Infections

(1993-2001) (N = 2002)

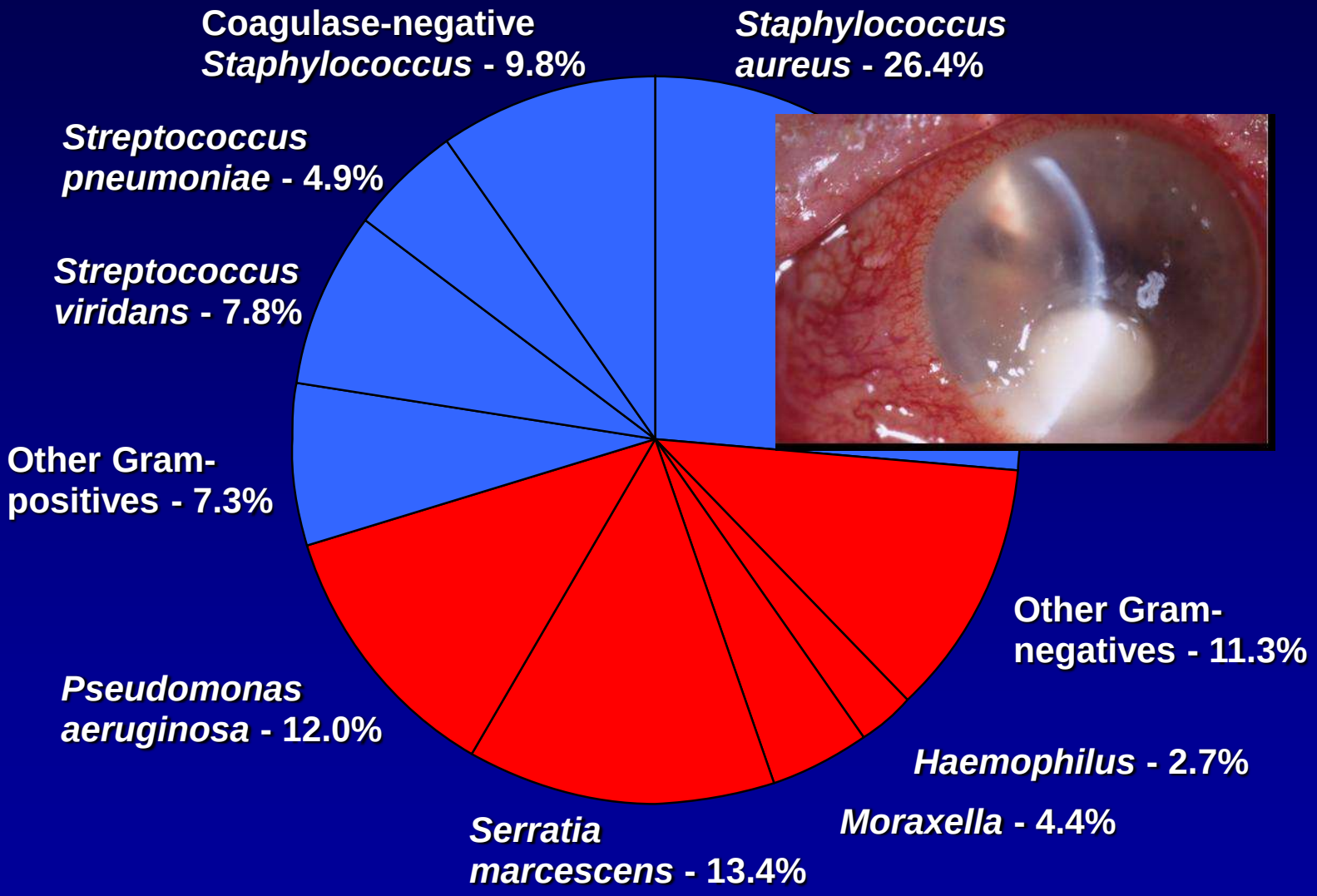


Gram-positive Bacteria
68% (1359/2002)

Gram-negative Bacteria
32% (643/2002)

Charles T. Campbell Eye Microbiology Lab. Unpublished Data.

Distribution of Bacterial Keratitis (1993-2001) (N = 841)

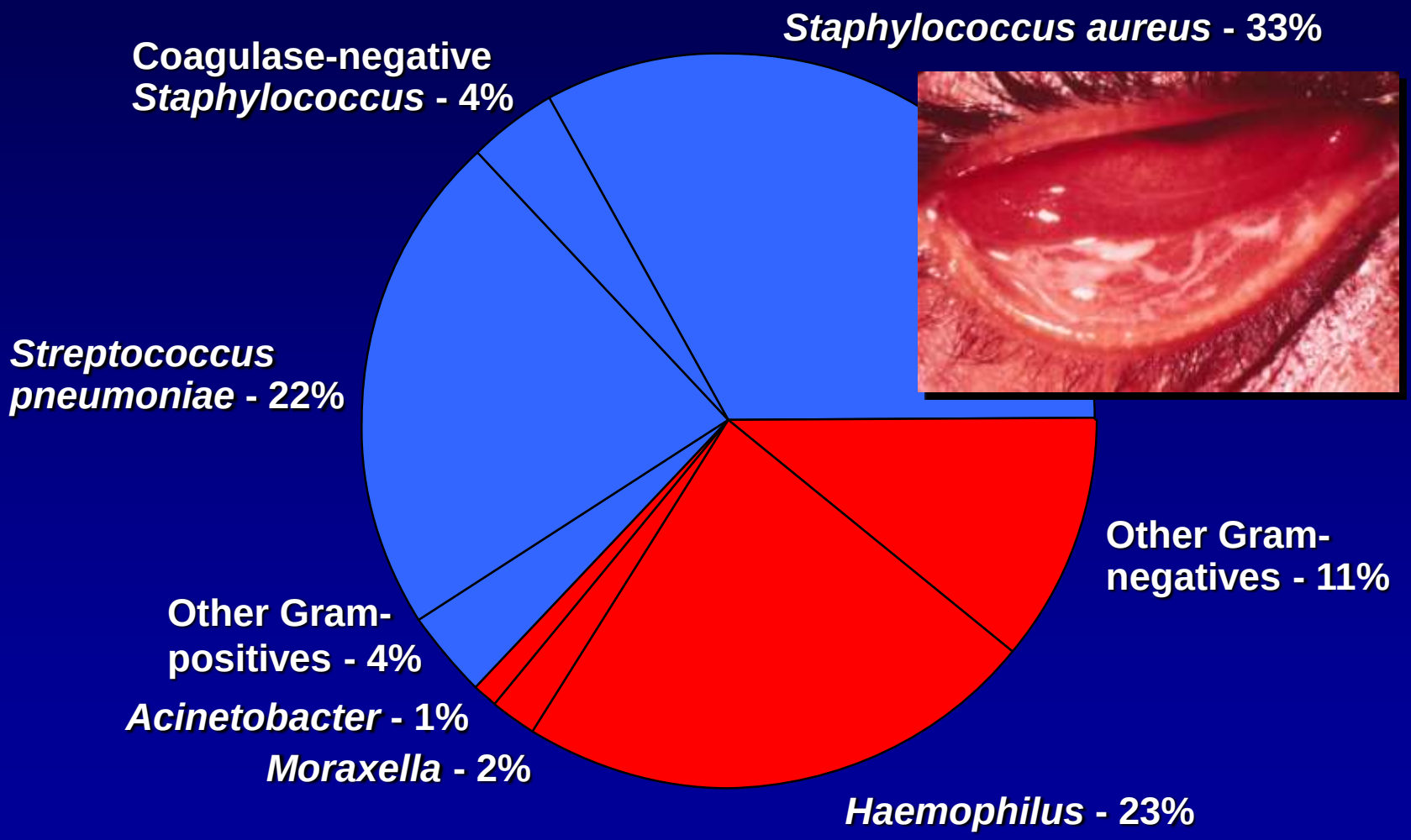


Kowalski, et al. Am J Ophthalmol. 2003. In press.

Distribution of Bacteria Isolated From Blepharitis (1993-2001) (N = 224)

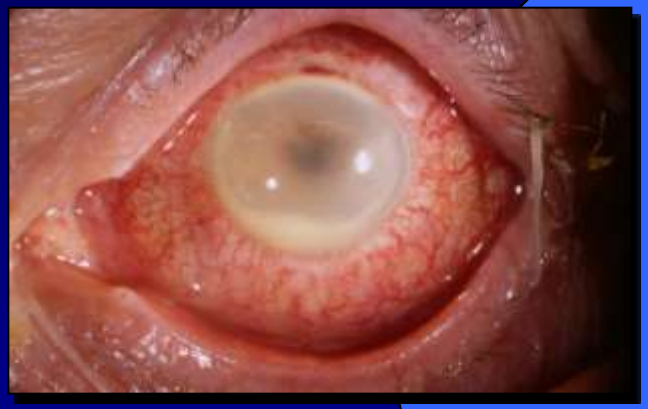
Charles T. Campbell Eye Microbiology Lab. Unpublished Data.

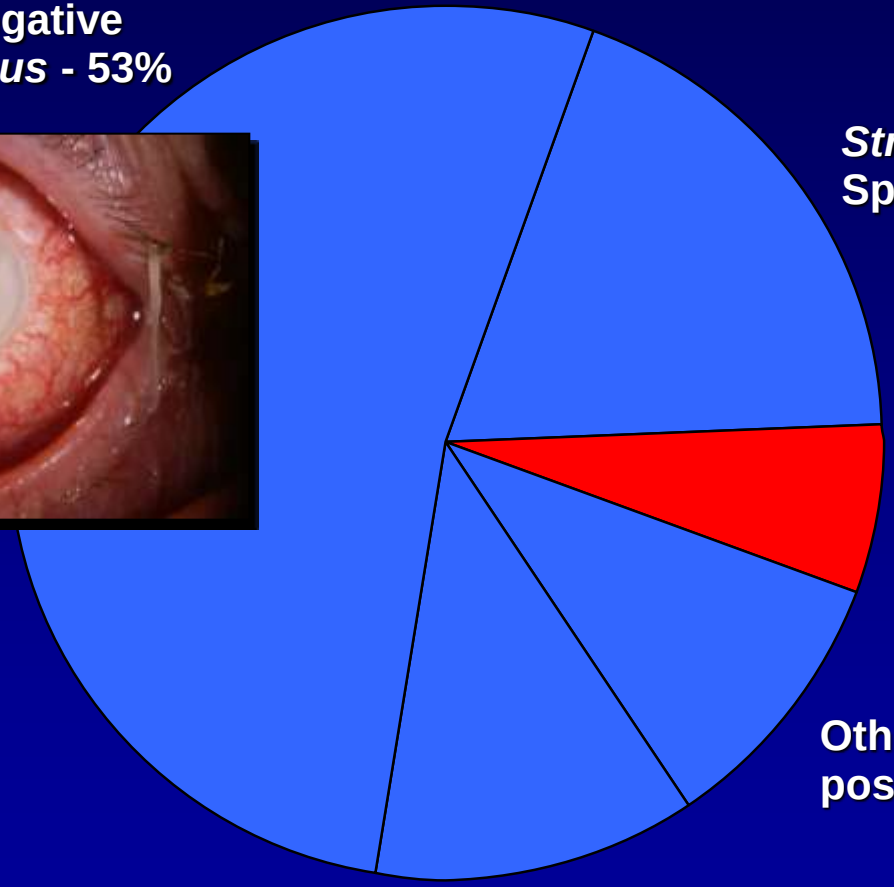
Distribution of Bacteria Isolated From Conjunctivitis (1993-2001) (N = 643)



Charles T. Campbell Eye Microbiology Lab. Unpublished Data.

Distribution of Bacteria Isolated From Endophthalmitis (1993-2001) (N = 294)





Charles T. Campbell Eye Microbiology Lab. Unpublished Data.

Clinical Implications of Endophthalmitis

- Devastating ocular complication associated with eye surgery
- Risk of significant vision loss largely determined by infecting pathogen
  - *P. aeruginosa*: 92% of infected eyes
  - All Gram-negatives sp.: 36%
  - Streptococcal sp.: 69%
  - *S. epidermidis*: 22%

Driebe WT, et al. Ophthalmology. 1986; Irvine WD, et al. Arch Ophthalmol. 1992; Mao LK, et al. Arch Ophthalmol. 1992.

Postoperative Infection Rates Appear to Be Rising

- Endophthalmitis rates are higher than past reports indicate:
  - Incidence rate once believed to be 1/1000
  - Recent data suggest an incidence as high as 1/400
- Infections following LASIK procedures are being seen more commonly
  - Up to 50% of infections seen following LVC are due to atypical bacteria

Jensen MK, Fiscella RG. Presented at: ARVO; 2002; Speaker MG, et al. Ophthalmology. 1991.

Endophthalmitis: Surgical Risk Factors

- Surgical complications
- Wound architecture
- Choice of prophylactic antimicrobial
  - Incidence of endophthalmitis significantly lower in patients treated with ofloxacin postoperatively than with ciprofloxacin (*P*<.004)
  - Decreased incidence with ofloxacin may be due to more favorable penetration, solubility, and pH

Mandelbaum S, Forster RK. In: Ocular Infection and Immunity. 1996;1298-1320;
Jensen MK, Fiscella RG. Presented at: ARVO; 2002.

Emerging Bacterial Resistance to Ophthalmic Anti-infective Therapy

What Is Resistance?

Tolerance vs resistance

- Tolerance
  - The ability of bacteria to survive in the presence of an anti-infective, but not to continue cell division
- Resistance
  - The ability of bacteria to both survive and to replicate in the presence of an anti-infective

What Causes Bacteria to Become Drug-resistant

- Vertical genetic exchange
  - Genetic information is passed down through generations as cells divide
- Horizontal genetic exchange
  - Movement of genetic material between bacteria other than by descent
  - Primary mechanism of evolution of antibiotic resistance
  - Sexual process that can take place through conjugation, transduction, or transformation

Transfer of Genetic Material

- Conjugation
  - Direct cell-to-cell contact of two bacterial cells and the subsequent transfer of DNA
  - Can occur between two unrelated bacterial species
  - Plays a large role in the spread of antibiotic resistance
- Transduction
  - A bacteriophage carries DNA from one species to another
- Transformation
  - Bacterial cells take up DNA from the surrounding environment and incorporate it into the host cell's chromosome

Bacterial Mechanisms of Antibiotic Resistance

- Altered target sites
  - Reduced binding of antibiotic
- Exclusion of the antibiotic from the cell
  - Altered permeation
    - Decreased entrance of antibiotic through smaller pores
  - Altered efflux
    - Increased antibiotic excretion from bacterium
- Drug inactivation
  - Alteration in antibiotic

Mechanisms of Antibiotic Resistance

- Expulsion of an antibiotic through an efflux pump
- Inactivation of an antibiotic through degradation and alteration of the drug by enzymatic activity

Factors Implicated in Growing Rates of Antibiotic Resistance

- Microbiological
  - Antibiotic misuse
- Environmental
  - Aging population
  - Social behavior
  - AIDS
  - International travel
- Technical
  - Increasing surgical intervention
  - Organ replacement
  - Life support systems

Blepharitis: A Model for Resistance

- Long/chronic treatment
- Subtherapeutic dosing
  - Lapses
  - Tapering
- Ophthalmologist "benign neglect"
- Erythromycin – 1st line for topical management
  - *S. aureus*: 37% isolates resistant in Campbell Lab Surveillance
  - Coagulase-negative *Staphylococcus*: 61% isolates resistant in Campbell Lab Surveillance

Mah F. Campbell Lab. Unpublished Data.

Resistance to Older Ocular Anti-infectives Is Increasing

- Jensen and associates tested 1291 ocular isolates from 12 laboratories in North and South America
- Tetracycline, gentamicin, erythromycin, and tobramycin were less effective against both Gram-negative and Gram-positive ocular isolates than were the currently available fluoroquinolones
  - For example: susceptibility of *Staph aureus* sp. ranged from 89% to 92% with the fluoroquinolones vs 57% to 78% with the older medications

Jensen HG, Felix C. Cornea. 1998.

Emerging Resistance to 3rd-Generation Fluoroquinolones

- Survey of 1053 isolates from 825 bacterial keratitis cases between 1993 to 1997
- Organisms tested for susceptibility to ciprofloxacin and ofloxacin
  - Ratio of Gram-positive to Gram-negative organisms changed from 81.8%:18.2% in 1993 to 51.4%:48.6% in 1997
  - Resistance of *Staph aureus* to ciprofloxacin increased from 5.8% in 1993 to 35% in 1997 ($P<.001$)
  - Resistance of *Staph aureus* to ofloxacin increased from 4.7% in 1993 to 35% in 1997 ($P<.001$)

Goldstein, et al. Ophthalmology. 1999.

Rapid Decline in Susceptibility of Endophthalmitis Isolates to 3rd-Generation Fluoroquinolones

- Two recent studies demonstrate a rapid decline in susceptibility
  - Ritterband et al reported a statistically significant decline in susceptibility to ciprofloxacin from the period 1991-1995 to 1996-2000 ($P<.004$)
  - Marangon et al reported that quinolone resistance among MSSA and MRSA isolates from keratitis and conjunctivitis cases increased significantly from 1990-1995 to 1996-2001
    - 166% increase in ciprofloxacin resistance
    - 100% increase in resistance for MSSA
    - 47% increase in resistance for MRSA

Marangon, et al. [abstract]. Invest Ophthalmol Vis Sci. 2002;
Ritterband, et al. [abstract]. Invest Ophthalmol Vis Sci. 2002.

Surgical Wound Architecture

Surgical Wounds as a Risk Factor for Endophthalmitis

- Postoperative endophthalmitis may occur after any surgical procedure during which the interior eye comes into contact with the external environment
- In most cases, the pathogen gains entrance to the interior of the eye at the time of the procedure
- Wound leaks that allow access to the interior of the eye after surgery, however, also increase the risk of endophthalmitis
  - Sutureless cataract surgery

Mandelbaum S, Forster RK. In: Ocular Infection and Immunity. 1996:1298-1320.

Scleral Tunnel Incisions

- Long scleral tunnel dissections can be sutureless
- Scleral incisions:
  - Reduce postoperative astigmatism and keratorefractive instability
  - May be associated with endophthalmitis because incisions may create a potential abscess cavity

Clear Corneal Incisions

- Smaller incisions allow for sutureless surgery
- Allow for rapid visual rehabilitation after phacoemulsification
- May be associated with an increased risk of postoperative infection
  - Risk may increase with transient reduction of intraocular pressure
    - May result in poor wound apposition and increase potential for fluid flow across the cornea and into the anterior chamber

Taban, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Clear Cornea vs Scleral Tunnel Incisions

- Greater incidence of endophthalmitis with clear corneal incision than with scleral tunnel incision
  - 2.6- to 3.5-fold increased risk of endophthalmitis with clear corneal incisions vs superior scleral tunnels (Colleaux and Hamilton, 2000; Lertsumitkul et al, 2001)
  - 4.6-fold increased risk of endophthalmitis with temporal corneal incisions vs superior sclerocorneal incisions (Nagaki et al, 2003)
  - 15-fold increase in risk with clear corneal vs scleral tunnel incisions (Buzard et al, 2001)
- Probable mechanism
  - Positive pressure during incision
  - Release of pressure “sucks” bacteria into incision from tear film

Colleaux KM, Hamilton WK, Can J Ophthalmol. 2000; Lertsumitkul S, et al. Clin Experiment Ophthalmol. 2001; Nagaki Y, et al. J Cataract Refract Surg. 2003. McDonnell PJ. Unpublished data.

Surgical Wound Architecture: Conclusions

- Preventing entry of microorganisms into the interior of the eye in the postsurgical period will reduce the risk of endophthalmitis
  - Maintain a closed globe postoperatively
    - Surgical incision must be closed, either by sutures or by an incision architecture that provides a watertight internal corneal seal without sutures
  - Eliminate potential pathogens with topical antimicrobial therapy

Mandelbaum S, Forster RK. In: Ocular Infection and Immunity. 1996:1298-1320.

Properties of an Effective Antimicrobial Agent

Properties of an Effective Topical Antimicrobial Agent

- Broad spectrum of antimicrobial activity
- High level of antimicrobial susceptibility
- Favorable pharmacokinetic profile
  - Effective penetration into ocular tissues and tear fluids
  - High degree of solubility
  - Favorable pH
- Safe and well-tolerated

Broad-Spectrum Anti-infective Therapy Should Include

- Gram-positive activity
  - *Streptococcus pneumoniae*
  - *Staphylococcus epidermidis*
  - *Staphylococcus aureus*
- Gram-negative activity
  - *Haemophilus influenzae*
  - *Pseudomonas aeruginosa*
  - *Serratia marcescens*
- Activity against “atypicals”
  - Anaerobes
  - Tuberculous and nontuberculous mycobacteria
  - *Chlamydia* sp.
  - *Neisseria gonorrhoeae*

Parameters for Measuring Antimicrobial Efficacy

- Broth dilutions
- Breakpoints
  - National Committee for Clinical Laboratory Standards (NCCLS) susceptibility guidelines
- MIC_{90}
  - Minimal inhibitory concentration required to inhibit growth of 90% of the isolates of a bacterial species tested
    - Lower MIC_{90} values indicate greater antibiotic potency
- Time kill curves
  - Chart plotted with % of surviving bacteria vs time

Optimal Pharmacokinetic Profile

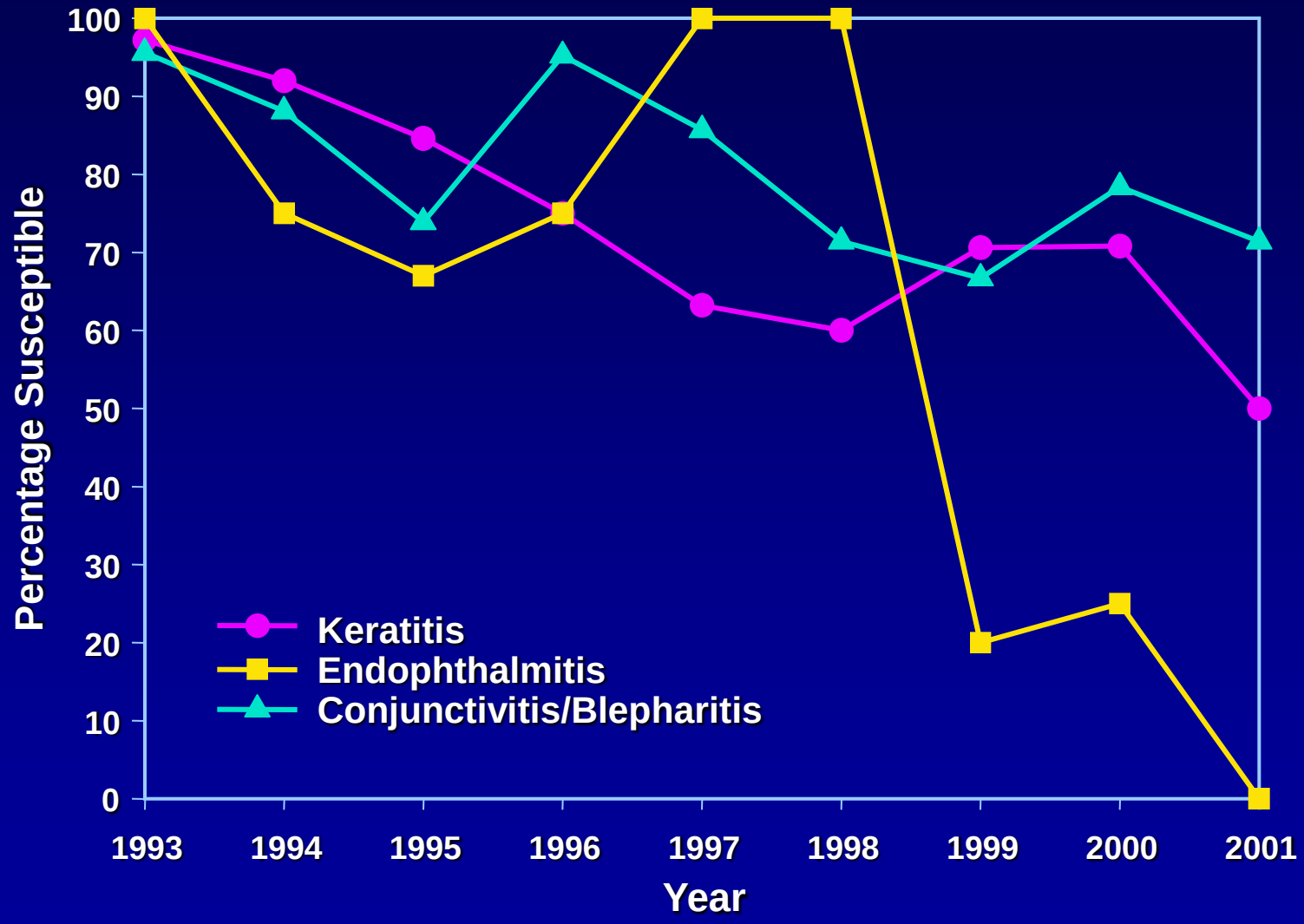
- Penetration
  - Ability of the antibiotic to reach the target tissue
- Solubility
  - Drug must be soluble to be effective
  - Precipitates may decrease efficacy
- pH
  - Acceptable pH range affects solubility

The Need for the 4th-Generation Fluoroquinolones

The Efficacy of Gatifloxacin

The Need for 4th-Generation FQs

In Vitro Susceptibility of *Staphylococcus aureus* to Currently Available Fluoroquinolones: A Study From the Campbell Laboratory



Kowalski et al. *Ophthal Clinics of N. America* 2003.

Historical Development of Quinolones

1st (1980s)	2nd (1980s)	3rd (1986-1996)	4th (1999)
• Nalidixic acid	• Oxolinic acid • Pipemidic acid • Cinoxacin	• Norfloxacin • Ciprofloxacin • Ofloxacin • Levofloxacin	• Gatifloxacin • Moxifloxacin
• Only effective for Gram-negative organisms; *Pseudomonas* resistant • Primarily used for UTI • An estimated 10,000 analogs generated from nalidixic acid	• Not clinically successful • Increased microbial activity, but with toxicity issues • Not soluble into ophthalmic formulations	• Excellent Gram-negative coverage; effective for many Gram-positive organisms • Increasing resistance • Works on a single enzyme	• Dual mechanism of action to interfere with DNA replication • Extended coverage of Gram-positive organisms • Effective against 3rd-generation resistant pathogens and atypical pathogens

Generation designation based on SAR (structure-activity-relationship)

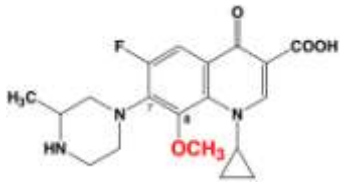
Generations of Fluoroquinolones

- 3rd-generation
  - Ofloxacin
  - Levofloxacin
  - Ciprofloxacin
- 4th-generation
  - Gatifloxacin & Moxifloxacin

Fluoroquinolone Structure

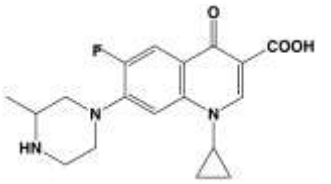
- Improved in vitro activity of 4th-generation fluoroquinolones against Gram-positives is due to the 8-methoxy group
- OCH_3 not present in the 3rd-generation

4th-Generation:

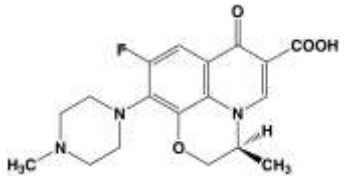


Gatifloxacin

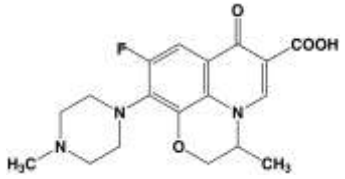
3rd-Generation:



Ciprofloxacin



Levofloxacin



Ofloxacin

Fukuda H, et al. Antimicrob Agents Chemother. 2001;
Ince D, Hooper DC. Antimicrob Agents Chemother. 2001.

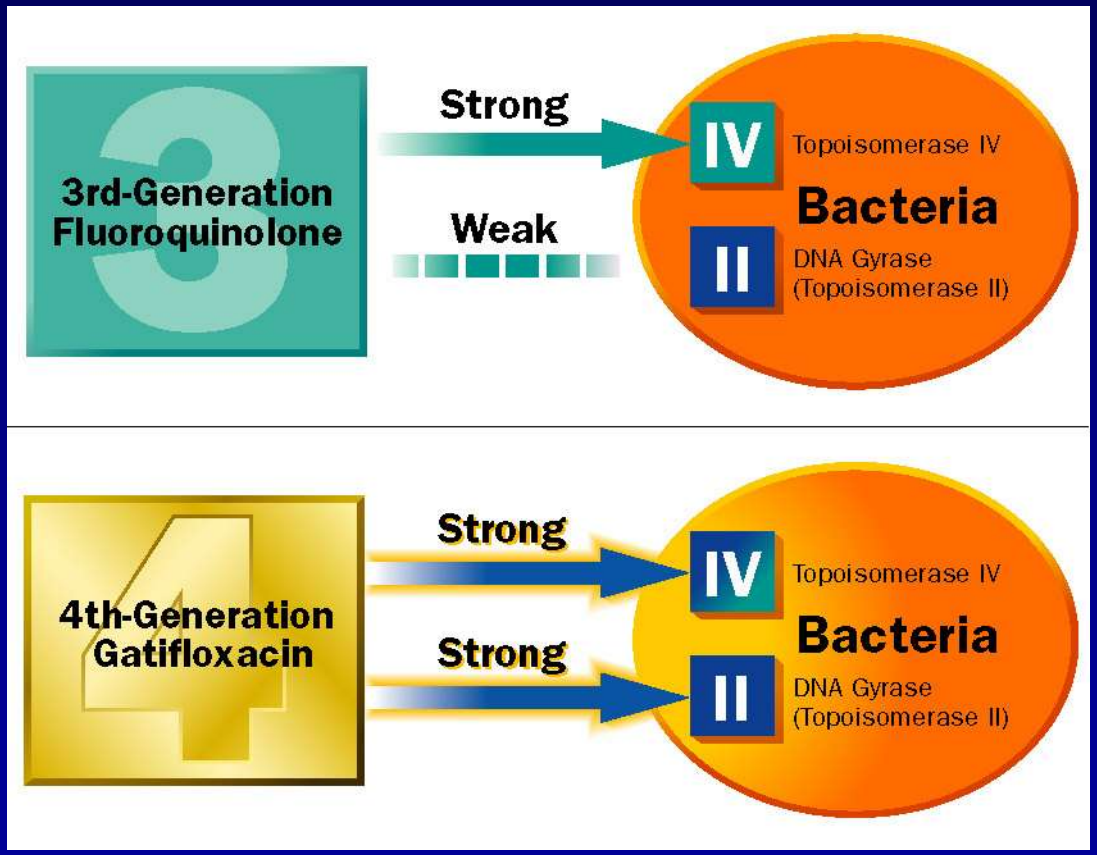
4th- vs 3rd-Generation Fluoroquinolones

- Expanded activity against Gram-positive pathogens
- Improved activity against atypical pathogens emerging as issues in refractive surgery
  - *Mycobacterium* sp., *Nocardia* sp.
- Decreased pathogen resistance because of the dual mechanism of action targeting multiple enzymes

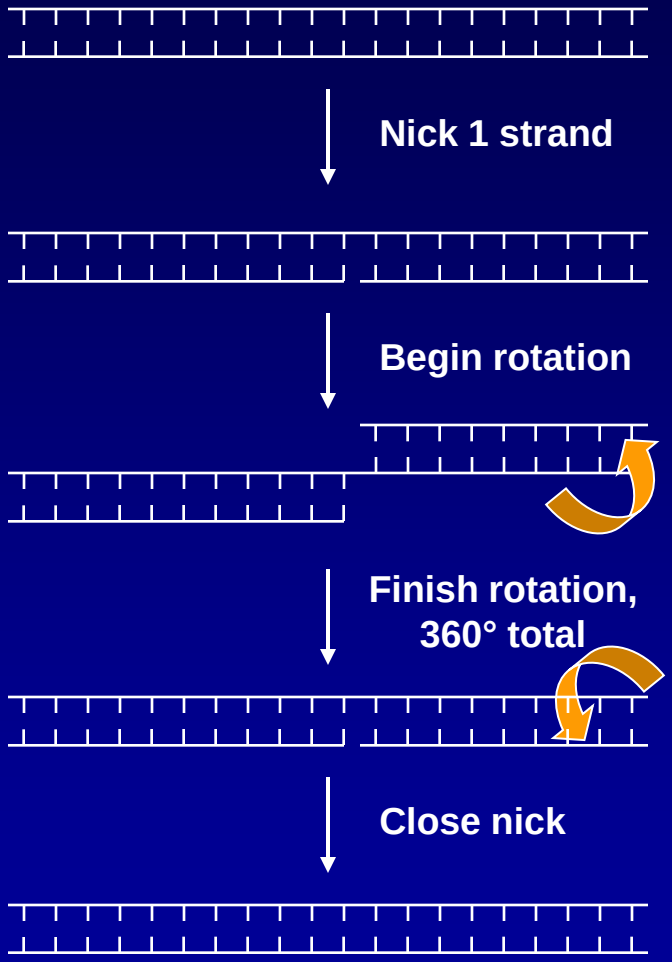
Mechanism of Action: Fluoroquinolones

- Bind DNA gyrase or topoisomerase IV in bacterial cells
- Cause lethal breaks in the bacterial chromosome
- Targets of 3rd-generation FQs
  - DNA gyrase in Gram-negatives
  - Topo IV in Gram-positives
- Targets of 4th-generation FQs
  - DNA gyrase AND topo IV in both Gram-positives and Gram-negatives

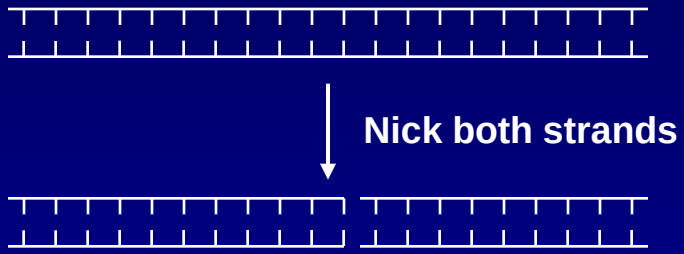
Gatifloxacin Binds to Both Topo IV and DNA Gyrase in Gram-positive Bacteria



Gyrase or Topo IV: Normal Activity



Gyrase or Topo IV: With Fluoroquinolone



Result: Chromosome Is Irretrievably Broken

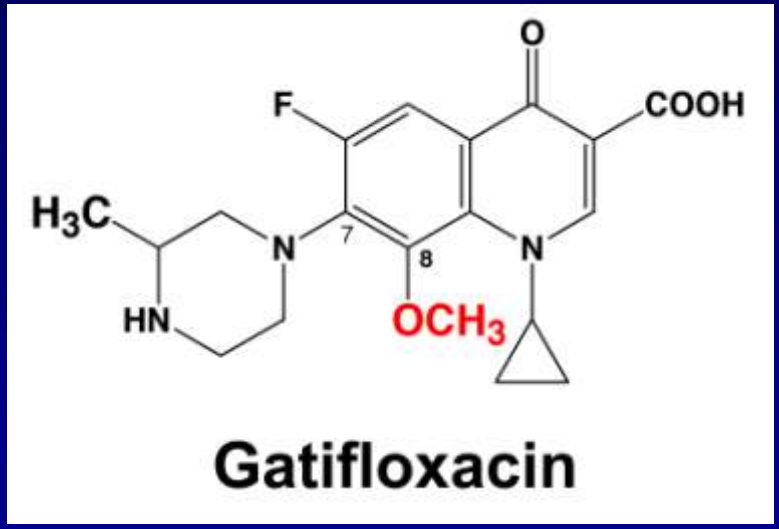
Khodursky AB, Cozzarelli NR. J Biol Chem. 1998; Hsiang YH, Liu LF. J Biol Chem. 1989; Hiasa H, et al. J Biol Chem. 1996.

Why Use 4th-Generation Fluoroquinolones Now?

- The 4th-generation of FQs require 2 steps for resistance
  - Once initial mutations have developed, second-step mutations facilitated, regardless of FQ used
- Avoid increasing consumption of less potent FQs that will rapidly lead to resistance in important pathogens
- Use the new FQs as the initial, more effective infection management tool

Gatifloxacin

- 8-methoxy group confers
  - Improved in vitro activity vs Gram-positives
  - Very low UV-induced toxicity
  - Methyl group on piperazinyl ring



Yamamoto T, et al. Toxicol in Vitro. 2001.

Improved Activity of Gatifloxacin vs Strains Resistant to Older FQs

Antibacterial activity (MIC_{90}), μg/mL

	n	Gatifloxacin	Levofloxacin	Ciprofloxacin
MRSA (Methicillin-resistant *Staphylococcus aureus*)				
FQ-susceptible	36	0.13	0.5	2
FQ-resistant	22	4	16	>32
Streptococcus pneumoniae				
FQ-susceptible	21	0.5	2	2
FQ-resistant	22	1	2	8

- Gatifloxacin: 2- to 8-fold greater activity against FQ-resistant strains

Fung-Tomc J, et al. Int J Antimicrob Agents. 2001.

Gatifloxacin: Less Likely to Cause Resistance Than Older FQs

Appearance of FQ Resistance (at 2 X MIC), Frequency Per Cell

Gatifloxacin	Levofloxacin	Ciprofloxacin
MRSA (Methicillin-resistant *S. aureus*)		
1.35×10^{-8}	16.5×10^{-8}	520×10^{-8}
S. pneumoniae		
$<0.24 \times 10^{-8}$		$>240 \times 10^{-8}$

- FQ resistance appears more frequent against 3rd-generation FQs than gatifloxacin
  - By 12- to 385-fold in *S. aureus*; 1000-fold in *S. pneumoniae*

Fung-Tomc J, et al. Int J Antimicrob Agents. 2001; Fukuda H, et al. Int J Antimicrob Agents. 2001.

Successful Treatment of Resistant *Staph. aureus* Keratitis

- 2 clinical isolates of *Staphylococcus aureus* resistant to gatifloxacin in vitro tested in vivo in animal model of keratitis with gatifloxacin 0.3%, levofloxacin 0.5%, and ciprofloxacin 0.3%
  - **MICs of 12 and 64 mg/mL**
- In vivo gatifloxacin treatment demonstrated significantly lower clinical corneal infiltrate score than that of all other treatment groups
- For both isolates, gatifloxacin demonstrated significantly lower decreases in colony counts compared with levofloxacin and ciprofloxacin

Mah. OMIG. 2003

Successful Treatment of Resistant *Staph. aureus* Keratitis With Gatifloxacin

- This study provided evidence that In vitro antibiotic resistance does not always correlate with in vivo resistance
- Aggressive treatment with gatifloxacin appears to overcome in vitro resistance

Mah. OMIG. 2003

Gatifloxacin Activity Against Ocular Pathogens

Gatifloxacin Activity Against Common Ocular Pathogens

- 2- to 4-fold improved antibacterial activity over 3rd-generation FQs
  - Against common Gram-positive ocular pathogens
    - *S. epidermidis, S. aureus, S. pneumoniae*
- Strong potency vs most Gram-negatives
  - Gatifloxacin activity vs *P. aeruginosa*
    - Within 2-fold of ciprofloxacin
    - Equivalent in rabbit model of keratitis

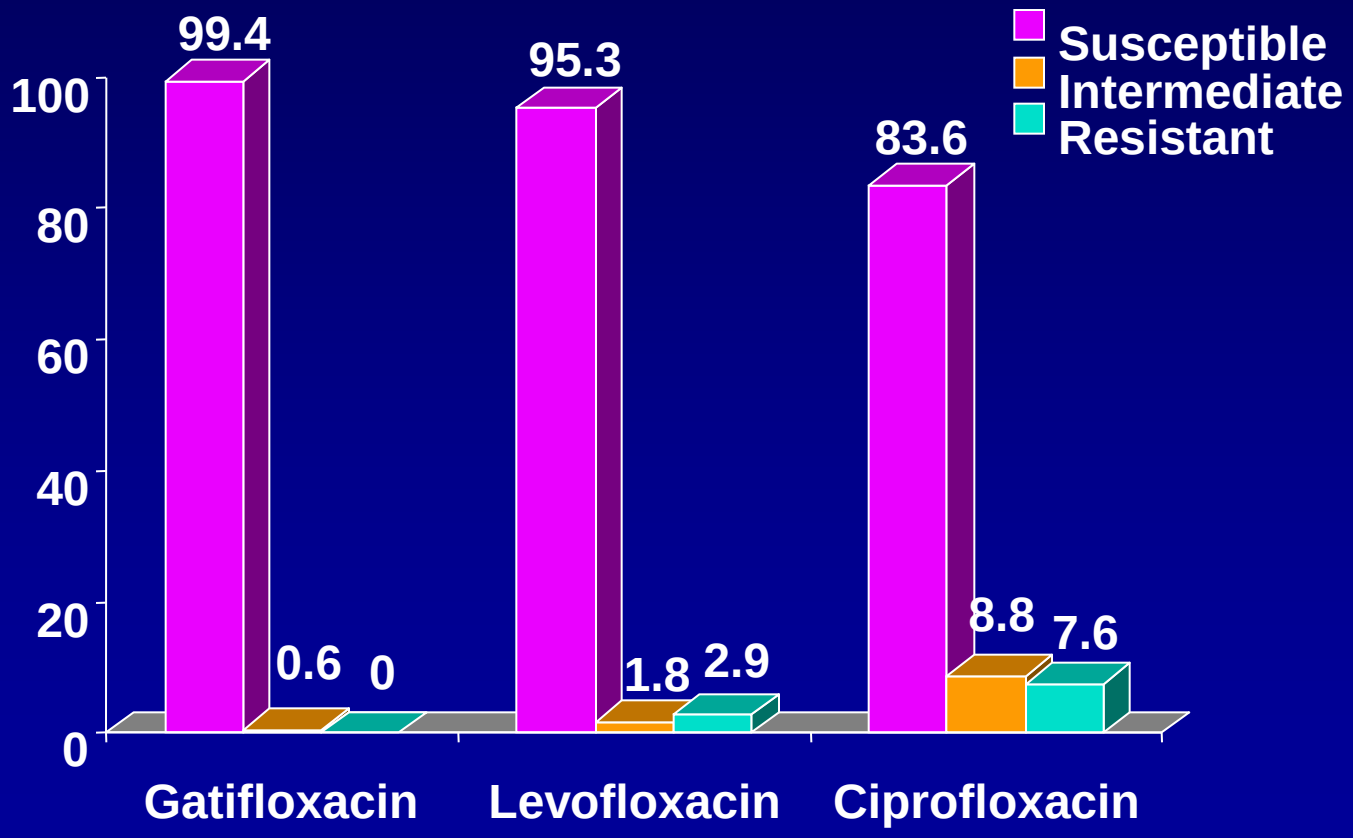
Fung-Tomc J, et al. J Antimicrob Chemother. 2000; Huczko E, et al. Int J Antimicrob Agents. 2000; McDonnell, Am J Ophthalmol. In press.

Efficacy of Gatifloxacin vs Older Fluoroquinolones

- Patients diagnosed with bacterial conjunctivitis were enrolled in a clinical trial for gatifloxacin therapy
- Conjunctival swabs for bacterial isolation were taken prior to any antibacterial therapy
- Minimal inhibitory concentrations (MICs) were determined by broth dilution
- MICs were classified using NCCLS susceptibility breakpoints

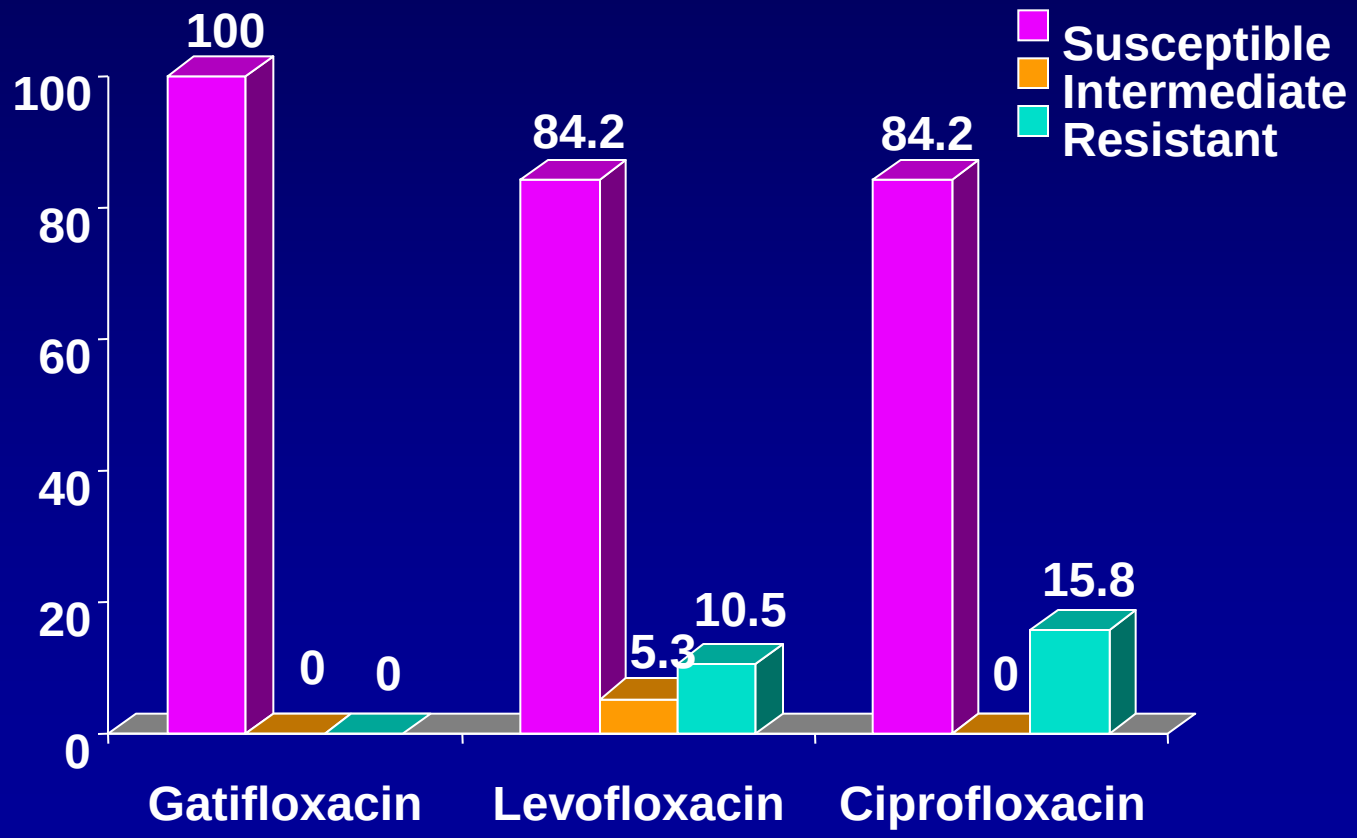
Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Susceptibility of All Gram-positive Ocular Isolates (n = 170)



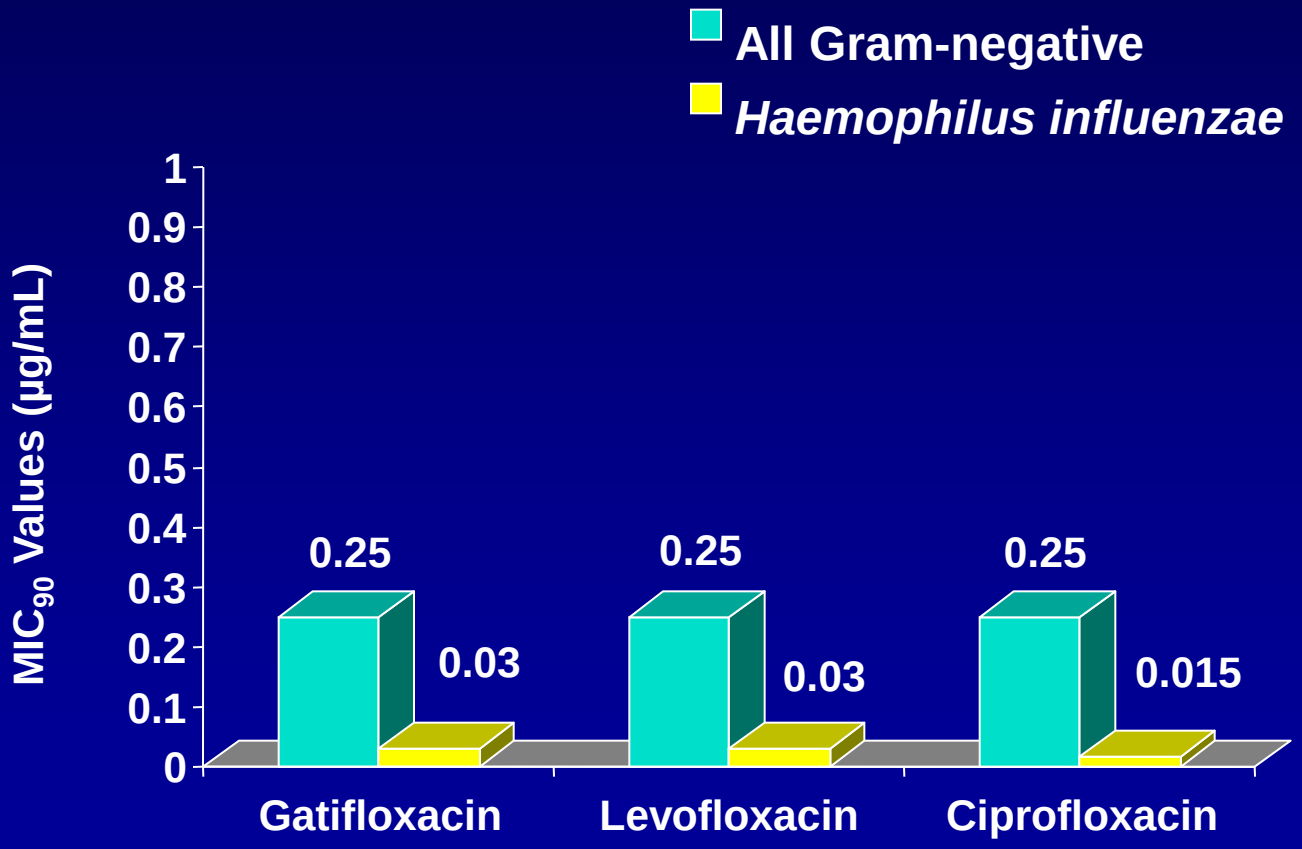
Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Susceptibility of *Staphylococcus epidermidis* Ocular Isolates (n = 38)



Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

MIC_{90} Values (μg/mL) Against Gram-negative Ocular Isolates



Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Summary: Gatifloxacin Activity vs Clinical Ocular Isolates

- All ocular isolates were susceptible to gatifloxacin (except a single strain of *S. haemolyticus*)
  - 4.7% of Gram-positive isolates were partially or fully resistant to levofloxacin, 16.4% to ciprofloxacin
- *S. epidermidis* strains resistant to 3rd-generation FQs were susceptible to gatifloxacin
  - Gatifloxacin MIC_{90} against *S. epidermidis*: 4- to 16-fold better than levofloxacin or ciprofloxacin

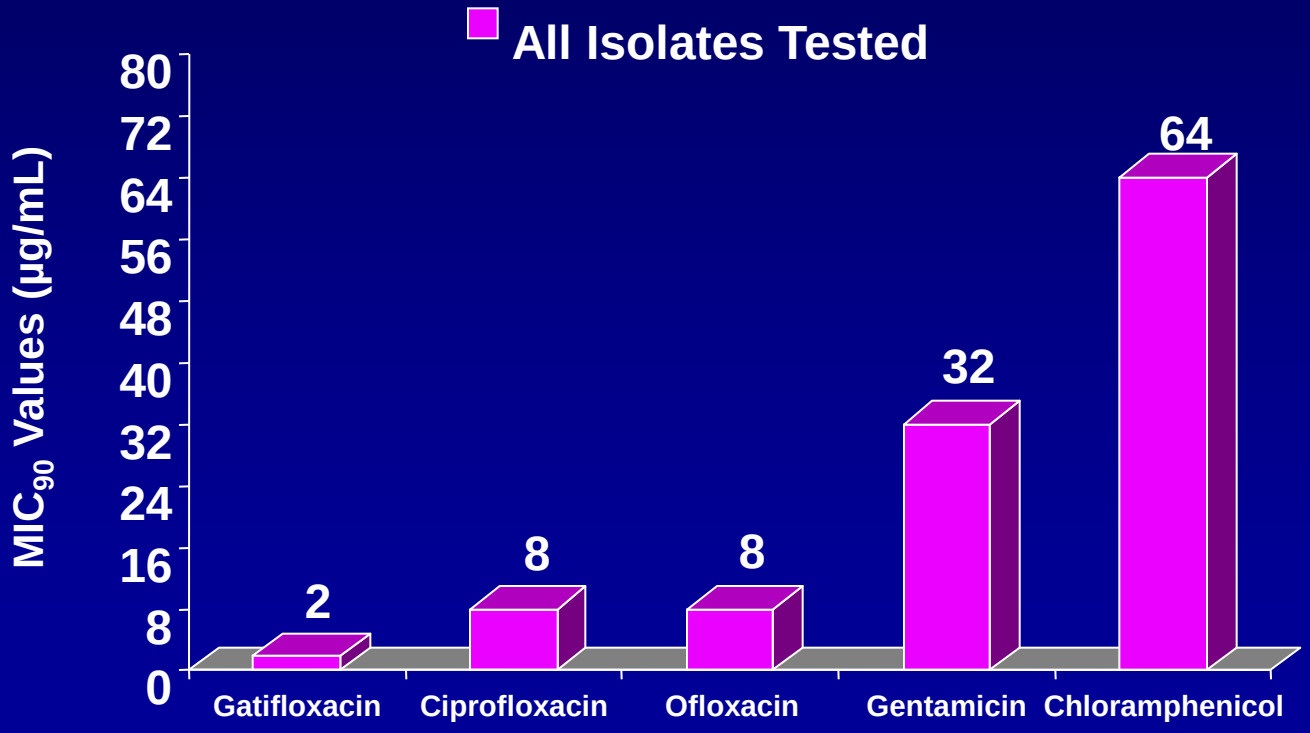
Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Summary: Susceptibility of Ocular Isolates to Gatifloxacin

- Substantial percentages of Gram-positive isolates resistant to older FQs were susceptible to gatifloxacin
- The 2- to 4-fold improvement in Gram-positive MIC_{90} values for gatifloxacin may be clinically significant
- Gatifloxacin displayed clearly improved activity vs Gram-positive ocular isolates compared with older fluoroquinolones

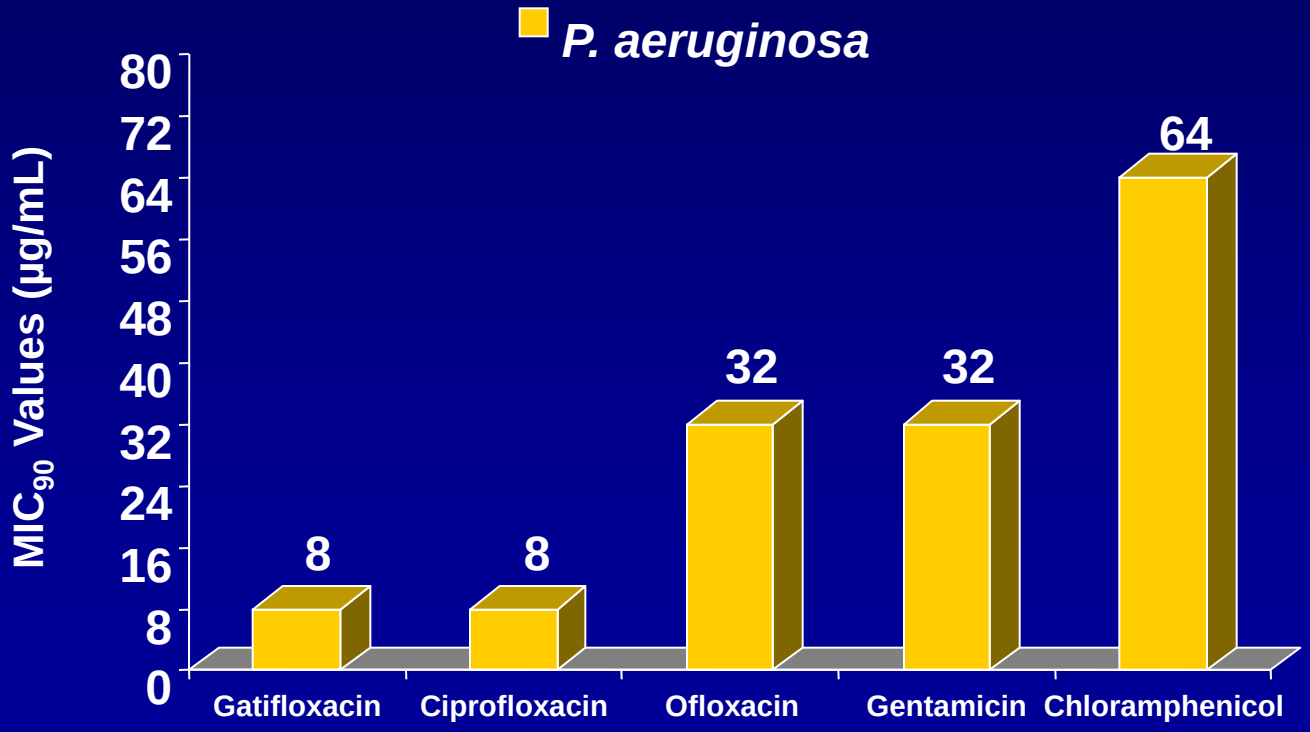
Long M, Jensen HG. [abstract]. Invest Ophthalmol Vis Sci. 2003.

MIC_{90} Values (µg/mL) Against Ocular Isolates (N = 532)



Data on file, Allergan, Inc.

MIC_{90} Values (µg/mL) Against *Pseudomonas aeruginosa*



Data on file, Allergan, Inc.

4th-Generation Fluoroquinolones

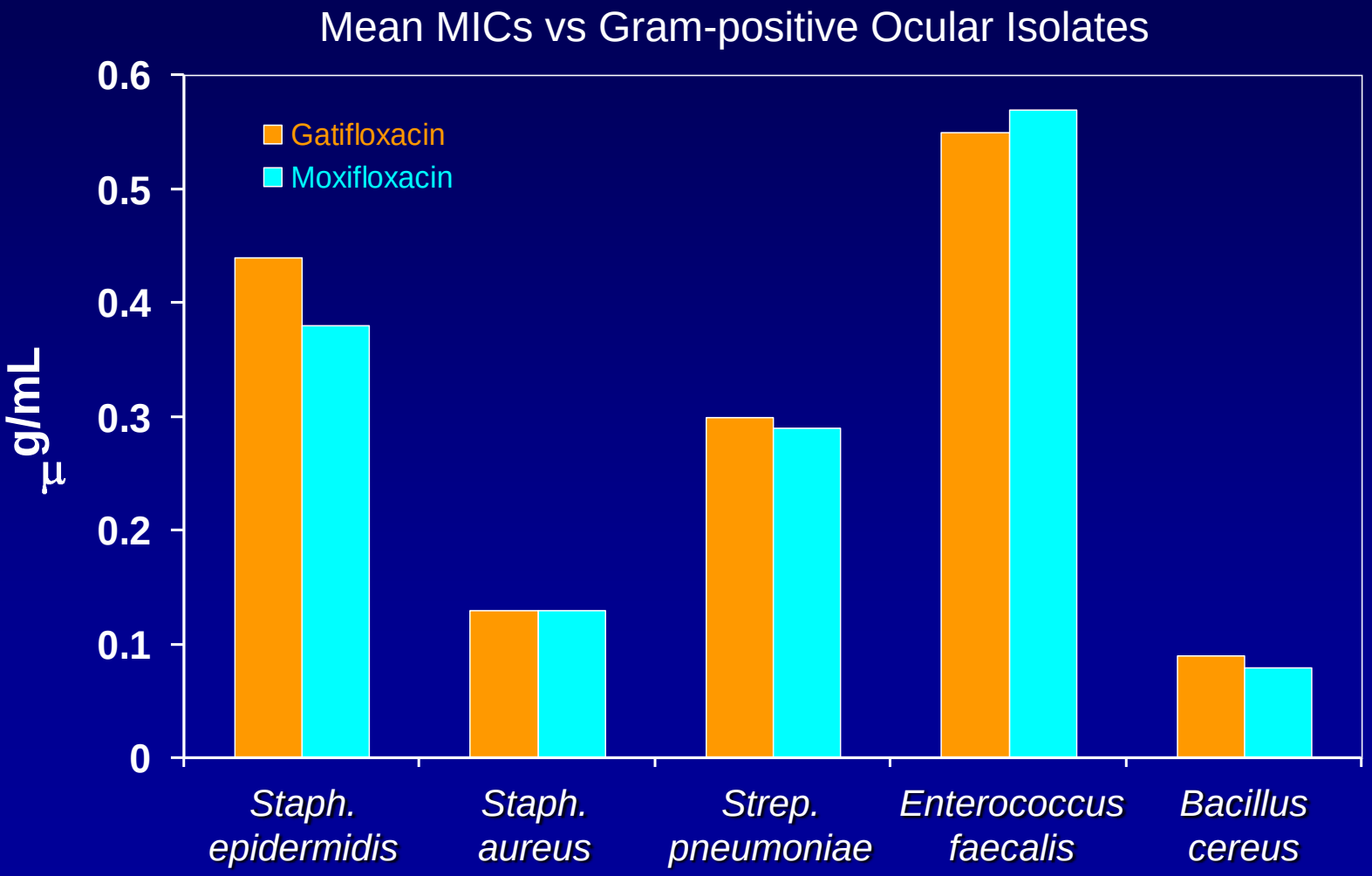
Gatifloxacin vs Moxifloxacin

Comparative MIC Study: 4th-Generation FQ Activity vs Ocular Isolates

- MICs against 6 independent ocular isolates of each species measured by broth dilution
  - MIC for each isolate determined in triplicate
  - Mean MIC computed
  - Exception: 4 strains of *Klebsiella pneumoniae* and *Enterobacter aerogenes* examined

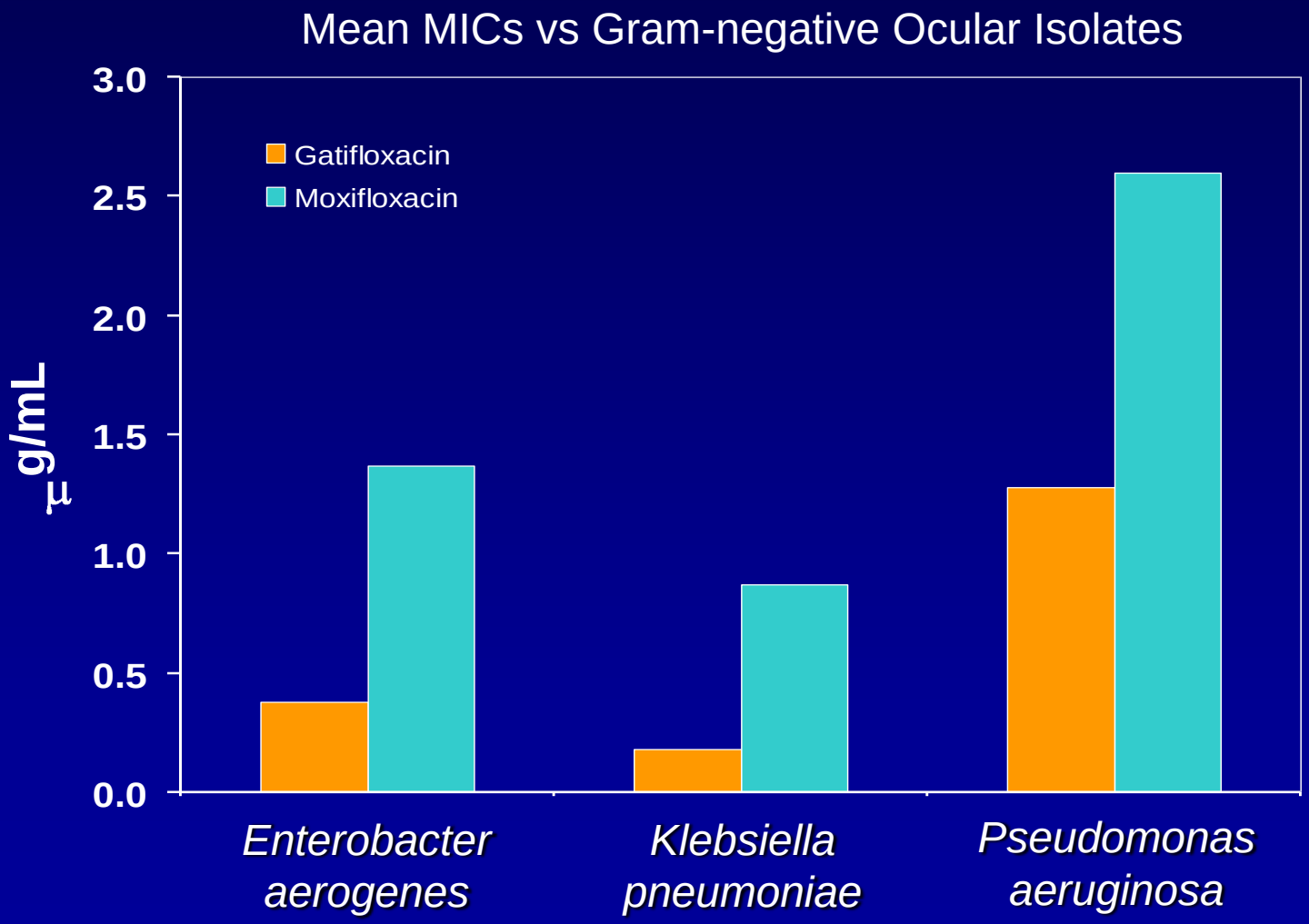
Callegan MC, et al. Presented at: ASCRS; 2003.

Comparing the 4th-Generation Fluoroquinolones



Callegan MC, et al. Presented at: ASCRS; 2003.

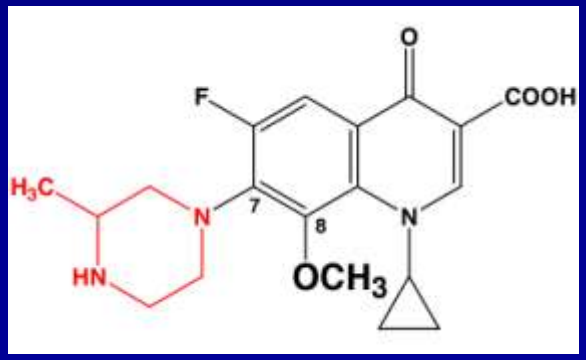
Comparing the 4th-Generation Fluoroquinolones



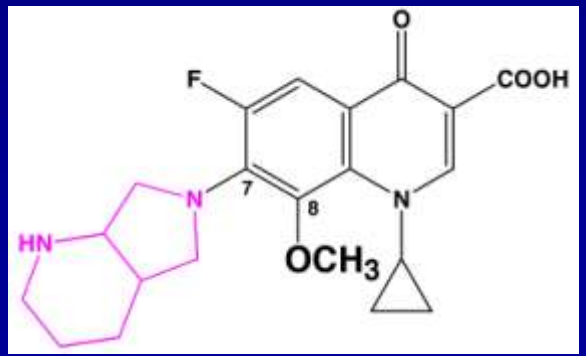
Callegan MC, et al. Presented at: ASCRS; 2003.

4th-Generation Fluoroquinolone Activity vs Clinical Ocular Isolates

- Gatifloxacin activity compared with moxifloxacin
  - Nearly identical vs Gram-positives
  - 2- to 6-fold better vs Gram-negatives
  - 2- to 6-fold better vs atypicals
- The addition of the methyl group on the piperazinyl ring may account for gatifloxacin's increased activity



Gatifloxacin



Moxifloxacin

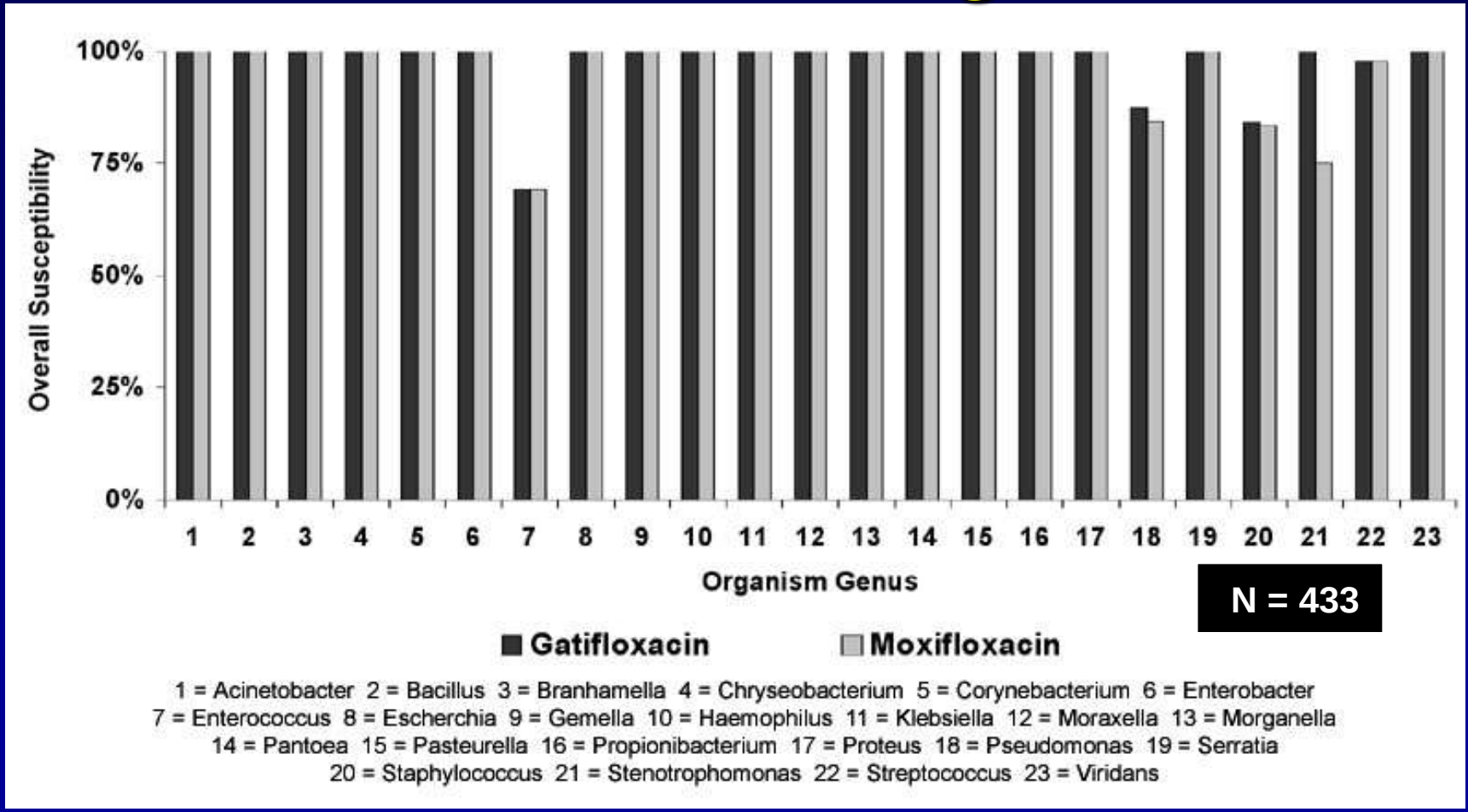
Callegan MC, et al. Presented at: ASCRS; 2003.

Large-scale In Vitro Susceptibility Study of Ocular Pathogens to Gatifloxacin and 4 Other Fluoroquinolones

- Comparison of the in vitro susceptibility of 433 isolates of ocular pathogens to gatifloxacin, moxifloxacin, ciprofloxacin, levofloxacin, and ofloxacin
- One of the most extensive in vitro studies of its kind
- Investigators
  - Bradley Fouraker[1]
  - Michelle Callegan[2]
  - Marc Desjardins[3]
  - Hank Perry[40]

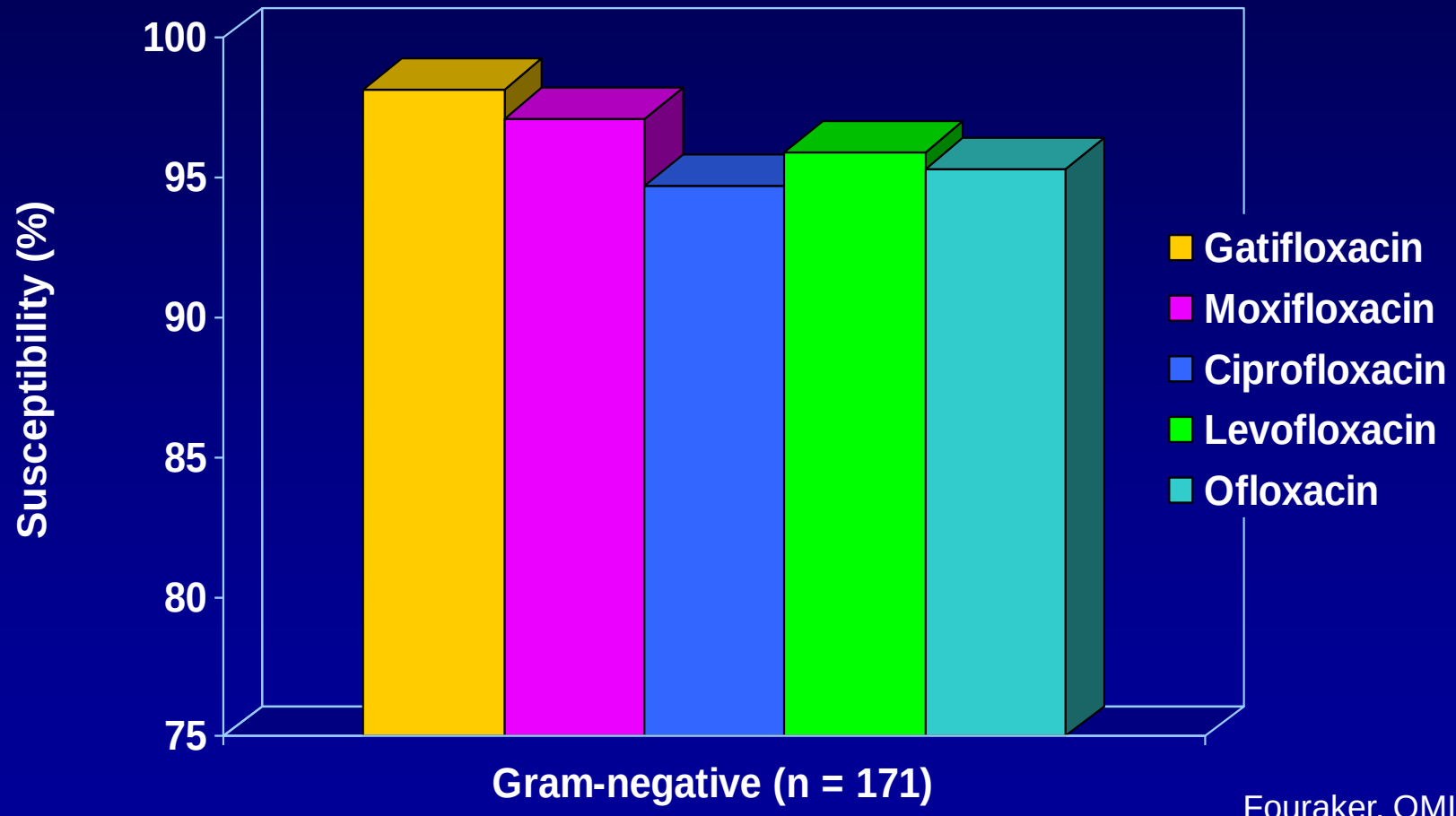
[1]Brandon Eye Clinic, Brandon, FL, USA
[2]Dean A. McGee Eye Institute, Oklahoma City, OK, USA
[3]Ottawa Hospital, Ottawa, Ontario, Canada
[4]Ophthalmic Consultants of Long Island, Rockville Center, NY,USA

Overall Susceptibility for Gatifloxacin Equal or Superior to Moxifloxacin in 23 Ocular Pathogens



Fouraker. OMIG. 2003.

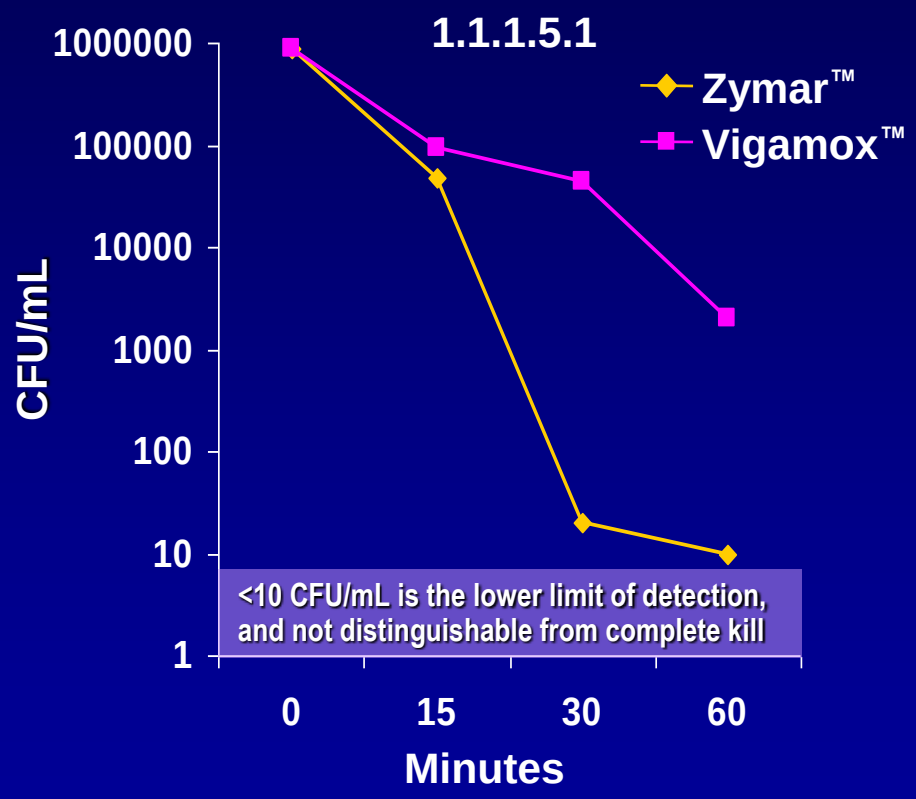
Gram-negative Organisms Show Greatest Susceptibility to Gatifloxacin Over Those of 4 Other Fluoroquinolones

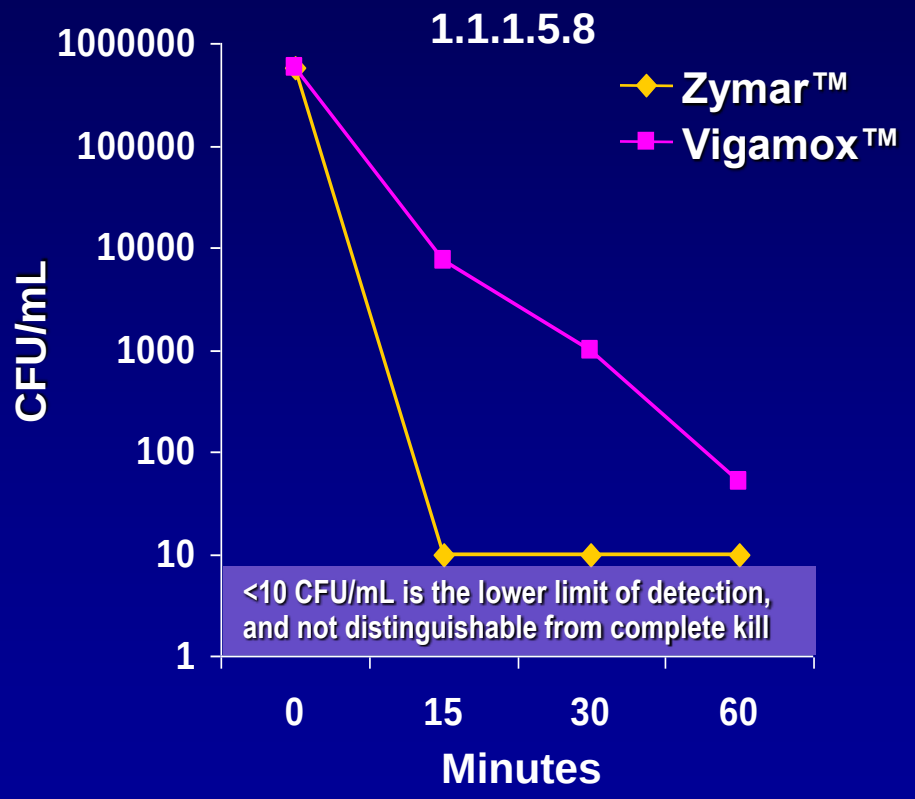


Fouraker. OMIG. 2003.

Kill Rates - Zymar™ vs Moxifloxacin Against 4 Strains of *Staph. aureus* Ocular Isolates (1)

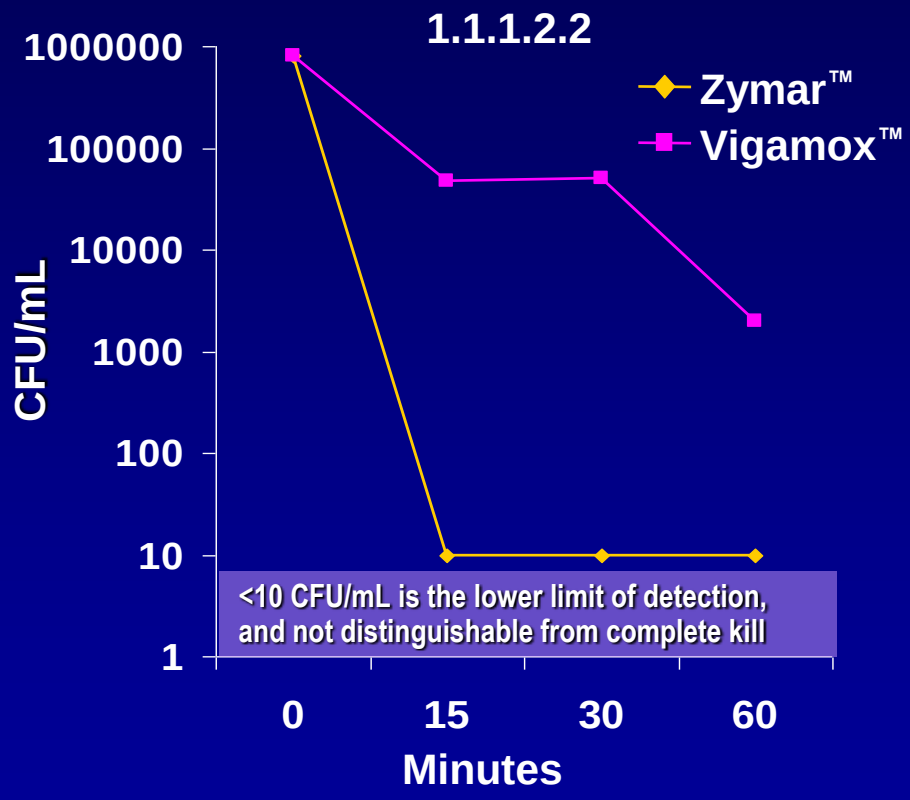
Kill Rates at Body Temperature

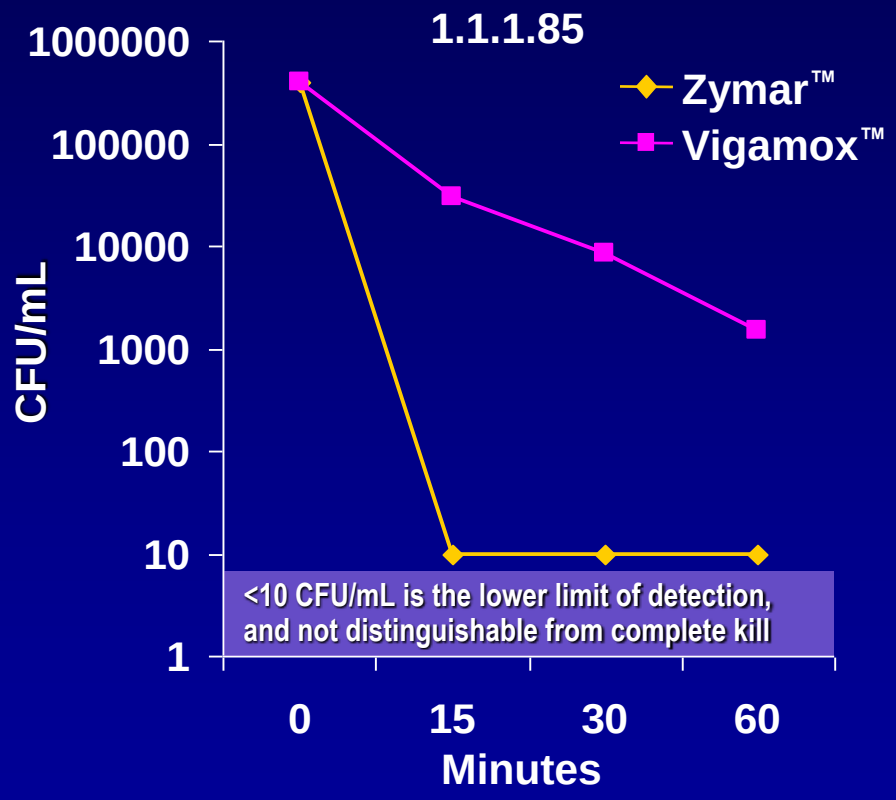




Kill rates - Zymar™ vs Moxifloxacin Against 4 Strains of *Staph. aureus* Ocular Isolates (2)

Kill Rates at Body Temperature





Preserved v/s Unpreserved

Preservative Use in Ophthalmic Anti-infectives

- Most topical ophthalmics contain preservatives, most commonly benzalkonium chloride (BAK)
- Prevents decomposition of active drug at room and elevated temperatures **(Abelson and Fink, 2002)**
- Provides antimicrobial activity against acanthamoeba and fungi within the bottle
- Enhances overall anti-microbial effectiveness

Abelson MB, Fink K. *Rev Ophthalmol*. 2002.

Why a Preservative Is Needed

- Touching the bottle tip to the ocular surface can cause contamination **(Schein et al, 1992)**
- Preservatives have been proven to limit bacterial, mycotic (fungal) **(Gupta, 2002)**, and amoebal (acanthamoebal) ocular infections **(Silvany, 1991)**

Despite differences in formulations, with short-term dosing neither gatifloxacin nor moxifloxacin appear to be toxic to the corneal epithelium in this rabbit model. (R. Noecker – ACT, 2003 Abstract)

Schein OD, et al. *Arch Ophthal*. 1992.; Gupta AK, et al. *Med Mycol*. 2002.; Silvany RE, et al. *Ophthalmol*. 1991.

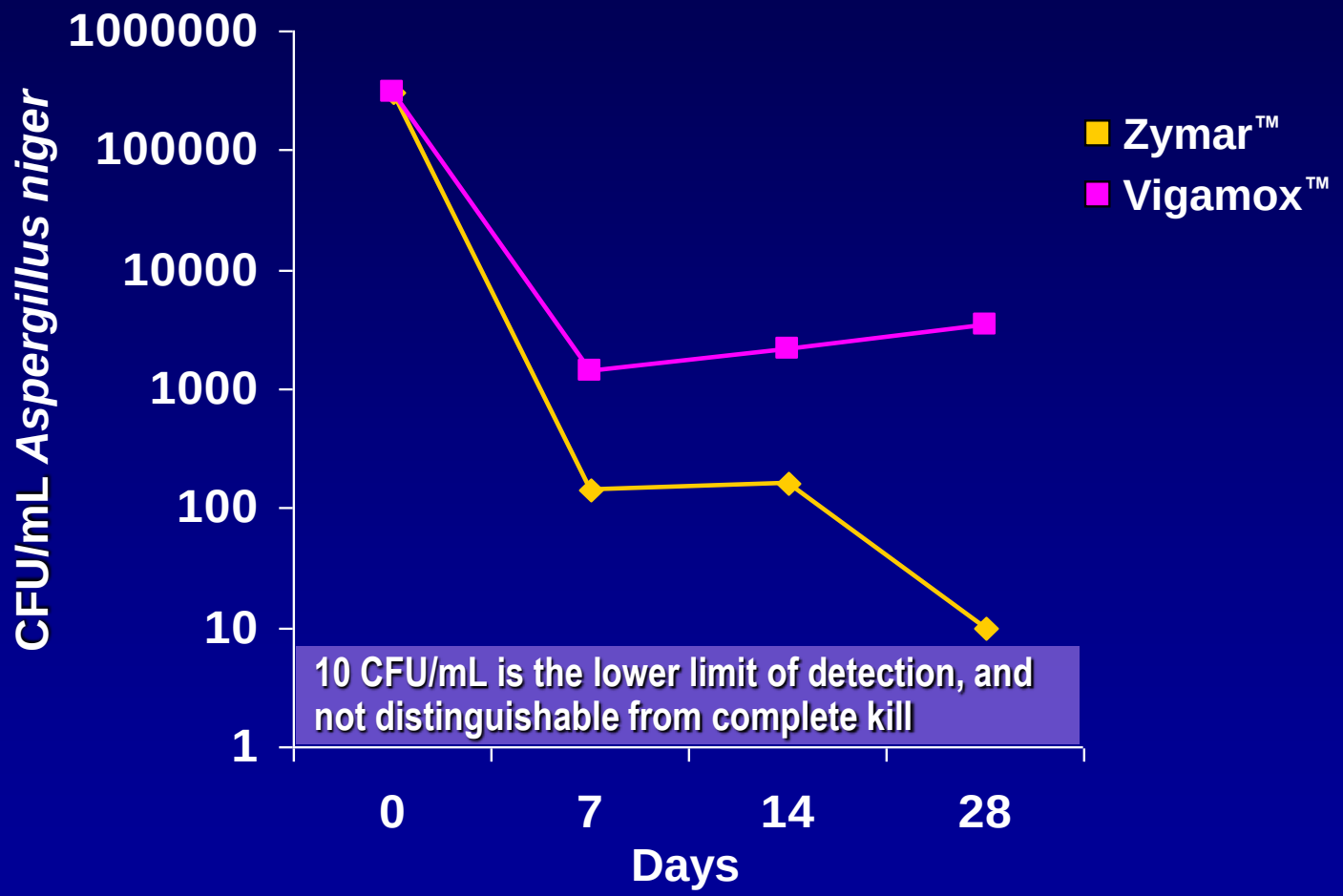
Antimicrobial Preservative Efficacy Against Yeast Zymar™ vs Moxifloxacin

- Study: Comparison of activity of gatifloxacin (with 0.005% BAK) vs moxifloxacin (no BAK) against 20 isolates of yeast (including *Candida* spp.)
- Results: Gatifloxacin solution inhibited all isolates while moxifloxacin solution failed to inhibit 11 of 20 isolates
- Conclusion: Presence of BAK reduces potential for introduction of yeast to the eye during surgery and patient usage of ophthalmic solutions

Rupp et al. OMIG. 2003.

Kill Rates at Room Temperature:

Zymar™ vs Moxifloxacin Against Fungus (*Aspergillus niger*)



Allergan Data on File.

Infections Associated with LASIK Surgery

The Efficacy of Gatifloxacin

LASIK Complications: Bacterial Infections

- Bacterial infection following LASIK is rare
  - Factors accounting for low frequency
    - Sterilization of equipment
    - Preop disinfectant washes off lids, conjunctiva
    - Widespread use of postop topical antibiotics

Sugar A, et al. Ophthalmology. 2002.

LASIK Complications: Bacterial Infections

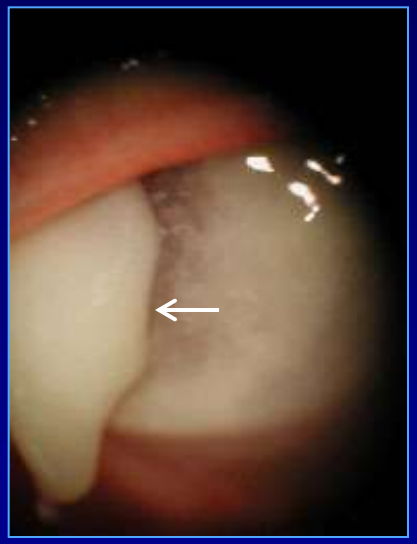
- Infections soon after LASIK
  - *Staphylococcus* and *Streptococcus* species
- Delayed onset infections: Mycobacteria
  - Difficult to control, aggressive therapy required

Gatifloxacin vs the Older FQs for the Treatment of MDR Staphylococcal Keratitis Post-LASIK

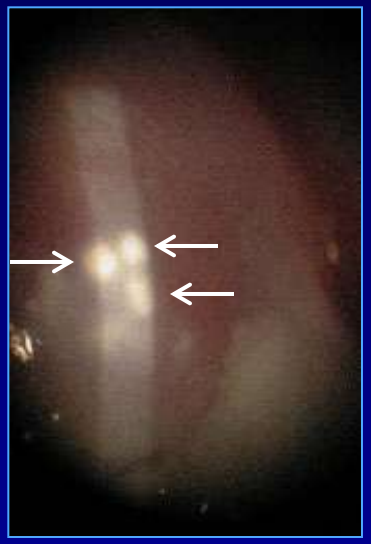
- 28 rabbits underwent lamellar keratectomy and injection of MDR *Staphylococcus aureus* in a single eye
  - Oxacillin-resistant, vancomycin-sensitive
- Eyes randomized to balanced salt solution, ciprofloxacin, levofloxacin, or gatifloxacin
  - One drop instilled immediately, then every 6 hours for 18 hours (4 drops total)

McDonnell PJ, et al. Am J Ophthalmol. In press.

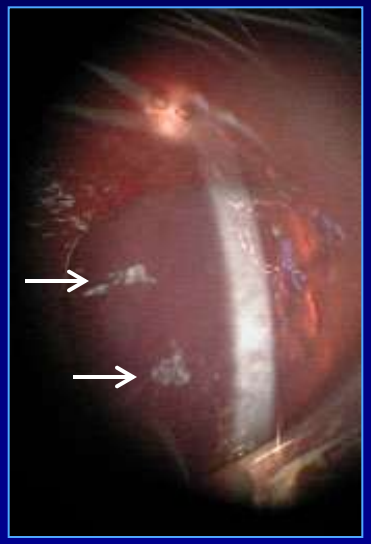
Gatifloxacin Is Superior to the Older FQs for the Prevention of MDR *S. aureus* Keratitis Post-LASIK



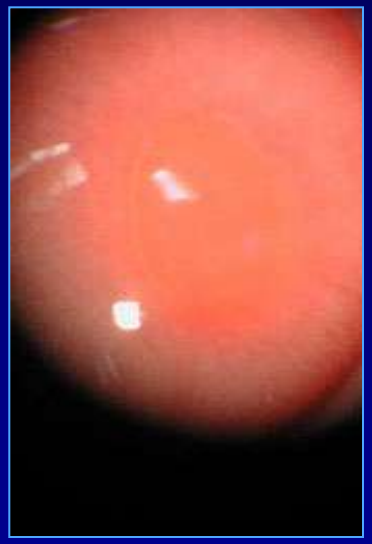
BSS (Control):
Flap & stromal bed fully infected, flap dislodged



Ciprofloxacin:
Stromal infiltrates indicate keratitis



Levofloxacin:
Corneal edema, stromal infiltrates



Gatifloxacin:
Clear cornea

McDonnell PJ, et al. Am J Ophthalmol. In press.
Clinical significance of these animal data is unknown.

Characteristics of Post-LASIK *Mycobacterium* Keratitis

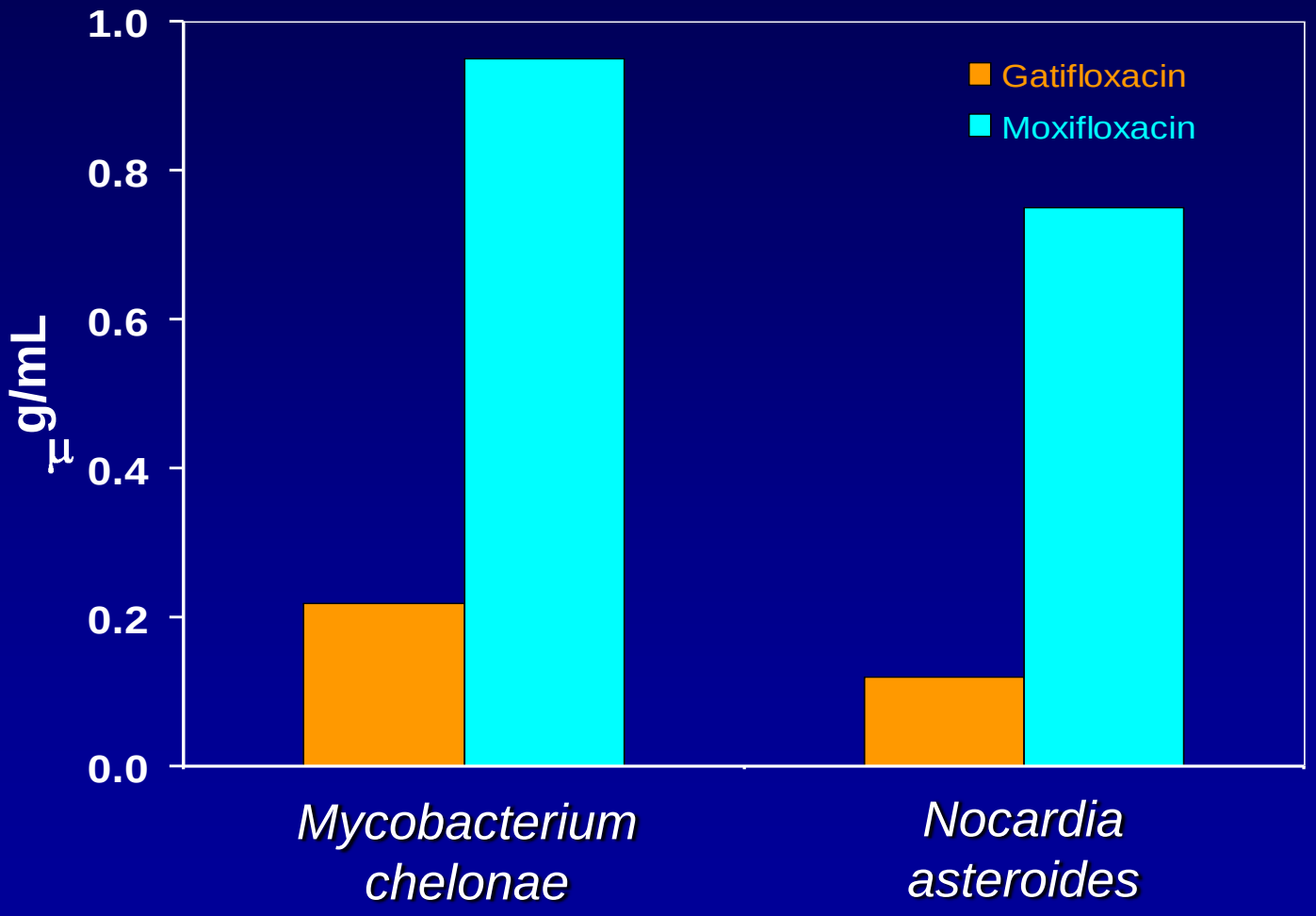
- Potentially vision-threatening
- Long latent period
- Delayed diagnosis
- Protracted course

Gatifloxacin Activity Against "Atypical" Pathogenic Species

- 4-fold improved activity compared with 3rd-generation FQs against:
  - *Propionibacterium acnes*
  - *Mycobacterium chelonae*

Fung-Tomc J, et al. J Antimicrob Chemother. 2000;
Brown-Elliott BA, et al. Antimicrob Agents Chemother. 2002.

Gatifloxacin Is 4-Fold as Active as Moxifloxacin Against Atypicals



Callegan M, et al. Presented at: ASCRS; 2003.

Gatifloxacin: Data on Pharmacokinetics, Toxicity, and Safety

Evaluation of Gatifloxacin's Ocular Pharmacokinetic Profile

- Objective: Evaluation of penetration into rabbit ocular tissue after instillation of gatifloxacin 0.3%
  - Comparator: ciprofloxacin 0.3%
- Topical application regimens
  - Single dose
  - Multiple dose (QID X 3 days)

Batoosingh AL, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003.

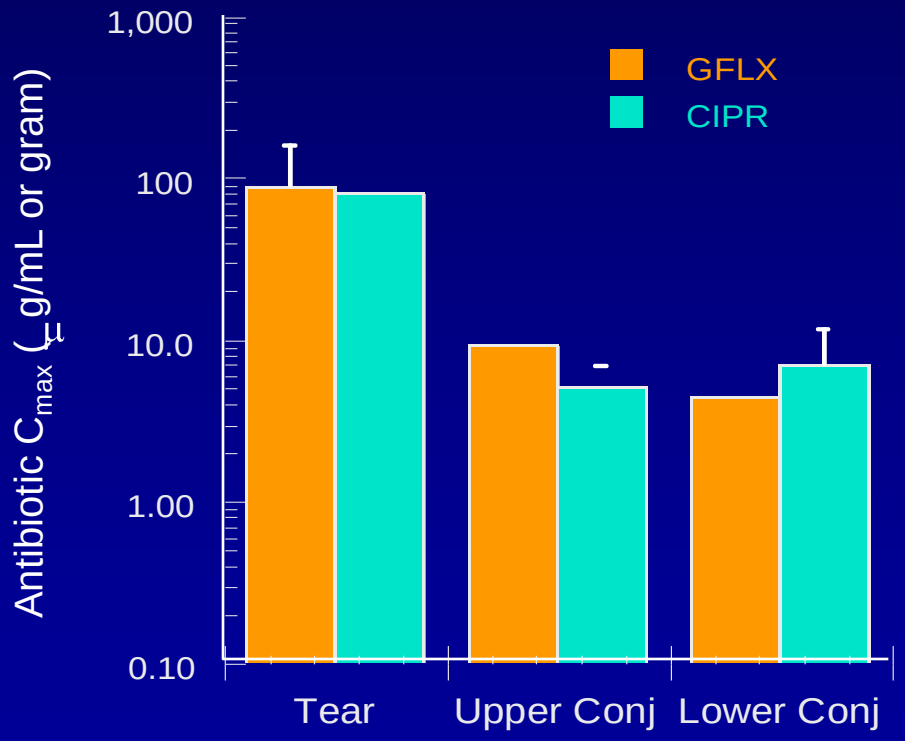
Methods for Ocular Pharmacokinetic Evaluation of Gatifloxacin

- Female N.Z. White rabbits
  - Gatifloxacin 0.3% or ciprofloxacin 0.3%, 1 drop/eye
    - Single dose or QID-3 days
- Tear, conjunctiva, cornea, aqueous humor sampled 9 times throughout the following 24 hours
- FQ concentrations measured by GLP-validated liquid chromatography tandem mass spectrometry

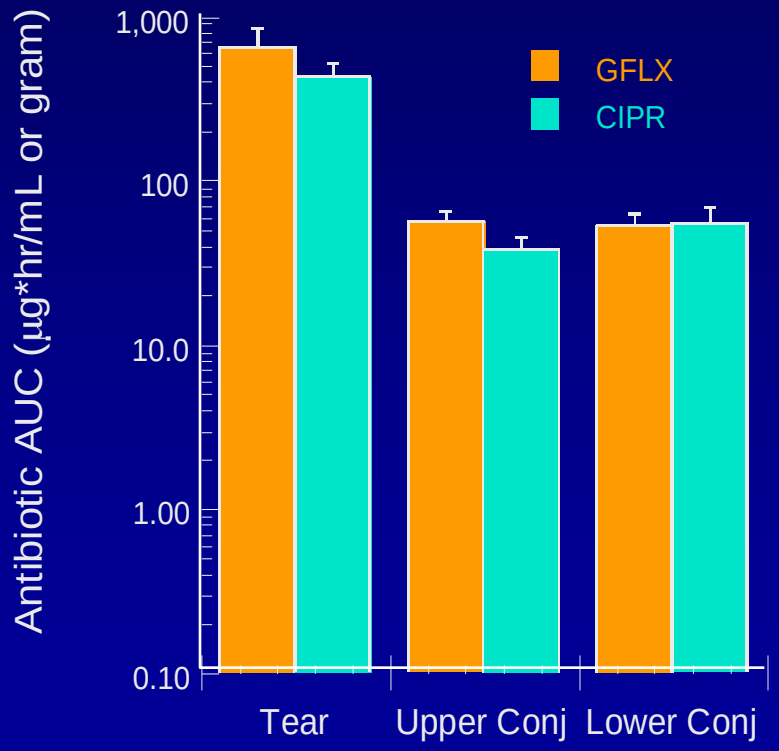
Batoosingh AL, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Gatifloxacin Concentration in Tears and Conjunctiva Greatly Exceeds MICs for Ocular Pathogens

C_{max} – QID for 3 Days

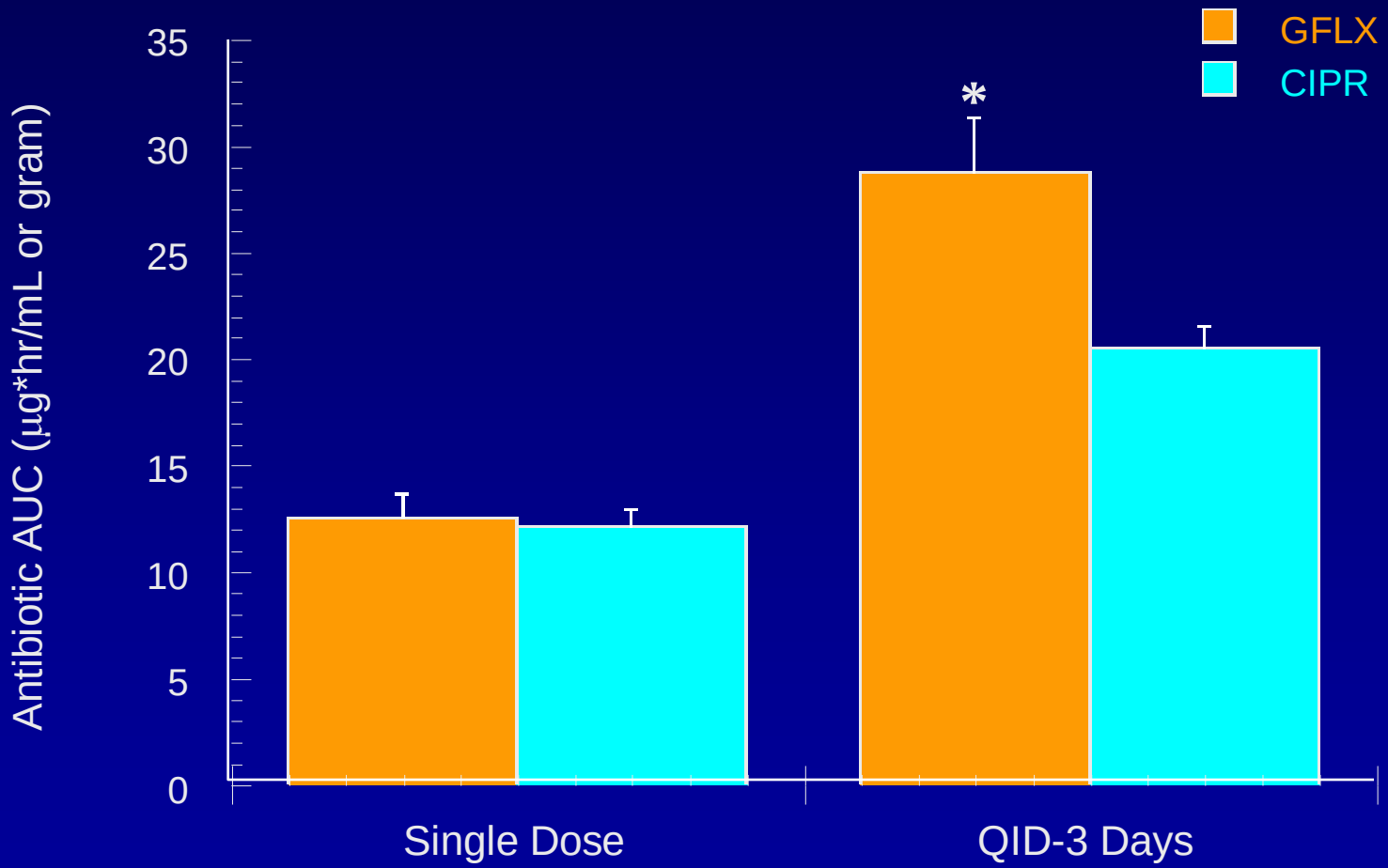


AUC – QID for 3 Days



Batoosingh AL, et al. Presented at: ARVO; 2003.

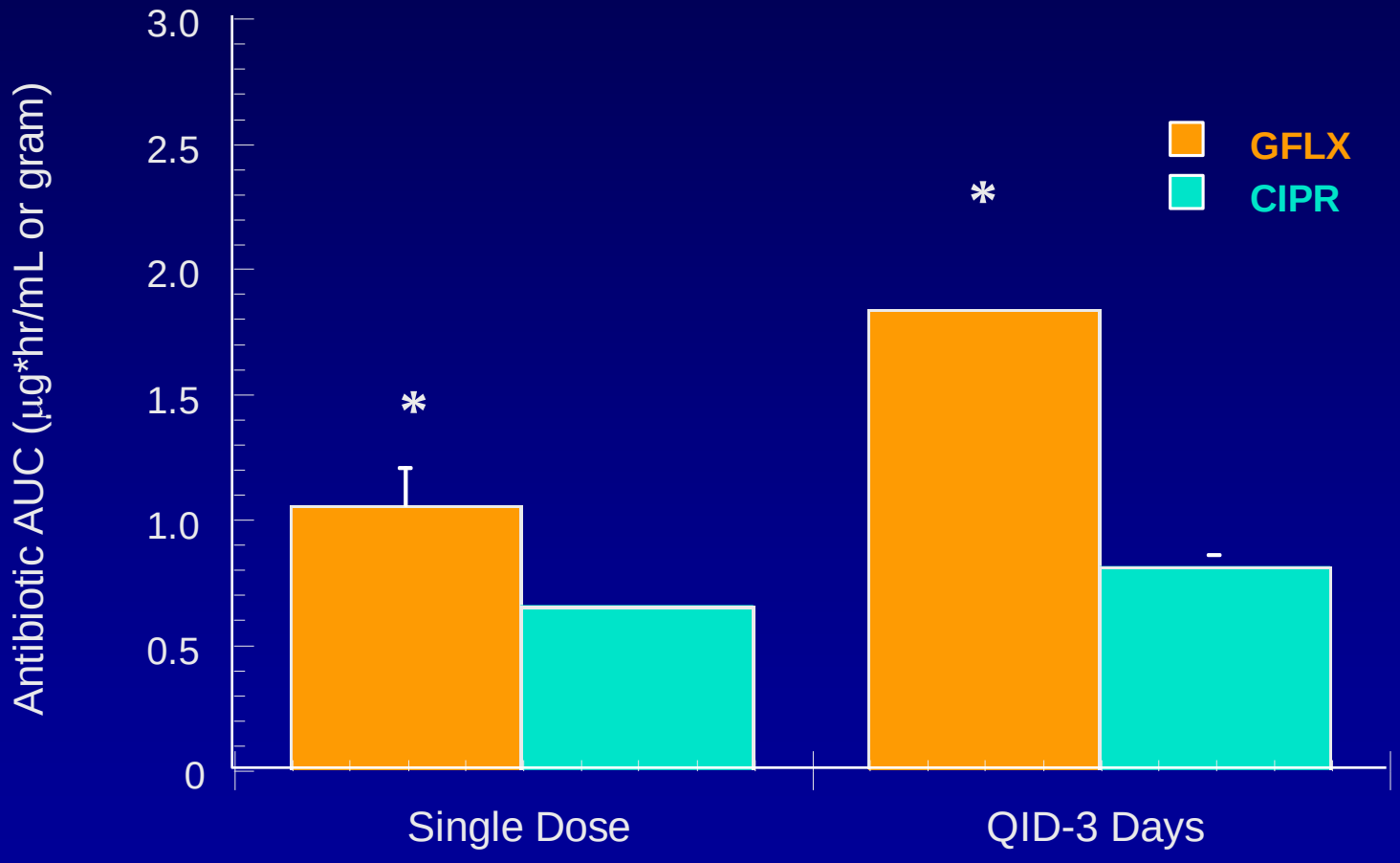
Gatifloxacin Concentration (AUC) Significantly Greater Than Ciprofloxacin in Cornea



**P* = .012 (QID-3 days)

Batoosingh AL, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003

Gatifloxacin Concentration (AUC) Significantly Greater Than Ciprofloxacin in Aqueous Humor



**P* = .045 (single dose); *P*<.0001 (QID-3 days)

Batoosingh AL, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Gatifloxacin Greatly Exceeds Corneal and Aqueous Penetration vs Ciprofloxacin

- Tears and conjunctiva (single and multiple dose)
  - Gatifloxacin and ciprofloxacin levels were similar
- Cornea (multiple dose)
  - Gatifloxacin AUC significantly greater than ciprofloxacin
- Aqueous humor (single and multiple dosing)
  - Gatifloxacin AUC significantly greater than ciprofloxacin

Batoosingh AL, et al. [abstract]. Invest Ophthalmol Vis Sci. 2003.

Gatifloxacin Is Less Phototoxic Than Older Fluoroquinolones

- UV exposure increased the toxicity of 3rd-generation FQs against cultured human corneal cells
- Ciprofloxacin and norfloxacin caused erythrocyte lysis following UV irradiation
- Gatifloxacin exhibited no cytotoxic or phototoxic effects following UV irradiation (Yamamoto et al, 2001)

Yamamoto T, et al. Toxicol in Vitro. 2001;
Domagala JM. J Antimicrob Chemother. 1994.

Safety of Gatifloxacin Evaluated Compared With Placebo

- Randomized (with age stratification), double-masked, parallel-group, placebo-controlled Phase 3 clinical trial
- Patients (N = 265) aged 1-90 years with acute bacterial conjunctivitis
- 1-2 drops of gatifloxacin or placebo, every 2 hours, 4-8 times on day 1, 6-8 times on day 2, and QID on days 3-5±1
- Clinical signs and symptoms scored, biomicroscopy and visual acuity examinations conducted

Data on file, Allergan, Inc.

Ocular Tolerability of 4th-Generation Fluoroquinolones

Gatifloxacin 0.3% vs Moxifloxacin 0.5%

- 30 healthy volunteers evaluated in blinded clinical trial
- Baseline evaluation for for conjunctival erythema, conjunctival vascularity, and pupil size
- Subjects received random drops in each eye from masked bottles of Zymar™ and Vigamox™ placed in either the right or left eye two times at a one minute interval
- Subjects waited for 5 minutes with eyes closed after drug administration

Allergan data on file.

Results

- Administration of Moxifloxacin was associated with statistically significant conjunctival erythema (P = .0005) and conjunctival vascularity (P = .0005) compared with baseline at 5 minutes
- No statistically significant difference in conjunctival erythema or vascularity associated with the administration of Zymar™
- Ocular irritation and pain was significantly less with Zymar™ as compared with Vigamox™ (P = .001)
- There was a significant reduction in pupil size in eyes receiving Moxifloxacin (P = .004)

Allergan data on file.

Summary: Gatifloxacin Safety and Tolerability

- Gatifloxacin was safe and well-tolerated by children and adults
- Most frequently reported adverse events (occurred in 5-10%) were conjunctival irritation, increased lacrimation, keratitis, and papillary conjunctivitis
- Adverse events were minimal with no significant differences between gatifloxacin and placebo

Challenges and Progress in the Treatment and Prevention of Ocular Infection

Summary

Challenges in the Treatment, Prevention of Ocular Infection

- While the causative agents of ocular infection are diverse, most infections are caused by Gram-positive bacteria
- Emerging ocular resistance poses a significant problem to the clinician
  - Many of the older classes of antibiotics are ineffective against many bacterial species
  - The 4th-generation fluoroquinolones act on DNA gyrase and topoisomerase IV
    - The presence of these two targets will require two simultaneous mutations for resistance

Progress in the Treatment, Prevention of Ocular Infection

- An effective anti-infective for the treatment of ocular infection should have a broad spectrum of activity, a high level of antimicrobial susceptibility, and a favorable pharmacokinetic profile
- The effect of the type of surgical wound on the incidence of postsurgical bacterial infection should be considered and appropriate prophylactic therapy initiated

Gatifloxacin, a New Therapeutic Agent for Ocular Infection

- Gatifloxacin is a highly effective 4th-generation fluoroquinolone
  - 2- to 6-fold better than moxifloxacin vs Gram-negatives
  - 2- to 6-fold better than moxifloxacin vs atypicals
- Decreased likelihood of resistance vs 3rd generation
- Gatifloxacin has a favorable pharmacokinetic profile
- Gatifloxacin is safe and well-tolerated

Thank you