CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-308

MICROBIOLOGY REVIEW(S)

Division of Anti-Infective and Ophthalmology Products
Clinical Microbiology Review

NDA: 022308 SN000

Date Company Submitted: 4 March 2009
Date Assigned: 6 March 2009
Date Completed: 25 March 2009
Reviewer: Kerry Snow MS

NAME AND ADDRESS OF APPLICANT:

Bausch & Lomb
1400 North Goodman Street
Rochester, NY 14609 USA

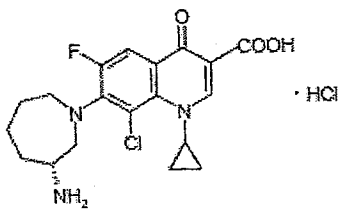
CONTACT PERSON:

Jennifer S. Knicley
Manager, Regulatory Affairs
585-338-6307

DRUG PRODUCT NAMES:

Proprietary Name: Besifloxacin
Proposed Trade Name: OPTURA™
Code Names: SS734, BOL-303224-A, FC-124
Established Name: Besifloxacin hydrochloride
Chemical Name: 3-quinolinecarboxylic acid, 7-[(3*R*)-3-aminohexahydro-1*H*-azepin-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-, monohydrochloride

STRUCTURAL FORMULA:



MOLECULAR FORMULA:
$C_{19}H_{21}ClFN_3O_3 \cdot HCl$

MOLECULAR WEIGHT:
430.30

DRUG CATEGORY:
Antimicrobial

PROPOSED DOSAGE FORM AND STRENGTH:
7.5 mL bottle containing 5 mL of besifloxacin hydrochloride ophthalmic suspension, 0.6% base (6 mg/mL)

ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
Instill one drop in the affected eye(s) 3 times a day for 7 days

DISPENSED:
Rx

PROPOSED INDICATION:
Treatment of bacterial conjunctivitis

RELATED DOCUMENTS:
IND 64335

TYPE OF SUBMISSION:
New Drug Application

PURPOSE OF SUBMISSION:

The Applicant has submitted an amendment to NDA 22308 which addresses requests made by the Agency (communicated 2 December 2008) for clarification and corrections of data and study summaries, presented in the original submission.

REMARKS:

Amendments to the original NDA have been reviewed. All requested clarifications and corrections have been addressed by the Applicant. These, and additional changes to the Microbiology Section of the NDA, are summarized in Table 1. Corrected tables and summary information do not require substantive changes to the clinical microbiology review of the original NDA submission (submitted 2 June 2008). Where discrepancies were noted between summarized information and data submitted by investigators or other researchers, the initial review was based on data from primary study reports.

CONCLUSION:

All requested corrections to the clinical microbiology section of NDA 22308 have been addressed. Responses from the Applicant are complete and appropriate.

FDA Request:
Please clarify and correct Table 29 in Section 2.7.2 of the Application. This table summarizes the data from all in vitro investigations of besifloxacin antimicrobial activity. Specific discrepancies include (but are not limited to) the following:
a. In Row 3, the "organism" column identifies "CDC coryneform group G" (C. pseudodiphtheriticum *and* C. striatum). *The referenced study* [redacted] *99K3020B), however, only lists the more general classification "*Corynebacterium *species" in the data tables. Please state whether identification to species level was performed on these isolates. If that identification was performed, please list MIC90 and MICrange for each species identified, and please include a complete description of the method used to identify these isolates. Since this is the only presented data that describes besifloxacin in vitro activity against* Corynebacterium *species, a line listing (including species identification, MIC against each antimicrobial tested, specimen source, specimen collection date) would be valuable for review purposes.* b(4)

B&L Response:
Identification to the species level was not performed. The organism title and corresponding footnote of the revised Section 2.7.2 Table 29 have been updated to reflect this clarification. Report [redacted] 99K3020B Appendix 1 Table 4 provides a line listing of individual MIC values for besifloxacin, norfloxacin, ofloxacin, ciprofloxacin, tobramycin, and gentamicin for each isolate. Isolates in this study were from ocular sources collected from clinical facilities throughout Japan between June 1997 and February 2000. b(4)

b. In Row 9, the "Organism" column appears to identify all Staphylococcus aureus *isolates tested in all in vitro investigations, summarized in this table. If that is the case, please review and make the appropriate corrections to all column entries (all are erroneous, with the possible exception of the right-most column). If that is not the case, please clarify the meaning of the "Organism" column for that data row.*

B&L Response:
Row 9 of revised Section 2.7.2 Table 29 has been updated with corrected values.

c. In Row 28, the "Organism" column lists "Streptococcus mitis group." Since members of this group are sought individually as indications for besifloxacin (and Streptococcus oralis, *presumably included in the* S. mitis *group, is listed again in the following row) please subdivide this column to list antimicrobial activity for each species tested in Study 500421 (the single study referenced for this group of ophthalmic pathogens).*

B&L Response:
The "Streptococcus mitis group" row 29 of Section 2.7.2 Table 29 includes 51 *S. mitis* isolates, 22 *S. oralis*, and 17 *S. sanguinis* (previously known as *S. sanguis*) isolates (thus 90 total isolates for all 3 species combined). Revised Section 2.7.2 Table 29 now includes separate rows for each of the 3 species within the *Streptococcus mitis* group (*S. mitis* in Row 28, *S. oralis* in Row 30, and *S. sanguinis* in Row 37.

d. In the footnotes, please include a definition for each resistance phenotype described in the table. The definition should include the breakpoint values used to define the particular phenotype (e.g. "penicillin-resistant Streptococcus pneumoniae": penicillin (nonmeningitis) = 8 µg/ml).

B&L Response:
Footnotes for the revised Section 2.7.2 Table 29 have been updated as requested. Resistance phenotypes were designated according to CLSI breakpoints.

e. Please make any additional corrections or clarifications, as appropriate.

B&L Response:
Revisions to correct and clarify Table 29 are listed in Table 1: Summary of changes to

Microbiology sections. Additional revisions were made to Section 2.7.2 as a result of revisions made to Table 29. These additional revisions are also listed in Table 1.

TABLE 1: Summary of changes to Microbiology sections

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_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Microbiology- 1

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Kerry Snow
3/25/2009 01:36:20 PM
MICROBIOLOGIST

Frederic Marsik
3/25/2009 03:35:46 PM
MICROBIOLOGIST

Division of Anti-Infective and Ophthalmology Products
Clinical Microbiology Review

NDA: 022308

Date Company Submitted: 2 June 2008:
Date Assigned: 9 June 2008
Date Completed: 31 December 2008
Reviewer: Kerry Snow MS

NAME AND ADDRESS OF APPLICANT:

Bausch & Lomb
1400 North Goodman Street
Rochester, NY 14609 USA

CONTACT PERSON:

Jennifer S. Knicley
Manager, Regulatory Affairs
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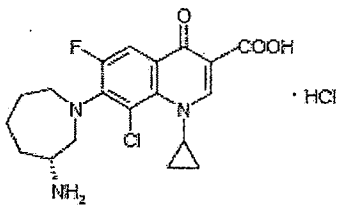

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ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
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DISPENSED:
Rx

PROPOSED INDICATION:
Treatment of bacterial conjunctivitis

RELATED DOCUMENTS:
IND 64335

TYPE OF SUBMISSION:
New Drug Application

PURPOSE OF SUBMISSION:

This New Drug Application (NDA) is submitted to seek approval for the use of Besifloxacin Hydrochloride Ophthalmic Suspension, 0.6% as base for the treatment of bacterial conjunctivitis in adults and pediatric patients one year or older. This review addresses the microbiologic efficacy of besifloxacin as a topical antibacterial. Supportive data, reviewed here, include in vitro drug characteristics (including mechanism of action, drug interaction, and development of resistance), pharmacokinetic/pharmacodynamic analysis, tentative quality control parameters employed during Phase 3 clinical trials, and correlation of in vitro activity with clinical outcomes

REMARKS:

This review is based on information submitted by the Applicant on May 30, 2008 (NDA 22308), including the Applicants summaries of studies performed to support the NDA, and microbiological data provided by the central testing laboratory, [redacted] b(4)
[redacted] This review does not address methods of besifloxacin susceptibility testing or quality control parameters that would be employed during those procedures. Breakpoints for antimicrobial susceptibility testing are not required for antimicrobials intended for topical administration, and these have not been proposed in this NDA. Criteria for inclusion of organisms in the proposed besifloxacin label was discussed with the Applicant at the End of Phase II meeting (December 2005) ["organisms that are cultured from an eye with conjunctivitis and treated with besifloxacin in 5 or more cases with a ≥ 80% eradication rate or cultured from an eye with conjunctivitis and treated with besifloxacin in 10 or more cases with a ≥ 50% eradication rate." These criteria have been used in this review to determine the appropriate organisms for inclusion in the proposed besifloxacin label.

SUMMARY AND RECOMMENDATIONS:

1) From the clinical microbiology perspective, this NDA submission may be approved, provided that the Applicant makes the changes in the microbiology subsection of the proposed label recommended by the Agency (below).

2) From the clinical microbiology perspective, submitted data and analysis supports the inclusion of the following organisms in the CLINICAL INDICATIONS section for besifloxacin:

CDC coryneform group G
*Corynebacterium pseudodiphtheriticum**
*Corynebacterium striatum**
Staphylococcus aureus
Staphylococcus epidermidis
*Staphylococcus hominis**
*Staphylococcus lugdunensis**
Streptococcus mitis
Streptococcus oralis
Streptococcus pneumoniae
*Streptococcus salivarius**
Haemophilus influenzae
*Moraxella lacunata**

*Efficacy of this organism was studied in fewer than 10 infections

3) From the clinical microbiology perspective, and based on the data presented by the Applicant, the Agency recommends the exclusion of the following organisms from the second list, for the reasons stated below.

Streptococcus mitis group
The Applicant has provided a rationale for including *S. mitis* group in the list of indicated pathogens, as opposed to *S. mitis* (species-level identification). The Applicant states that "the sponsor chose to focus on the latter group [*S. mitis* group] to avoid redundancy. The Agency agrees that the inclusion of "group" nomenclature, in addition to species-level nomenclature will result in redundancy and possible confusion. The rationale, however, must include all redundant species, in order to avoid such confusion, and would logically have to include both *S. pneumoniae* and *S. oralis* in the S. mitis group. Since both of these pathogens represent "key isolates", the Agency prefers species-level identification for all bacteria listed in the proposed indications. The preferred label eliminates *S. mitis* group and replaces it with *S. mitis*. There were 20 isolates of *S. mitis* (identified to species level) recovered in the three clinical trials, with eradication rates similar to that seen for S. mitis group (exceeding criteria for inclusion).

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EXECUTIVE SUMMARY

I. IN VITRO INFORMATION

MECHANISM OF ACTION

Data from two studies have been presented to describe the mechanism of action of besifloxacin and the possibility of interactions with human topoisomerase IV. These investigations support the claim that the mechanism of action for besifloxacin is similar to that of other fluoroquinolones, i.e. the inhibition of bacterial DNA gyrase and topoisomerase IV. While both enzymes appear to be targeted by the antimicrobial, DNA gyrase is the probably the preferred target in the tested microorganisms (*S. pneumoniae*, *S. aureus*, and *E. coli*). At high concentrations, besifloxacin interacts with human topoisomerase IV.

ANTIMICROBIAL SPECTRUM OF ACTIVITY

The Applicant has submitted data and summary information from nine studies designed to investigate the in vitro antimicrobial activity of besifloxacin against pathogens associated with ocular infections. Data from these studies suggest that besifloxacin is generally active against the isolates tested in these studies, including isolates of *S. aureus* (including methicillin-resistant *S.aureus*), *S. epidermidis*, *S. pneumoniae* (including penicillin-resistant *P. pneumoniae*), and *H. influenzae* (all sought in the proposed indications). Cross resistance was noted to methicillin-resistant isolates of staphylococcus species (quinolone and methicillin-resistant *S. aureus* MIC_{90} = 4 mcg/ml; methicillin-resistant *S. epidermidis* MIC_{90} = 4 mcg/ml; levofloxacin-resistant *S. pneumoniae* MIC_{90} = 4 mcg/ml). Activity against *P. aeruginosa* and *Acinetobacter baumanii-calcoaceticus* was poor (MIC_{90} values of >8 mcg/ml against both pathogens). The in vitro data in this submission does not support the indication of antimicrobial activity against *Corynebacterium* species, since no reviewable data is included in the NDA.

RESISTANCE STUDIES

The Applicant has submitted study data that supports chromosomal mutations, primarily in the *gyrA* gene, as a primary mechanism of besifloxacin resistance. Spontaneous mutations in this gene, as well as mutations in *gyrB* (and the topoisomerase IV genes, *parC* and *parE*, observed in pre-selected mutant isolates) occur a low frequencies ($< 1 \times 10^{-10}$ in all tested species); hence the likelihood of resistance via this mechanism is purportedly low. Chromosomal mutations result in cross-resistance to other fluoroquinolones (ciprofloxacin and moxifloxacin). The Applicant has noted that these studies have suggested "uncharacterized mechanisms" of besifloxacin resistance that may explain a significant proportion of besifloxacin non-susceptible isolates observed in these experiments. These mechanisms have not been further investigated.

BACTERICIDAL ACTIVITY

Studies submitted in support of this NDA have shown that besifloxacin demonstrates bactericidal activity against specific ocular pathogens, including *S. aureus*, *S. pneumoniae*, *S. epidermidis*, and *H. influenzae*. The measured MBC:MIC ratio for most isolates was ≤ 2. Time-kill experiments demonstrated rapid bactericidal activity against most tested ocular pathogens that was similar to or exceeded that of comparator fluoroquinolones (including ciprofloxacin, norfloxacin, and ofloxacin). Against *P. aeruginosa*, besifloxacin was less active than fluoroquinolone comparators.

II. HUMAN AND ANIMAL STUDIES

ANIMAL DISEASE MODELS

Study reports from two animal models of infection, including a study of a systemic *S. pneumoniae* infection in ICR mice, and a study of *S. aureus* endophthalmitis in New Zealand white rabbits support the in vivo efficacy of besifloxacin. In the mouse study, oral besifloxacin was shown to be protective, compared to ofloxacin and control, extending the survival of mice intraperitoneally inoculated with *S. pneumoniae* IID 553. In the rabbit study, topical besifloxacin (0.6%) was proven superior to gatifloxacin (0.3%) and saline control in limiting ophthalmic inflammation and conjunctival discharge.

PHARMACOKINETIC / PHARMACODYNAMIC STUDIES

The Applicant has submitted data from pharmacokinetic studies that demonstrates that besifloxacin 0.6%, delivered as a topical ocular application, results in very low plasma levels in both healthy individuals (< 0.35 ng/ml) and in subjects with presumptive bacterial conjunctivitis (< 0.5 ng/ml). Based on published pharmacodynamic targets for fluoroquinolones, and the presumption that besifloxacin is bound by tear fluid proteins to a degree similar to that demonstrated in human serum (~40 %), PK/PD modeling predicts that achievable free besifloxacin tear fluid concentrations exceed the therapeutic concentrations necessary for antibacterial activity against *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*.

III. CLINICAL TRIALS

The Applicant has presented data from three clinical trials designed to investigate the safety and efficacy of besifloxacin in the treatment of bacterial conjunctivitis. Two of the trials (Study 373 and 433) compared besifloxacin to vehicle. One trial (Study 434) compared besifloxacin to moxifloxacin (for non-inferiority). Besifloxacin microbial eradication rates exceeded those of Vehicle in Trials 373 and 433, and exceeded or were similar to moxifloxacin in Trail 434. Eradication rates for all key pathogens (including CDC coryneform group G, *Corynebacterium pseudodiphtheriticum*, *C. striatum*, *Haemophilus influenzae*, *Moraxella lacunata*, *Staphylococcus aureus*, *S. epidermidis*, *S. hominis*, *S. lugdunensis*, *Streptococcus mitis* group, *Streptococcus oralis*, *Streptococcus pneumoniae*, and *S. salivarius*) at Visit 2 (the primary endpoint defined in the study protocols) met or exceeded criteria for inclusion in the proposed besifloxacin indications. Overall species-specific microbial eradication by besifloxacin at Visit 2 was 92.2%.

Besifloxacin in vitro activity against key ocular pathogens (listed above) collected during the clinical trials was comparable to activity determined in pre-clinical studies. MIC_{90} values (or MIC_{50} values, in situations where fewer than 10 isolates were collected in the combined clinical trials) were within the therapeutic range predicted by PK/PD studies. The overall MIC_{90} value for isolates analyzed by the Applicant (n = 1324) was 0.5 mcg/ml. Of the key ocular pathogens isolated in the clinical trials, no single isolate demonstrated an MIC value greater than 8 mcg/ml. Elevated MIC values were observed (up to 8 mcg/ml) in ciprofloxacin-resistant staphylococcal isolates.

Susceptibility testing on microbiological failures (persistent isolates confirmed by pulsed field gel electrophoresis) indicated no development of resistance during the clinical trials (≥ 4-fold increase in baseline MIC).

Quality control was performed on each day of testing, according to procedures published by

CLSI. Tentative quality control parameters were determined during Study 373 and were employed by the central testing laboratory for susceptibility testing of isolates recovered in Studies 433 and 434. All quality control data was included in the submission and was reviewed for this report.

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INTRODUCTION

BACTERIAL CONJUNCTIVITIS

Microbial conjunctivitis may be caused by a wide variety of pathogens, including viruses, bacteria, parasites, and fungi. Viral etiology is probably the most common form of acute conjunctivitis, with the majority of cases caused by adenoviruses. Bacterial conjunctivitis is frequently associated with a compromised conjunctival epithelium [Mandell 2005], and is most commonly caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Haemophilus influenzae*.

Laboratory identification of the etiologic agents associated with cases of conjunctivitis is rarely performed. Diagnosis is usually performed by the patient, and differential diagnosis (differentiating bacterial etiology from viral etiology) is marginally significant. Most cases are self-resolving, with symptoms disappearing before bacterial culture results would be available.

There is a high rate of cure in cases of acute bacterial conjunctivitis when no treatment is given (65% within 2-5 days) [Rose 2007]. Recent meta-analysis studies have shown, however, that antibiotic treatment is associated with improved rates of clinical remission, and early and late microbiological remission [Sheikh 2001].

Treatment, if given, usually involves topical administration of a broad-spectrum antibiotic. Aminoglycosides, fluoroquinolones, and sulfacetamide are frequently prescribed as first-line agents. If antibacterials are prescribed, treatment should be guided by laboratory findings. Appropriate procedures for laboratory diagnosis of bacterial conjunctivitis include a conjunctival scraping for culture and Gram stain (and/or Giemsa stain), taken with a calcium alginate swab. Inoculation media should include blood and chocolate agar, and a fungal growth medium.

FLUOROQUINOLONE CLASS OF ANTIBIOTICS

The fluoroquinolones are concentration-dependent bactericidal antimicrobials that act by disrupting the bacterial enzymes DNA gyrase and topoisomerase IV. The fluoroquinolone class is considered "broad spectrum", but activity against specific pathogens is structure-related, with particular substituent groups providing enhanced coverage against certain bacteria [Bryskier 2005]. Fourth generation fluoroquinolones (e.g. gatifloxacin and moxifloxacin, both possessing a C-OCH_3 group at position 8) have increased activity against Gram positive pathogens and fluoroquinolone-resistant isolates [Scoper 2008].

Topical fluoroquinolones for ophthalmic indications have been used since 1990, when ciprofloxacin hydrochloride (ophthalmic drops) was approved (Ciloxan; NDA 019992). Fluoroquinolones currently marketed for ophthalmic infections (conjunctivitis and/or corneal ulcers) include ciprofloxacin (Ciloxan solution and ointment), gatifloxacin (Zymar), levofloxacin (Quixin and Iquix), moxifloxacin (Vigamox), and ofloxacin (Ocuflox).

Besifloxacin is described as a fourth-generation quinolone, intended initially for the treatment of bacterial conjunctivitis. The Applicant has developed besifloxacin (0.6% as base) as a long acting ophthalmic suspension to be dosed three times daily (TID), which will purportedly represent added convenience and compliance compared to currently marketed competitors. The formulation includes a proprietary delivery vehicle (DuraSite ®)

IN VITRO INFORMATION

MECHANISM OF ACTION

The 4-quinolones act by disrupting two bacterial enzymes, DNA gyrase and DNA topoisomerase IV (both categorized as type 2 topoisomerases). DNA gyrase is responsible for introducing negative supercoils into bacterial DNA. Topoisomerase IV (a homolog of DNA gyrase) is responsible for decatenation of DNA following replication to allow integration into daughter cells. Both enzymes are composed of two groups of two identical subunits (A and B subunits in DNA gyrase, and their homolog C and E subunits in topoisomerase IV). Specific quinolones may have greater affinity for a particular enzyme or subunit homolog, forming reversible complexes consisting of the antimicrobial, the enzyme, and the bacterial DNA. The bactericidal activity of the 4-quinolones is most likely related to the release of DNA fragments into the cellular matrix [Drlica 1997].

The Applicant has presented data from two studies designed to investigate the mechanism of action of besifloxacin.

In a study (Study PHA-005) conducted by [redacted] investigators assayed the ability of besifloxacin (SS734) to inhibit DNA gyrase-related supercoiling, decatenation by topoisomerase IV, and the promotion of cleavable complexes. b(4)

In these experiments, DNA gyrase and topoisomerase IV from *E. coli* and *S. pneumoniae* were used. Ciprofloxacin and moxifloxacin were assayed as comparators. In the DNA supercoiling assay and the decatenation assay, the concentration of fluoroquinolone required to inhibit 50% of enzyme activity (IC_{50}) was determined. In the DNA cleavage assay, 25% of maximum DNA cleavage (CC_{25}) was determined. The results of these assays are summarized in Table 1. Against *S. pneumoniae*, besifloxacin was more potent in all assays than fluoroquinolone comparators. Against *E. coli*, besifloxacin activity was comparable to comparators.

Table 1: Inhibitory activity (IC_{50}) and potency in stabilizing the cleavable complex (CC_{25}) of *S. pneumoniae* and *E. coli* DNA gyrases and topoisomerases IV

Quinolones	*S. pneumoniae* enzymes				*E. coli* enzymes			
	IC_{50}		CC_{25}		IC_{50}		CC_{25}	
	Gyrase	Topo IV	Gyrase	Topo IV	Gyrase	Topo IV	Gyrase	Topo IV
Ciprofloxacin								
-µM	40	5	40-80	2.5-5	1	27	0.15	1.5
-µg/mL	15	2	15-25	1-2	0.3	9	0.05	0.5
Moxifloxacin								
-µM	10	2.5	10-20	2.5	1.6	20	0.2	2.3
-µg/mL	4	1	4-8	1.5	0.7	9	0.07	1
Besifloxacin								
-µM	2.5	1	2.5	1	2.3	23	0.1	1.4
-µg/mL	1	0.4	1	0.4	1	10	0.04	0.6

Topo IV = topoisomerase IV

CC_{25} is the drug concentration that produces 25% linearization of the DNA under the reaction conditions used.

Source: Study PHA-005, Table 1

In the same study, quinolone-resistant mutant isolates of *S. aureus*, *E. coli*, and *S. pneumoniae* were selected in a two-step procedure, with analysis of the quinolone-resistance determination region (QRDR) following each isolation step. From these studies, the sponsor determined that

DNA gyrase is the primary target for besifloxacin in *E. coli* (gyrA mutations observed in first- and second-step selection), with no proven target role for topoisomerase IV. Resistant *E. coli* isolates lacking QRDR mutations were also identified in these investigations, which the Applicant proposes may be related to decreased permeability, increased efflux, or other mutations.

In *S. pneumoniae*, DNA gyrase was identified as the primary target (first-step selection mutants), with topoisomerase IV identified as a secondary target (mutants identified in second-step selection). No other mutations (non-QRDR-associated resistance) were identified in this investigation.

The investigators in this study were unable to identify the primary target for besifloxacin in isolates of *S. aureus*. Two successive selection steps produced no resistant mutants. In other experimental conditions (i.e. tests using previously selected *parC* mutants) the investigators were able to demonstrate the selection of *gyrA* mutants. The researchers conclude that the data supports a contention that besifloxacin exerts dual targeting of both DNA gyrase and topoisomerase IV, with a possible preference for DNA gyrase is certain conditions.

In a study (Study PHA-006) of the mechanism of action of besifloxacin conducted by [redacted] investigators assayed besifloxacin inhibition of ATP-dependent DNA decatenation by topoisomerase IIα, the effect of besifloxacin on DNA relaxation by topoisomerase IIα, and the formation of drug-stimulated cleavable complexes. The study intended to investigate the action of besifloxacin on human topoisomerase activity. Etoposide, a known topoisomerase IIα inhibitor was used as a control. Interactions of besifloxacin with human topoisomerase IV were compared to interactions with *Streptococcus pneumoniae* gyrase and topoisomerase IV, with ciprofloxacin and moxifloxacin assayed as comparator fluoroquinolones. The investigators concluded that besifloxacin interacted with human topoisomerase IV at high concentrations, and that bacterial gyrase and topoisomerase IV were more sensitive by several orders of magnitude. The results are summarized in Tables 2 and 3.

b(4)

Table 2: Comparison of Inhibitory Activity (IC_{50}) and potency in stabilizing the Cleavable Complex (CC_{15}) of human topoisomerase IIα

Drug	IC_{50} (μM)		CC_{15} (μM)[a]
	Decatenation	Relaxation	Cleavage
Besifloxacin	1000 - 1250	500	>2000
Etoposide	424	100	850

[a] CC_{15} is the drug concentration that produces 15% linearization of the DNA under the reaction conditions used.

Source: Study PHA-006, Table 1

Table 3: Inhibitory Activity (IC_{50}) and potency in stabilizing the Cleavable Complex (CC_{25}) of *S. pneumoniae* and topoisomerase IV

Drug	IC_{50} (μM)		CC_{25} (μM)[a]	
	Gyrase (Supercoiling)	Topo IV (Decatenation)	Gyrase	Topo IV
Ciprofloxacin	40	5	40-80	2.5-5
Moxifloxacin	10	2.5	10-20	2.5
Besifloxacin	2.5	1	2.5	1

[a] CC_{25} is the drug concentration that produces 25% linearization of the DNA under the reaction conditions used.

Source: Study PHA-006, Table 2

In Summary:
Study PHA-005 demonstrated that besifloxacin inhibits the activity of DNA gyrase and topoisomerase IV in isolates of *S. pneumoniae* and *E. coli*, and that detectable levels of cleavage products are formed in the presence of the antimicrobial. The study also provided supportive data for the contention that DNA gyrase is the primary target for besifloxacin against *S. pneumoniae* and *E.coli*, and against *S. aureus* in certain conditions.
Study PHA-006 demonstrated that at high drug levels, besifloxacin interacts with human topoisomerase IV. This activity is greater than that determined for the comparator fluoroquinolones (ciprofloxacin and moxifloxacin), but the sensitivity of human enzymes to the antimicrobial is lower by several orders of magnitude than for bacterial gyrase and topoisomerase IV.

Mechanism of Action Studies ~ Conclusions:
Data from two studies have been presented to describe the mechanism of action of besifloxacin and the possibility of interactions with human topoisomerase IV. These investigations generally support the claim that the mechanism of action for besifloxacin is similar to that of other fluoroquinolones, i.e. the inhibition of bacterial DNA gyrase and topoisomerase IV. While both enzymes appear to be targeted by the antimicrobial, DNA gyrase is the probably the preferred target in the tested microorganisms (*S. pneumoniae*, *S. aureus*, and *E. coli*). At high concentrations, besifloxacin interacts with human topoisomerase IV.

ANTIMICROBIAL SPECTRUM OF ACTIVITY

The Applicant has submitted data from a number of studies designed to investigate the in vitro antimicrobial activity of besifloxacin. They include:

- Study [redacted] 99K3020B [redacted] b(4)
  an investigation of the in vitro antimicrobial activity of besifloxacin, compared to fluoroquinolone and aminoglycoside comparators, against ophthalmic isolates (including Gram positive, Gram negative, aerobic, anaerobic, and drug-resistant isolates) collected in Japan from 1997 until 2000. In addition to the determination of MIC values, the investigation included time-kill studies of besifloxacin and fluoroquinolones comparators.
- Study SS734PRE-003 [redacted] b(4)
  a study of the in vitro activity of (±) SS734 (besifloxacin) and its enantiomers, (+)SS734 and (-)SS734, and the fluoroquinolone comparator ofloxacin, against a reference collection of Gram positive and Gram negative pathogens associated with ophthalmic infections.
- Study BL-MIC-001B [redacted] b(4)
  an investigation of the in vitro activity of besifloxacin, compared to ofloxacin against isolates associated with bacterial conjunctivitis (100 each: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*). All isolates were collected in 2000, except the *M. catarrhalis* isolates, which were collected between 1996 and 1998.
- Study BL-MIC-002B [redacted] b(4)
  an investigation of the in vitro activity of besifloxacin, compared to ofloxacin against 100 isolates each of *Acinetobacter* sp. *Enterobacter* sp., *Proteus mirabilis*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. Isolates were acquired between 1998 and 2005.
- Study [redacted] 06-15RB [redacted] b(4)
  a study designed to assess the performance of 2 mcg, 5 mcg, and 10 mcg besifloxacin disks, using a CLSI-approved disk diffusion susceptibility test method against recent isolates (n ≥ 100) of *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *H. influenzae*, *Enterobacter cloacae*, and *Neisseria gonorrhoeae*, and to propose besifloxacin disk diffusion breakpoints. Comparators were gatifloxacin and moxifloxacin.
- Study [redacted] 07-12R2 [redacted] b(4)
  an investigation of the in vitro activity of besifloxacin against at least 100 isolates each of *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *H. influenzae*, *E. cloacae*, and *N. gonorrhoeae*. Comparators were gemifloxacin, azithromycin, and tobramycin (and gatifloxacin and moxifloxacin for most *N. gonorrhoeae* isolates).
- Study 07-MIC-392 [redacted] b(4)
  : a study performed in support of B&L Report Number PH06164 "Efficacy in a Rabbit Model of *S. aureus* Endophthalmitis", to determine the minimum inhibitory concentrations of besifloxacin (SS734) and comparators (gatifloxacin, moxifloxacin, levofloxacin) against ATCC strains of *S. aureus* used in the animal efficacy model.
- Study 500421 [redacted] b(4)
  : an investigation of the in vitro activity of besifloxacin against 1179 aerobic and anaerobic isolates of Gram negative and Gram positive bacteria. Comparators included azithromycin, ceftazidime (Gram-negative spp only), clindamycin (anaerobes only), ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, metronidazole (anaerobes only), penicillin (streptococci only), oxacillin (staphylococci only) and tobramycin.
- Study 500510 [redacted] b(4)
  an investigation to determine the minimum inhibitory concentration and minimum bactericidal concentration of besifloxacin against recent isolates of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*.

These studies are discussed in detail below.

b(4)

Study [redacted] 99K3020B, "Antimicrobial Activity of SS732 and (+)SS734"

In this investigation, [redacted] investigated the in vitro activity of SS734 (besifloxacin) and SS732 (a fluoroquinolone antimicrobial not discussed in this NDA). The researchers determined MICs by agar dilution methods using procedures described by CLSI. Tested isolates were collected from clinical facilities in Japan from 1997 through 2000. Comparators included ciprofloxacin, norfloxacin, ofloxacin, gentamicin, and tobramycin. The study report includes no information describing quality control procedures used in the course of these investigations. The study results are summarized in Tables 4 through 6. The study also included time-kill studies designed to investigate the bactericidal activity of the two fluoroquinolones. The results of the time-kill studies are discussed elsewhere in this review.

b(4)

Table 4: MIC of besifloxacin and comparators against Gram positive aerobic and anaerobic pathogens

Organism	Drug	MIC (µg/ml)		
		50%	90%	Range
S. pneumoniae n=30	Besifloxacin	0.13	0.13	≤ 0.06 - 0.13
	Ciprofloxacin	1	1	0.5 - 2
	Norfloxacin	4	8	1 - 16
	Ofloxacin	2	2	1 - 2
	Gentamicin	4	8	1 - 8
	Tobramycin	16	16	1 - 16
S. aureus N=30	Besifloxacin	≤ 0.06	1	≤ 0.06-2
	Ciprofloxacin	0.25	64	0.25-128
	Norfloxacin	1	>128	0.5->128
	Ofloxacin	0.25	32	0.25-128
	Gentamicin	0.25	32	0.13-128
	Tobramycin	0.5	128	0.13->128
Coagulase Negative *Staphylococcus* n=30	Besifloxacin	≤ 0.06	0.5	≤ 0.06 - 0.5
	Ciprofloxacin	0.25	16	0.13 - 64
	Norfloxacin	1	128	0.25 - 128
	Ofloxacin	0.5	8	0.25 - 16
	Gentamicin	0.13	64	≤ 0.06 - >128
	Tobramycin	0.13	32	≤ 0.06 - >128
Corynebacterium species n=30	Besifloxacin	0.25	2	≤ 0.06 - 2
	Ciprofloxacin	0.5	8	≤ 0.06 - 64
	Norfloxacin	2	16	0.5 - 64
	Ofloxacin	1	32	0.13 - 128
	Gentamicin	≤ 0.06	0.13	≤ 0.06 - 4
	Tobramycin	≤ 0.06	0.13	≤ 0.06 - 8
Anaerobic Organisms				
Organism	Drug	MIC (µg/ml)		
		50%	90%	Range
Propionibacterium acnes n=30	Besifloxacin	0.25	0.25	0.25 - 0.5
	Ciprofloxacin	0.5	0.5	0.5
	Norfloxacin	4	4	2 - 8
	Ofloxacin	1	1	0.5 - 1
	Gentamicin	16	16	8 - 16
	Tobramycin	64	128	32 - 128

Source: Table 10 Section 2.7.2; This submission

Table 5: MIC of besifloxacin and comparators against Gram negative pathogens

Organism	Drug	MIC (µg/ml)		
		50%	90%	Range
H.influenzae n=30	Besifloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Norfloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Ofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Gentamicin	2	2	0.5 - 2
	Tobramycin	1	2	1 - 4
Moraxella species n=30	Besifloxacin	≤ 0.06	0.13	≤ 0.06 - 0.13
	Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Norfloxacin	0.25	0.25	0.13 - 0.25
	Ofloxacin	≤ 0.06	0.13	≤ 0.06 - 0.13
	Gentamicin	0.25	0.25	≤ 0.06 - 0.25
	Tobramycin	0.25	0.25	≤ 0.06 - 0.5
*N. gonorrhoeae** n=30	Besifloxacin	≤ 0.06	0.5	≤ 0.06 - 4
	Ciprofloxacin	≤ 0.06	0.5	≤ 0.06 - 16
	Norfloxacin	≤ 0.06	4	≤ 0.06 - 32
	Ofloxacin	≤ 0.06	0.5	≤ 0.06 - 16
	Gentamicin	2	4	1 - 4
	Tobramycin	2	4	1 - 4
P. aeruginosa n=30	Besifloxacin	2	4	1 - 8
	Ciprofloxacin	0.25	0.5	0.13 - 2
	Norfloxacin	1	4	0.5 - 8
	Ofloxacin	1	4	0.5 - 8
	Gentamicin	4	8	1 - >128
	Tobramycin	1	2	0.5 - 64

***Obstetric and Gynecologic Isolates**
Source: Table 11 Section 2.7.2; This submission

Table 6: MIC of besifloxacin and comparators against drug-resistant Gram-positive and Gram-negative pathogens

Gram Positive Organism	Drug	MIC (µg/ml)		
		50%	90%	Range
Ofloxacin-Resistant *S. aureus* n=10	Besifloxacin	2	8	0.5 - 8
	Ciprofloxacin	>128	>128	32 - >128
	Norfloxacin	>128	>128	64 - >128
	Ofloxacin	>128	>128	16 - >128
Gentamicin-Resistant *S. aureus* n=10	Besifloxacin	0.5	4	≤ 0.06 - 8
	Ciprofloxacin	32	>128	0.25 - >128
	Norfloxacin	128	>128	0.5 - >128
	Ofloxacin	32	>128	0.25 - >128
Penicillin-Resistant *S. pneumoniae* n=10	Besifloxacin	0.13	0.13	≤ 0.06 – 0.13
	Ciprofloxacin	1	1	0.5 - 2
	Norfloxacin	4	8	2 - 8
	Ofloxacin	2	2	1 - 2
Gram Negative Organism	**Drug**	**50%**	**90%**	**Range**
Gentamicin-Resistant *P. aeruginosa* n=10	Besifloxacin	4	32	0.5 - 32
	Ciprofloxacin	1	32	0.13 - 32
	Norfloxacin	2	128	0.5 - 128
	Ofloxacin	4	64	0.5- 128
Ampicillin-Resistant *H. influenzae* n=10	Besifloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Ciprofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Norfloxacin	≤ 0.06	≤ 0.06	≤ 0.06
	Ofloxacin	≤ 0.06	≤ 0.06	≤ 0.06
Ofloxacin-Resistant Enterobacteriaceae n=10	Besifloxacin	8	16	4 - 32
	Ciprofloxacin	8	32	4 - 32
	Norfloxacin	32	128	16 - 128
	Ofloxacin	16	64	8 - 64

Source: Table 12 Section 2.7.2; This submission

In this study, bacteria commonly associated with ophthalmic infections were tested against besifloxacin and selected comparators. Thirty isolates of each species were analyzed, with some species sub-classified by resistance phenotype (10 isolates of each identified resistance phenotype were tested). Against *S. pneumoniae* (besifloxacin MIC_{90} = 0.13 mcg/ml), *S. aureus* (besifloxacin MIC_{90} = 1 mcg/ml), and coagulase-negative staphylococci (besifloxacin MIC_{90} = 0.5 mcg/ml), besifloxacin was more active than all comparators. Against *Corynebacterium* species (besifloxacin MIC_{90} = 2 mcg/ml), besifloxacin was more active than fluoroquinolone comparators but was less active than aminoglycoside comparators. Against *Propionibacterium acnes* (besifloxacin MIC_{90} = 0.25 mcg/ml), besifloxacin was more active than all comparators. Activity of besifloxacin against Gram positive isolates in summarized in Table 4.

In tests involving Gram negative pathogens (Table 5), besifloxacin activity was comparable (MIC_{90} within 2 doubling dilutions) to fluoroquinolones comparators against *H. influenzae* (besifloxacin $MIC_{90} \leq 0.06$ mcg/ml), *Moraxella* sp. (besifloxacin MIC_{90} = 0.13 mcg/ml), *N. gonorrhoeae* (besifloxacin MIC_{90} = 0.5 mcg/ml) , and *P. aeruginosa* (besifloxacin MIC_{90} = 4 mcg/ml), and was more active than aminoglycoside comparators (MIC_{90} > 2 doubling dilutions) against *H. influenzae* and *N. gonorrhoeae*.

Against isolates with specific resistance phenotypes (Table 6), Besifloxacin activity (measured as MIC_{90}) was equal to or greater than all fluoroquinolone comparators. Activity against ofloxacin- and gentamicin-resistant *S. aureus* was at least 5-fold greater than fluoroquinolone comparators. MIC_{90} values for besifloxacin against gentamicin-resistant *P. aeruginosa* (32 mcg/ml) and ofloxacin-resistant Enterobacteriaceae (16 mcg/ml), however, exceeded the susceptible range for fluoroquinolones listed in CLSI document M100-S18 (CLSI 2008).

Corynebacterium species tested in this study (n = 30) were not identified to species level. In summary tables, compiled from all preclinical in vitro investigations, Study [redacted] 99K3020B is the only investigation listed that includes data for Corynebacterium species. The Applicant is seeking indications for besifloxacin treatment of ophthalmic infections by CDC coryneform Group G, *Corynebacterium pseudodiphtheriticum*, and *C. striatum.* b(4)

The usefulness of Study [redacted] 99K3020B in understanding the in vitro efficacy of besifloxacin is limited by several factors. First, all isolates were collected outside of the United States. Second, no information has been provided detailing the specific source of the tested isolates or their date of collection. Finally, no information is available describing quality control procedures used in the course of these investigations. b(4)

Study SS734PRE-003 "In vitro antibacterial activities of (±)SS734, (+)SS734 and (-)SS734 b(4)

In this investigation, conducted by [redacted] in 1993, the SS734 enantiomers (± SS734, -SS734, and +SS734) were tested to determine their antimicrobial activity against selected reference isolates, including isolates with known resistance phenotypes (methicillin- and penicillin-resistant *S. aureus*). The (+)SS734 enantiomer is being developed as the proposed ophthalmic product, besifloxacin. The researchers used agar dilution methods, based on guidelines approved by the Japanese Society of Chemotherapy. No information has been provided in the study report regarding quality control procedures, ranges, or organisms. The results of these studies are summarized in Tables 7 through 10.

Table 7: Antibacterial activities of (±)SS734, (+)SS734), (-)SS734) and ofloxacin against Gram positive bacteria

Strain	MIC (µg/mL)			
	(±)SS734	(+)SS734	(–)SS734	Ofloxacin
Bacillus subtilis ATCC 6633	0.025	0.012	0.025	0.1
S. aureus ATCC 25923	0.012	0.012	0.05	0.2
S. aureus FDA 209P	0.05	0.05	0.1	0.39
S. aureus Terajima	0.2	0.1	0.2	0.78
S. aureus Smith	0.025	0.012	0.025	0.2
S. aureus IID 980	0.025	0.025	0.05	0.2
S. aureus IID 5220	0.05	0.025	0.1	0.39
S. epidermidis ATCC 12228	0.1	0.05	0.1	0.78
Sarcina lutea ATCC 9341	0.1	0.1	0.2	3.13
Enterococcus faecalis IFO 12964 [a]	0.2	0.2	0.39	1.56
E. faecalis ATCC 29212 [a]	0.2	0.2	0.39	1.56
Micrococcus lysodeikticus IFO 3333	0.2	0.2	0.39	1.56

Source: Table 13 Section 2.7.2; This submission

Table 8: Antibacterial activities of (±)SS734, (+)SS734, (-)SS734 and ofloxacin against methicillin or penicillin resistant *S. aureus*

Strain	MIC (µg/mL)			
	(±)SS734	(+)SS734	(–)SS734	Ofloxacin
Methicillin resistant strains				
S. aureus No. 395	0.025	0.025	0.1	0.39
S. aureus No. 415	0.025	0.012	0.05	0.2
S. aureus No. 419	0.05	0.025	0.1	0.39
S. aureus No. 420	0.025	0.012	0.05	0.2
S. aureus No. 421	0.05	0.025	0.1	0.39
S. aureus ATCC 33591	0.05	0.025	0.1	0.39
S. aureus ATCC 33592	0.025	0.012	0.05	0.2
S. aureus ATCC 33593	0.025	0.025	0.05	0.2
Penicillin resistant strains of				
S. aureus ATCC 11632	0.025	0.025	0.05	0.2
S. aureus ATCC 13301	0.025	0.012	0.05	0.2

Source: Table 14 Section 2.7.2; This submission

Table 9: Antibacterial activities of (±)SS734, (+)SS734 (-)SS734 and ofloxacin against *S. pneumoniae* and *Streptococcus pyogenes*

Strain	MIC (µg/mL)			
	(±)SS734	(+)SS734	(–)SS734	Ofloxacin
S. pneumoniae IID 552	0.2	0.1	0.2	1.56
S. pneumoniae IID 553	0.2	0.1	0.2	1.56
S. pneumoniae IID 554	0.1	0.1	0.2	0.78
S. pneumoniae IID 555	0.1	0.1	0.2	1.56
S. pneumoniae IID 557	0.1	0.1	0.2	1.56
S. pneumoniae GIFU 3192	0.1	0.1	0.2	0.78
S. pyogenes IID 693	0.2	0.1	0.2	1.56
S. pyogenes IID 698	0.1	0.05	0.1	0.78
S. pyogenes Cook	0.1	0.05	0.1	0.78

Source: Table 15 Section 2.7.2; This submission

Table 10: Antibacterial activities of (±)SS734, (+)SS734, (-)SS734 and ofloxacin against Gram negative bacteria

Strain	MIC (µg/mL)			
	(±)SS734	(+)SS734	(–)SS734	Ofloxacin
E. coli O-1	0.1	0.1	0.1	0.1
E. coli ATCC 25922	0.1	0.1	0.1	0.05
E. coli K-12	0.1	0.1	0.1	0.1
Salmonella typhi TD	0.05	0.05	0.05	0.05
Shigella flexneri 2b	0.012	0.012	0.012	0.012
P. aeruginosa IFO 12582	3.13	1.56	3.13	3.13
P. aeruginosa IFO 13736	1.56	1.56	1.56	1.56
P. aeruginosa P_2	1.56	1.56	1.56	1.56
P. aeruginosa ATCC 27853	3.13	1.56	3.13	3.13
P. aeruginosa IID 5086	1.56	1.56	1.56	0.78
Klebsiella pneumoniae ATCC 10031	0.012	0.012	0.012	0.012
K. pneumoniae IFO 13541	0.025	0.025	0.05	0.025
Proteus vulgaris OXK	0.05	0.025	0.05	0.05
Proteus rettgeri	0.2	0.2	0.39	0.2
Serratia marcescens NHL	0.2	0.2	0.2	0.1

Source: Table 16 Section 2.7.2; This submission

The (+)SS734 enantiomer is being developed as the proposed ophthalmic product, besifloxacin. In these studies, this enantiomer was the most active against tested isolates, and was as active or more active against all tested Gram positive pathogens (including resistant phenotypes) than the comparator, ofloxacin. Against Gram negative isolates, (+)SS734 was as active or more active than the other forms of SS734, and was comparable to ofloxacin overall (all MIC values were within 1 doubling dilution). Activity of the (+)SS734 enantiomer against resistant S. aureus phenotypes was comparable to that against the other tested S. aureus isolates.

Analysis of Study SS734PRE-003 is limited by several factors. The method employed for agar dilution is not a method approved by CLSI. No information was provided in the study report, documenting quality control procedures, organisms, or acceptable interpretive ranges. Although SS734 enantiomers and the fluoroquinolone control (ofloxacin) were tested against isolates described as "methicillin-resistant strains" and "penicillin-resistant strains", the susceptibility patterns of these isolates, with regard to penicillin and methicillin, were not confirmed or reported.

Study BL-MIC-001B "MIC50 and MIC90 Determinations of SS734 against Selected Bacterial Conjunctivitis Target Pathogen Populations"

Study BL-MIC-001 was performed by [redacted] in 2005 and amended as Study BL-MIC-001*B* to include additional analysis tables. [redacted] tested 100 isolates each of 5 bacterial species commonly associated with ophthalmologic infection, including *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. Isolates were further characterized by resistance phenotype (e.g. methicillin-resistant *S. aureus*, penicillin-resistant *S. pneumoniae*, and ß-lactamase positive *H. influenzae*).
Testing was performed using microbroth dilution techniques approved by CLSI. Microtiter plates were produced in-house. Quality control procedures were performed on each day of testing. The sponsor has provided all quality control results (with selected QC organisms and associated ranges of acceptable response). Tested isolates were primarily collected from respiratory sources between 1996 and 2000. The geographic origin of the isolates was not reported. Data

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from this study is summarized in Tables 11 and 12.

Table 11: MIC of besifloxacin and ofloxacin against Gram positive and Gram negative pathogens associated with bacterial conjunctivitis

Organism	N	Besifloxacin		Ofloxacin	
		MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
S. aureus (All)	100	0.06	2	1	>8
S. aureus (MSSA)	49	0.06	0.12	0.5	2
S. aureus (MRSA)	51	1	4	≥ 8	≥ 8
S. pyogenes	100	0.06	0.12	1	4
S. pneumoniae	100	0.12	0.12	2	2
H. influenzae	100	0.03	0.06	0.03	0.06
Moraxella catarrhalis	100	0.06	0.12	0.12	0.12

Source: Table 17 Section 2.7.2; this submission

Table 12: Comparison of the activity of besifloxacin and ofloxacin against drug-resistant pathogens associated with bacterial conjunctivitis

Phenotype and Species	N	Drug	MIC Values (µg/mL)			
			Minimum	Maximum	MIC_{50}	MIC_{90}
Methicillin-Resistant *S. aureus*	51	Besifloxacin	0.03	8	1	4
		Ofloxacin	0.25	>8	≥8	≥8
Methicillin-Susceptible *S. aureus*	49	Besifloxacin	0.015	4	0.06	0.12
		Ofloxacin	0.25	>8	0.5	2
Penicillin-Resistant *S. pneumoniae*	33	Besifloxacin	0.06	0.25	0.12	0.12
		Ofloxacin	1	4	2	2
Penicillin-Susceptible *S. pneumoniae*	67	Besifloxacin	0.06	1	0.12	0.12
		Ofloxacin	0.5	>8	2	2
β Lactamase Positive *H. influenzae*	50	Besifloxacin	0.015	0.12	0.03	0.06
		Ofloxacin	0.03	0.12	0.03	0.06
β Lactamase Negative *H. influenzae*	50	Besifloxacin	0.008	0.12	0.03	0.06
		Ofloxacin	≤0.004	0.12	0.03	0.06

Source: Table 18 Section 2.7.2; this submission

The results of this investigation suggest that besifloxacin is more active against selected Gram positive pathogens than the fluoroquinolone comparator (ofloxacin). Against tested Gram negative pathogens (*H. influenzae* and *M. catarrhalis*), besifloxacin activity is similar to the comparator. Besifloxacin was active against resistant isolates, although the MIC_{90} against methicillin-resistant *S. aureus* was five-fold higher than for methicillin-susceptible *S. aureus*.

Study BL-MIC-002B "MIC50 and MIC90 determinations of SS734 against selected bacterial conjunctivitis target pathogen populations"

Study BL-MIC-002 was performed by [redacted] in 2005 and was amended as BL-MIC-002B to incorporate additional data analysis. The

b(4)

investigators tested 100 isolates each of five bacterial genera. Some genera (*Acinetobacter, Enterobacter, Pseudomonas*) were sub-classified by species or resistance phenotype for additional analysis.

Isolates were tested using microbroth dilution methods approved by CLSI. Microtiter test plates were produced in-house by [redacted] Quality control was performed on every day of testing, using QC organisms and ranges listed in CLSI document M100-S15 for ofloxacin testing. Tested isolates were collected by [redacted] between 1999 and 2004. The majority of isolates were not identified by collection source, and no information concerning geographic origin was provided. The results of the study are summarized in Table 13. b(4) b(4)

Table 13: MIC of besifloxacin and ofloxacin against Gram negative pathogens associated with bacterial conjunctivitis

Organism	N	Besifloxacin		Ofloxacin	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Acinetobacter species	100	0.5	>8	0.5	>8
Acinetobacter lwoffii	13	0.5	0.5	0.25	0.5
Acinetobacter baumanii	48	1	2	0.5	2
Acinetobacter baumanii-calcoaceticus	33	0.5	>8	0.5	>8
Enterobacter species	100	0.25	2	0.12	1
Enterobacter cloacae	59	0.25	0.5	0.12	1
Enterobacter aerogenes	39	0.25	0.25	0.12	2
Proteus mirabilis	100	0.5	1	0.12	0.5
P. aeruginosa[a]	100	4	>8	4	>8
P. aeruginosa, CR[b]	10	>8	>8	>8	>8
S. marcescens	100	1	2	0.5	1

[a]*P. aeruginosa* was also tested against levofloxacin resulting in MIC_{50} and MIC_{90} values of 1 and >8 µg/mL
[b]Ciprofloxacin-resistant
Source: Table 19 Section 2.7.2; this submission

In Study BL MIC—002B, besifloxacin activity against selected Gram negative pathogens was similar to that of ofloxacin, with MIC_{90}s within 1 doubling dilution against all tested species. Both fluoroquinolones were inactive (MIC_{90} values outside of the upper testing limit) against *Acinetobacter* sp., *Acinetobacter baumanii-calcoaceticus*, and *P. aeruginosa*.

Study [redacted] 06-15RB "Relative in vitro antimicrobial potency of SS734 and determination of optimal disk mass" b(4)

Study [redacted] 06-15R was performed by the [redacted] in 2006 and amended as [redacted] 06-15RB to include information concerning isolate receipt dates and besifloxacin preparation methods. The study was conducted to investigate the in vitro activity of besifloxacin against isolates of *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *H. influenzae*, *E. cloacae*, and *N. gonorrhoeae* (at least 100 isolates of each), and to determine an optimal disk mass for disk diffusion antimicrobial susceptibility testing. Disk diffusion susceptibility testing for besifloxacin is not proposed in this application, and data regarding the development of disk diffusion breakpoints was not submitted in the study summaries. Tested isolates were received by [redacted] from 1997 through 2006 (with the majority received between 2002 and 2006). The study b(4)

report includes no information regarding the clinical source of the isolates and no information regarding the geographic origin of the isolates.

Susceptibility testing was performed by broth microdilution and disk diffusion techniques using methods approved by CLSI. Quality control was performed on each day of testing, using reference isolates appropriate for the organisms being tested. All quality control results were submitted with the study report, and all results were within acceptable QC ranges, as published in CLSI guidelines. Study results are summarized in Tables 14 and 15.

Table 14: MIC of besifloxacin, gatifloxacin, and moxifloxacin against Gram positive and Gram negative pathogens associated with bacterial conjunctivitis

Organism	n	MIC_{50}/MIC_{90} (µg/mL)		
		Besifloxacin	Gatifloxacin	Moxifloxacin
H. influenzae	105	0.03/0.06	0.015/0.03	0.03/0.06
H. influenzae, ß-lactamase-negative	27	0.03/0.03	0.015/0.03	0.03/0.03
H. influenzae, ß-lactamase positive	53	0.03/0.03	0.015/0.03	0.03/0.06
N. gonorrhoeae	107	0.008/0.015	0.004/0.008	0.015/0.015
E. cloacae	92	0.25/8	0.06/2	0.12/4
E. cloacae-ESBL+	15	8/>8	2/>8	4/>8
S. aureus (All)	103	0.06/2	0.25/8	0.12/8
S. epidermidis (All)	102	0.06/4	0.12/>8	0.12/>8
S. pneumoniae (All)	103	0.12/1	0.25/8	0.25/4

ESBL+= extended spectrum β-lactamase
Source: Table 20 Section 2.2.2; this submission

Table 15: MIC of besifloxacin, gatifloxacin, and moxifloxacin against drug-resistant Gram positive and Gram negative pathogens associated with bacterial conjunctivitis

Organism	n	MIC_{50}/MIC_{90} (µg/mL)		
		Besifloxacin	Gatifloxacin	Moxifloxacin
H. influenzae, ß-lactamase + ampicillin-R	25	0.03/0.12	0.03/0.03	0.06/0.12
S. aureus, MR, CR	24	1/8	8/>8	8/>8
S. aureus, MR, CS	25	0.03/0.06	0.06/0.12	0.06/0.12
S. aureus, MS, CS	28	0.03/0.06	0.12/0.25	0.06/0.12
S. aureus, vancomycin-I	23	1/2	4/8	4/8
S. epidermidis, MR	64	0.5/8	2/>8	1/>8
S. epidermidis, MS	38	0.03/0.5	0.12/2	0.06/2
S. pneumoniae, levofloxacin-R	25	1/2	4/>8	2/>8
S. pneumoniae, penicillin-S	26	0.12/0.12	0.25/0.5	0.25/0.25
S. pneumoniae, penicillin-I	26	0.12/0.12	0.25/0.25	0.12/0.25
S. pneumoniae, penicillin-R	26	0.12/0.12	0.25/0.5	0.25/0.25

MR = methicillin resistant; MS = methicillin susceptible; CR = ciprofloxacin resistant
CS = ciprofloxacin susceptible; S = susceptible, I = intermediate, R = resistant
Source: Table 21 Section 2.7.2; this submission

Besifloxacin was active against most tested staphylococci and streptococci, with MIC_{90} values lower than both fluoroquinolone comparators (gatifloxacin and moxifloxacin). Activity was

decreased against ciprofloxacin-resistant MRSA (MIC_{90} = 8 mcg/ml) and methicillin-resistant *S. epidermidis* (MIC_{90} = 8 mcg/ml). The investigators noted a stepwise increase in besifloxacin MIC_{90} values against *S. aureus* isolates with increasing resistance to vancomycin (the $MIC_{(geometric\ mean)}$ for vancomycin-susceptible isolates was 0.03 mcg/ml; for vancomycin-intermediate isolates, 0.652 mcg/ml; and for vancomycin-resistant isolates, 1.587 mcg/ml). The stepwise increase in MIC values was similar in the fluoroquinolone comparators. Against *Enterobacter cloacae*, besifloxacin activity was less than comparators and above the measurable range (≥ 8 mcg/ml) against isolates of *E. cloacae* positive for extended spectrum ß-lactamase (ESBL).

Study [redacted] 07-12R2 "Potency of SS734 vs. Gemifloxacin, Azithromycin, and Tobramycin b(4)

Study [redacted] 07-12R2 was performed by the [redacted] b(4)
in 2007. Researchers determined the activity of besifloxacin against 600 bacterial isolates (essentially the same set of organisms tested in Study [redacted] 06-15RB, reviewed above), using gemifloxacin, azithromycin, and tobramycin as comparators.

The tested isolates were received by [redacted] between 1997 and 2006. No information was provided in the study report, regarding the clinical source or geographic origin of the isolates. b(4)
Susceptibility testing was performed using broth microdilution methods approved by CLSI (M7-A7, 2006). Quality control was performed on each day of testing, using organisms and ranges published by CLSI. All QC results were within acceptable ranges with one exception, which was within the acceptable range on repeat testing. All quality control data was submitted with the study report.

Data from Study [redacted] 07-12R2 is summarized in Tables 16 and 17. b(4)

Table 16: MIC of besifloxacin, gemifloxacin, azithromycin, and tobramycin against Gram positive and Gram negative pathogens associated with bacterial conjunctivitis

Organism	n	MIC_{50}/MIC_{90} (µg/mL)			
		Besifloxacin	Gemifloxacin	Azithromycin	Tobramycin
H. influenzae	104	0.03/0.12	0.008/0.03	1/4	2/4
H. influenzae, ß-lactamase-negative	27	0.03/0.03	0.008/0.015	1/2	2/2
H. influenzae, ß-lactamase positive	52	0.03/0.03	0.008/0.015	1/2	2/2
N. gonorrhoeae	103	0.015/0.015	0.004/0.008	0.12/0.25	8/8
E. cloacae	92	0.25/4	0.06/4	32/>32	0.5/1
E. cloacae-ESBL+	15	8/>8	4/>8	32/>32	16/16
S. aureus (All)	103	0.12/2	0.12/>8	>32/>32	1/>32
S. epidermidis (All)	100	0.06/4	0.06/>8	>32/>32	0.12/>32
S. pneumoniae (All)	101	0.12/1	0.03/0.5	8/>32	16/32

ESBL+= extended spectrum β-lactamase
Source: Table 22 Section 2.7.2; this submission

Table 17: MIC of besifloxacin, gemifloxacin, azithromycin, and tobramycin against drug-resistant Gram positive and Gram negative pathogens associated with bacterial conjunctivitis

Organism	n	MIC_{50}/MIC_{90} (µg/mL)			
		Besifloxacin	Gemifloxacin	Azithromycin	Tobramycin
H. influenzae, ß-lactamase + ampicillin-R	25	0.12/0.25	0.03/0.06	4/4	4/4
S. aureus, MR, CR	24	1/4	>8/>8	>32/>32	>32/>32
S. aureus, MR, CS	25	0.03/0.06	0.03/0.06	>32/>32	1/2
S. aureus, MS, CS	26	0.03/0.06	0.06/0.06	2/>32	0.5/1
S. aureus, vancomycin-I	23	1/2	4/>8	>32/>32	>32/>32
S. aureus, vancomycin-R	5	1/4	4/>8	>32/>32	>32/>32
S. epidermidis, MR	60	0.25/4	1/>8	>32/>32	4/>32
S. epidermidis, MS	40	0.03/0.12	0.03/0.12	>32/>32	0.12/4
S. pneumoniae, levofloxacin-R	23	1/4	0.5/8	4/>32	16/32
S. pneumoniae, penicillin-S	26	0.12/0.12	0.03/0.06	0.12/0.12	16/>32
S. pneumoniae, penicillin-I	26	0.12/0.12	0.03/0.03	8/>32	16/32
S. pneumoniae, penicillin-R	26	0.12/0.12	0.03/0.06	>32/>32	16/32

S = susceptible, I = intermediate, R = resistant
Source: Table 23 Section 2.7.2; this submission

In Study [redacted] 07-12R2, besifloxacin was more active than comparators against Gram positive isolates, including isolates with specific resistance phenotypes (methicillin-resistant *S. aureus* and *S. epidermidis*, vancomycin-non-susceptible *S. aureus*, levofloxacin-resistant *S. pneumoniae*, and penicillin-non-susceptible *S. pneumoniae*). Against levofloxacin-resistant *S. pneumoniae*, and methicillin-resistant *S. aureus* and *S. epidermidis*, the MIC_{90} for besifloxacin was 4 mcg/ml. b(4)

Besifloxacin was active against both *H. influenzae* (MIC_{90} = 0.12 mcg/ml) and *N. gonorrhoeae* (MIC_{90} = 0.015 mcg/ml), with potency comparable to gemifloxacin and exceeding both azithromycin and tobramycin. Against *E. cloacae* (the only other Gram negative pathogen tested), the besifloxacin MIC_{90} was 4 mcg/ml (for ESBL+ *E. cloacae*, the MIC_{90} was >8 mcg/ml).

Study 07-MIC-392 "Minimum Inhibitory Testing of Besifloxacin, Gatifloxacin, Moxifloxacin, and Levofloxacin against Methicillin-Resistant *S. aureus* (MRSA) and Methicillin-Susceptible *S. aureus* (MSSA) in Support of B&L Report PH06164

Study 07-MIC-392 was conducted by Bausch & Lomb (Rochester NY) in 2007, to determine the MIC of besifloxacin against reference isolates (*S. aureus* ATCC strains) used in an animal efficacy study (B&L Report Number PH06164 "Efficacy in a Rabbit Model of *S. aureus* Endophthalmitis"). Four isolates were tested, comparing the activity of besifloxacin (BOL-303224-A), gatifloxacin, moxifloxacin, levofloxacin, tetracycline, and oxacillin. No information was provided in the study report, regarding quality control procedures employed during the study. The results of the investigation are summarized in Table 18.

Besifloxacin was active against all four tested isolates (including methicillin- and tetracycline-resistant isolates), with activity exceeding that of all comparators. MIC values ranged from ≤0.031 to 0.016 mcg/ml.

Table 18: MIC values for besifloxacin (BOL-30224-A), and comparators against four strains **of *S. aureus***

ATCC #	Replicate #	Gatifloxacin (µg/mL)	Besifloxacin (µg/mL)	Moxifloxacin (µg/mL)	Levofloxacin (µg/mL)	Tetracycline* (µg/mL)	Oxacillin (µg/mL)
43300	Rep 1	0.25	≤0.031	0.063	0.25	0.5	>32.0
	Rep 2	0.25	0.031	0.063	0.25	0.5	>32.0
	Rep 3	0.125	0.031	0.063	0.125	0.5	>32.0
	Rep 4	0.125	0.031	0.063	0.5	0.5	>32.0
25923	Rep 1	0.25	≤0.031	0.063	0.25	1.0	0.5
	Rep 2	0.25	0.031	0.125	0.25	1.0	0.5
	Rep 3	0.125	0.031	0.063	0.125	1.0	0.5
	Rep 4	0.125	0.031	0.063	0.25	1.0	0.5
29213	Rep 1	0.125	≤0.031	0.063	0.25	1.0	0.5
	Rep 2	0.125	0.031	0.063	0.25	1.0	0.5
	Rep 3	0.125	0.031	0.063	0.25	1.0	0.5
	Rep 4	0.125	0.016	0.063	0.25	2.0	0.5
33591	Rep 1	0.125	≤0.031	0.125	0.125	>32.0	>32.0
	Rep 2	0.25	0.031	0.063	0.25	>32.0	>32.0
	Rep 3	0.125	0.031	0.063	0.125	>32.0	>32.0
	Rep 4	0.25	0.031	0.125	0.25	>32.0	>32.0

ATCC = American Type Culture Collection number, Rep = replication

S. aureus ATCC 43300 = methicillin-resistant strain

S. aureus ATCC 25923 = Quality Control strain

S. aureus ATCC 29213 = Quality Control strain, methicillin-susceptible strain

S. aureus ATCC 33591 = methicillin-resistant and tetracycline-resistant strain

* Tetracycline replications 2 - 4 from same organism preparation on 7/19/07

Source: Table 24 Section 2.7.2; this submission

Study 500421 "In Vitro Activity of Besifloxacin Relative to Comparator Fluoroquinolones against Recent US and European Clinical Isolates of Select Gram-Positive and Gram-Negative Pathogens including Anaerobes"

b(4)

Study 500421 was conducted by [redacted] in 2007. The investigators tested 1179 isolates against besifloxacin and selected comparators. Isolates included aerobic and anaerobic bacteria, with both Gram positive and Gram negative pathogens represented. Although the applicant states that isolates were "ocular/respiratory isolates wherever possible", the majority of isolates in the study report line list are classified as "other" or "unknown." Isolates were collected between 2004 and 2007 with a predominance overall, of isolates being collected from sites in the U.S.

Aerobic bacteria were tested using broth microdilution methods approved by CLSI (CLSI M7-A7). Anaerobic bacteria were tested using agar dilution methods approved by CLSI (CLSI M11-A6). Quality control was performed each day of testing. QC organisms and acceptable ranges were based on criteria published in the appropriate CLSI documents. Quality control results were submitted with the study report, and were within acceptable limits.

The results of Study 500421 are summarized in Table 19 through 21.

Table 19: Summary of MIC data for besifloxacin and comparators against selected Gram-positive pathogens

Organism	Antimicrobial Agent	N	MIC (µg/mL)[a]			
			Range	Mode	MIC_{50}	MIC_{90}
Staphylococci						
S. sapropinyticus	Besifloxacin	101	0.015-0.25	0.12	0.06	0.12
	Levofloxacin	101	0.06-0.5	0.5	0.5	0.5
	Moxifloxacin	101	0.03-0.25	0.12	0.12	0.12
	Gatifloxacin	101	0.03-0.25	0.12	0.12	0.25
	Ciprofloxacin	101	0.06-0.5	0.25	0.25	0.5
	Tobramycin	101	≤0.008-32	0.015	0.015	0.06
	Azithromycin	101	0.12->8	>8	1	>8
	Oxacillin	101	≤0.06->8	0.5	0.5	1
S. haemolyticus	Besifloxacin	101	0.015-4	0.03	0.5	1
	Levofloxacin	101	0.06->8	>8	4	>8
	Moxifloxacin	101	0.015->8	0.03	1	8
	Gatifloxacin	101	0.03->8	0.06	2	8
	Ciprofloxacin	101	0.06->8	>8	>8	>8
	Tobramycin	101	0.015->32	0.03	2	32
	Azithromycin	101	0.25->8	>8	>8	>8
	Oxacillin	101	≤0.06->8	>8	>8	>8
S. hominis	Besifloxacin	50	0.015-2	0.25	0.25	1
	Levofloxacin	50	0.06->8	8	8	>8
	Moxifloxacin	50	0.03->8	2	1	4
	Gatifloxacin	50	0.03->8	1	1	4
	Ciprofloxacin	50	0.06->8	>8	8	>8
	Tobramycin	50	0.015->32	16	16	32
	Azithromycin	50	0.12->8	>8	>8	>8
	Oxacillin	50	≤0.06->8	>8	>8	>8
S. warneri	Besifloxacin	50	0.015-2	0.06	0.06	1
	Levofloxacin	50	0.06->8	0.12	0.12	>8
	Moxifloxacin	50	0.015->8	0.06	0.06	4
	Gatifloxacin	50	0.03->8	0.12	0.12	4
	Ciprofloxacin	50	0.06->8	0.25	0.25	>8
	Tobramycin	50	0.015->32	0.03	0.06	8
	Azithromycin	50	0.12->8	>8	>8	>8
	Oxacillin	50	≤0.06->8	>8	0.5	>8
Staphylococcus lugdunensis	Besifloxacin	15	0.015-2	0.06	0.06	0.5
	Levofloxacin	15	0.06->8	0.25	0.25	>8
	Moxifloxacin	15	0.03->8	0.03	0.12	2
	Gatifloxacin	15	0.03-8	0.06	0.12	2
	Ciprofloxacin	15	0.06->8	0.12	0.12	>8
	Tobramycin	15	0.03->32	0.03	0.12	32
	Azithromycin	15	0.25->8	>8	>8	>8
	Oxacillin	15	≤0.06->8	>8	0.5	>8
Streptococci						
Streptococcus agalactiae	Besifloxacin	100	0.03-0.12	0.06	0.06	0.06
	Levofloxacin	100	0.25-4	0.5	0.5	1
	Moxifloxacin	100	0.06-1	0.12	0.12	0.25
	Gatifloxacin	100	0.12-1	0.25	0.25	0.25
	Ciprofloxacin	100	0.5-8	0.5	0.5	1
	Tobramycin	100	8->128	32	32	64
	Azithromycin	100	0.015->8	0.06	0.06	>8
	Penicillin	100	≤0.015-0.06	0.03	0.03	0.06

Table 19: Summary of MIC data for besifloxacin and comparators against selected Gram-positive pathogens (cont'd)

Organism	Antimicrobial Agent	N	MIC (µg/mL)[a]			
			Range	Mode	MIC_{50}	MIC_{90}
S. pyogenes	Besifloxacin	101	0.03-0.06	0.03	0.03	0.06
	Levofloxacin	101	0.25-2	0.5	0.5	0.5
	Moxifloxacin	101	0.06-0.5	0.12	0.12	0.25
	Gatifloxacin	101	0.06-0.5	0.12	0.12	0.25
	Ciprofloxacin	101	0.12-2	0.5	0.5	0.5
	Tobramycin	101	4-64	16	16	16
	Azithromycin	101	0.03->8	0.06	0.06	8
	Penicillin	101	≤0.015-0.06	≤0.015	≤0.015	≤0.015
C,F,G streptococci	Besifloxacin	50	0.015-0.25	0.03	0.03	0.06
	Levofloxacin	50	0.12-8	0.5	0.5	0.5
	Moxifloxacin	50	0.03-1	0.12	0.12	0.12
	Gatifloxacin	50	0.06-2	0.12	0.12	0.25
	Ciprofloxacin	50	0.12->8	0.5	0.5	0.5
	Tobramycin	50	2-32	8	8	16
	Azithromycin	50	0.008->8	0.06	0.06	>8
	Penicillin	50	≤0.015-0.06	≤0.015	≤0.015	0.06
Viridans streptococci[b]	Besifloxacin	156	0.015-2	0.06	0.06	0.12
	Levofloxacin	156	0.12->8	1	1	1
	Moxifloxacin	156	0.03-4	0.12	0.12	0.25
	Gatifloxacin	156	0.03-8	0.25	0.25	0.5
	Ciprofloxacin	156	0.12->8	1	1	4
	Tobramycin	156	0.5-128	8	16	32
	Azithromycin	156	0.008->8	0.06	0.06	>8
	Penicillin	156	≤0.015->4	≤0.015	0.06	1
Streptococcus mitis group[c]	Besifloxacin	90	0.015-2	0.06	0.06	0.12
	Levofloxacin	90	0.25->8	1	1	1
	Moxifloxacin	90	0.03-4	0.12	0.12	0.25
	Gatifloxacin	90	0.03-8	0.25	0.25	0.25
	Ciprofloxacin	90	0.12->8	2	1	4
	Tobramycin	90	2-128	16	16	32
	Azithromycin	90	0.008->8	0.015	2	8
	Penicillin	90	≤0.015->4	0.12	0.12	2

[a] MIC_{50} = MIC for 50% of strains tested; MIC_{90} = MIC for 90% of strains tested

[b] For this study viridans group streptococci consists of 2 *S. anginosus*, 13 *S. bovis*, 7 *S. constellatus*, 28 *S. intermedius*, 51 *S. mitis*, 22 *S. oralis*, 2 *S. salivarius*, 17 *S. sanguinis*, and 14 other viridans group species

[c] For this study *S. mitis* group consists of 51 *S. mitis*, 22 *S. oralis* and 17 *S. sanguinis* (also included among the viridans group streptococci)

Source: Table 25 Section 2.7.2; this submission

Table 20: Summary of MIC data for besifloxacin and comparators against selected Gram-negative pathogens

Organism	Antimicrobial agent	Total n	MIC (µg/mL) [a] Range	Mode	MIC_{50}	MIC_{90}
Enterobacteriaceae						
Klebsiella oxytoca	SS734	50	0.06-8	0.12	0.12	1
	Levofloxacin	50	0.015-8	0.03	0.03	0.5
	Moxifloxacin	50	0.03-8	0.06	0.06	2
	Gatifloxacin	50	0.015-8	0.03	0.03	0.5
	Ciprofloxacin	50	0.008->8	0.015	0.015	0.5
	Tobramycin	50	0.25-8	0.5	0.5	1
	Azithromycin	50	8->8	>8	>8	>8
	Ceftazidime	50	0.03-1	0.12	0.12	0.5
Citrobacter koseri	SS734	100	0.03->8	0.06	0.06	0.25
	Levofloxacin	100	0.015->8	0.015	0.03	0.12
	Moxifloxacin	100	0.015->8	0.03	0.03	0.25
	Gatifloxacin	100	0.008->8	0.015	0.015	0.12
	Ciprofloxacin	100	0.004->8	0.008	0.008	0.06
	Tobramycin	100	0.25-16	0.5	0.5	1
	Azithromycin	100	2->8	8	8	>8
	Ceftazidime	100	0.06-4	0.12	0.12	0.5
M. morganii	SS734	51	0.03->8	0.12	0.12	4
	Levofloxacin	51	0.015->8	0.03	0.06	8
	Moxifloxacin	51	0.03->8	0.12	0.25	>8
	Gatifloxacin	51	0.015->8	0.06	0.12	>8
	Ciprofloxacin	51	0.004->8	0.008	0.015	>8
	Tobramycin	51	0.25-32	1	1	4
	Azithromycin	51	8->8	>8	>8	>8
	Ceftazidime	51	0.03->32	0.06	0.12	16
Non-enterobacteriaceae						
M. catarrhalis	SS734	101	0.015-0.12	0.03	0.03	0.03
	Levofloxacin	101	0.015-0.5	0.015	0.015	0.03
	Moxifloxacin	101	0.015-0.12	0.03	0.03	0.03
	Gatifloxacin	101	0.008-0.25	0.015	0.015	0.015
	Ciprofloxacin	101	0.008-0.25	0.015	0.015	0.015
	Tobramycin	101	0.03-0.5	0.25	0.25	0.25
	Azithromycin	101	0.015-0.06	0.03	0.03	0.03
	Oxacillin	101	0.25->8	8	4	8
L. pneumophila	SS734	50	0.015-0.06	0.03	0.03	0.03
	Levofloxacin	50	0.015-0.06	0.03	0.03	0.03
	Moxifloxacin	50	0.015-0.06	0.03	0.03	0.06
	Gatifloxacin	50	0.015-0.06	0.03	0.03	0.06
	Ciprofloxacin	50	0.015-0.06	0.03	0.03	0.03
	Tobramycin	50	0.25-4	1	1	2
	Azithromycin	50	0.03-1	0.12	0.12	1

Source: Table 26 Section 2.7.2; this submission

Table 21: Summary of MIC data for besifloxacin and comparators against anaerobic pathogens

Organism	Antimicrobial agent	Total n	MIC (µg/mL) [a] Range	Mode	MIC_{50}	MIC_{90}
Gram-positive *Clostridium perfringens*	Besifloxacin	21	0.12-0.25	0.25	0.25	0.25
	Moxifloxacin	21	0.25-0.5	0.5	0.5	0.5
	Gatifloxacin	21	0.5-1	1	1	1
	Clindamycin	21	0.06-4	2	2	4
	Metronidazole	21	1-4	2	2	4
Propionibacterium acnes	Besifloxacin	21	0.12-0.25	0.25	0.25	0.25
	Moxifloxacin	21	0.25-0.25	0.25	0.25	0.25
	Gatifloxacin	21	0.25-0.5	0.25	0.25	0.5
	Clindamycin	21	≤0.03-2	0.06	0.06	0.12
	Metronidazole	21	>16->16	>16	>16	>16
Gram-negative *Bacteroides fragilis*	Besifloxacin	20	0.25-2	0.25	0.5	1
	Moxifloxacin	20	0.25-8	0.5	0.5	2
	Gatifloxacin	20	1-16	4	2	4
	Clindamycin	20	0.5->8	>8	2	>8
	Metronidazole	20	2-2	2	2	2
Fusobacterium spp.	Besifloxacin	21	0.12-8	0.25	0.25	1
	Moxifloxacin	21	0.25->16	1	1	2
	Gatifloxacin	21	0.5->16	1	1	4
	Clindamycin	21	0.06-8	0.06	0.06	2
	Metronidazole	21	≤0.12-2	0.25	0.25	1
Prevotella spp.	Besifloxacin	20	0.06-16	2	1	4
	Moxifloxacin	20	0.12->16	4	4	8
	Gatifloxacin	20	0.25->16	8	8	16
	Clindamycin	20	≤0.03->8	≤0.03	≤0.03	>8
	Metronidazole	20	0.25-8	4	4	4

Source: Table 27 Section 2.7.2; this submission

Against isolates of coagulase negative staphylococci (*S. saprophyticus* n=101, *S. haemolyticus* n=101, *S. hominis* n=50, *S. warneri* n=50, and *S. lugdunensis* n=15), besifloxacin was as active or more active than all comparators (levofloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, tobramycin, azithromycin, and oxacillin). Against two of the coagulase negative staphylococci sought in the proposed indications for besifloxacin, *S. hominis* and *S. lugdunensis*, MIC_{90} values were ≤0.25 mcg/ml. No isolates of *S. epidermidis* (also sought in the proposed indications for besifloxacin) or of *S. aureus* were tested in these investigations.

Besifloxacin MIC_{90} values were ≤0.06 mcg/ml against all streptococcal species tested (including *S. agalactiae*, *S. pyogenes*, "C,F,G streptococci", "viridans streptococci", and "*Streptococcus mitis* group." Identical isolates have been identified in both the "viridans streptococci" and "*Streptococcus mitis* group" headings. For review purposes, each isolate will be considered based on its identification to species level (e.g. *Streptococcus oralis*, n=22; *S. sanguinis*, n=17; *S. mitis*, n=51).

Against *Klebsiella oxytoca*, *Citrobacter koseri*, and *Morganella morganii*, besifloxacin MIC_{90}

values were 1 mcg/ml, 0.25 mcg/ml, and 4 mcg/ml, respectively. The results were similar to fluoroquinolone comparators. Besifloxacin was active against *M. catarrhalis* (MIC_{90} = 0.03 mcg/ml) and *Legionella pneumophila* (MIC_{90} = 0.03 mcg/ml).

Against tested Gram positive anaerobic bacteria (*Clostridium perfringens* and *Propionibacterium acnes*), besifloxacin MIC_{90} values were 0.25 mcg/ml. Against Gram negative anaerobes, besifloxacin more active than fluoroquinolone comparators (moxifloxacin and gatifloxacin) with MIC_{90} values of 1 mcg/ml against *Bacteroides fragilis* and *Fusobacterium* spp, and 4 mcg/ml against *Prevotella* spp.

Study 500510 "MIC and MBC of SS734 and comparators against ocular isolates of *S. aureus, S. epidermidis, S. pneumoniae*, and *H. influenzae*

Study 500510 was conducted by [redacted] in 2008. The investigators tested 30 *S. aureus* isolates (including methicillin- and ciprofloxacin-resistant isolates), 30 *S. epidermidis* isolates (including methicillin- and ciprofloxacin-resistant isolates), 40 *H. influenzae* isolates (including ß-lactamase positive isolates) and 35 *S. pneumoniae* isolates (including penicillin-non-susceptible isolates and a levofloxacin-resistant isolate). All isolates were collected from ocular infections from 2006 through 2007. Susceptibility was performed using broth microdilution methods (frozen panels) according to standards published in CLSI document M23-A. Quality control was performed on each day of testing, and was within acceptable range (QC results were submitted with the study report). b(4)

Against *S. aureus*, besifloxacin was the most active (MIC_{90} = 4mcg/ml) of antimicrobials tested, including moxifloxacin, gatifloxacin, ciprofloxacin, levofloxacin, azithromycin, tobramycin, and oxacillin. The tested isolates included methicillin-resistant phenotypes (n=11) and ciprofloxacin non-susceptible phenotypes (n=16). Against both of these phenotypes, the MIC_{90} of besifloxacin was elevated (compared to besifloxacin activity against oxacillin- or ciprofloxacin-susceptible isolates), but was lower (4 mcg/ml) than any comparator.

Against *S. epidermidis*, besifloxacin was the most active (MIC_{90} = 4mcg/ml) of the antimicrobials tested (besifloxacin > gatifloxacin/moxifloxacin > ciprofloxacin/levofloxacin). Activity against resistant phenotypes was decreased (MIC_{90} was 4 mcg/ml for methicillin-resistant and ciprofloxacin-resistant isolates compared to 0.25 mcg/ml for methicillin susceptible isolates and o.03 mcg/ml for ciprofloxacin-susceptible isolates).

Against *S. pneumoniae*, besifloxacin was the most active (MIC_{90} = 0.06 mcg/ml) of all antimicrobials tested (besifloxacin > moxifloxacin > gatifloxacin > ciprofloxacin/levofloxacin). Activity against penicillin-resistant isolates was within one doubling-dilution of the activity measured against penicillin-susceptible isolates.

Besifloxacin was active against isolates of *H. influenzae* (MIC_{90} = 0.015 mcg/ml), with activity comparable to that of the other tested fluoroquinolones (ciprofloxacin MIC_{90} = 0.015 mcg/ml, gatifloxacin MIC_{90} = 0.008 mcg/ml, levofloxacin MIC_{90} = 0.015 mcg/ml, and moxifloxacin MIC_{90} = 0.03 mcg/ml). Activity against ß-lactamase positive isolates was within one doubling dilution of that against ß-lactamase negative isolate.

In Summary:

The applicant has provided study reports and summary data from nine investigations of the in vitro activity of besifloxacin. The compiled study results are summarized in Tables 22 and 23.

Two of the nine studies were performed in Japan [redacted] using agar dilution methods for susceptibility testing. In both studies, recent ocular isolates, collected in Japan, were used. Study [redacted] 99K3020B employed methods approved by the Clinical and Laboratory Standards Institute, and compared besifloxacin activity to 5 other antimicrobials (ciprofloxacin, norfloxacin, ofloxacin, gentamicin, and tobramycin) against relatively small numbers of ocular pathogens (≤30 each of *S. pneumoniae*, *S. aureus*, coagulase-negative staphylococci, *Corynebacterium* species, *P. acnes*, *H. influenzae*, *Moraxella* species, *N. gonorrhoeae*, and *P. aeruginosa*). Besifloxacin activity was generally similar to (within 1 doubling dilution) or greater than the comparators against all tested pathogens (ciprofloxacin was more active against both *Moraxella* species and *P. aeruginosa*). Activity against resistant phenotypes was diminished compared to that against susceptible phenotypes, but still exceeded that of most comparators. In the second study performed by [redacted] (Study SS734PRE-003), besifloxacin enantiomers were tested and compared for their in vitro activity against 34 specific ocular pathogens (including isolates with known resistance phenotype). No information was provided in the study report concerning the source or date of the tested isolates, or concerning quality control procedures employed during the tests. Disk diffusion methods were employed, but were not methods approved by CLSI for antimicrobial susceptibility testing. The besifloxacin (+)SS734 enantiomer was shown to be the most active, although all racemic mixtures displayed greater activity against the tested pathogens than the comparator fluoroquinolone (ofloxacin). It is noted that Study [redacted] 99K3020B is the only source of "pre-clinical" in vitro data for *Corynebacterium* species. Although the study purports to comply with CLSI recommendations for susceptibility testing, no disk diffusion methods are currently recommended for the testing of *Corynebacterium* species, and no discussion of quality control is included in the study report. For these reasons, the data concerning the in vitro activity of besifloxacin against isolates of Corynebacterium species is not adequate to support this NDA. Similarly, in Study SS734PRE-003 the methods used for susceptibility testing were not approved by CLSI, yet no test validation or quality control information was included in the report.

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Two of the studies were performed by [redacted] Both of these studies were performed using broth microdilution techniques approved by CLSI. Both studies used recently collected clinical isolates (primarily from respiratory sources), and quality control was performed on each day of testing. In the first study (BL-MIC-001B), 100 isolates of five ocular pathogens were tested (*S. aureus*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*). In the second study (BL-MIC-002B), 100 isolates of five Gram negative ocular pathogens were tested (*Acinetobacter* species, *Enterobacter* species, *P. mirabilis*, *P. aeruginosa*, and *S. marcescens*). In the first study, besifloxacin activity exceeded that of the fluoroquinolone comparator (ofloxacin) against all Gram positive isolates and was comparable against *H. influenzae* and *M. catarrhalis*). In the second study, besifloxacin activity was comparable to ofloxacin (within one doubling dilution). Activity of both tested fluoroquinolones against *P. aeruginosa* was poor (MIC_{90} > 8 mcg/ml).

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Two studies were performed by the [redacted] The same set of 600 reference isolates from the [redacted] collection was used for both studies (no specimen source or date of isolation was provided in the study report). Broth microdilution susceptibility testing was performed in both of the studies, according to methods approved by CLSI. Quality control was performed on each day of testing. In Study [redacted] 06-15RB, the isolates were tested against besifloxacin, gatifloxacin, and moxifloxacin. In Study [redacted] 07-12R2, isolates were tested against besifloxacin, gemifloxacin, azithromycin, and tobramycin. In these studies, besifloxacin was active against *H. influenzae*, *S. aureus*, *S. epidermidis*, and *S. pneumoniae*, with activity

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similar to or exceeding all comparators except gemifloxacin (gemifloxacin MIC_{90} values were generally 2 2-fold dilutions lower than besifloxacin against these pathogens). Cross resistance to other antimicrobials was noted in some isolates, including methicillin-resistant *S. aureus*, methicillin-resistant *S. epidermidis*, and fluoroquinolone-resistant isolates of *S. pneumoniae* and staphylococcus species. No change in MIC_{90} values were noted in ß-lactamase positive *H. influenzae* isolates compared to ß-lactamase negative isolates, and MIC_{90} values were identical in penicillin-nonsusceptible and –susceptible isolates of *S. pneumoniae*.

Two studies were performed by [redacted] Both studies employed broth microdilution procedures approved by CLSI. Quality control was performed on each day of testing, and a complete line list of study isolates (including date of collection, geographic origin, and specimen source) was included in the study reports. Study 500421 (2007) tested 1179 aerobic and anaerobic isolates against besifloxacin and a variety of comparators appropriate for the tested isolate (including moxifloxacin and at least 1 other comparator fluoroquinolone). Besifloxacin was active against all tested isolates, with activity generally exceeding that of comparators (Tables 19-21). Isolates of *Pseudomonas aeruginosa*, *Acinetobacter* species, or *Proteus* species were not tested in this study. Study 500510 (2008) tested 30 isolates each of S. aureus, S. epidermidis, S. pneumoniae, and H. influenzae against besifloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, levofloxacin, azithromycin, tobramycin, and oxacillin. Besifloxacin was active against all tested isolates, with activity exceeding that of fluoroquinolone comparators against all isolates except *H. influenzae* (all fluoroquinolones were similarly active against these isolates). Cross resistance was noted in tests against methicillin-resistant staphylococcus species.

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One study (Study 07-MIC-392) was performed by the Applicant (B&L Biological Test Center, Irvine CA) in support of animal efficacy studies (described below). In this study, besifloxacin activity against four reference S. aureus isolates (ATCC strains 43300, 25923, 29213, and 33591) was compared to gatifloxacin, moxifloxacin, levofloxacin, tetracycline, and oxacillin. Besifloxacin MICs (≤ 0.016 mcg/ml) against all tested isolates were lower than comparators.

Antimicrobial Spectrum of Activity Studies ~ Conclusions:
The Applicant has submitted data and summary information from nine studies designed to investigate the in vitro antimicrobial activity of besifloxacin against pathogens associated with ocular infections. Data from these studies suggest that besifloxacin is generally active against the isolates tested in these studies, including isolates of *S. aureus* (including methicillin-resistant *S. aureus*), *S. epidermidis*, *S. pneumoniae* (including penicillin-resistant *S. pneumoniae*), and *H. influenzae* (all sought in the proposed indications). Decreased susceptibility was noted against methicillin-resistant isolates of staphylococcus species (quinolone and methicillin-resistant *S. aureus* MIC_{90} = 4 mcg/ml; methicillin-resistant *S. epidermidis* MIC_{90} = 4 mcg/ml; levofloxacin-resistant *S. pneumoniae* MIC_{90} = 4 mcg/ml). Decreased susceptibility was noted against *P. aeruginosa* and *Acinetobacter baumanii-calcoaceticus*. (MIC_{90} values of >8 mcg/ml against both pathogens). The in vitro data in this submission does not support the indication of antimicrobial activity against *Corynebacterium* species, since no reviewable data is included in the NDA.

Table 22: Summary of besifloxacin MIC data in non-clinical in vitro studies against pathogens associated with bacterial conjunctivitis

Organism	No. of Studies	Total N	Besifloxacin			Study Reference(s)
			MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range	
All Isolates[a]	5	1205	0.06	1	≤ 0.04 - >8	500421, 500510 BL-MIC-001B BL-MIC-002B 07-12R2-B
All Isolates[a] Quinolone-S[b]	5	920	0.06	0.12	≤ 0.04 - >8	500421, 500510 BL-MIC-001B BL-MIC-002B 07-12R2-B
CDC coryneform group G *Corynebacterium pseudodiptheriticum* *Corynebacterium striatum*	1	30[c,d]	0.25	2	≤ 0.06 - 2	MBC99K3020B
Haemophilus influenzae	3	243	0.03	0.06	≤ 0.004 – 0.25	500510 BL-MIC-001B 07-12R2-B
β-lactamase +	3	118	0.03	0.06	0.008 – 0.12	500510 BL-MIC-001B 07-12R2-B
β-lactamase -	3	100	0.03	0.03	≤ 0.004 – 0.12	500510 BL-MIC-001B 07-12R2-B
β-lactamase - Ampicillin-R	1	25	0.12	0.25	0.015 – 0.25	07-12R2-B
Moraxella lacunata	1	30[d,e]	≤ 0.06	0.13	≤ 0.06 - 0.13	MBC99K3020B
Staphylococcus aureus	2	36	0.03	0.06	0.015 – 0.12	500510 BL-MIC-001B 07-12R2-B
MSSA[f]	3	93	0.03	0.25	0.015 - 4	500510 BL-MIC-001B 07-12R2-B
MSSA Ciprofloxacin-S	2	36	0.03	0.06	0.015 – 0.12	500510 07-12R2-B
MRSA Quinolone– R[g]	3	73	1	4	0.25 - 4	500510 BL-MIC-001B 07-12R2-B
MRSA Quinolone – S[g]	3	37	0.03	0.12	0.015 - 1	500510 BL-MIC-001B 07-12R2-B
Quinolone-S[g]	3	93	0.03	0.06	0.015 – 0.25	500510 BL-MIC-001B 07-12R2-B

Table 22: Summary of besifloxacin MIC data in non-clinical in vitro studies against pathogens associated with bacterial conjunctivitis (cont'd)

Staphylococcus epidermidis	2	115	0.06	4	0.015 - 8	500510 07-12R2-B
Ciprofloxacin-R[h]	1	6	2.5	N/A	0.25 - 4	500510
Ciprofloxacin-S	1	9	0.03	N/A	0.015 – 0.03	500510
MRSE Ciprofloxacin-S	1	4	N/A	N/A	0.015 – 0.03	500510
MRSE Ciprofloxacin-R[h]	1	2	2	N/A	0.25 - 4	500510
MSSE	2	46	0.03	0.25	0.015 - 1	500510 07-12R2-B
MRSE	2	69	0.25	4	0.015 - 8	500510 07-12R2-B
Staphylococcus hominis	1	50	0.25	1	0.015 - 2	500421
Ciprofloxacin-S	1	15	0.03	0.06	0.015 – 0.06	500421
Ciprofloxacin-R[h]	1	35	0.25	1	0.125 - 2	500421
Staphylococcus lugdunensis	1	15	0.06	0.5	0.015 – 2	500421
Ciprofloxacin-S	1	10	0.03	0.06	0.015 – 0.06	500421
Ciprofloxacin-R[h]	1	5	0.5	2	0.125 - 2	500421
Streptococcus mitis group	1	90	0.06	0.12	0.015 – 2	500421
Streptococcus oralis	1	22	0.06	0.12	0.03 - 2	500421
Streptococcus pneumoniae	3	235	0.12	0.5	0.015 - >8	500510 BL-MIC-001B 07-12R2-B
Levofloxacin-R	1	23	1	4	0.5 - >8	07-12R2-B
Penicillin-S	3	123	0.12	0.12	0.015 - 1	500510 BL-MIC-001B 07-12R2-B
Penicillin-I	2	28	0.12	0.12	0.03 – 0.25	500510 07-12R2-B
Penicillin-R	3	61	0.12	0.12	0.03 – 0.25	500510 BL-MIC-001B 07-12R2-B
Streptococcus salivarius	1	2	N/A	N/A	0.06	500421

[a] *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus lugdunensis, Streptococcus pneumoniae, Streptococcus oralis,* Streptococcus mitis group, Streptococcus *salivarius, Haemophilus influenzae,* and *Moraxella catarrhalis* tested by broth dilution.

[b] Ciprofloxacin or ofloxacin resistant isolates were not included

[c] Corynebacterium species

[d] Study MBC99K3020B MIC values were obtained by the agar dilution method. MIC values were obtained by broth dilution for all other listed studies

[e] Moraxella species

[f] Isolates are MSSA-CS in Study 07-12R2-B

[g] Ciprofloxacin (Studies 500510 and 07-12R2-B) and ofloxacin (Study BL-MIC-001B)

[h] Isolates were ciprofloxacin non-susceptible in Study 500510

Source Table 29 Section 2.7.2; this submission

Table 23: Summary of besifloxacin MIC data in non-clinical in vitro studies against other selected pathogens

Organism	No. of Studies	Total N	Besifloxacin			Study Reference(s)
			MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range	
S. pyogenes	2	201	0.06	0.12	0.03 – 0.12	500421 BLMIC001B
M. catarrhalis	2	201	0.06	0.12	0.015 – 0.12	500421 BLMIC001B
N. gonorrhoeae	1	103	0.015	0.015	0.004 - 2	[redacted] 07-12R2B
P. aeruginosa	1	100	4	>8	0.5 - >8	BLMIC002B
A. lwoffii	1	13	0.5	0.5	0.12 - 2	BLMIC002B
A. baumanii	1	48	1	2	0.25 - >8	BLMIC002B
A. baumannii-calcoaceticus	1	33	0.5	>8	0.12 - >8	BLMIC002B
E. cloacae	1	59	0.25	0.5	0.12 - >8	BLMIC002B
E. aerogenes	1	39	0.25	2	0.12 - >8	BLMIC002B
P. mirabilis	1	100	0.5	1	0.25 - >8	BLMIC002B
S. marcescens	1	100	1	2	0.25 - >8	BLMIC002B
K. oxytoca	1	50	0.12	1	0.06 - 8	500421
C. koseri	1	100	0.06	0.25	0.03 - >8	500421
M. morganii	1	51	0.12	4	0.03 - >8	500421
L. pneumophila	1	50	0.03	0.03	0.015 - 0.06	500421

MIC_{50} = MIC for 50% of strains tested; MIC_{90} = MIC for 90% of strains tested
Source: Section 2.7.2, Table 30, this submission

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RESISTANCE STUDIES

Quinolone resistance most frequently occurs by chromosomal mutations in the genes encoding the principle quinolone targets, DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*). Additional mechanisms of resistance include the expression of multi-drug efflux pumps [Mazzariol 2000] and the transfer of plasmid-borne resistance determinants, including *qnr* genes, aac(6')-IB-cr, and *qepA* [Ma 2008]. Not all members of the fluoroquinolone class are affected by all mechanisms. Quinolones with multiple targets (e.g. gatifloxacin and levofloxacin) are generally less affected by certain chromosomal mutations, and the molecular structure of the specific quinolone may result in dramatic differences in MICs against fluoroquinolone-resistant isolates [Becnel 2008]. Some studies have suggested that unknown resistance mechanisms may be present in a high percentage of quinolone-resistant bacteria [Morgan-Linnell 2008]. Recent investigations of pathogens collected from ocular infections have identified frequent mutations in the quinolone resistance determining region (QRDR) in *Staphylococcus epidermidis* [Yamada 2008], while separate investigations have demonstrated high levels of quinolone resistance in *Corynebacterium macginleyi*, a recently recognized ocular pathogen [Eguchi 2008].

The sponsor has conducted studies to determine the possible mechanisms of besifloxacin resistance in target pathogens and studies designed to investigate the in vitro emergence of resistance to besifloxacin.

In Study PHA-005, conducted by the [redacted] investigators examined the role of chromosomal mutations affecting the *gyrA*, *gyrB*, *parC*, and *parE* genes, using a stepwise mutation process. Other mechanisms of fluoroquinolone-resistance (e.g. active efflux, plasmid-mediated determinants) were not studied, although resistant isolates of E. coli were identified at both first- and second-step selections that did not demonstrate QRDR mutations. Chromosomal mutations observed in tested isolates of *E. coli* and S. pneumoniae are summarized in Table 24. DNA gyrase was determined to be the primary target in *E. coli* (mutations observed at both first- and second-step selection). DNA gyrase was determined to be the primary target in *S. pneumoniae*, with topoisomerase IV

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determined to be a secondary target. Similar experiments with isolates of *S. aureus* could not identify first- or second-stage mutants. *S. aureus* was shown to target DNA gyrase in *parC* mutants selected by other fluoroquinolones. In separate experiments (using liquid media), *S. aureus* was shown to select a *gyrA* mutant without prior *parC* mutations. The Applicant contends that this evidence supports dual targeting of DNA gyrase and topoisomerase in isolates of *S. aureus*. Analysis of resistant isolates selected in these studies demonstrated mutations in either the *gyrA* or *gyrB* genes.

Table 24: Topoisomerase gene mutations in resistant mutants of *E. coli* and *S. pneumoniae* selected in vitro by besifloxacin (SS734)

Bacterial species (N mutants studied)	*gyrA* (n mutants)	*gyrB*	*parC*	*parE*	No QRDR mutation	MIC SS734 (µg/mL)
E. coli						
-first step (10)	wt Ser83Leu (1) Gly81Asp (1) Asp87Tyr (1)	no	no	no	7	0.12 1 1 1
- second step from a mutant without QRDR mutation (10)	Ser83Leu (5) Gly81Asp (4)	no	no	no	1	8 – 16 16
S. pneumoniae						
-first step (10)	wt Ser81Phe (5) Ser81Tyr (4) Glu85Lys (1)	no	no	no	0	0.12 0.5 0.5 1
- second step from *gyrA* mutants Ser81Phe or Ser81Tyr (10)	Ser81Phe[a] Ser81Tyr[a]	no no	Ser79Tyr (2) Ser79Phe (1) Ser79Tyr (2) Ser79Phe (1)	 Glu474Lys (1) Glu474Lys (1)	2	1 1 1 1 1 1

QRDR = quinolone resistance determining region, MIC = minimum inhibitory concentration
[a] mutation selected at the first step
Source: Study PHA-005 Table 2

The investigators determined mutational prevention concentrations (MPC) and the mutant selection window (MSW) for besifloxacin against *E. coli* KL16, *S. aureus* ATCC 15752 and *S. pneumoniae* 7785, using data from stepwise development of resistance studies. The results are summarized in Table 25. Based on this data, compared to currently approved breakpoints for fluoroquinolones, the calculated MPC for all tested isolates suggests a low likelihood for the development of resistance at clinically achievable besifloxacin concentrations (besifloxacin pharmacokinetics/pharmacodynamics is discussed below). All first-step mutations were seen at very low proportions at 4x MIC.

Study PHA-005 also included an investigation of the mechanisms of besifloxacin resistance in isolates with known topoisomerase mutations. Table 26 summarizes the data from this investigation. In isolates of *S. aureus*, besifloxacin was less affected by both single (*parC*) mutations and double (*gyrA* and *parC*) mutations than ciprofloxacin and moxifloxacin. Activity in isolates of *S. pneumoniae* against both types or mutants (single and double mutations) was comparable to moxifloxacin (exceeding that of ciprofloxacin). In isolates of *E.coli*, besifloxacin was somewhat less active against both types of mutants, compared to both comparators.

Table 25: Summary of the results on proportion of resistant mutants and mutational prevention concentration (MPC) of besifloxacin at two steps selection by besifloxacin (SS734)

Bacterial strains and step selection	SS734 MIC (µg/mL)	Inoculum	MPC (µg/mL)	MPC/ MIC	MSW (µg/mL)	Proportion at 4x MIC
E. coli KL16						
- first step	0.12	1.5×10^{10}	4	32	0.12 - 2	3.8×10^{-8}
- second step	2	3.4×10^{9}	16	8	2 - 8	6×10^{-9}
S. aureus ATCC 15752						
- first step	0.03	3×10^{10}	0.12	4	0.03 – 0.06	$< 3.3 \times 10^{-10}$
- second step	0.25	1.3×10^{10}	0.25	1	No mutant obtained	
- second step from *parC* mutants *parC* mutant Cip-R *parC* mutant Lev-R	0.06 0.06	3×10^{10} 2.5×10^{10}	1 1	16 16	0.06 – 0.5 0.06 – 0.5	1.7 to 2.9 x 10^{-8} 1.7×10^{-8}
S. pneumoniae 7785						
- first step	0.125	1.4×10^{10}	0.5	4	0.12 - 0.25	$< 7 \times 10^{-10}$
- second step	0.5	1.0×10^{10}	2 - 4	8 - 16	0.5 - 2	2.4×10^{-8}

MPC = mutant prevention concentration, MSW = mutant selection window, MIC = minimum inhibitory concentration

Source: Study PHA-005 Table 3

Table 26: Activity of besifloxacin against defined topoisomerase mutants

Topoisomerase mutants	MIC (µg/mL)		
	Besifloxacin	Ciprofloxacin	Moxifloxacin
S. pneumoniae			
wild type	0.12	1	0.25
parC S79Y	0.25	8	0.25
gyrA S81F	0.5	1	0.5
parC S79Y + gyrA S81F	1	64	4
S. aureus			
wild type	0.03	1	0.06
parC S80F	0.06	8	0.5
parC E84K	0.06	8	0.25
parC S80F + gyrA S84L	0.5	64	2
E. coli			
wild type	0.12	0.008	0.06
gyrA D87Y	0.5	0.12	0.5
gyrA S83L	0.5	0.25	0.5
gyrB D426N	0.5	0.03	0.12
gyrA S83L + parE H445L	1	0.12	0.5
gyrA S83L + parC S80R	16	4	4

MIC = minimum inhibitory concentration

Source: Study PHA-005 Tables 4, 5, 6

In summary:

The Applicant has submitted data from a study (PHA-005) designed to investigate the mechanism of resistance to besifloxacin and to evaluate the emergence of in vitro resistance of specific pathogens to the antimicrobial. Mutations in the gyrase genes (primarily *gyrA*), the primary target for besifloxacin against *S. aureus*, *S. pneumoniae*, and *E. coli*, appear to represent the main mechanism of resistance in these species. Mutations involving topoisomerase genes were only noted in isolates with selected mutations in one or more of the DNA gyrase genes. Specific mutations resulted in cross-resistance among the tested fluoroquinolones (notably moxifloxacin and ciprofloxacin). The spontaneous emergence of resistance determined in this study was low (comparable to other tested fluoroquinolones). The Mutation Prevention Concentration (MPC) for besifloxacin in first-step selection experiments against *E. coli* was 4 mcg/ml (high, compared to CLSI approved breakpoints for the fluoroquinolone class). The MPCs for *S. aureus* (0.12 mcg/ml) and *S. pneumoniae* (0.5 mcg/ml) both suggest a low probability for the emergence of besifloxacin resistance in these species.

Resistance Studies ~ Conclusions:
The Applicant has submitted study data that supports chromosomal mutations, primarily in the *gyrA* gene, as a primary mechanism of besifloxacin resistance. Spontaneous mutations in this gene, as well as mutations in *gyrB* (and the topoisomerase IV genes, *parC* and *parE*, observed in pre-selected mutant isolates) occur a low frequencies ($< 1 \times 10^{-10}$ in all tested species), hence the likelihood of resistance via this mechanism is purportedly low. Chromosomal mutations result in cross-resistance to other fluoroquinolones (ciprofloxacin and moxifloxacin). The Applicant has noted that these studies have suggested "uncharacterized mechanisms" of besifloxacin resistance that may explain a significant proportion of besifloxacin non-susceptible isolates observed in these experiments. These mechanisms have not been further investigated.

BACTERICIDAL ACTIVITY

I. Minimum Bactericidal Concentration Studies

The Applicant has presented data from a study designed to investigate the bactericidal activity of besifloxacin tested against recent clinical isolates of *S. aureus* (n = 30), *S. epidermidis* (n = 15), *S. pneumoniae* (n = 35), and *H. influenzae* (n = 40) compared to gatifloxacin, moxifloxacin, ciprofloxacin, levofloxacin, penicillin, tobramycin, and azithromycin. Study 500510, "Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SS734 and comparators against ocular isolates of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*" was conducted in 2008 by [redacted] Clinical isolates collected from ocular sources between 2006 and 2007 were analyzed, using methods approved by CLSI (documents M100-S18 and M26-A). Quality control was performed on each day of testing. A complete line list of all tested isolates (including date of recovery, geographic origin, resistance phenotype, and clinical source) was included in the study report. The study results are summarized in Tables 27 – 20. Against *S. aureus* isolates, 80% had an MBC:MIC ratio of ≤ 2, while 13% had an MBC:MIC ration of 8. Against *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*, the percentage of isolates with MBC:MIC ≤ 2 was 93, 97, and 93 respectively.

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Table 27: In vitro MBC range and MIC:MBC ratio results for besifloxacin (SS734) and comparators against *S. aureus*

Phenotype*	Agent	Total n	MBC Range (µg/mL)	MBC:MIC n (%) 1		2		4		8		>8		ND	
Alt	Besifloxacin	30	0.015-8	17	(56.7)	7	(23.3)	2	(6.7)	4	(13.3)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015->8	16	(53.3)	4	(13.3)	1	(3.3)	4	(13.3)	0	(0.0)	5	(16.7)
	Gatifloxacin		0.03->8	16	(53.3)	6	(20.0)	2	(6.7)	1	(3.3)	1	(3.3)	4	(13.3)
	Ciprofloxacin		0.12->8	11	(36.7)	4	(13.3)	3	(10.0)	2	(6.7)	0	(0.0)	10	(33.3)
	Azithromycin		1->8	0	(0.0)	1	(3.3)	1	(3.3)	2	(6.7)	5	(16.7)	21	(70.0)
	Tobramycin		0.25->32	13	(43.3)	5	(16.7)	4	(13.3)	1	(3.3)	1	(3.3)	6	(20.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
OXA S	Besifloxacin	19	0.015-4	11	(57.9)	3	(15.8)	1	(5.3)	4	(21.1)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015->8	13	(68.4)	2	(10.5)	1	(5.3)	2	(10.5)	0	(0.0)	1	(5.3)
	Gatifloxacin		0.03->8	10	(52.6)	6	(31.6)	1	(5.3)	0	(0.0)	1	(5.3)	1	(5.3)
	Ciprofloxacin		0.12->8	10	(52.6)	4	(21.1)	2	(10.5)	0	(0.0)	0	(0.0)	3	(15.8)
	Azithromycin		1->8	0	(0.0)	1	(5.3)	1	(5.3)	2	(10.5)	4	(21.1)	11	(57.9)
	Tobramycin		0.25->16	10	(52.6)	4	(21.1)	3	(15.8)	0	(0.0)	1	(5.3)	1	(5.3)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
OXA R	Besifloxacin	11	0.03-8	6	(54.5)	4	(36.4)	1	(9.1)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.03->8	3	(27.3)	2	(18.2)	0	(0.0)	2	(18.2)	0	(0.0)	4	(36.4)
	Gatifloxacin		0.06->8	6	(54.5)	0	(0.0)	1	(9.1)	1	(9.1)	0	(0.0)	3	(27.3)
	Ciprofloxacin		0.5->8	1	(9.1)	0	(0.0)	1	(9.1)	2	(18.2)	0	(0.0)	7	(63.6)
	Azithromycin		8->8	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(9.1)	10	(90.9)
	Tobramycin		0.5->32	3	(27.3)	1	(9.1)	1	(9.1)	1	(9.1)	0	(0.0)	5	(45.5)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
CIP S	Besifloxacin	14	0.015-0.25	7	(50.0)	4	(28.6)	1	(7.1)	2	(14.3)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015-0.5	9	(64.3)	1	(7.1)	0	(0.0)	4	(28.6)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.03-4	6	(42.9)	5	(35.7)	1	(7.1)	1	(7.1)	1	(7.1)	0	(0.0)
	Ciprofloxacin		0.12-4	6	(42.9)	3	(21.4)	3	(21.4)	2	(14.3)	0	(0.0)	0	(0.0)
	Azithromycin		1->8	0	(0.0)	1	(7.1)	1	(7.1)	2	(14.3)	2	(14.3)	8	(57.1)
	Tobramycin		0.25->16	7	(50.0)	4	(28.6)	1	(7.1)	0	(0.0)	1	(7.1)	1	(7.1)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
CIP NS	Besifloxacin	16	0.12-8	10	(62.5)	3	(18.8)	1	(6.3)	2	(12.5)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.12->8	7	(43.8)	3	(18.8)	1	(6.3)	0	(0.0)	0	(0.0)	5	(31.3)
	Gatifloxacin		0.5->8	10	(62.5)	1	(6.3)	1	(6.3)	0	(0.0)	0	(0.0)	4	(25.0)
	Ciprofloxacin		2->8	5	(31.3)	1	(6.3)	0	(0.0)	0	(0.0)	0	(0.0)	10	(62.5)
	Azithromycin		8->8	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	3	(18.8)	13	(81.3)
	Tobramycin		0.25->32	6	(37.5)	1	(6.3)	3	(18.8)	1	(6.3)	0	(0.0)	5	(31.3)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	

NT = not tested, MBC = minimum bactericidal concentration, MIC = minimum inhibitory concentration
Source: Table 31; Section 2.7.2, this submission

Table 28: In vitro MBC range and MIC:MBC ratio results for besifloxacin (SS734) and comparators against *S. epidermidis*

Phenotype*	Agent	Total	MBC Range	MBC:MIC n (%)											
				1		2		4		8		>8		ND	
		n	(µg/mL)												
All	Besifloxacin	15	0.015-4	11	(73.3)	3	(20.0)	1	(6.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.06->8	8	(53.3)	3	(20.0)	1	(6.7)	0	(0.0)	0	(0.0)	3	(20.0)
	Gatifloxacin		0.06->8	6	(40.0)	4	(26.7)	2	(13.3)	0	(0.0)	0	(0.0)	3	(20.0)
	Ciprofloxacin		0.12->8	5	(33.3)	4	(26.7)	1	(6.7)	0	(0.0)	0	(0.0)	5	(33.3)
	Azithromycin		1->8	0	(0.0)	1	(6.7)	0	(0.0)	0	(0.0)	2	(13.3)	12	(80.0)
	Tobramycin		0.03->32	3	(20.0)	9	(60.0)	2	(13.3)	0	(0.0)	0	(0.0)	1	(6.7)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
OXA S	Besifloxacin	6	0.015-1	3	(50.0)	2	(33.3)	1	(16.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.06-2	3	(50.0)	3	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.06-4	2	(33.3)	3	(50.0)	1	(16.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.12->8	2	(33.3)	2	(33.3)	1	(16.7)	0	(0.0)	0	(0.0)	1	(16.7)
	Azithromycin		1->8	0	(0.0)	1	(16.7)	0	(0.0)	0	(0.0)	2	(33.3)	3	(50.0)
	Tobramycin		0.03-0.25	1	(16.7)	3	(50.0)	2	(33.3)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
OXA R	Besifloxacin	9	0.03-4	8	(88.9)	1	(11.1)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.06->8	5	(55.6)	0	(0.0)	1	(11.1)	0	(0.0)	0	(0.0)	3	(33.3)
	Gatifloxacin		0.06->8	4	(44.4)	1	(11.1)	1	(11.1)	0	(0.0)	0	(0.0)	3	(33.3)
	Ciprofloxacin		0.12->8	3	(33.3)	2	(22.2)	0	(0.0)	0	(0.0)	0	(0.0)	4	(44.4)
	Azithromycin		4->8	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	9	(100.0)
	Tobramycin		0.06->32	2	(22.2)	6	(66.7)	0	(0.0)	0	(0.0)	0	(0.0)	1	(11.1)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
CIP S	Besifloxacin	9	0.015-0.06	6	(66.7)	3	(33.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.06-0.12	5	(55.6)	3	(33.3)	1	(11.1)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.06-0.25	4	(44.4)	4	(44.4)	1	(11.1)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.12-0.5	4	(44.4)	4	(44.4)	1	(11.1)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		1->8	0	(0.0)	1	(11.1)	0	(0.0)	0	(0.0)	2	(22.2)	6	(66.7)
	Tobramycin		0.03-16	2	(22.2)	6	(66.7)	1	(11.1)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	
CIP NS	Besifloxacin	6	0.25-4	5	(83.3)	0	(0.0)	1	(16.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		1->8	3	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	3	(50.0)
	Gatifloxacin		1->8	2	(33.3)	0	(0.0)	1	(16.7)	0	(0.0)	0	(0.0)	3	(50.0)
	Ciprofloxacin		2->8	1	(16.7)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	5	(83.3)
	Azithromycin		>8->8	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	6	(100.0)
	Tobramycin		0.12->32	1	(16.7)	3	(50.0)	1	(16.7)	0	(0.0)	0	(0.0)	1	(16.7)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Oxacillin		NT	NT		NT		NT		NT		NT		NT	

*OXA, oxacillin; CIP, ciprofloxacin; S, susceptible; NS, non-susceptible
n <10 isolates, ND - MBC:MIC ratio not determined; results exceed panel range, NT - not tested
Source: Table 32; Section 2.7.2, this submission

Table 29: In vitro MBC range and MIC:MBC ratio results for besifloxacin (SS734) and comparators against *S. pneumoniae*

Phenotype*	Agent	Total n	MBC Range (µg/mL)	MBC:MIC n (%) 1		2		4		8		>8		ND	
All	Besifloxacin	35	0.015-0.5	21	(60.0)	13	(37.1)	1	(2.9)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.03-2	18	(51.4)	14	(40.0)	3	(8.6)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.03-4	17	(48.6)	18	(51.4)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.03-2	25	(71.4)	9	(25.7)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.9)
	Azithromycin		0.06->8	16	(45.7)	1	(2.9)	6	(17.1)	2	(5.7)	0	(0.0)	10	(28.6)
	Tobramycin		16-128	16	(45.7)	15	(42.9)	4	(11.4)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Penicillin		NT	NT		NT		NT		NT		NT		NT	
PEN S	Besifloxacin	31	0.015-0.5	17	(54.8)	13	(41.9)	1	(3.2)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.03-2	14	(45.2)	14	(45.2)	3	(9.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.03-4	14	(45.2)	17	(54.8)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.03->8	22	(71.0)	8	(25.8)	0	(0.0)	0	(0.0)	0	(0.0)	1	(3.2)
	Azithromycin		0.06->8	15	(48.4)	1	(3.2)	6	(19.4)	2	(6.5)	0	(0.0)	7	(22.6)
	Tobramycin		16-64	14	(45.2)	14	(45.2)	3	(9.7)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Penicillin		NT	NT		NT		NT		NT		NT		NT	
PEN I	Besifloxacin	2	0.06-0.12	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.12-0.12	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.25-0.5	1	(50.0)	1	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		1-1	1	(50.0)	1	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		>8->8	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	2	(100.0)
	Tobramycin		32-128	0	(0.0)	1	(50.0)	1	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Penicillin		NT	NT		NT		NT		NT		NT		NT	
PEN R	Besifloxacin	2	0.03-0.06	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.06-0.25	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.12-0.25	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.5-1	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		0.06->8	1	(50.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(50.0)
	Tobramycin		16-32	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
	Penicillin		NT	NT		NT		NT		NT		NT		NT	

*PEN, penicillin; S, susceptible; I, intermediate; R, resistant
n <10 isolates; ND - MBC:MIC ratio not determined, results exceed panel range; NT - not tested
Source: Table 33; Section 2.7.2, this submission

Table 30: In vitro MBC range and MIC:MBC ratio results for besifloxacin (SS734) and comparators against *H. influenzae*

Phenotype	Agent	Total n	MBC Range (µg/mL)	MBC:MIC n (%) 1		2		4		8		>8		ND	
All	Besifloxacin	40	0.015-0.03	17	(42.5)	20	(50.0)	3	(7.5)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015-0.06	24	(60.0)	15	(37.5)	1	(2.5)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.008-0.015	20	(50.0)	19	(47.5)	1	(2.5)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.008-0.03	19	(47.5)	21	(52.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		0.03-8	6	(15.0)	22	(55.0)	6	(15.0)	3	(7.5)	1	(2.5)	2	(5.0)
	Tobramycin		0.12-4	27	(67.5)	13	(32.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
β-lactamase neg	Besifloxacin	24	0.015-0.03	12	(50.0)	9	(37.5)	3	(12.5)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015-0.03	14	(58.3)	9	(37.5)	1	(4.2)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.008-0.15	13	(54.2)	10	(41.7)	1	(4.2)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.008-0.03	13	(54.2)	11	(45.8)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		0.03->4	4	(16.7)	13	(54.2)	3	(12.5)	1	(4.2)	1	(4.2)	2	(8.3)
	Tobramycin		0.12-4	13	(54.2)	11	(45.8)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	
β-lactamase pos	Besifloxacin	16	0.015-0.03	5	(31.3)	11	(68.8)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Moxifloxacin		0.015-0.06	10	(62.5)	6	(37.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Gatifloxacin		0.008-0.015	7	(43.8)	9	(56.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Ciprofloxacin		0.008-0.015	6	(37.5)	10	(62.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Azithromycin		0.5-8	2	(12.5)	9	(56.3)	3	(18.8)	2	(12.5)	0	(0.0)	0	(0.0)
	Tobramycin		1-4	14	(87.5)	2	(12.5)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Levofloxacin		NT	NT		NT		NT		NT		NT		NT	

*CLSI recommended breakpoints not available for susceptible (S), intermediate (I), and/or resistant (R) interpretation
ND - MBC:MIC ratio not determined, results exceed panel range; NT - not tested
Source: Table 34; Section 2.7.2, this submission

II. Time-kill Studies

The Applicant has submitted data from a study intended to describe the bactericidal activity of besifloxacin. Study [redacted] 99K3020B, "Antimicrobial Activity of SS732 and (+)SS734", was performed by the [redacted] in 2000. Time-kill studies were performed using recent Japanese clinical isolates of *S. aureus*, *S. pneumoniae*, and *H. influenzae*. MIC determinations were made using agar dilution techniques approved by CLSI. Isolates were tested at MIC, 2x MIC, and 4x MIC, and were sampled at 1, 15, 30, 45, 60, 90, 120 and 180 minutes. Isolates for the time-kill investigation were chosen based on their phenotypic profile (summarized in Table 31). No information was provided in the study report regarding quality control procedures performed during these investigations. b(4)

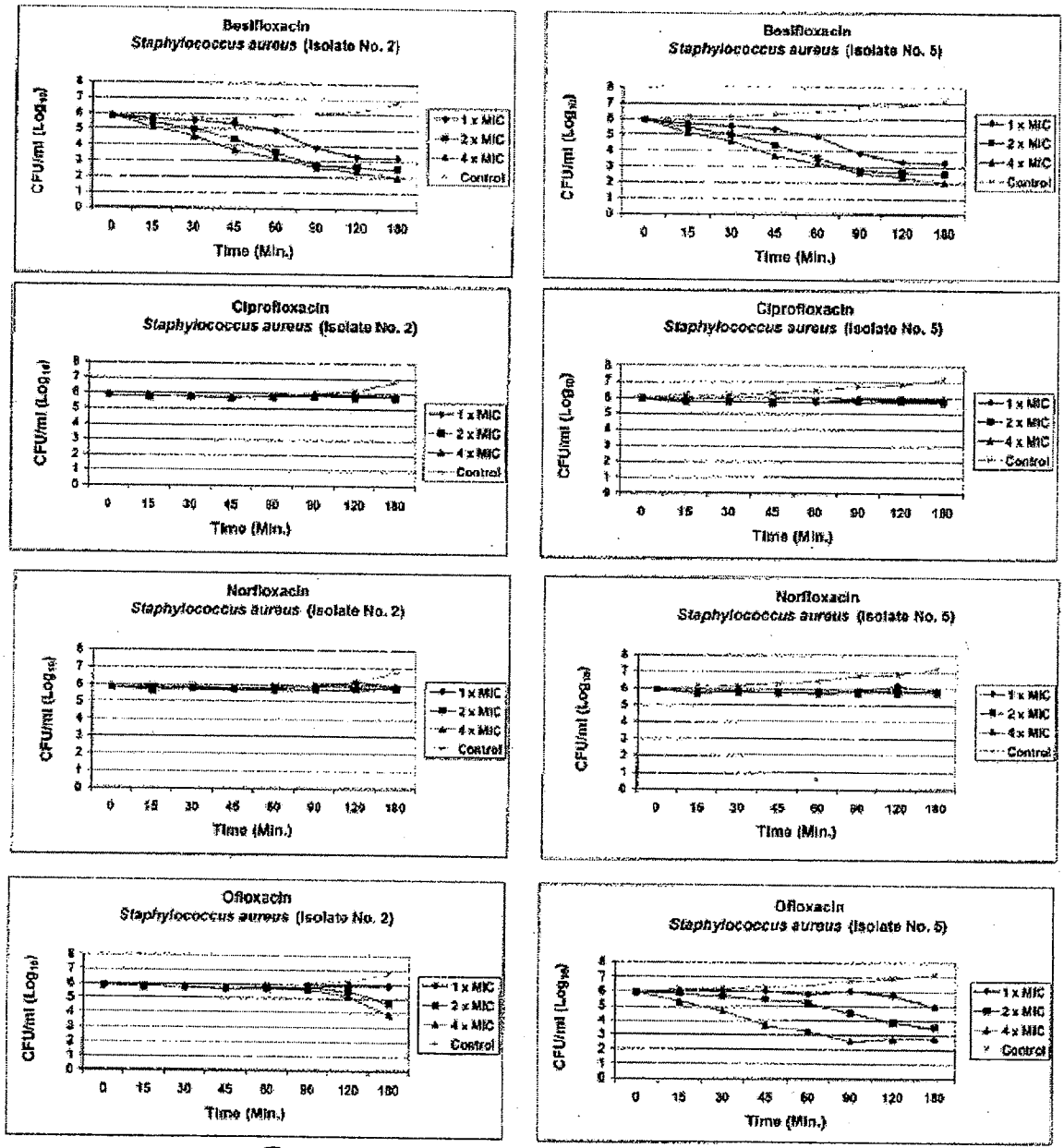
Table 31: In vitro Activity (mcg/ml) of Besifloxacin, Ciprofloxacin, Ofloxacin, and Norfloxacin Against Ocular Isolates Chosen for Time-Kill Experiments

Organism	Isolate	Besifloxacin	Ciprofloxacin	Ofloxacin	Norfloxacin
Streptococcus pneumoniae	3	0.13	1	2	4
	21	0.13	2	2	8
Staphylococcus aureus	2	0.5	8	8	32
	5	0.015	0.25	0.25	0.25
Coagulase negative Staphylococcus	2	0.06	0.25	0.25	0.25
	9	0.25	8	4	16
Haemophilus influenzae	1	0.06	0.015	0.06	0.13
	2	0.03	0.015	0.06	0.06
Pseudomonas aeruginosa	1	1	0.5	0.5	1
	2	4	2	2	4

Source: Table 5-9, Study [redacted] 99K3020B study report b(4)

The study results are summarized in Figures 1 though 3. Data is not summarized for coagulase negative staphylococci, since controls in this experiment were invalid (insufficient growth). Study results are also not presented *P. aeruginosa* determinations. Besifloxacin was less active than all comparators against the fluoroquinolone-susceptible isolate of *P. aeruginosa* (*P. aeruginosa* is not sought in the proposed indications for besifloxacin). Against fluoroquinolone-resistant isolates of *S. aureus*, besifloxacin activity was greater than norfloxacin and ciprofloxacin, and was bactericidal (3-log kill) at 90 minutes at 4x MIC (for both fluoroquinolone-susceptible and –resistant isolates). Against isolates of *S. pneumoniae* and *H. influenzae*, activity of besifloxacin was comparable to other fluoroquinolones.

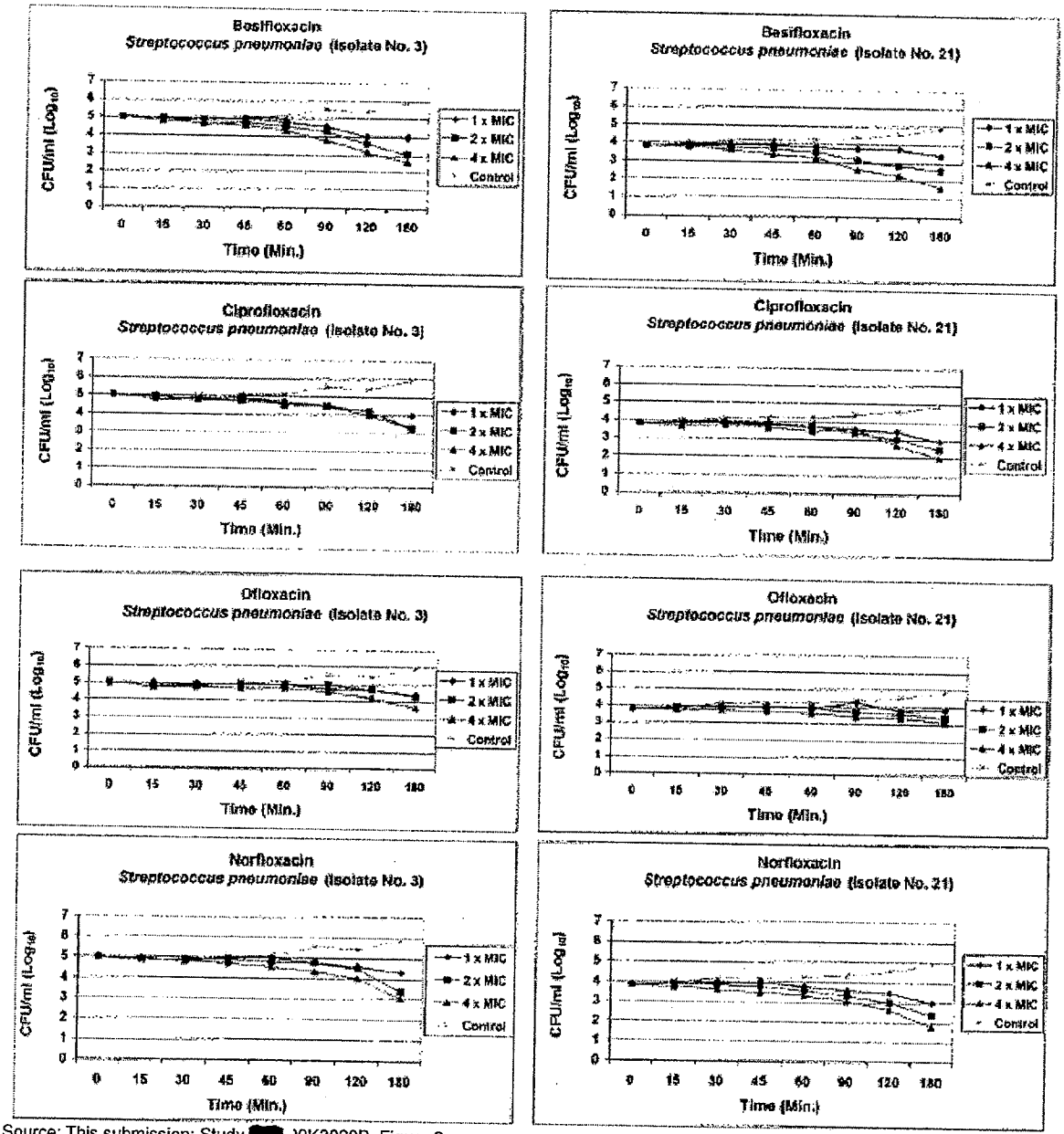
Figure 1: *S. aureus* reduction in CFU/ml (Log_{10}) for besifloxacin, ciprofloxacin, norfloxacin, and ofloxacin



Source: This submission; Study [redacted] 99K3020B, Figure 3

b(4)

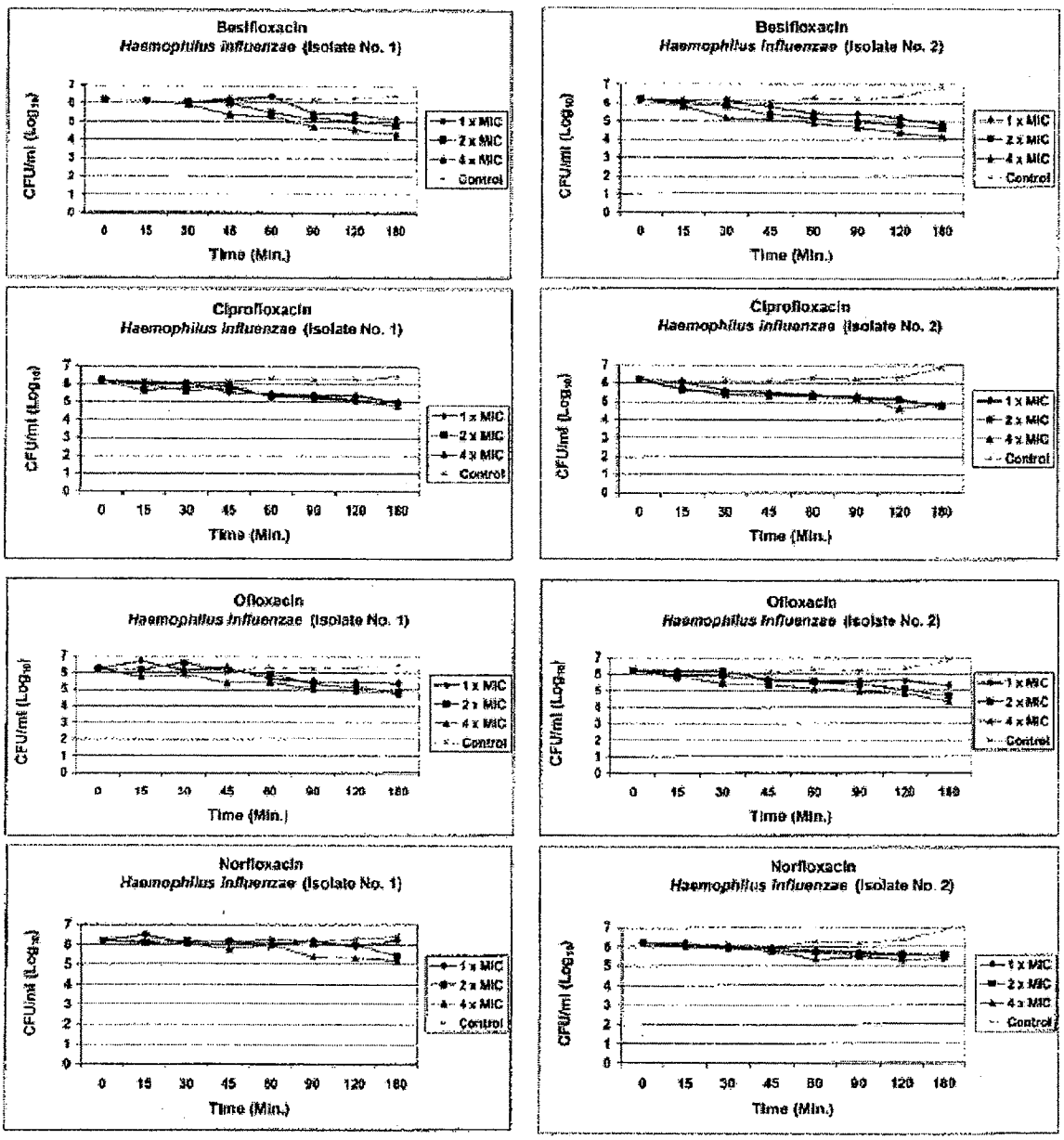
Figure 2: *S. pneumoniae* reduction in CFU/ml (Log_{10}) for besifloxacin, ciprofloxacin, norfloxacin, and ofloxacin



Source: This submission; Study [redacted] J9K3020B, Figure 2

b(4)

Figure 3: *H. influenzae* reduction in CFU/ml (Log_{10}) for besifloxacin, ciprofloxacin, norfloxacin, and ofloxacin



Source: This submission; Study [redacted]99K3020B, Figure 1

b(4)

In Summary:
The Applicant has submitted two studies to support the contention that besifloxacin displays bactericidal activity against ocular pathogens. In Study 500510, "Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SS734 and comparators against ocular isolates of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*", investigators demonstrated bactericidal activity (MBC:MIC ration ≤ 2) of besifloxacin against *S. aureus* (80% of isolates), *S. pneumoniae* (97% of isolates), *S. epidermidis* (93% of isolates), and *H. influenzae* (93% of isolates). Bactericidal activity against these pathogens, in this study, was similar to that of comparator fluoroquinolones. In Study [redacted]99K3020B, "Antimicrobial Activity of SS732 and (+)SS734", time-kill studies were performed, comparing the bactericidal activity of besifloxacin to ciprofloxacin, norfloxacin, and ofloxacin against isolates chosen for specific resistance phenotypes (including fluoroquinolone resistance). In this study, besifloxacin demonstrated rapid bactericidal activity against tested isolates (except *P. aeruginosa*), with activity greater than or similar to fluoroquinolone comparators. b(4)

Bactericidal Activity Studies ~ Conclusions:
Studies submitted in support of this NDA have shown that besifloxacin demonstrates bactericidal activity against specific ocular pathogens, including *S. aureus*, *S. pneumoniae*, *S. epidermidis*, and *H. influenzae*. The measured MBC:MIC ratio for most isolates was ≤ 2. Time-kill experiments demonstrated rapid bactericidal activity against most tested ocular pathogens that was similar to or exceeded that of comparator fluoroquinolones (including ciprofloxacin, norfloxacin, and ofloxacin). Against *P. aeruginosa*, besifloxacin was less active than fluoroquinolone comparators.

HUMAN AND ANIMAL STUDIES

ANIMAL EFFICACY STUDIES

The Applicant has submitted data from two studies designed to investigate the in vivo efficacy of besifloxacin in animal infections.

Study SS734PRE-001. "Protective effect of FC-124, a new quinolone compound, in a systemic *Streptococcus pneumoniae* infection in mice", was performed at [redacted] in 1991. In this study, male ICR mice were inoculated intraperitoneally with 0.5 ml (6 x 10^4 CFU) of a suspension of S. pneumoniae IID 553 in brain heart infusion broth (with 5% horse blood). Mice (5 mice per treatment arm) were treated orally with a single 0.5 ml dose of either besifloxacin, tosulfloxacin, ofloxacin, or placebo control. The study results are summarized in Table 32. All tested antibiotics extended survival, compared to the placebo control. Besifloxacin and tosufloxacin, dosed at 25 mg/kg extended survival of all animals to the study endpoint (day 6)

b(4)

Table 32: Protective effect of besifloxacin, tosufloxacin and ofloxacin in a systemic S. pneumoniae infection model in mice

Drug	Dose (mg/kg)	No. of survivors at day 6/ No. of mice tested	Mean survival day (mean ± S.D.)
Placebo control	-	0/5	1.6 ± 0.5
Besifloxacin	12.5	2/5	4.0 ± 0.5[a]
	25	5/5	6.0 ±0.0[b]
	50	5/5	6.0 ± 0.0[b]
Tosufloxacin	12.5	0/5	3.0 ± 0.7[a]
	25	5/5	6.0 ± 0.0[b]
	50	5/5	6.0 ± 0.0[b]
Ofloxacin	25	0/5	2.0 ± 0.0
	50	0/5	2.2 ± 0.4
	100	0/5	3.4 ± 1.5[a]

[a] $p < 0.05$;
[b] $p < 0.01$ (Kaplan-Meier method [Cox-Mantel test])
Source: Figure 36, Section 2.7.2; this submission

Study BL07001, "Evaluation of the Efficacy of Four Antibiotic Formulations in a Prophylactic Endophthalmitis Model in New Zealand White Rabbits," was performed in 2007 by the Applicant at the B&L Biological Test Center (Irvine CA). In this study, bacterial *S. aureus* endophthalmitis was induced in 51 female rabbits. All rabbits were administered topical antibiotics or placebo according to the same regimen (60, 45, 30, and 15 minutes pre-inoculation, and 0, 6, 12, 18, and 24 hours post-inoculation). The right eyes of the rabbits were inoculated with suspensions of *S. aureus* ATCC 33591 in three separate experiments with varying protocols (Groups A-C, Groups D-F, Groups G-I), described in Table 33. All rabbits were examined after treatment and ophthalmic findings were graded for severity. Rabbits were euthanized after examination. Right eye aqueous and vitreous humors were collected and plated for bacterial colony count.

Table 33: Rabbit Endophthalmitis Study: Treatment Groups

Group	No.	Topical Antibiotic Treatment (Right Eye)	Dose Volume	Bacteria Dosing (Right eye)	*S. aureus* ATCC 33591 Dose Volume	Necropsy
A	6	Saline	50 µL	Intracameral	25 µL	Day 2
B	6	0.6% BOL-303224-A	50 µL	Intracameral	25 µL	Day 2
C	6	Zymar® (0.3% Gatifloxacin)	50 µL	Intracameral	25 µL	Day 2
D	6	Quixin® (0.5% Levofloxacin)	50 µL	Intracameral	25 µL	Day 2
E	6	Vigamox® (0.5% Moxifloxacin)	50 µL	Intracameral	25 µL	Day 2
F[1]	3	Untreated	N/A	Intracameral	25 µL	Day 2
G	6	Saline	50 µL	Intracameral	25 µL	Day 2
H	6	Quixin® (0.5% Levofloxacin)	50 µL	Intracameral	25 µL	Day 2
I	6	Vigamox® (0.5% Moxifloxacin)	50 µL	Intracameral	25 µL	Day 2

N/A = Not applicable.

[1] Note: In Table 1 of the original protocol, Group F contained 6 rabbits designated to receive AzaSite™ (1% Azithromycin) treatment. This treatment group was removed per Amendment 3.

Mean ophthalmic severity scores were lower for besifloxacin-treated rabbits than for rabbits treated with any comparator. Differences in severity scores for the same comparators in the separate experiments were noted. No conjunctival discharge was noted in besifloxacin-treated subjects (all other subjects produced a discharge). No viable bacteria were recovered from the vitreous humor or aqueous humor of treated subjects, with the exception of Group H (two samples grew 5 CFU/ml). A third of the control groups (saline or untreated) also failed to produce viable bacteria in the aqueous humor (no bacteria were recovered from the vitreous humor of any animal).
Reviewer's Note: The study design of this investigation resulted in data that is difficult to integrate. Results from the three test groups differ in significant aspects, including differences in growth of *S. aureus* in aqueous humor (including controls) and the noted difference in ophthalmic severity scores between the three experimental groups (presumably associated with the three separate inoculation concentrations). These design aspects, combined with the numerous protocol deviations noted in the study report, suggest that conclusions drawn to support this NDA should be cautiously limited to experimental subgroup 1 (Groups A-C).

In summary:
The Applicant has presented data from two studies intended to investigate in vivo efficacy of besifloxacin in animal models of infection. Study SS734PRE-001. "Protective effect of FC-124, a new quinolone compound, in a systemic *Streptococcus pneumoniae* infection in mice" (1991) examined the effect of oral dosing of besifloxacin in mice intraperitoneally inoculated with *S. pneumoniae*. Pre- and post-inoculation doses of Besifloxacin at 25 mg/kg extended survival of mice, compared to control, through the experimental protocol (6 days). Study BL07001, "Evaluation of the Efficacy of Four Antibiotic Formulations in a Prophylactic Endophthalmitis Model in New Zealand White Rabbits" examined the role of besifloxacin in controlling ocular inflammation and discharge in rabbits intracamerally injected with *S. aureus*. Besifloxacin (0.6% 50 µL) was shown to decrease conjunctival discharge, compared to saline control and gatifloxacin (0.3%).

Animal Efficacy Studies ~ Conclusions:
Study reports from two animal models of infection, including a study of a systemic *S. pneumoniae* infection in ICR mice, and a study of *S. aureus* endophthalmitis in New Zealand white rabbits support the in vivo efficacy of besifloxacin. In the mouse study, oral besifloxacin was shown to be protective, compared to ofloxacin and control, extending the survival of mice intraperitoneally

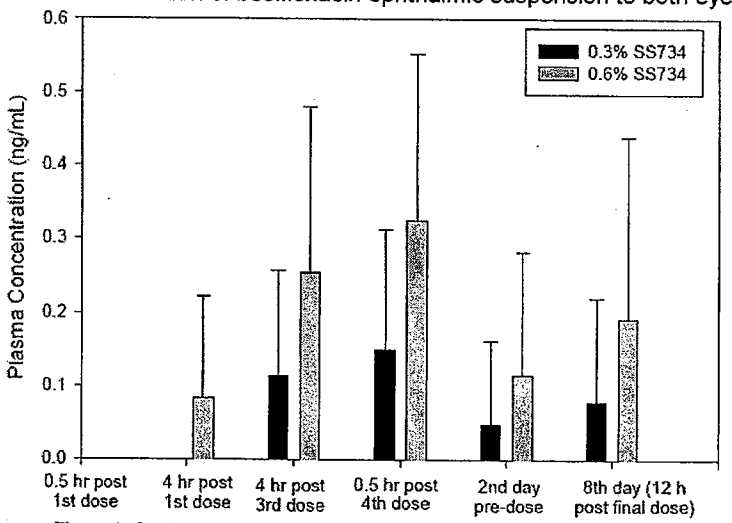
inoculated with *S. pneumoniae* IID 553. In the rabbit study, topical besifloxacin (0.6%) was proven superior to gatifloxacin (0.3%) and saline control in limiting ophthalmic inflammation and conjunctival discharge.

PHARMACOKINETIC / PHARMACODYNAMIC STUDIES

The Applicant has provided data from investigations designed to evaluate systemic exposure resulting from topical administration of besifloxacin, both in healthy patients and in patients with ocular infection.

Study C-02-403-001, "A Study to Evaluate the Systemic Safety and Ocular Safety/Tolerability of Topical Administration of 0.3% and 0.6% ISV-403 Compared to Vehicle When Dosed QID for 7 Days in Normal Volunteers," was sponsored by [redacted] and performed in 2003 at [redacted] The study was a single site, randomized, double-masked, parallel clinical trial. Two ascending doses (0.3 % and 0.6% besifloxacin, compared to vehicle) were studied in two separate groups of approximately 14 subjects per arm (12 subjects were treated in the 3% besifloxacin arm). The treatments were dosed QID for 7 days. Blood samples were drawn according to the protocol illustrated in Figure 4. Plasma level for both dosage groups averaged less than 0.35 ng/ml. b(4)

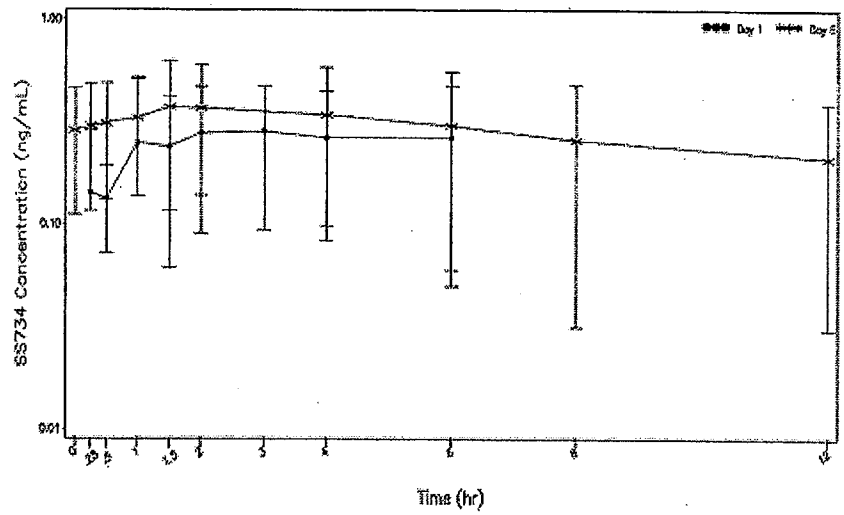
Figure 4: Mean (±SD) plasma concentrations of besifloxacin following single and repeated topical ocular administration of besifloxacin ophthalmic suspension to both eyes of healthy subjects



Source: Figure 1, Section 2.7.2; this submission

Study 478, "Systemic Pharmacokinetics of SS-734 after Single and Multiple TID Instillations of 0.6% ISV-403 Ophthalmic Suspension in Subjects with Suspected Bacterial Conjunctivitis," was performed by the Applicant in 2008. The objective of the study was to investigate systemic exposure of besifloxacin 0.6%, following topical administration (TID), in patients with suspected bacterial conjunctivitis. The study was designed as a multicenter, open-label, single-dose/multiple-dose, pharmacokinetics study. Twenty-four subjects with a diagnosis of bilateral bacterial conjunctivitis were treated with 1 drop TID for 5 days. Blood samples were drawn from an indwelling catheter at regular intervals. The study results are summarized in Figures 5 and 6. The average C_{max} was below 0.5 ng/ml, with slight accumulation observed after repeated dosing.

Figure 5: Mean (±SD) plasma concentration-time profiles for besifloxacin (SS734) after single (Day 1) and repeated (Day 6) TID topical ocular administration of besifloxacin ophthalmic suspension (0.6%)



Source: Figure 2, Section 2.7.2; this submission

Table 33: PK Parameter Values for Besifloxacin after the First Dose (Day 1) and at Steady-State (Day 6) Following Topical Ocular Administration of Besifloxacin Ophthalmic Suspension (0.6%)

Parameter	Units	Day 1				Day 6			
		N	Mean	SD	%CV	N	Mean	SD	%CV
C_{max}	(ng/mL)	22	0.368	0.274	75	22	0.428	0.299	70
T_{max}	(h)	22	3.17	1.74	55	22	2.41	2.4	100
AUC_{0-6}	(ng*h/mL)	20	1.45	0.865	60	22	1.95	1.31	67
$t_{1/2}$	(h)	8	4.27	2.22	52	14	6.75	2.14	32
C_{max} Accumulation Ratio		-	-	-	-	22	1.45	0.656	45
AUC Accumulation Ratio		-	-	-	-	20	1.60	0.742	46

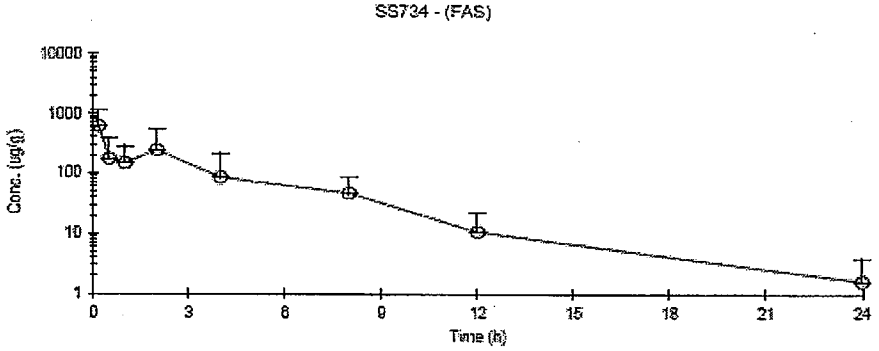
C_{max} Accumulation Ratio was calculated as the ratio of C_{max} for Day 6/Day1
AUC Accumulation Ratio was calculated as the ratio of AUC_{0-6} for Day 6/Day1
-=Not calculated.

Source: Table 2, Section 2.7.2; this submission

The Applicant has presented data from studies conducted to investigate the pharmacokinetics of besifloxacin in human tear fluid. Study 424, "Ocular Pharmacokinetics of 0.6% ISV-403 Eye Drops After a Single Instillation and During Repeated Instillations for 5 Days in Healthy Volunteers", was conducted at the [redacted] in 2006 (report issued in 2008). The investigation was a single-center, open-label, prospective study, conducted on 64 healthy subjects, aged 18 to 39 years, who each received a single instillation (37 µL) or besifloxacin 0.6% in the conjunctival sac of each eye. Tear fluid was collected with a Schirmer tear strep, with 8 samples collected over a 24 hour period. The results (mean concentration by sample time) are summarized in Figure 6.

b(4)

Figure 6: Mean (+SD) besifloxacin (SS734) concentration-time profile in tears after single topical ocular administration of besifloxacin ophthalmic suspension (0.6%) to healthy subjects.



Source: Figure 3, Section 2.7.2; this submission

Noncompartmental PK analysis was performed, using the determined mean besifloxacin concentration. Total exposure of besifloxacin (AUC_{0-24}) was determined to be 1232 µg*h/g, with an estimated half-life of 3.4 hours. MIC_{90} values of 1 mcg/ml for *S. aureus* and ≤ 0.06 mcg/ml for *H. influenzae* (determined in separate in vitro experiments) were used to calculate C_{max}/MIC_{90} and AUC_{24}/MIC_{90} values for each ocular pathogen. For *S. aureus*, the C_{max}/MIC_{90} ratio was calculated to be 610 and the AUC_{24}/MIC_{90} ratio was 1523. For *H. influenzae*, the C_{max}/MIC_{90} ratio was ≥ 13517 and the AUC_{24}/MIC_{90} ratio was ≥ 25383. These results are summarized in Table 34.

Table 34: PK/PD Parameter Values For Besifloxacin in Tears Following Single Topical Ocular Administration of Besifloxacin Ophthalmic Suspension (0.6%) to Healthy Subjects

	N	T_{max} (h)	C_{max} (µg/g)	AUC_{24} (µg * h/g)	$T_{1/2}$ (h)	C_{max}/MIC_{90}[a]	C_{max}/MIC_{90}[b]	AUC_{24}/MIC_{90}[a]	AUC_{24}/MIC_{90}[b]
FAS[c]	64	0.17	610	1232	3.43	610	≥10167	1232	≥20533
PP[d]	51	0.17	811	1523	3.51	811	≥13517	1523	≥25383

[a] MIC_{90} *S. aureus* = 1 µg/mL
[b] MIC_{90} *H. influenzae* ≤ 0.06 µg/mL
[c] FAS: Full Analysis Set
[d] PP: Per Protocol set
Source: Table 3, Section 2.7.2; this submission

The Applicant also used the experimental data to calculate a two-compartment PK model. With the assumption that protein binding of besifloxacin in ocular tissue is similar to that determined in human plasma (~ 40%), and using the experimentally derived C_{max} (as opposed to the modeled C_{max}), PK/PD ratios were determined for total and free besifloxacin. The results are summarized in Table 35.

Table 35: Predicted PK/PD ratios for besifloxacin in tears after repeated (TID) topical administration of besifloxacin ophthalmic suspension 0.6% to human subjects

Organism	MIC_{90} (µg/mL)	C_{max}/MIC_{90} [a]		AUC_{24}/MIC_{90} [b]	
		Total [c]	Free [d]	Total [c]	Free [d]
Gram-positive					
S. aureus	0.5	1220	732	7602	4561
S. pneumoniae	0.125	4880	2928	30408	18245
S. epidermis	0.5	1220	732	7602	4561
Gram-negative					
H. influenzae	0.06	10167	6100	63350	38010

[a] Calculations based on besifloxacin Cmax (observed) of 610 µg/g.
[b] Calculations based on besifloxacin AUC_{24} (predicted, TID) of 3801 µg*h/g
[c] PK/PD ratios calculated based on total (bound and free) besifloxacin
[d] PK/PD ratios calculated based on free besifloxacin levels, which were calculated using the measured value of besifloxacin binding to human plasma proteins (40% bound)
TID = three times daily, MIC_{90} = minimum inhibitory concentration for 90% of strains tested
Source: Table 35, Section 2.7.2; this submission

In summary:
The Applicant has presented data from three studies designed to describe the pharmacokinetics and pharmacodynamics of besifloxacin in human tears. Study C-02-403-001, "A Study to Evaluate the Systemic Safety and Ocular Safety/Tolerability of Topical Administration of 0.3% and 0.6% ISV-403 Compared to Vehicle When Dosed QID for 7 Days in Normal Volunteers," demonstrated very low plasma levels (< 0.35 ng/ml) following topical administration in healthy volunteers. In Study 478, "Systemic Pharmacokinetics of SS-734 after Single and Multiple TID Instillations of 0.6% ISV-403 Ophthalmic Suspension in Subjects with Suspected Bacterial Conjunctivitis," topical administration of besifloxacin in infected eyes likewise resulted in very low levels of systemic exposure (average C_{max} < 0.5 ng/ml). Study 424, "Ocular Pharmacokinetics of 0.6% ISV-403 Eye Drops After a Single Instillation and During Repeated Instillations for 5 Days in Healthy Volunteers" provided data for the determination of PK/PD parameters for besifloxacin in human tear fluid. This study demonstrated that topical administration of besifloxacin resulted in high levels that exceeded the MIC_{90} values for specific ocular pathogens (*S. aureus*, *S. pneumoniae*, *S. epidermidis*, and *H. influenzae*), persisting for at least 24 hours (mean C_{24h} = 1.60 ± 2.28 mcg/ml). Based on this model, free besifloxacin levels were higher than quinolone targets (C_{max}/MIC_{90} ratio of > 10, AUC/MIC_{90} ratio of >100-125) for the listed pathogens.

Pharmacokinetic / Pharmacodynamic Studies ~ Conclusions:
The Applicant has submitted data from pharmacokinetic studies that demonstrates that besifloxacin 0.6%, delivered as a topical ocular application, results in very low plasma levels in both healthy individuals (< 0.35 ng/ml) and in subjects with presumptive bacterial conjunctivitis (< 0.5 ng/ml). Based on published pharmacodynamic targets for fluoroquinolones, and the presumption that besifloxacin is bound by tear fluid proteins to a degree similar to that demonstrated in human serum (~40 %), PK/PD modeling predicts that achievable free besifloxacin tear fluid concentrations exceed the therapeutic concentrations necessary for antibacterial activity against *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*.

CLINICAL TRIALS

The Applicant conducted three Phase 3 clinical trials in support of the NDA. All three were multi-center, randomized, double-masked trials designed to investigate the efficacy and safety of 0.6% besifloxacin ophthalmic solution (TID x 5 days). Studies 373 and 433 were vehicle-controlled trials. Study 434 was an active-controlled, non-inferiority trial comparing besifloxacin to moxifloxacin (Vigamox). The primary endpoint assessment for Studies 433 and 434 was clinical resolution (absence of ocular discharge and bulbar injection) at Visit 2 (Day 5 ± 1). The primary endpoint assessment for Study 373 was clinical resolution (absence of ocular discharge, bulbar injection, and palpebral injection) at Visit 3 (Day 8 (9)). Specimens for microbiologic analysis (bacterial and viral) were collected at the Baseline Visit, Visit 2 (Day 4 ± 1 for Study 373, and Day 5 ± 1 for Studies 433 and 434), and Visit 3 (Day 8 +1). Microbial eradication was defined as "the absence of all accepted ocular bacterial species that were ≥ threshold at baseline." Species-specific microbial eradication was "determined independently for each baseline (Visit 1) bacterial isolate." Analysis performed for the purpose of describing besifloxacin activity against specific ocular pathogens was generally reported using the mITT population and "species-specific study eyes" (and "species-specific fellow eyes"), except as noted.

Threshold criteria were based on published recommendations for judging culture positive specimens collected from ocular infections [Leibowitz 1991, Cagle 1981]. The employed criteria did not categorically describe the isolation of multiple organisms from a single study eye. In such cases, each isolated organism was categorized and analyzed separately. Many subjects in all three Phase 3 trials produced more than a single isolate from a study eye (often as many as three isolates, all analyzed as baseline pathogens), and in some cases, subjects produced both positive bacterial and viral cultures. As discussed below, bacteria present in fellow eyes that exceeded threshold levels, but were not present in the designated study eye, were also analyzed as baseline pathogens.

The major inclusion and exclusion criteria were similar for all trials.

Studies 373 and 433 were conducted in the United States. Study 434 included both US sites and Asian sites.

Study populations were defined as follows, for purposes of statistical analysis and data tabulation (source: this Application; Report M1240, page 29):

- The Intent to Treat (ITT) study population (n=2387) included all randomized subjects. Analyses performed on the ITT study population were according to treatments 'as randomized'. In these analyses, either observed data and/or data imputed for missing values/discontinued subjects were employed.
- The mITT study population (n=1041) presented in this integrated clinical microbiology report and corresponding appendix tables include all ITT subjects from Study M373 and all mITT subjects from Study M433 and M434, and included all subjects in the study population for whom baseline cultures in at least 1 eye indicated bacteria levels at or above threshold for any accepted ocular species. Analyses performed on the mITT study population were according to treatments 'as randomized' unless specified 'as treated'. In these analyses, either observed data and/or data imputed for missing values/discontinued subjects were employed.
- The Per Protocol (PP) study population (n=705) included those subjects in the ITT study population for whom no major protocol violations were noted.

In Study 373, the ITT population included subjects with bacterial conjunctivitis confirmed by culture. In Studies 433 and 434, the ITT population included subjects with bacterial conjunctivitis diagnosed clinically, and the mITT populations in these studies included subjects with culture-confirmed diagnosis.

Bacterial isolates were collected and analyzed from both baseline-designated Study Eyes and Fellow Eyes, dependent on criteria published in the study protocol (identical for all three protocols). These criteria included (source: this Application, Section 2.7.2, page 141):

- At baseline (Visit 1), subjects included in mITT and PP populations had at least one eye that (i) met clinical criteria for acute conjunctivitis, (ii) was treated with besifloxacin or control, and (iii) yielded bacterial cultures at or above defined threshold levels for that pathogen.
- If only one eye met criteria (i)-(iii), then this eye was designated as the Study Eye. The terms Baseline-Designated Study Eye and Study Eye are used interchangeably.
- If both eyes met criteria (i)-(iii), then the eye with the highest clinical score was designated as the Study Eye. If both eyes met criteria (i)-(iii) with the same clinical score, then the right eye (OD) was designated as the Study Eye. The eye that was not the Study Eye was designated as the Fellow Eye.
- In all cases, any baseline (Visit 1) bacterial species isolated at or above threshold from an individual Study Eye was used in any Species-Specific Study Eye tabulations for that species.
- If both subject eyes met criteria (i)-(iii), and the baseline-designated Fellow Eye yielded baseline cultures at or above threshold for an additional species not present at or above threshold in the Study Eye, then the additional bacterial species isolated at or above threshold from that subject's Fellow Eye was also included in tabulations of Species-Specific Study Eyes for that species.
- Note that all tabulations of baseline bacterial pathogens using the Species- Specific Study Eye designation therefore included isolates from a subject's Fellow Eye only if that species was not present at or above threshold in that subject's Study Eye. Thus, the Species-Specific Eye designation ensured that each bacterial species was counted only once per subject in any tables or summaries presenting an analysis by species.
- In summary, the Study Eye and Fellow Eye designations were used to evaluate data at the eye level, whereas the Species-Specific Study Eye and Species-Specific Fellow Eye designations were used to evaluate microbiological data at the species level.

All specimens were shipped to [redacted] as culture swabs suspended in transport media (PBS plus 20% glycerol for bacterial culture, M4RT for viral culture). Specimens collected in Asia (Study 434) were shipped first to a Contract Research Organization (CRO) in [redacted] and then to [redacted] Vortexed media was plated onto Sheep blood agar (BAP), Chocolate agar (CAP), and Sabouraud agar (Emmons) for confluent growth, and to BAP and CAP for quantitative culture. Swabs were plated to CAP and BAP and streaked for isolation. Gram stains were not performed. Plates were incubated in appropriate conditions and interpreted at 24 and 48 hours for bacterial growth, and at 2-5 days for fungal growth. Viral transport media was cultured for recovery of Varicella Zoster Virus, Herpes Simplex Virus, Adenovirus, and Enterovirus. Microorganisms were identified according to the [redacted] protocol submitted with the study reports. The protocol was reviewed and is appropriate as submitted. Strain typing by pulsed field gel electrophoresis (PFGE) was performed for all bacteria of the same species that were recovered at both the baseline and subsequent visits. All tested isolates were stored in duplicate.

b(4)
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Susceptibility testing for isolates from cultures that passed threshold criteria was performed by minimum inhibitory test procedures following CLSI guideline M7-A6 (2003), using microtiter plates prepared by [redacted] Antibiotics were added to the plates in 2-fold dilutions (0.004 – 8 mcg/ml for besifloxacin, azithromycin, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin, 0.015 – 8 mcg/ml for penicillin, and 0.03 – 8 mcg/ml for oxacillin). Interpretive standards for all comparator antimicrobials were based on CSLI document M100-S16 (2006). Besifloxacin tentative interpretive standards and quality control are discussed below. Quality control measures were performed according to CLSI guidelines (M7-A6, 2003), with corrective

b(4)

actions and repeat testing performed as required. No disk diffusion testing was performed.

Conjunctival cultures were measured on the following 0-3 scale:

- 0a. Eradication (infecting organism originally present at or above threshold on Day 1 and absent in follow-up culture without new isolate at or above threshold)
- 0b. Eradication (infecting organism originally present at or above threshold on Day 1 and absent in follow-up culture with new isolate present at or above threshold)
- 1a. Reduction (infecting organism originally present at or above threshold on Day 1 and reduced to a count below threshold in follow-up culture without a new isolate at or above threshold)
- 1b. Reduction (infecting organism originally present at or above threshold on Day 1 and reduced to a count below threshold in follow-up culture with a new isolate present at or above threshold)
- 2a. Persistence (infecting organism originally present at or above threshold on Day 1 and not exceeding Day 1 count, but remains above or equal to threshold in follow-up culture without new isolate at or above threshold)
- 2b. Persistence (infecting organism originally present at or above threshold on Day 1 and not exceeding Day 1 count, but remains above or equal to threshold in follow-up culture with new isolate present at or above threshold)
- 3a. Proliferation (infecting organism originally present at or above threshold on Day 1 and is increased above Day 1 count in follow-up culture) without a new isolate at or above threshold
- 3b. Proliferation (infecting organism originally present at or above threshold on Day 1 and is increased above Day 1 count in follow-up culture) with a new isolate present at or above threshold

The overall bacterial eradication results from the integrated study analysis for the mITT (as treated) and PP populations are summarized in Tables 36 and 37.

Table 36: Overall microbiological eradication rates for mITT (as treated) population

	Study 373*		Study 433		Study 434	
	Besifloxacin N=76	Vehicle N=69	Besifloxacin N=251	Vehicle N=229	Besifloxacin N=329	Vigamox N=370
Visit 2 (Day 5, ±1 Day)*						
Eradicated	70 (92.1%)	36 (52.2%)	228 (90.8%)	147 (64.2%)	307 (93.3%)	339 (91.6%)
Non-eradicated	6 (7.9%)	33 (47.8%)	23 (9.2%)	82 (35.8%)	22 (6.7%)	31 (8.4%)
Visit 3 (Day 8, ±1 Day)						
Eradicated	69 (90.8%)	44 (63.8%)	224 (89.2%)	172 (75.1%)	287 (87.2%)	317 (85.7%)
Non-eradicated	7 (9.2%)	25 (36.2%)	27 (10.8%)	57 (24.9%)	42 (12.8%)	53 (14.3%)

- For Study 373 Visit 2 was Day 4, ±1 Day

Source: Table 41, Section 2.7.2; this submission

Table 37: Overall microbiological eradication rates for PP population

	Study 373*		Study 433		Study 434	
	Besifloxacin N=56	Vehicle N=46	Besifloxacin N=191	Vehicle N=162	Besifloxacin N=205	Vigamox N=235
Visit 2 (Day 5, ±1 Day)*						
Eradicated	51 (91.1%)	27 (58.7%)	176 (92.1%)	107 (66.0%)	201 (98.0%)	217 (92.3%)
Non-eradicated	5 (8.9%)	19 (41.3%)	15 (7.9%)	55 (34.0%)	4 (2.0%)	18 (7.7%)
Visit 3 (Day 8, ±1 Day)						
Eradicated	52 (92.9%)	36 (78.3%)	178 (93.2%)	131 (80.9%)	188 (91.7%)	206 (87.7%)
Non-eradicated	4 (7.1%)	10 (21.7%)	13 (6.8%)	31 (19.1%)	17 (8.3%)	29 (12.3%)

* For Study 373 Visit 2 was Day 4, ±1 Day
Source: Table 42 Section 2.7.2; this submission

STUDY 373

Study 373 was conducted between December 2004 and June 2005 at 35 U.S. sites. The study randomized 269 subjects (137 received besifloxacin, 132 received vehicle), with 44% (118/269) culture confirmed for bacterial conjunctivitis. The ITT population included 116 subjects. Treatment (besifloxacin vs. vehicle) distribution between demographic groups was balanced.

90% of subjects randomized to besifloxacin treatment experienced bacterial resolution at Visit 3, compared to 69.1% of subjects randomized to vehicle. At Visit 2, 90% of subjects randomized to besifloxacin experienced bacterial resolution, compared to 51.8% of subjects randomized to vehicle. At baseline, 145 bacterial isolates were identified from species-specific study eyes (76 from subjects randomized to besifloxacin treatment, and 69 from subjects randomized to vehicle). In the per-protocol population, 102 bacterial isolates (from 80 subjects) were recovered. Of these, 54.9% were recovered from subjects randomized to besifloxacin treatment, and 45.1% from subjects randomized to vehicle. The distribution of key pathogens is summarized in Table 38 (baseline) and Table 39 (per-protocol population).

Bacterial isolates recovered at Visit 3 that met or exceeded threshold criteria for analysis, were tested by pulsed field electrophoresis to distinguish new infection from recurrence/persistence. Isolate pairs in the besifloxacin group were 100% concordant (11/11), while isolate pairs in the vehicle group were 94.7% concordant (36/38). No concordant isolates, recovered at Visits 2 or 3, had an MIC value ≥ 4-fold the baseline value.

Besifloxacin activity against the primary pathogens (*S. aureus*, *S. epidermidis*, *H. influenzae*, and *S. pneumoniae*) recovered in Study 373 was generally greater than or comparable to other fluoroquinolones tested. Besifloxacin MIC values for these pathogens were lower than the achievable therapeutic levels predicted by PK/PD analysis (discussed above). Besifloxacin MIC values were elevated against ciprofloxacin-resistant isolates of staphylococcal species, but these MIC values did not exceed predicted therapeutic levels of besifloxacin, and all ciprofloxacin-resistant isolates were eradicated by Visit 3. No differences in MIC values were noted against isolates with resistance to the ß-lactam class of antimicrobials, or against ß-lactamase positive isolates compared non-resistant and ß-lactamase negative isolates.

Table 38: Baseline distribution of key pathogens across Study 373 treatment groups: ITT population, as treated: species-specific study eye: sorted by overall organism frequency

Organism	Phenotype[a]	Besifloxacin		Vehicle		Overall	
		N	%[b]	N	%[b]	N	%[b]
Haemophilus influenzae	All	25	32.9	21	30.4	46	31.7
Streptococcus pneumoniae	All	24	31.6	16	23.2	40	27.6
Staphylococcus aureus	All	10	13.2	10	14.5	20	13.8
	MRSA-CR	1	1.3	2	2.9	3	2.1
	MSSA-CS	9	11.8	8	11.6	17	11.7
Staphylococcus epidermidis	All	3	3.9	4	5.8	7	4.8
	MRSE-CR	2	2.6	0	0.0	2	1.4
	MSSE-CR	1	1.3	0	0.0	1	0.7
	MSSE-CS	0	0.0	4	5.8	4	2.8
Streptococcus oralis	All	2	2.6	2	2.9	4	2.8
CDC coryneform group G	All	2	2.6	0	0.0	2	1.4
Corynebacterium pseudodiphtheriticum	All	1	1.3	0	0.0	1	0.7
Streptococcus mitis group	All	1	1.3	0	0.0	1	0.7

[a] Phenotype distribution shown only for *S. aureus* and *S. epidermidis* in this table. CR = ciprofloxacin resistant, CS = ciprofloxacin susceptible, MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus*, MSSE = methicillin-susceptible *S. epidermidis* MRSE = methicillin-resistant *S. epidermidis*

[b] Percents for the eye cultures with the given pathogen are calculated out of the overall number of pathogens within each treatment group.

Source: Table 44 Section 2.7.2; this submission

Table 39: Baseline distribution of key pathogens across Study 373 treatment groups: PP population: species-specific study eye: sorted by overall organism frequency

Organism	Phenotype[a]	Besifloxacin		Vehicle		Overall	
		N	%[b]	N	%[b]	N	%[b]
Haemophilus influenzae	All	17	30.4	14	30.4	31	30.4
Streptococcus pneumoniae	All	16	28.6	8	17.4	24	23.5
Staphylococcus aureus	All	8	14.3	6	13.0	14	13.7
Staphylococcus epidermidis	All	3	5.4	3	6.5	6	5.9
Streptococcus oralis	All	2	3.6	2	4.3	4	3.9
CDC coryneform group G	All	1	1.8	0	0.0	1	1.0
Streptococcus mitis Group	All	1	1.8	0	0.0	1	1.0

[a] Phenotype distribution not shown in this table.

[b] Percents for the eye cultures with the given pathogen are calculated out of the overall number of pathogens within each treatment group.

STUDY 433

Study 433 was conducted between June 2006 and November 2007 at 58 U.S. sites, with 957 subjects randomized (475 to besifloxacin, 482 to vehicle). There were 390 (41%) subjects with culture-confirmed bacterial conjunctivitis (199 randomized to besifloxacin, 191 randomized to vehicle), and 480 bacterial isolates analyzed from species-specific study eyes (mITT population). The Per Protocol population included 284 subjects. There were 353 isolates from species-specific study eyes in the Per Protocol (PP) population. The baseline distributions of key pathogens in the mITT and PP populations are summarized in Table 40 and 41. The overall distribution of the principle pathogens isolated in Study 433 (*S. pneumoniae*, *H. influenzae*, *S. aureus*, and *S. epidermidis*) was similar between the mITT and PP populations. The distribution of recovered pathogens seen in the besifloxacin group was similar to that seen in the vehicle group.

At Visit 2 (Day 5, ±1 day), 91.5% (182/199) and 59.7% (114/191) of the mITT population

randomized to besifloxacin and vehicle, respectively, had microbial resolution in a species-specific eye. At Visit 3 (Day 8, +1) 88.4% of subjects randomized to besifloxacin demonstrated bacterial resolution, compared to 71.7% of subjects randomized to vehicle.

PFGE analysis of microbiological failures (isolates of a particular "ocular bacterial species" present at baseline that were not eradicated at Visit 2) indicated that 79.3% (23/29) of isolate pairs were concordant. MIC values for the concordant isolates recovered at Visit 2 did not change by ≥ 4-fold, indicating no development of resistance during the trial.

Microbial eradication rates for besifloxacin against the key pathogens recovered in this study (*H. influenzae*, *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *S. mitis* group, and CDC coryneform group G) were all greater than 86%. Besifloxacin MIC values were within the therapeutic range predicted by PK/PD analysis. Activity against ciprofloxacin-resistant staphylococci (*S. aureus* and *S. epidermidis*) was decreased compared to ciprofloxacin-susceptible isolates. No differences in MIC values were noted against isolates with resistance to the ß-lactam class of antimicrobials, or against ß-lactamase positive isolates compared non-resistant and ß-lactamase negative isolates.

Table 40: Baseline distribution of key pathogens across Study 433 treatment groups: mITT population, as treated: species-specific study eye: sorted by overall organism frequency

		Besifloxacin		Vehicle		Overall	
Organism	Phenotype[a]	N	%[b]	N	%[b]	N	%[b]
Streptococcus pneumoniae	All	73	29.1	67	29.3	140	29.2
Haemophilus influenzae	All	63	25.1	66	28.8	129	26.9
Staphylococcus aureus	All	24	9.6	31	13.5	55	11.5
	MRSA-CR	1	0.4	2	0.9	3	0.6
	MRSA-CS	1	0.4	2	0.9	3	0.6
	MSSA-CR	1	0.4	1	0.4	2	0.4
	MSSA-CS	21	8.4	26	11.4	47	9.8
	None	0	0.0	0	0.0	0	0.0
Staphylococcus epidermidis	All	18	7.2	16	7.0	34	7.1
	MRSE-CR	4	1.6	3	1.3	7	1.5
	MRSE-CS	6	2.4	5	2.2	11	2.3
	MSSE-CR	0	0.0	0	0.0	0	0.0
	MSSE-CS	8	3.2	8	3.5	16	3.3
	None	0	0.0	0	0.0	0	0.0
Streptococcus mitis group	All	7	2.8	12	5.2	19	4.0
CDC coryneform group G	All	7	2.8	2	0.9	9	1.9
Streptococcus salivarius	All	3	1.2	2	0.9	5	1.0
Moraxella lacunata	All	1	0.4	3	1.3	4	0.8
Staphylococcus hominis	All	2	0.8	2	0.9	4	0.8
Streptococcus oralis	All	3	1.2	1	0.4	4	0.8
Corynebacterium striatum	All	3	1.2	0	0.0	3	0.6
Staphylococcus lugdunensis	All	1	0.4	0	0.0	1	0.2

[a] Phenotype distribution shown only for *Staphylococcus aureus* and *Staphylococcus epidermidis* in this table. CR = ciprofloxacin resistant, CS = ciprofloxacin susceptible, MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus*, MSSE = methicillin-susceptible *S. epidermidis* MRSE = methicillin-resistant *S. epidermidis*

[b] Percents for the eye cultures with the given pathogen are calculated out of the overall number of pathogens within each treatment group.

Source: Table 50 Section 2.7.2; this submission

Table 41: Baseline pathogen distribution across Study 433 treatment groups: PP population: species-specific study eye: sorted by overall organism frequency

Organism	Phenotype[a]	Besifloxacin N	Besifloxacin % [b]	Vehicle N	Vehicle % [b]	Overall N	Overall % [b]
Streptococcus pneumoniae	All	58	30.4	46	28.4	104	29.5
Haemophilus influenzae	All	41	21.5	46	28.4	87	24.6
Staphylococcus aureus	All	19	9.9	24	14.8	43	12.2
Staphylococcus epidermidis	All	13	6.8	12	7.4	25	7.1
Streptococcus mitis group	All	5	2.6	7	4.3	12	3.4
CDC coryneform group G	All	6	3.1	1	0.6	7	2.0
Moraxella lacunata	All	1	0.5	3	1.9	4	1.1
Staphylococcus hominis	All	2	1.0	1	0.6	3	0.8
Streptococcus salivarius	All	2	1.0	1	0.6	3	0.8
Corynebacterium striatum	All	2	1.0	0	0.0	2	0.6
Streptococcus oralis	All	2	1.0	0	0.0	2	0.6
Staphylococcus lugdunensis	All	1	0.5	0	0.0	1	0.3

[a] Phenotype distribution not shown in this table.
[b] Percents for the eye cultures with the given pathogen are calculated out of the overall number of pathogens within each treatment group.
Source: Table 51 Section 2.7.2; this submission

STUDY 434

Study 434 was conducted between June 2006 and July 2007 at 84 U.S. and Asian sites. Unlike the other trials (Study 373 and Study 433), Study 434 was active-controlled (non-inferiority), comparing besifloxacin to moxifloxacin (Vigamox). There were 1161 patients randomized, of whom 533 (46%) had culture confirmed bacterial conjunctivitis (252 randomized to besifloxacin, 281 randomized to moxifloxacin). The primary pathogens isolated in Study 434 (≥ 10) included *H. influenzae*, *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *S. mitis* group, CDC coryneform group G, and *S. oralis* (not included in *S. mitis* group). The baseline distributions of key pathogens are summarized in Tables 42 and 43. Of the 699 isolates from species-specific study eyes, collected from the mITT population, 604 were from U.S. subjects and 95 were from Asian subjects.

At Visit 2 (Primary Endpoint Assessment), 94.5 (241/255) of subjects randomized to besifloxacin demonstrated bacterial eradication, compared to 89.9% (250/278) of subjects randomized to moxifloxacin.

PFGE analysis of microbiological failures (Visit Two eradication failures) demonstrated concordance in 58 of 65 isolate pairs (23 of 26 in the besifloxacin group and 35 of 39 in the moxifloxacin group). No MIC values for all concordant pairs were ≥ 4-fold baseline MICs, indicating no development of resistance during the study.

Microbial eradication rates for subjects randomized to besifloxacin treatment were ≥ 86% against all principle pathogens recovered in the study. Eradication rates were similar or identical against these pathogens for the subjects randomized to moxifloxacin treatment.

As seen in Studies 373 and 433, besifloxacin activity was diminished against ciprofloxacin-resistant staphylococcal species, compared to ciprofloxacin-susceptible isolates of the same species (*S. aureus* and *S. epidermidis*). No cross resistance was noted between besifloxacin and ß-lactam antimicrobials (i.e. activity was similar against both penicillin-susceptible and penicillin-non-susceptible isolates of *S. pneumoniae*) and activity against ß-lactamase-positive phenotypes (e.g. ß-lactamase-positive *H. influenzae*) was similar to that demonstrated against ß-lactamase-negative isolates of the same species. Besifloxacin MICs against all key pathogens were within therapeutic levels predicted by PK/PD studies.

Table 42: Baseline distribution of key pathogens across Study 434 treatment groups: mITT population, as treated: species-specific study eye: sorted by overall global organisms frequency

Organism	Phenotype[a]	Region	Besifloxacin N	Besifloxacin % [b]	Vigamox N	Vigamox % [b]	Overall N	Overall % [b]
Haemophilus influenzae	All	US	72	91.1	85	94.4	157	92.9
		Asia	7	8.9	5	5.6	12	7.1
		Global	79	24.0	90	24.3	169	24.2
Streptococcus pneumoniae	All	US	56	100.0	63	95.5	119	97.5
		Asia	0	0.0	3	4.5	3	2.5
		Global	56	17.0	66	17.8	122	17.5
Staphylococcus aureus	All	US	51	86.4	44	78.6	95	82.6
		Asia	8	13.6	12	21.4	20	17.4
		Global	59	17.9	56	15.1	115	16.5
	MRSA-CR	US	6	100.0	5	100.0	11	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	6	1.8	5	1.4	11	1.6
	MRSA-CS	US	1	100.0	5	100.0	6	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	1	0.3	5	1.4	6	0.9
	MSSA-CR	US	1	20.0	1	10.0	2	13.3
		Asia	4	80.0	9	90.0	13	86.7
		Global	5	1.5	10	2.7	15	2.1
	MSSA-CS	US	41	93.2	33	91.7	74	92.5
		Asia	3	6.8	3	8.3	6	7.5
		Global	44	13.4	36	9.7	80	11.4
	None	US	2	66.7	0	0.0	2	66.7
		Asia	1	33.3	0	0.0	1	33.3
		Global	3	0.9	0	0.0	3	0.4
Staphylococcus epidermidis	All	US	27	93.1	38	92.7	65	92.9
		Asia	2	6.9	3	7.3	5	7.1
		Global	29	8.8	41	11.1	70	10.0
	MRSE-CR	US	6	100.0	8	88.9	14	93.3
		Asia	0	0.0	1	11.1	1	6.7
		Global	6	1.8	9	2.4	15	2.1
	MRSE-CS	US	5	100.0	10	90.9	15	93.8
		Asia	0	0.0	1	9.1	1	6.3
		Global	5	1.5	11	3.0	16	2.3
	MSSE-CR	US	4	100.0	5	100.0	9	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	4	1.2	5	1.4	9	1.3
	MSSE-CS	US	12	85.7	15	93.8	27	90.0
		Asia	2	14.3	1	6.3	3	10.0
		Global	14	4.3	16	4.3	30	4.3
	None	US	0	0.0	0	0.0	0	0.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	0	0.0	0	0.0	0	0.0
Streptococcus mitis group	All	US	9	81.8	13	92.9	22	88.0
		Asia	2	18.2	1	7.1	3	12.0
		Global	11	3.3	14	3.8	25	3.6
CDC coryneform group G	All	US	4	57.1	7	63.6	11	61.1
		Asia	3	42.9	4	36.4	7	38.9
		Global	7	2.1	11	3.0	18	2.6
Streptococcus oralis	All	US	4	66.7	4	100.0	8	80.0
		Asia	2	33.3	0	0.0	2	20.0
		Global	6	1.8	4	1.1	10	1.4
Corynebacterium pseudodiphtheriticum	All	US	4	80.0	2	100.0	6	85.7
		Asia	1	20.0	0	0.0	1	14.3
		Global	5	1.5	2	0.5	7	1.0
Staphylococcus lugdunensis	All	US	4	100.0	3	100.0	7	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	4	1.2	3	0.8	7	1.0
Corynebacterium striatum	All	US	2	100.0	0	0.0	2	40.0
		Asia	0	0.0	3	100.0	3	60.0
		Global	2	0.6	3	0.8	5	0.7
Moraxella lacunata	All	US	2	50.0	0	0.0	2	40.0
		Asia	2	50.0	1	100.0	3	60.0
		Global	4	1.2	1	0.3	5	0.7
Staphylococcus hominis	All	US	4	100.0	1	100.0	5	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	4	1.2	1	0.3	5	0.7
Streptococcus salivarius	All	US	2	100.0	2	100.0	4	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	2	0.6	2	0.5	4	0.6

[a] Phenotype distribution shown only for *S. aureus* and *S. epidermidis* in this table. CR = ciprofloxacin resistant, CS = ciprofloxacin susceptible, MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus*, MSSE = methicillin-susceptible *S. epidermidis* MRSE = methicillin-resistant *S. epidermidis*

[b] Percents for US and Asia are calculated out of the Global number of eye cultures with the given pathogen, within each treatment group. Percents for the Global eye cultures with the given pathogen are calculated out of the overall Global number of pathogens within each treatment group.

Source: Table 55 Section 2.7.2; this submission

Table 43: Baseline distribution of key pathogens across Study 434 treatment groups: PP population: species-specific study eye: sorted by overall global organism frequency

Organism	Phenotype[a]	Region	Besifloxacin N	Besifloxacin % [b]	Vigamox N	Vigamox % [b]	Overall N	Overall % [b]
Haemophilus influenzae	All	US	39	86.7	49	94.2	88	90.7
		Asia	6	13.3	3	5.8	9	9.3
		Global	45	22.0	52	22.1	97	22.0
Streptococcus pneumoniae	All	US	35	100.0	37	92.5	72	96.0
		Asia	0	0.0	3	7.5	3	4.0
		Global	35	17.1	40	17.0	75	17.0
Staphylococcus aureus	All	US	25	75.8	26	68.4	51	71.8
		Asia	8	24.2	12	31.6	20	28.2
		Global	33	16.1	38	16.2	71	16.1
Staphylococcus epidermidis	All	US	17	89.5	24	92.3	41	91.1
		Asia	2	10.5	2	7.7	4	8.9
		Global	19	9.3	26	11.1	45	10.2
CDC coryneform group G	All	US	2	66.7	5	55.6	7	58.3
		Asia	1	33.3	4	44.4	5	41.7
		Global	3	1.5	9	3.8	12	2.7
Corynebacterium pseudodiphtheriticum	All	US	3	75.0	2	100.0	5	83.3
		Asia	1	25.0	0	0.0	1	16.7
		Global	4	2.0	2	0.9	6	1.4
Streptococcus oralis	All	US	3	75.0	2	100.0	5	83.3
		Asia	1	25.0	0	0.0	1	16.7
		Global	4	2.0	2	0.9	6	1.4
Corynebacterium striatum	All	US	2	100.0	0	0.0	2	40.0
		Asia	0	0.0	3	100.0	3	60.0
		Global	2	1.0	3	1.3	5	1.1
Moraxella lacunata	All	US	2	50.0	0	0.0	2	40.0
		Asia	2	50.0	1	100.0	3	60.0
		Global	4	2.0	1	0.4	5	1.1
Staphylococcus hominis	All	US	4	100.0	1	100.0	5	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	4	2.0	1	0.4	5	1.1
Staphylococcus ingalumensis	All	US	3	100.0	2	100.0	5	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	3	1.5	2	0.9	5	1.1
Streptococcus salivarius	All	US	1	100.0	0	0.0	1	100.0
		Asia	0	0.0	0	0.0	0	0.0
		Global	1	0.5	0	0.0	1	0.2

[a] Phenotype distribution not shown in this table.

[b] Percents for US and Asia are calculated out of the Global number of eye cultures with the given pathogen, within each treatment group. Percents for the Global eye cultures with the given pathogen are calculated out of the overall Global number of pathogens within each treatment group.

Source: Table 56 Section 2.7.2; this submission

SUMMARY OF CLINICAL TRIALS

Integrated analysis of the three clinical trials was confounded by several factors. Studies 373 and 433 were vehicle controlled, while Study 434 was active controlled. Clinical resolution in Study 373 was determined on Day 4, ±1 (based on 3 clinical signs), while in Studies 433 and 434 clinical resolution was determined on Day 5, ±1. The Applicant has concluded that these confounding factors did not impact analysis of species-specific microbial eradication rates. This reviewer concurs. Data summaries linking MIC values of specific ocular pathogens to clinical resolution were not useful, however, since "species-specific" eradication could not be directly correlated to clinical resolution.

Overall, the ITT study population for the three besifloxacin clinical trials included 2387 subjects. The mITT population (as analyzed in the Applicant's integrated summary) included 1041 subjects, and the PP population included 705 subjects. There were 1324 bacterial isolates recovered in the three trials (145 from Study 373, 480 from Study 433, and 699 from Study 434). Asian sites were responsible for 95 of the isolates recovered in Study 434 (all other isolates were from U.S. subjects). Subjects randomized to besifloxacin treatment contributed 656 of the analyzed bacterial isolates (49.5%), with 370 (27.9%) and 298 (22.5%) from the moxifloxacin- and vehicle-treated subjects, respectively.

Species-specific microbial eradication rates are summarized in Tables 44-46. Against all isolates

recovered from the mITT population, species-specific microbial eradication in besifloxacin treated subjects was 92.2% at Visit 2 (88.4% at Visit 3). Besifloxacin was active against the principle ocular pathogens recovered in the clinical trials, listed in Table 47, with activity similar to or exceeding comparator fluoroquinolones (moxifloxacin and gatifloxacin). Besifloxacin activity (as measured by in vitro susceptibility testing) could not be generally correlated to species-specific eradication, however, since for most species tested, isolates with higher MIC values (albeit, recovered in lower numbers) were completely eradicated, while a percentage of isolates with lower MICs persisted.

Table 44: Besifloxacin species-specific microbiological eradication results across clinical trials

Organism	Study 373		Study 433		Study 434	
	Visit 2	Visit 3	Visit 2	Visit 3	Visit 2	Visit 3
All species	70/76 (92.1%)	69/76 (90.8%)	228/251 (90.8%)	224/251 (89.2%)	307/329 (93.3%)	287/329 (87.2%)
Gram positive	44/47 (93.6%)	41/47 (87.2%)	159/173 (91.9%)	157/173 (90.8%)	209/227 (92.1%)	194/227 (85.5%)
Gram negative	26/29 (89.7%)	28/29 (96.6%)	69/78 (88.5%)	67/78 (85.9%)	98/102 (96.1%)	93/102 (91.2%)
CDC coryneform group G	2/2 (100.0%)	2/2 (100.0%)	7/7 (100.0%)	7/7 (100.0%)	6/7 (85.7%)	6/7 (85.7%)
Corynebacterium pseudodiphtheriticum	1/1 (100.0%)	1/1 (100.0%)	0/0 -	0/0 -	5/5 (100.0%)	5/5 (100.0%)
Corynebacterium striatum	0/0 -	0/0 -	3/3 (100.0%)	3/3 (100.0%)	2/2 (100.0%)	2/2 (100.0%)
Haemophilus influenzae	22/25 (88.0%)	24/25 (96.0%)	55/63 (87.3%)	52/63 (82.5%)	75/79 (94.9%)	72/79 (91.1%)
Moraxella lacunata	0/0 -	0/0 -	1/1 (100.0%)	1/1 (100.0%)	4/4 (100.0%)	3/4 (75.0%)
Staphylococcus aureus	8/10 (80.0%)	9/10 (90.0%)	23/24 (95.8%)	21/24 (87.5%)	50/59 (84.7%)	48/59 (81.4%)
Staphylococcus epidermidis	3/3 (100.0%)	3/3 (100.0%)	17/18 (94.4%)	17/18 (94.4%)	27/29 (93.1%)	24/29 (82.8%)
Staphylococcus hominis	0/0 -	0/0 -	1/2 (50.0%)	2/2 (100.0%)	4/4 (100.0%)	4/4 (100.0%)
Staphylococcus lugdunensis	0/0 -	0/0 -	1/1 (100.0%)	1/1 (100.0%)	4/4 (100.0%)	4/4 (100.0%)
Streptococcus mitis group	1/1 (100.0%)	1/1 (100.0%)	6/7 (85.7%)	5/7 (71.4%)	10/11 (90.9%)	10/11 (90.9%)
Streptococcus oralis	2/2 (100.0%)	2/2 (100.0%)	2/3 (66.7%)	2/3 (66.7%)	6/6 (100.0%)	4/6 (66.7%)
Streptococcus pneumoniae	23/24 (95.8%)	19/24 (79.2%)	66/73 (90.4%)	65/73 (89.0%)	53/56 (94.6%)	48/56 (85.7%)
Streptococcus salivarius	0/0 -	0/0 -	3/3 (100.0%)	3/3 (100.0%)	2/2 (100.0%)	1/2 (50.0%)

Source: Table 77 Section 2.7.2; this submission

Table 45: Species-specific microbiological eradication in the mITT (as treated) population - global

	Besifloxacin		Vehicle		Vigamox	
Organism	**Visit 2**	**Visit 3**	**Visit 2**	**Visit 3**	**Visit 2**	**Visit 3**
All species	605/656 (92.2%)	580/656 (88.4%)	183/298 (61.4%)	216/298 (72.5%)	339/370 (91.6%)	317/370 (85.7%)
Gram positive	412/447 (92.2%)	392/447 (87.7%)	114/195 (58.5%)	140/195 (71.8%)	219/244 (89.8%)	211/244 (86.5%)
Gram negative	193/209 (92.3%)	188/209 (90.0%)	69/103 (67.0%)	76/103 (73.8%)	120/126 (95.2%)	106/126 (84.1%)
CDC coryneform group G	15/16 (93.8%)	15/16 (93.8%)	1/2 (50.0%)	2/2 (100.0%)	11/11 (100.0%)	11/11 (100.0%)
C. pseudodiphtheriticum	6/6 (100.0%)	6/6 (100.0%)	0/0 (--)	0/0 (--)	2/2 (100.0%)	2/2 (100.0%)
C. striatum	5/5 (100.0%)	5/5 (100.0%)	0/0 (--)	0/0 (--)	2/3 (66.7%)	3/3 (100.0%)
H. influenzae	152/167 (91.0%)	148/167 (88.6%)	56/87 (64.4%)	64/87 (73.6%)	85/90 (94.4%)	79/90 (87.8%)
M. lacunata	5/5 (100.0%)	4/5 (80.0%)	2/3 (66.7%)	3/3 (100.0%)	1/1 (100.0%)	1/1 (100.0%)
S. aureus	81/93 (87.1%)	78/93 (83.9%)	16/41 (39.0%)	20/41 (48.8%)	48/56 (85.7%)	46/56 (82.1%)
S. epidermidis	47/50 (94.0%)	44/50 (88.0%)	11/20 (55.0%)	15/20 (75.0%)	36/41 (87.8%)	32/41 (78.0%)
S. hominis	5/6 (83.3%)	6/6 (100.0%)	1/2 (50.0%)	1/2 (50.0%)	1/1 (100.0%)	1/1 (100.0%)
S. lugdunensis	5/5 (100.0%)	5/5 (100.0%)	0/0 (--)	0/0 (--)	3/3 (100.0%)	2/3 (66.7%)
S. mitis group	17/19 (89.5%)	16/19 (84.2%)	10/12 (83.3%)	10/12 (83.3%)	13/14 (92.9%)	13/14 (92.9%)
S. oralis	10/11 (90.9%)	8/11 (72.7%)	2/3 (66.7%)	2/3 (66.7%)	3/4 (75.0%)	3/4 (75.0%)
S. pneumoniae	142/153 (92.8%)	132/153 (86.3%)	47/83 (56.6%)	61/83 (73.5%)	60/66 (90.9%)	57/66 (86.4%)
S. salivarius	5/5 (100.0%)	4/5 (80.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)

Source: Table 61 Section 2.7.2; this submission

Table 46: Species-specific microbiological eradication in the mITT (as treated) population – US sites

	Besifloxacin		Vehicle		Vigamox	
Organism	Visit 2	Visit 3	Visit 2	Visit 3	Visit 2	Visit 3
All species	561/611 (91.8%)	540/611 (88.4%)	183/298 (61.4%)	216/298 (72.5%)	296/320 (92.5%)	270/320 (84.4%)
Gram positive	386/420 (91.9%)	369/420 (87.9%)	114/195 (58.5%)	140/195 (71.8%)	192/210 (91.4%)	180/210 (85.7%)
Gram negative	175/191 (91.6%)	171/191 (89.5%)	69/103 (67.0%)	76/103 (73.8%)	104/110 (94.5%)	90/110 (81.8%)
CDC coryneform group G	12/13 (92.3%)	12/13 (92.3%)	1/2 (50.0%)	2/2 (100.0%)	7/7 (100.0%)	7/7 (100.0%)
C. pseudodiphtheriticum	5/5 (100.0%)	5/5 (100.0%)	0/0 (--)	0/0 (--)	2/2 (100.0%)	2/2 (100.0%)
C. striatum	5/5 (100.0%)	5/5 (100.0%)	0/0 (--)	0/0 (--)	0/0 (--)	0/0 (--)
H. influenzae	145/160 (90.6%)	141/160 (88.1%)	56/87 (64.4%)	64/87 (73.6%)	80/85 (94.1%)	74/85 (87.1%)
M. lacunata	3/3 (100.0%)	2/3 (66.7%)	2/3 (66.7%)	3/3 (100.0%)	0/0 (--)	0/0 (--)
S. aureus	73/85 (85.9%)	71/85 (83.5%)	16/41 (39.0%)	20/41 (48.8%)	39/44 (88.6%)	35/44 (79.5%)
S. epidermidis	45/48 (93.8%)	42/48 (87.5%)	11/20 (55.0%)	15/20 (75.0%)	34/38 (89.5%)	30/38 (78.9%)
S. hominis	5/6 (83.3%)	6/6 (100.0%)	1/2 (50.0%)	1/2 (50.0%)	1/1 (100.0%)	1/1 (100.0%)
S. lugdunensis	5/5 (100.0%)	5/5 (100.0%)	0/0 (--)	0/0 (--)	3/3 (100.0%)	2/3 (66.7%)
S. mitis group	15/17 (88.2%)	14/17 (82.4%)	10/12 (83.3%)	10/12 (83.3%)	12/13 (92.3%)	12/13 (92.3%)
S. oralis	8/9 (88.9%)	7/9 (77.8%)	2/3 (66.7%)	2/3 (66.7%)	3/4 (75.0%)	3/4 (75.0%)
S. pneumoniae	142/153 (92.8%)	132/153 (86.3%)	47/83 (56.6%)	61/83 (73.5%)	57/63 (90.5%)	54/63 (85.7%)
S. salivarius	5/5 (100.0%)	4/5 (80.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)

Source: Table 62 Section 2.7.2; this submission

Table 47: In vitro (MIC90) activity vs. clinical isolates; Studies 373, 433, 434

		MIC_{90} (mcg/ml)			
Pathogen	**N**	**Besifloxacin**	**Moxifloxacin**	**Gatifloxacin**	**Azithromycin**
All isolates	1324	0.25	0.5	0.5	>8
Gram +	886	0.25	0.5	1.0	>8
Gram -	438	0.5	0.25	0.25	>8
H. influenzae	344	0.06	0.06	0.03	4
S. aureus	190	0.5	2	4	>8
S. epidermidis	111	0.5	4	2	>8
S. pneumoniae	302	0.125	0.125	0.5	>8

Source: adapted from Slide CE-38, Applicant's presentation at Advisory Committee Meeting 2008 Dec 5

Besifloxacin MIC values for "key pathogens" (ocular pathogens that were recovered five or more times in the combined clinical trials) versus species-specific microbiological eradication at Visits 2 and 3 (all trials) are summarized in Tables 48 through 53.

Haemophilus influenzae was the most frequently isolated bacterial species (n = 344). The overall besifloxacin MIC_{range} for isolates recovered in the three clinical trials was 0.008 – 0.5 mcg/ml, and the MIC_{90} was 0.06 mcg/ml. Eradication rates were not apparently affected by ß-lactamase production, and MIC values were similar between ß-lactamase-positive and ß-lactamase-negative isolates. Besifloxacin MIC versus species-specific eradication of H. influenzae isolates is summarized in Tables 48 (Visit 2) and 49 (Visit 3).

CDC coryneform group G was recovered from 29 subjects in the three clinical trials (16 from besifloxacin-treated subjects). The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.008 - 2 mcg/ml and 0.125 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of CDC coryneform group G isolates is summarized in Tables 48 (Visit 2) and 49 (Visit 3).

Corynebacterium pseudodiphtheriticum was isolated from 8 subjects in the three clinical trials (6 in the besifloxacin group). The overall besifloxacin MIC_{range} and MIC_{50} against these isolates were 0.015 – 0.25 mcg/ml and 0.25 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *C. pseudodiphtheriticum* isolates is summarized in Tables 48 (Visit 2) and 49 (Visit 3). All isolates of *C. pseudodiphtheriticum* were listed as eradicated at Visits 2 and 3.

Corynebacterium striatum was isolated from 8 subjects (5 from subjects randomized to besifloxacin treatment). The overall besifloxacin MIC_{range} and MIC_{50} against these isolates were 0.015 – 0.25 mcg/ml and 0.015 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *C. striatum* isolates is summarized in Tables 48 (Visit 2) and 49 (Visit 3). All isolates of *C. striatum* were listed as eradicated at Visits 2 and 3.

Table 48: Besifloxacin MIC versus species-specific microbiological eradication at Visit 2: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*H. influenzae*			CDC coryneform group G			*Corynebacterium pseudodiphtheriticum*			*Corynebacterium striatum*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC	1	1	100.0%	1	1	100.0%						
<=0.004												
0.008	1	1	100.0%	1	1	100.0%						
0.015	12	12	100.0%	9	10	90.0%	1	1	100.0%	5	5	100.0%
0.03	114	127	89.8%	1	1	100.0%						
0.06	21	23	91.3%	1	1	100.0%						
0.125	2	2	100.0%	1	1	100.0%						
0.25	1	1	100.0%				5	5	100.0%			
0.5												
1												
2				1	1	100.0%						
4												
8												
>8												
Total	152	167	91.0%	15	16	93.8%	6	6	100.0%	5	5	100.0%

Source: Table 64 Section 2.7.2; this submission

Table 49: Besifloxacin MIC versus species-specific microbiological eradication at Visit 3: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*H. influenzae*			CDC coryneform group G			*Corynebacterium pseudodiphtheriticum*			*Corynebacterium striatum*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC	1	1	100.0%	1	1	100.0%						
<=0.004												
0.008		1	0.0%	1	1	100.0%						
0.015	12	12	100.0%	9	10	90.0%	1	1	100.0%	5	5	100.0%
0.03	112	127	88.2%	1	1	100.0%						
0.06	20	23	87.0%	1	1	100.0%						
0.125	2	2	100.0%	1	1	100.0%						
0.25	1	1	100.0%				5	5	100.0%			
0.5												
1												
2				1	1	100.0%						
4												
8												
>8												
Total	148	167	88.6%	15	16	93.8%	6	6	100.0%	5	5	100.0%

Source: Table 65 Section 2.7.2; this submission

Staphylococcus aureus was isolated from 190 subjects in the three clinical trials (93 from subjects randomized to besifloxacin treatment). The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.008 - 8 mcg/ml and 0.5 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *S. aureus* isolates is summarized in Tables 50 (Visit 2) and 51 (Visit 3). Besifloxacin MIC values against MRSA and MSSA were comparable, but MIC values against ciprofloxacin-resistant staphylococcal isolates (ciprofloxacin ≥ 4 mcg/ml) were higher than MICs against ciprofloxacin-susceptible isolates.

Staphylococcus epidermidis was isolated from 111 subjects in the three clinical trials (50 from subjects randomized to besifloxacin treatment). The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.03 - 4 mcg/ml and 0.5 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *S. epidermidis* isolates is summarized in Tables 50 (Visit 2) and 51 (Visit 3). As observed in testing of *S. aureus* isolates, besifloxacin MIC values against MRSE and MSSE were comparable, but MIC values against ciprofloxacin-resistant staphylococcal isolates (ciprofloxacin ≥ 4 mcg/ml) were higher than MICs against ciprofloxacin-susceptible isolates.

Staphylococcus hominis was isolated from 9 subjects in the three clinical trials (6 from subjects randomized to besifloxacin treatment). The overall besifloxacin MIC_{range} and MIC_{50} against these isolates were 0.03 – 0.5 mcg/ml and 0.06 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *S. hominis* isolates is summarized in Tables 50 (Visit 2) and 51 (Visit 3).

Staphylococcus lugdunensis was isolated from 8 subjects in the three clinical trials (5 from subjects randomized to besifloxacin treatment). The overall besifloxacin MIC_{range} and MIC_{50} against these isolates were 0.06 – 0.5 mcg/ml and 0.125 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *S. lugdunensis* isolates is summarized in Tables 50 (Visit 2) and 51 (Visit 3).

Table 50: Besifloxacin MIC versus species-specific microbiological eradication at Visit 2: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*Staphylococcus aureus*			*Staphylococcus epidermidis*			*Staphylococcus hominis*			*Staphylococcus lugdunensis*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC							1	1	100.0%			
<=0.004												
0.008												
0.015	3	4	75.0%									
0.03	37	40	92.5%	6	6	100.0%	1	1	100.0%			
0.06	29	32	90.6%	24	27	88.9%	2	3	67%	1	1	100.0%
0.125	2	3	66.7%							2	2	100.0%
0.25										1	1	100.0%
0.5	5	8	62.5%	14	14	100.0%	1	1	100.0%	1	1	100.0%
1	2	3	66.7%									
2	2	2	100.0%	1	1	100.0%						
4				2	2	100.0%						
8	1	1	100.0%									
>8												
Total	81	93	87.1%	47	50	94.0%	5	6	83.3%	5	5	100.0%

Source: Table 66 Section 2.7.2; this submission

Table 51: Besifloxacin MIC versus species-specific microbiological eradication at Visit 3: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*Staphylococcus aureus*			*Staphylococcus epidermidis*			*Staphylococcus hominis*			*Staphylococcus lugdunensis*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC							1	1	100.0%			
<=0.004												
0.008												
0.015	3	4	75.0%									
0.03	37	40	92.5%	6	6	100.0%	1	1	100.0%			
0.06	24	32	75.0%	23	27	85.2%	3	3	100.0%	1	1	100.0%
0.125	2	3	66.7%							2	2	100.0%
0.25										1	1	100.0%
0.5	7	8	87.5%	12	14	85.7%	1	1	100.0%	1	1	100.0%
1	2	3	66.7%									
2	2	2	100.0%	1	1	100.0%						
4				2	2	100.0%						
8	1	1	100.0%									
>8												
Total	78	93	83.9%	44	50	88.0%	6	6	100.0%	5	5	100.0%

Source: Table 67 Section 2.7.2; this submission

In the three clinical trials, there were 20 isolates identified as *Streptococcus mitis* (10 in subjects randomized to besifloxacin treatment) and 45 isolates identified as *Streptococcus mitis* group (19 from subjects randomized to besifloxacin treatment). Besifloxacin in vitro activity and eradication rates were similar for isolates identified as *S. mitis* and *S. mitis* group. The Applicant has chosen to focus on organisms identified as *S. mitis* group, in order to avoid redundancy, and this decision is reflected in the proposed indications. The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.03 - 1 mcg/ml and 0.25 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *S. mitis* group isolates is summarized in Tables 52 (Visit 2) and 53 (Visit 3).

Streptococcus oralis was recovered from 18 subjects in the three clinical trials (11 from besifloxacin-treated subjects). The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.015 – 0.25 mcg/ml and 0.25 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *Streptococcus oralis* isolates is summarized in Tables 52 (Visit 2) and 53 (Visit 3).

Streptococcus pneumoniae was recovered from 302 subjects in the three clinical trials (153 from besifloxacin-treated subjects). The overall besifloxacin MIC_{range} and MIC_{90} against these isolates were 0.03 – 0.25 mcg/ml and 0.125 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *Streptococcus pneumoniae* isolates is summarized in Tables 52 (Visit 2) and 53 (Visit 3). Isolates identified as penicillin susceptible (PSSP: penicillin MIC ≤ 2 mcg/ml), penicillin intermediate (PISP: penicillin MIC = 4 mcg/ml) and penicillin-resistant (PRSP: penicillin MIC ≥ 8 mcg/ml) all had similar besifloxacin MIC values.

Streptococcus salivarius was recovered from 9 subjects in the three clinical trials (5 from besifloxacin-treated subjects). The overall besifloxacin MIC_{range} and MIC_{50} against these isolates were 0.06 – 0.25 mcg/ml and 0.125 mcg/ml, respectively. Besifloxacin MIC versus species-specific eradication of *Streptococcus salivarius* isolates is summarized in Tables 52 (Visit 2) and 53 (Visit 3).

Table 52: Besifloxacin MIC versus species-specific microbiological eradication at Visit 2: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*Streptococcus mitis* group			*Streptococcus oralis*			*Streptococcus pneumoniae*			*Streptococcus salivarius*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC												
<=0.004												
0.008												
0.015				1	1	100.0%						
0.03	2	2	100.0%				3	3	100.0%			
0.06	3	3	100.0%				119	128	93.0%	1	1	100.0%
0.125	11	13	84.6%	5	6	83.3%	18	19	94.7%	3	3	100.0%
0.25	1	1	100.0%	4	4	100.0%	2	3	66.7%	1	1	100.0%
0.5												
1												
2												
4												
8												
>8												
Total	17	19	89.5%	10	11	90.9%	142	153	92.8%	5	5	100.0%

Source: Table 68 Section 2.7.2; this submission

Table 53: Besifloxacin MIC versus species-specific microbiological eradication at Visit 3: Global results for the integrated bacterial conjunctivitis clinical studies – mITT As Treated Population – Besifloxacin treatment group

Besifloxacin MIC (µg/ml)	*Streptococcus mitis* group			*Streptococcus oralis*			*Streptococcus pneumoniae*			*Streptococcus salivarius*		
	n	N	%	n	N	%	n	N	%	n	N	%
no MIC												
<=0.004												
0.008												
0.015				1	1	100.0%						
0.03	2	2	100.0%				2	3	66.7%			
0.06	3	3	100.0%				112	128	87.5%	1	1	100.0%
0.125	10	13	76.9%	4	6	66.7%	17	19	89.5%	2	3	66.7%
0.25	1	1	100.0%	3	4	75.0%	1	3	33.3%	1	1	100.0%
0.5												
1												
2												
4												
8												
>8												
Total	16	19	84.2%	8	11	72.7%	132	153	86.3%	4	5	80.0%

Source: Table 69 Section 2.7.2; this submission

Moraxella lacunata was isolated from 9 subjects in the clinical trials (5 from patients randomized to besifloxacin treatment). The besifloxacin MIC_{range} for *M. lacunata* isolates recovered in the clinical trials was 0.03 – 0.06 mcg/ml. [redacted] was unable to obtain susceptibility results against these isolates, due to insufficient growth in the MIC panels employed at the central laboratory. Additional testing, using techniques described for testing *M. catarrhalis* in CLSI document 45-A (May 2006) employing cation-adjusted Mueller-Hinton broth, also failed to produce adequate growth for interpretation of results. The [redacted] central laboratory amended the standard procedure for testing *M. catarrhalis*, to include the addition of 3-5% lysed horse blood, with incubation at 35°C (ambient air) for 24-48 hours, based on methods developed for the testing of *S. pneumoniae* (CLSI M100-S18). This method proved successful in ensuring sufficient growth for test interpretation. Quality control was performed each day of testing (using methods appropriate for testing of *S. pneumoniae*). All QC results were within the ranges published by CLSI (CLSI M100-S18) for the tested antibiotics (azithromycin, gatifloxacin, levofloxacin, moxifloxacin, ofloxacin, and penicillin) and within the tentative range proposed for besifloxacin (see discussion above). Microbial eradication rates of *M. lacunata* by besifloxacin were 100% at Visit 2 (5/5) and 80% at Visit 3 (4/5).

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b(4)

Besifloxacin was active against antibiotic-resistant phenotypes recovered in the three clinical trials. Against ciprofloxacin-susceptible, methicillin-resistant *S. aureus* (MRSA) (n = 9), the highest besifloxacin MIC was 0.06 mcg/ml. Against ciprofloxacin-resistant MRSA (n = 17), the MIC_{90} was 4 mcg/ml. Against ciprofloxacin-susceptible methicillin-resistant *S. epidermidis* (MRSE) (n = 27), the besifloxacin MIC_{90} was 0.06 mcg/ml. Against ciprofloxacin-resistant MRSE (n = 24), the besifloxacin MIC_{90} was 4 mcg/ml. Besifloxacin was active against both penicillin-non-susceptible isolates of *S. pneumoniae* and ß-lactamase positive isolates of *H. influenzae* (with activity similar to susceptible/ß-lactamase-negative isolates of the same species). PFGE analysis of concordant isolate pairs indicated no development of resistance in any isolates recovered in the besifloxacin clinical trials.

QUALITY CONTROL PROCEDURES DURING CLINICAL STUDIES

Tentative quality control ranges (QC) for susceptibility testing of besifloxacin by minimum inhibitory concentration (MIC) methods, were developed by [redacted]. ATCC strains were tested for 30 days against besifloxacin and comparators, prior to testing of patient isolates, using methods approved by CLSI (M100-S15). Patient isolates from Study 373 were used to set the tentative besifloxacin QC ranges. The quality control ranges are summarized in Table 53. Tentative besifloxacin QC ranges met the requirements for the development of such criteria, outlined in CLSI document M23-A2.

b(4)

Table 54: Besifloxacin MIC Tier I Quality Control Ranges used by

Organism	ATCC No.[a]	Range (µg/mL)[b]
Staphylococcus aureus	29213	
Enterococcus faecalis	29212	
Escherichia coli	25922	
Pseudomonas aeruginosa	27853	
Streptococcus pneumoniae	49619	
Haemophilus influenzae	49247	

b(4)

[a] Strains designation according to the American Type Culture Collection (ATCC)
[b] Acceptable Minimum inhibitory concentration (MIC) ranges established by [redacted] (Memo 31 March 2008)
Source: Table 37 Section 2.7.2; this submission

The Tier 1 QC ranges determined by [redacted] were compared to results from three independent laboratories, including [redacted] Besifloxacin lots varied between testing laboratories. In the comparison studies conducted by [redacted] all tested isolates fell within the tentative QC ranges established by [redacted]. In the [redacted] study, all isolates except those of *H. influenzae* were within the [redacted] tentative QC ranges. The MIC value for *H. influenzae* in the [redacted] The results of the comparisons of QC values, between the four testing laboratories are summarized in Table 54.

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During the clinical trials, quality control testing was performed on each day that susceptibility tests (MIC) were run, in addition to 30-day validation procedures and weekly QC in accordance with CLSI guidelines (M7-A6). Corrective actions were taken and noted in cases of aberrant QC results, with repeat MIC testing as required.

Quality control results for besifloxacin were not reported for isolates tested in Study 373. In this study, results from comparator antibiotics were required to be in range. Results from MIC testing of besifloxacin performed against clinical isolates from Study373 were used to determine the tentative quality control ranges employed in the subsequent studies (433 and 434).

SUMMARY OF CLINICAL STUDIES

The Applicant has presented data from three clinical trials designed to investigate the safety and efficacy of besifloxacin in the treatment of bacterial conjunctivitis. Two of the trials (Study 373 and 433) compared besifloxacin to vehicle. One trial (Study 434) compared besifloxacin to moxifloxacin (for non-inferiority). Besifloxacin microbial eradication rates exceeded those of Vehicle in Trials 373 and 433, and exceeded or were similar to moxifloxacin in Trail 434. Eradication rates for all key pathogens (including CDC coryneform group G, *Corynebacterium pseudodiphtheriticum*, *C. striatum*, *Haemophilus influenzae*, *Moraxella lacunata*, *Staphylococcus aureus*, *S. epidermidis*, *S. hominis*, *S. lugdunensis*, *Streptococcus mitis* group, *Streptococcus oralis*, *Streptococcus pneumoniae*, and *S. salivarius*) at Visit 2 (the primary endpoint defined in the study protocols) met or exceeded criteria for inclusion in the proposed besifloxacin indications. Overall species-specific microbial eradication by besifloxacin at Visit 2 was 92.2%.

Besifloxacin in vitro activity against key ocular pathogens (listed above) collected during the clinical trials was comparable to activity determined in pre-clinical studies. MIC_{90} values (or MIC_{50} values, in situations where fewer than 10 isolates were collected in the combined clinical trials) were within the therapeutic range predicted by PK/PD studies. The overall MIC_{90} value for isolates analyzed by the Applicant (n = 1324) was 0.5 mcg/ml. Of the key ocular pathogens isolated in the clinical trials, no single isolate demonstrated an MIC value greater than 8 mcg/ml. Elevated MIC values were observed (up to 8 mcg/ml) in ciprofloxacin-resistant staphylococcal isolates.

Susceptibility testing on microbiological failures (persistent isolates confirmed by pulsed field gel electrophoresis) indicated no development of resistance during the clinical trials (≥ 4-fold increase in baseline MIC).

Quality control was performed on each day of testing, according to procedures published by CLSI. Tentative quality control parameters were determined during Study 373 and were employed by the central testing laboratory for susceptibility testing of isolates recovered in Studies 433 and 434. All quality control data was included in the submission and was reviewed for this report.

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X Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Microbiology- 4

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F. Marsik
Clin Micro/ TL/ HFD-520
8 Jan 09 FIN FJM

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/s/

Kerry Snow
2/11/2009 02:15:09 PM
MICROBIOLOGIST

Frederic Marsik
2/11/2009 02:17:40 PM
MICROBIOLOGIST

Clinical Microbiology: 45-Day Meeting Checklist NDA - Fileability
NDA 22-308: Besifloxacin Hydrochloride Ophthalmic Solution for Bacterial Conjunctivitis
Reviewer: Kerry Snow Date Review completed: 5 July 2008

On **initial** overview of the NDA application for RTF:

No.	Item	Yes	No	Comments
1	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	✓		
2	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA indexed, paginated, and/or linked in a manner to allow substantive review to begin?	✓		
3	Is the clinical microbiology information (preclinical/nonclinical and clinical) in different sections of the NDA legible so that substantive review can begin?	✓		
4	On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/ isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	✓		
5	Has the applicant submitted draft provisional breakpoint and interpretive criteria, along with quality control (QC) parameters, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA studies, and in a manner to allow substantive review to begin?	✓		
6	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	✓		
7	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	✓		

Clinical Microbiology: 45-Day Meeting Checklist NDA - Fileability
NDA 22-308: Besifloxacin Hydrochloride Ophthalmic Solution for Bacterial Conjunctivitis
Reviewer: Kerry Snow Date Review completed: 5 July 2008

8	Has the applicant submitted the clinical microbiology datasets in a format which intends to correlate baseline pathogen with clinical and microbiologic outcomes exhibited by relevant pathogens isolated from test of cure or end of treatment?	✓		
9	Has the applicant submitted a clinical microbiology dataset in a format which intends to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline relevant pathogen with clinical and microbiologic outcome as exhibited by relevant pathogens isolated from test of cure or end of treatment?	✓		
10	Has the applicant used standardized methods or if non-standardized methods were used has the applicant included full details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	✓		
11	Is the clinical microbiology draft labeling consistent with 21 CFR Parts 201, 314, 601 and current Divisional policy.	✓		
12	FROM A CLINICAL MICROBIOLOGY PERSPECTIVE, IS THIS NDA FILEABLE? **IF NO, GIVE REASONS BELOW.**	✓		

Any Additional Clinical Microbiology Comments:
No additional comments.

Reviewing Clinical Microbiologist: Kerry Snow

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/s/

Kerry Snow
7/9/2008 01:34:09 PM
MICROBIOLOGIST

Product Quality Microbiology Review

07 January 2009

NDA: 22-308/N-000

Drug Product Name
Proprietary: N/A.
Non-proprietary: Besifloxacin hydrochloride.
Drug Product Priority Classification: S.

Review Number: 1.

Dates of Submission(s) Covered by this Review

Letter	Stamp	Review Request	Assigned to Reviewer
30 MAY 2008	02 JUN 2008	09 JUN 2008	09 JUN 2008
14 NOV 2008	17 NOV 2008	N/A	N/A
17 DEC 2008	18 DEC 2008	N/A	N/A

Applicant/Sponsor
Name: Bausch & Lomb Inc.
Address: 1400 North Goodman St.
Rochester, NY 14609
Representative: Jennifer S. Knicley
Telephone: 585-338-6307

Name of Reviewer: John W. Metcalfe, Ph.D.

Conclusion: Recommend approval.

Product Quality Microbiology Data Sheet

A. 1. **TYPE OF SUBMISSION:** Original NDA.

2. **SUBMISSION PROVIDES FOR:** A new drug product.

3. **MANUFACTURING SITE:**
Bausch & Lomb, Inc.
8500 Hidden River Parkway
Tampa, FL 33637

4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:**
   - Sterile suspension (5 mL fill in 7.5 mL LDPE bottle and 2 mL fill in 4 mL LDPE bottle).
   - Topical ophthalmic.
   - 0.6%.

5. **METHOD(S) OF STERILIZATION:** [redacted] b(4)

6. **PHARMACOLOGICAL CATEGORY:** Indicated for the treatment of bacterial conjunctivitis.

B. **SUPPORTING/RELATED DOCUMENTS:** None.

C. **REMARKS:**
The NDA is submitted electronically in the CTD format.

An Initial Quality Assessment of the application was performed by the ONDQA PAL (dated 30 SEP 2008). No issues pertaining to product quality microbiology were identified in the IQA.

An information request regarding sterility assurance of the drug product was forwarded to the applicant on 10 October 2008. Following is the information request:

A sterility assurance review of NDA 22-308 is on-going. Please provide the following information, or reference to its location in the subject submission:

- The methods used and data sets from the 1997 and 2004 container closure integrity tests.
- A narrative describing the environmental microbiological monitoring program which includes information regarding the sampling and testing methods, incubation conditions, alert and action limits and routine production monitoring frequency.
- [redacted] b(4)

b(4)

[redacted]

support of these holding periods.

- A description of the method used for sterility testing along with verification data that the sterility test method is suitable for use with the subject drug product.
- A description of the method used for bacterial endotoxins testing along with verification data that the bacterial endotoxins test method is suitable for use with the subject drug product (reference to 03 SEP 2008 electronic mail from the Agency to the applicant regarding the addition to the drug product specification of an endotoxin test method and an acceptance criterion).

The applicant amended the application on 14 November 2008 with responses to all but the fifth bullet point of this request. On 17 December 2008 the applicant amended the application with a response to the fifth bullet point. The responses are summarized and reviewed in relevant sections of this review.

APPEARS THIS WAY ON ORIGINAL

File Name: N022308R1.doc

Executive Summary

I. Recommendations

A. Recommendation on Approvability – NDA 22-308/N-000 is recommended for approval on the basis of product quality microbiology.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – Not applicable.

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology -

b(4)

B. Brief Description of Microbiology Deficiencies – There are no microbiology deficiencies identified.

C. Assessment of Risk Due to Microbiology Deficiencies – Not applicable.

III. Administrative

A. Reviewer's Signature _____
John W. Metcalfe, Ph.D.

B. Endorsement Block_____
Stephen Langille, Ph.D.

C. CC Block
N/A

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/s/

John Metcalfe
1/7/2009 01:26:51 PM
MICROBIOLOGIST

Stephen Langille
1/7/2009 01:38:19 PM
MICROBIOLOGIST

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 22-308 **Applicant:** Bausch & Lomb **Letter Date:** 30 MAY 2008

Drug Name: Besifloxacin hydrochloride **NDA Type:** Standard **Stamp Date:** 02 JUNE 2008

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	X		
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?	X		
4	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	
5	Has the applicant submitted preservative effectiveness studies (if applicable) and container-closure integrity studies?	X		
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		
7	Has the applicant submitted the results of analytical method verification studies?	X		
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?	X		
9	Is this NDA fileable? If not, then describe why.	X		

Additional Comments: None.

John W. Metcalfe, Ph.D.
Reviewing Microbiologist Date

Stephen Langille, Ph.D.
Microbiology Secondary Reviewer/Team Leader Date

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/s/

John Metcalfe
8/19/2008 11:03:32 AM
MICROBIOLOGIST

Stephen Langille
8/19/2008 11:12:34 AM
MICROBIOLOGIST