CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206307Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

Division of Anti-Infective Products
Clinical Microbiology Review
Division of Ophthalmology and Transplant Products Consult

NDA:	**206-307 (Orig: SDN001,002, 009, 011, 013, 015)**
Date Company Submitted:	4/25/2014, 5/7/2014, 8/21/2014, 9/11/2014, 9/19/2014, 9/24/2014
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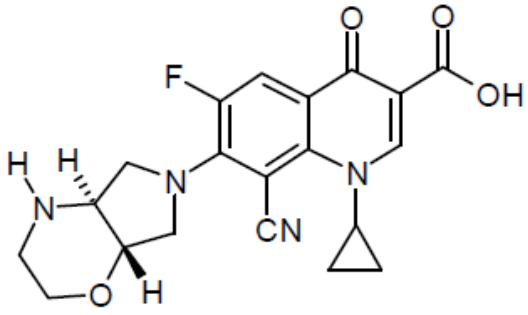
Date Assigned:	5/7/2014

NAME AND ADDRESS OF APPLICANT:
Alcon Research Limited
6201 South Freeway
Fort Worth, TX 76134-2099

DRUG PRODUCT NAMES:

Proprietary Name:	Finafloxacin
Code Name:	AL60371, BAY 35-3377, BAY 14-1881, Gastroquinolone, BYK 60621
Established Name:	Finafloxacin hydrochloride
Chemical Name:	8-cyano-1-cyclopropyl-6- fluoro-7- [(4aS,7aS) hexahydropyrrolo -[3,4-b]-1,4- oxazin-6 (2H)-yl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid monohydrochloride
Molecular Weight:	(b) (4)
Molecular Formula:	$C_{20}H_{19}FN_4O_4HCl$
Structural Formula:	

DRUG CATEGORY
Anti-bacterial

PROPOSED INDICATION
Treatment of Acute Otitis Externa

PROPOSED DOSAGE FORM, STRENGTH AND ROUTE OF ADMINISTRATION:

Dosage Form	Suspension
Route of Administration	Ototopical
Dose Strength	0.3% Finafloxacin
Frequency	4 drops twice daily
Duration	7 days

DISPENSED:
Prescription Product

RELATED DOCUMENTS:
IND (b) (4)

REMARKS
The applicant in this submission is seeking approval to market Finafloxacin otic suspension, 0.3%. The applicant has conducted two pivotal phase 3 efficacy studies to support the safety and efficacy of Finafloxacin otic suspension, 0.3% in the treatment of acute otitis externa in subject (b) (4) of age or older.

SUMMARY AND RECOMMENDATIONS
The Division of Ophthalmology and Transplant Products (DTOP) requested the following consult:

From a clinical microbiology perspective the information provided by the Applicant supports the efficacy of finafloxacin otic suspension 0.3% for the treatment of acute otitis externa due to Pseudomonas aeruginosa and Staphylococcus aureus. Please refer to Section 8 of this review for the proposed labeling of the clinical microbiology subsection of the finafloxacin otic suspension 0.3% package insert. There are several recommendations proposed that should be communicated to the sponsor.

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EXECUTIVE SUMMARY

Alcon Research, Ltd is seeking approval to market Finafloxacin otic suspension, 0.3% for the treatment of acute otitis externa in subjects (b) (4) of age or older. Acute otitis externa are usually caused by bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Finafloxacin has been shown to share many basic chemical and biological characteristics with the fluoroquinolone class of antibacterial agents. Its mechanism of action is similar to other fluoroquinolones by inhibiting the bacterial type II topoisomerases, DNA gyrase and topoisomerase IV which are essential for bacterial DNA replication, transcription, recombination and repair.

Studies were conducted that evaluated the *in vitro* activity of finafloxacin against gram-positive, gram-negative and atypical microorganisms by the determining the minimum inhibitory concentrations (MIC) under conditions of varying pH. The optimal pH range for finafloxacin was between pH 5.8 and pH 6.2 where finafloxacin MICs were shown to be 2 – 4-fold lower than the MICs under standard testing conditions. In contrast, comparator fluoroquinolones (i.e., ciprofloxacin, levofloxacin, ofloxacin and moxifloxacin) at lower pH had reduced activity (MICs were elevated) as compared to normal testing conditions. Under acidic conditions (pH 5.8), at least 50% of the isolates exhibited finafloxacin MICs (MIC_{50}) that ranged from 0.008 µg/mL to 32 µg/mL. For gram-positive organisms, finafloxacin MICs were 4- to 64-fold lower than ciprofloxacin or ofloxacin. For gram-negative organisms, finafloxacin MICs were equivalent or higher (0.008 – 16 µg/mL) than ciprofloxacin or ofloxacin. At neutral pH (7.2 – 7.4) finafloxacin exhibited a susceptibility profile comparable with other fluoroquinolones. Finafloxacin is bactericidal at or above 4x the MIC.

Similar to other fluoroquinolones, resistance to finafloxacin occurs by chromosomal mutations in the genes encoding the principle targets (DNA gyrase and topoisomerase IV), due to decreased outer membrane permeability, over-expression of efflux pumps or plasmids carrying extra-chromosomal mutations. No specific modifying or degrading enzymes have been found to be a mechanism of bacterial resistance to finafloxacin or other fluoroquinolones. Overall, the spontaneous mutation frequency to finafloxacin was low ($<10^{-9}$) at 2x MIC for both gram-positive and gram-negative organisms. Multiple mutations in both DNA gyrase and topoisomerase IV were shown as the predominant mechanism to finafloxacin against gram-positive and gram-negative aerobes. Cross resistance was observed between finafloxacin and other fluoroquinolones suggesting that these drugs share similar targets.

In guinea pig experimental animal models of acute otitis externa *P. aeruginosa* infection, application of a single dose of finafloxacin otic suspension 0.3% reduced the colony count below the limit of detection at 24 hours. No re-growth was observed in the finafloxacin treated animals at 48 hours. Overall, the therapeutic efficacy of finafloxacin otic suspension 0.3% was similar in activity to ciprofloxacin (Ciloxan®) but superior to ofloxacin. In a variety of different infection animal models, finafloxacin was shown to be effective in treating systemic infections caused by gram-positive and gram-negative bacteria. In general, systemic exposure to finafloxacin otic suspension 0.3% was shown to be extremely low (<0.05 ng/mL) and within safety margins of the drug.

Based on the two phase 3 efficacy studies, the primary endpoint was met which showed that a higher proportion of subjects in the finafloxacin otic suspension 0.3% arm had "clinical cure" compare to subjects in the vehicle group. A "clinical cure" was attained if the sum of the numerical scores of the 3 signs and symptoms of AOE (i.e., tenderness, erythema and edema) was 0 at Day 11 (TOC). "Microbiological success" was also evaluated as a secondary endpoint. "Microbiological success" was based on the intent-to-treat population which included all patients who were randomized and had a baseline microbiological specimen that was pathogen positive for *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* which was absent from the exit specimen on Day 11 (TOC). The overall proportion of subjects with "microbiological success" was significantly higher among the finafloxacin otic suspension 0.3% treated subjects (89.7%) compared to vehicle treated subjects (59.7%). Finafloxacin otic suspension 0.3% was shown to be effective in eradicating *P. aeruginosa* (90.5%) and *S. aureus* (87.8%) in the clinical trials.

The following is the proposed recommendation for labeling (only the sections pertinent to Clinical Microbiology are provided below):

12.1 Mechanism of Action

(b) (4)

12.4 Microbiology

Mechanism of Action

(b) (4)

6 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

1. INTRODUCTION

The subject of this NDA is finafloxacin otic suspension 0.3% for the proposed treatment of acute otitis externa in pediatric (age (b) (4) and older), adult and elderly patients. Acute otitis externa reflect disorders of the ear canal skin and subdermis with acute inflammation and variable edema[1-5]. Patients with AOE may exhibit various symptoms such as otalgia, itching, fullness, hearing loss, jaw pain and signs including tenderness of the tragus and pinna, diffuse ear canal edema and erythema, otorrhea, regional lymphadenitis, tympanic membrane erythema, cellulitis of the pinna and adjacent skin[2]. The infection may be localized or diffuse. Localized acute otitis externa occurs in the form of a pustule/furuncle or erysipela which may involve the external ear canal and the soft tissue of the ear itself and is usually caused by *Staphylococcus aureus* or group A streptococci. Diffuse otitis externa (often referred to as swimmer's ear) is related to maceration or softening of tissue of the ear caused by gram-negative bacilli, particularly *Pseudomonas aeruginosa*[1-5]. Contaminating skin bacterial microbiota (corynebacteria and staphylococci) can be present, which are not significant[5]. More invasive infections are caused by extension of bacteria into the adjacent soft tissue and bone. Chronic otitis externa is usually caused by bacterial infection and predominantly caused by *P. aeruginosa*[1-5].

1.1. REGULATORY HISTORY

Finafloxacin otic suspension is formulated for ototopical administration. Finafloxacin hydrochloride is a novel fluoroquinolone originally being developed as an oral formulation by MerLion Pharmaceutial GmbH (MerLion).

2. *IN VITRO* ACTIVITY

The fluoroquinolones directly inhibit DNA synthesis through the interaction of the drug complexes with one or both of the target bacterial type II topoisomerase enzymes, DNA gyrase and topoisomerase IV. DNA gyrase is a heterotetrameric enzyme (A_2B_2) consisting of two subunits, GyrA and GyrB encoded by the *gryA* and *gyrB* enzymes. Like DNA gyrase, topoisomerase IV enzyme is composed of two subunits, the *parC* and *parE* genes, respectively. These bacterial type II topoisomerases play essential roles in bacterial DNA replication, transcription, recombination and repair of DNA. Fluoroquinolones appear to trap the enzyme on DNA during topoisomerization reaction, resulting in barrier to the movement of the replication fork. These complexes trigger poorly defined events within the bacterial cell resulting in double stranded DNA breaks, damage and ultimately cell death.

2.1. Mechanism(s) of action

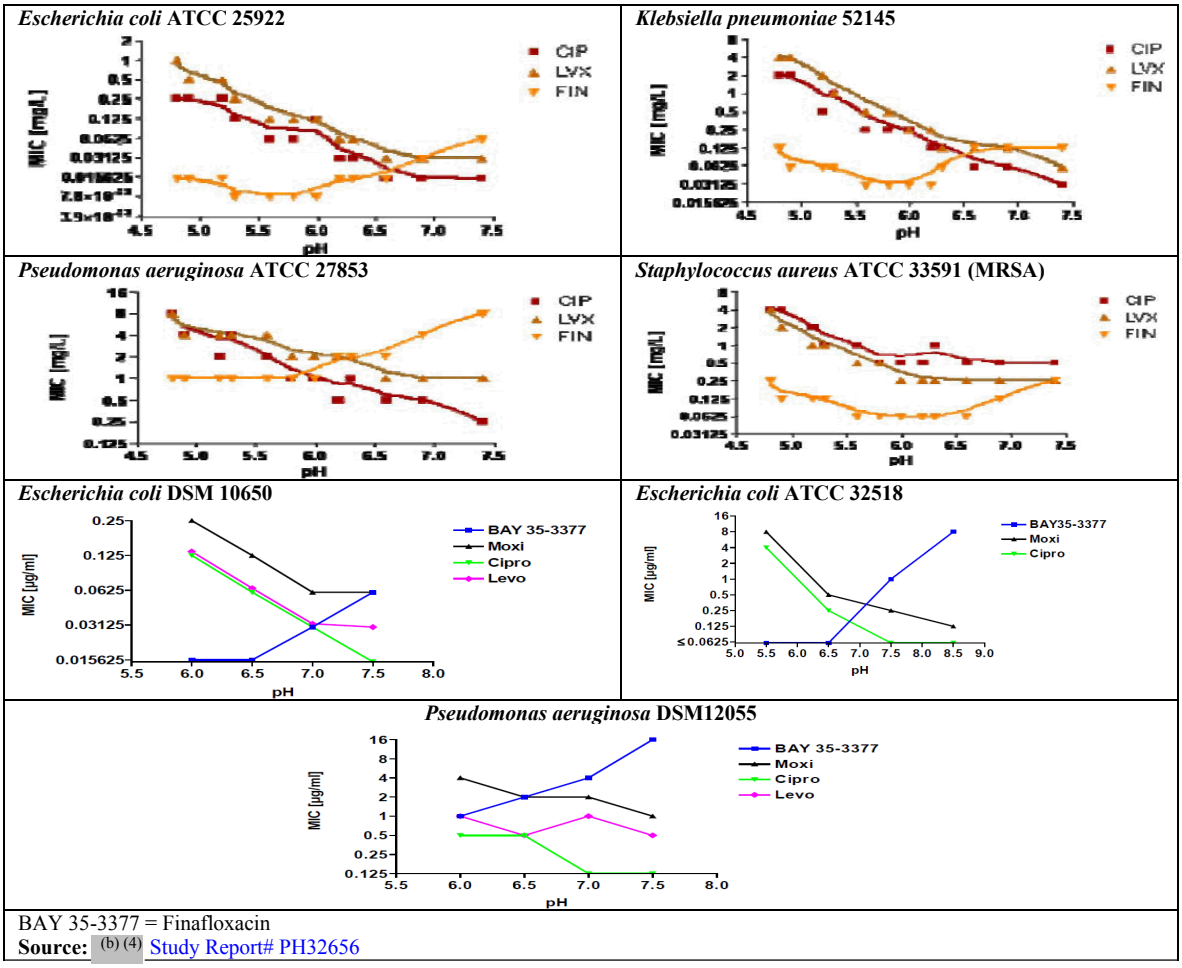
Finafloxacin has been shown to share many basic chemical and biological characteristics with the fluoroquinolone class of antibacterial agents. However, studies showing the

inhibitory activity of finafloxacin against bacterial topoisomerase IV enzymes were not provided.

2.2. Antimicrobial spectrum of activity

Fluoroquinolones are amphoteric molecules and hence their antibacterial activity is influenced by the pH of the surrounding medium. Studies were conducted that evaluated the activity of finafloxacin against gram-positive and gram-negative isolates by determining the minimum inhibitory concentration (MIC) under conditions of varying pH concentrations. The optimal pH range for finafloxacin was between pH 5.0 – 6.0 where finafloxacin MICs were shown to be 2 – 3 fold lower that the MICs at neutral pH (Figure 1). In contrast, ciprofloxacin and levofloxacin at lower pH had reduced activity (MICs were elevated) as compared to neutral pH.

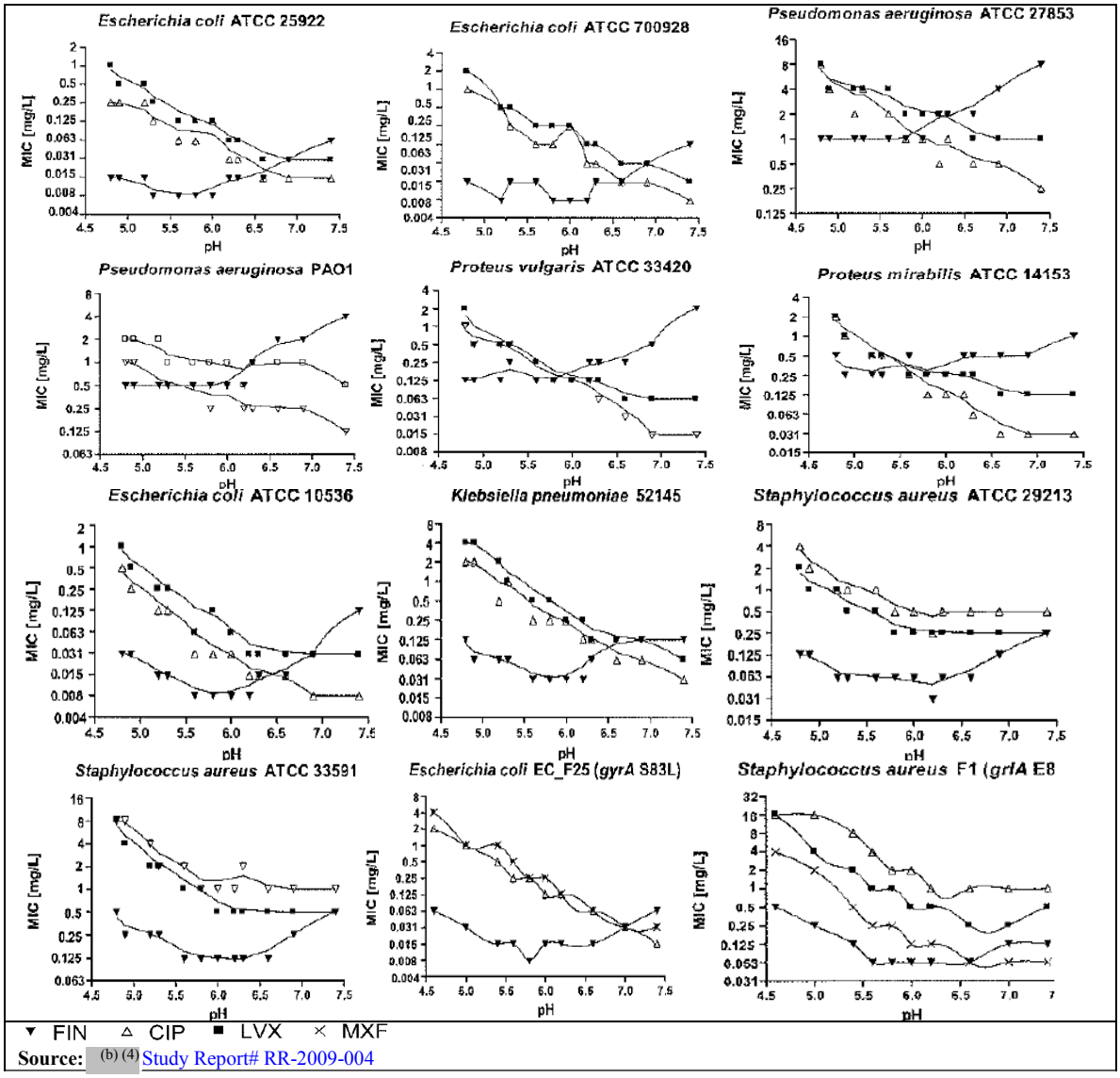
Figure 1: Activity of finafloxacin and comparator fluoroquinolones against selected pathogens at varying pH concentrations



BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

Against several bacterial species, the finafloxacin MICs determined over pH ranges of 4.5 to 7.5 with increments of approximately 0.25 pH units, showed a pH dependent increase in activity (decrease in MIC) against all tested strains at pH values below 7.4. These trends were also observed with fluoroquinolone mutants of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 which harbored target mutations in *gyrA* and *grlA*, respectively.

Figure 2: MIC profiles of finafloxacin and comparator fluoroquinolones against bacterial strains depending on the pH



Source: (b) (4) Study Report# RR-2009-004

The *in vitro* activity of finafloxacin and comparators were determined against a variety of gram-positive and gram-negative clinical isolates. The finafloxacin MIC values and comparators were determined under neutral (pH 7.3) and acidic conditions (pH 5.8) in Mueller Hinton (MH) agar or broth. For typical bacteria, the methods used in susceptibility testing were in accordance with Clinical Laboratory Standards Institute M07 guidelines. For *Streptococcus pneumoniae*, bovine serum was added to final concentration of 20%. Otherwise, any modifications to the standard CLSI methods or special methods used for determination of antimicrobial susceptibility testing were described.

2.2.1. Gram-positive organisms

2.2.1.1. *Staphylococcus aureus*

Under acidic conditions, the highest finafloxacin MIC observed for *S. aureus* was 32 µg/mL, with MICs that ranged from 0.008 – 32 µg/mL (Table 1). The finafloxacin MICs was higher against ciprofloxacin-resistant *S. aureus* (range, 0.125 to 32 µg/mL) compared to ciprofloxacin susceptible *S. aureus* (range, 0.008 to 0.125 µg/mL). Finafloxacin showed 32 - >64-fold greater activity than ciprofloxacin and 16 – 128-fold greater than ofloxacin. At neutral pH, finafloxacin exhibited a susceptibility profile comparable to other fluoroquinolones.

Table 1: Activity of finafloxacin and comparators against *S. aureus* clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
Ciprofloxacin-resistant *S. aureus* (N=12)[1]	Finafloxacin	4	8	0.5 - 8	16	16	2 - 16	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	128	>256	16 - >256	128	128	4 - 128	
	Ofloxacin	64	>512	16 - >512	32	256	2 - 256	
	Moxifloxacin	8	64	2 - 64	4	16	1 - 16	
Ciprofloxacin-susceptible *S. aureus* (N=8)[1]	Finafloxacin	0.016	NA	0.008 – 0.125	0.125	NA	0.016 – 0.25	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	1	NA	0.5 – 4	0.25	NA	0.125 – 1	
	Ofloxacin	1	NA	0.5 – 64	0.25	NA	0.125 – 0.5	
	Moxifloxacin	0.031	NA	0.031 – 1	0.016	NA	≤0.008 – 0.03	
Ciprofloxacin-resistant *S. aureus* (N=22)[2]	Finafloxacin	4	8	0.125 – 16	16	16	0.5 – 32	**Broth Microdilution (CLSI)[B]**
	Ciprofloxacin	512	1024	8 – 1024	64	1024	4 - >2048	
	Ofloxacin	64	256	4 - > 512	32	64	1 – 128	
	Moxifloxacin	--	--	--	--	--	--	
Ciprofloxacin-susceptible *S. aureus* (N=78)[2]	Finafloxacin	0.06	0.125	0.03 – 0.125	0.125	0.25	0.06 – 0.5	**Broth Microdilution (CLSI)[B]**
	Ciprofloxacin	1	1	0.25 – 2	0.25	1	0.125 – 1	
	Ofloxacin	1	1	0.5 – 2	0.25	0.5	0.125 – 1	
	Moxifloxacin	--	--	--	--	--	--	
***S. aureus* (N=8)**	Finafloxacin	≤0.03	0.06	≤0.03 - 0.06	0.125	0.125	0.06 - 0.125	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	1	1	0.5 - 1	0.25	0.5	0.125 - 0.5	
	Levofloxacin	0.5	1	0.25 - 1	0.125	0.25	0.06 - 0.5	
	Moxifloxacin	0.125	0.25	0.125 - 0.25	≤0.03	0.06	≤0.03 - 0.06	
Ciprofloxacin-resistant *S. aureus* (N=30)	Finafloxacin	1	4	0.25 - 32	2	16	0.25 - 32	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	32	>64	16 - >64	32	>64	4 - > 64	
	Levofloxacin	16	>64	4 - >64	16	64	4 - >64	
	Moxifloxacin	8	32	0.5 - >64	2	32	0.125 - 32	
***S. aureus* community associated MRSA [CA-MRSA] (N=41)**	Finafloxacin	0.06	1	0.03 - 1	0.125	2	0.125 - 4	**Broth Microdilution (CLSI)[D]**
	Ciprofloxacin	1	>32	1 - >32	0.5	>32	0.25 - >32	
	Levofloxacin	ND	ND	ND	ND	ND	ND	
	Moxifloxacin	0.25	8	0.125 - 8	0.06	2	0.03 - 2	
References: A = TDOC0011241; B = TDOC0017294; C = PH32656; D = RR-2009-007								

2.2.1.2. *Coagulase negative staphylococci*

Under acidic conditions, finafloxacin was more active than other fluoroquinolones against *S. epidermidis* with MICs that ranged from 0.008 – 16 µg/mL (Table 2). The finafloxacin MICs were higher against ciprofloxacin-resistant *S. epidermidis* (MIC_{50}, 2 µg/mL) than ciprofloxacin-susceptible isolates (MIC_{50}, 0.016 µg/mL). Against other coagulase negative staphylococci finafloxacin MIC values ranged from 0.008 - 4 µg/mL.

Table 2: Activity of finafloxacin and comparators against coagulase negative staphylococci clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
Ciprofloxacin-resistant *S. epidermidis* (N=12)[1]	Finafloxacin	2	16	0 5 – 16	8	512	2 – 512	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	128	256	8 – 256	32	64	4 – 64	
	Ofloxacin	32	>512	2 - >512	16	>512	0 5 - >512	
	Moxifloxacin	8	128	0 5 – 128	2	32	11689	
Ciprofloxacin-susceptible *S. epidermidis* (N=8)[1]	Finafloxacin	0 016	NA	0 008 – 0 125	0 125	NA	0 016 – 0 25	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	1	NA	0 5 – 4	0 25	NA	0 125 – 1	
	Ofloxacin	1	NA	0 5 – 64	0 25	NA	0 125 – 0 5	
	Moxifloxacin	0 03	NA	0 03 – 1	0 016	NA	≤0 008 – 0 03	
Coagulase negative staphylococci (N=20)[1]	Finafloxacin	0 016	4	0 008 – 4	0 25	16	0 016 – 16	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	4	128	0 25 – 128	1	64	0 06 – 64	
	Ofloxacin	--	--	--	--	--	--	
	Moxifloxacin	0 064	16	0 03 – 16	0 031	8	0 016 - 8	
References: A = TDOC0011241; B = TDOC0017294; C = PH32656								

2.2.1.3. *Streptococcus species*

Under acidic conditions the finafloxacin MIC values against *S. pneumoniae* isolates ranged from 0.125 – 0.5 µg/mL (Table 3). Against beta-hemolytic streptococci, finafloxacin MIC values were 4-16-fold lower than ciprofloxacin and ofloxacin.

Table 3: Activity of finafloxacin and comparators against streptococcal clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
***S. pneumoniae* (N=10)**	Finafloxacin	0 25	0 5	0 125 – 0 5	0 5	2	0 25 – 2	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	1	1	0 5 – 4	0 5	2	0 5 – 2	
	Ofloxacin	2	2	1 – 4	1	4	0 5 – 4	
	Moxifloxacin	0 25	0 25	0 125 – 0 25	0 125	0 125	0 06 – 0 125	
***S. pneumoniae* (N=21)**	Finafloxacin	--	--	--	1	2	0 5 - 4	**Agar Microdilution (CLSI)[C]**
	Ciprofloxacin	--	--	--	2	4	1 - 4	
	Levofloxacin	--	--	--	1	2	0 5 - 2	
	Moxifloxacin	--	--	--	0 25	0 5	0 125 - 0 5	
Beta-hemolytic streptococci (N=10)[1]	Finafloxacin	0 125	1	0 125 – 1	0 5	1	0 25 – 1	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	0 5	8	0 5 – 8	0 25	2	0 125 – 2	
	Ofloxacin	--	--	--	--	--	--	
	Moxifloxacin	0 25	2	0 125 – 1	0 125	0 5	0 125 – 0 5	
Non-pneumococcal streptococci (N=13)	Finafloxacin	--	--	--	0 5	0 5	0 25 - 0 5	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	--	--	--	0 5	1	0 25 - 1	
	Moxifloxacin	--	--	--	0 125	0 125	0 03 - 0 125	
References: A = TDOC0011241; B = TDOC0017294; C = PH32656								

2.2.1.4. *Enterococcus spp.*

Finafloxacin demonstrated good activity against *E. faecalis* compared to other fluoroquinolones under acidic and neutral conditions (Table 4). Finafloxacin MICs was generally 4->512-fold lower compared to ciprofloxacin and 16- 32-fold lower than ofloxacin.

Table 4: Activity of finafloxacin and comparators against *Enterococci* clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
E. faecalis (N=10)[1]	Finafloxacin	8	16	0.25 – 16	4	32	0.5 – 32	Broth Microdilution (CLSI)[A]
	Ciprofloxacin	128	>512	2 - >512	64	128	1 – 128	
	Ofloxacin	16	256	4 – 256	128	512	1 – 512	
	Moxifloxacin	16	32	0.5 – 32	8	16	0.03 - 16	
Enterococcus species (N=21)[1]	Finafloxacin	--	--	--	1	2	1 - 4	Agar Dilution (CLSI)[C]
	Ciprofloxacin				2	4	2 - 4	
	Levofloxacin				2	4	1 - 4	
	Moxifloxacin				0.5	1	0.25 - 1	
References: A = TDOC0011241; B = TDOC0017294; C = PH32656								

2.2.2. Gram-negative bacteria

2.2.2.1. *Haemophilus and Moraxella* spp.

Finafloxacin showed activity against *H. influenzae* and *M. catarrhalis* with MIC_{90} values of 0.008 µg/mL for both species (Table 5). The activity of finafloxacin was similar to ciprofloxacin and ofloxacin under neutral and acidic conditions.

Table 5: Activity of finafloxacin and comparators against *H. Influenzae* and *M. catarrhalis* isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
Moraxella catarrhalis (N=10)[1]	Finafloxacin	--	--	--	0 008	1	0 008 – 1	Broth Microdilution (CLSI)[A]
	Ciprofloxacin	--	--	--	0 016	1	≤0 008 – 1	
	Ofloxacin	--	--	--	0 03	2	0 03 – 2	
	Moxifloxacin	--	--	--	0 016	0 5	0 016 – 0 5	
Haemophilus influenzae (N = 10)[1]	Finafloxacin	0 008	0 125	≤0 004 – 0 125	0 008	0 125	≤0 004 – 0 125	Broth Microdilution (CLSI)[A]
	Ciprofloxacin	0 016	0 5	≤0 008 – 0 25	0 016	0 125	≤0 008 – 0 125	
	Ofloxacin	0 03	1	0 016 – 1	0 03	0 5	≤0 008 – 0 5	
	Moxifloxacin	0 25	2	0 5 – 16	0 06	0 5	0 03 - 1	
References: A = TDOC0011241; B = TDOC0017294;								

2.2.2.2. *Enterobacteriaceae*

Under acidic conditions, the finafloxacin MIC_{90} values were < 1 µg/mL for most of the *Enterobacteriaceae* isolates with the exception of *E. coli* and *P. mirabilis* that had MIC_{90} values of 8 µg/mL (Table 6). Against ciprofloxacin-sensitive *E. coli* isolates, the

finafloxacin MIC values ranged from 0.008 – 0.25 µg/mL; whereas ciprofloxacin-resistant *E. coli* isolates had MIC values that ranged from 4 – 8 µg/mL.

Table 6: Activity of finafloxacin and comparators against Enterobacteriaceae clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
Enterobacter cloacae **(N=10)**	Finafloxacin	0 125	0 5	0 016 – 0 5	1	2	0 25 – 2	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	1	2	0 25 – 2	0 06	0 25	0 016 – 0 25	
	Ofloxacin	--	--	--	--	--	--	
	Moxifloxacin	--	--	--	--	--	--	
Enterobacter **spp. (N=10)**	Finafloxacin	≤0 03	≤0 03	≤0 03 - 0 125	0 125	0 125	0 06 - 0 5	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	0 125	0 25	0 06 - 0 5	≤0 03	≤0 03	≤0 03	
	Levofloxacin	0 25	0 5	0 125 - 0 5	≤0 03	0 06	≤0 03 - 0 06	
	Moxifloxacin	0 25	0 5	0 125 - 2	≤0 03	0 06	≤0 03 - 0 06	
Escherichia coli **(N=10)**	Finafloxacin	4	8	0 008 – 8	32	64	0 008 – 64	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	256	>1024	0 06 - >1024	16	128	≤0 008 – 128	
	Ofloxacin	512	512	0 25 – 512	16	16	0 016 – 16	
	Moxifloxacin	128	128	0 03 – 128	16	16	0 008 - 16	
Escherichia coli, **Fluoroquinolone Resistant (N=75)**	Finafloxacin	8	32	2 - 64	128	256	16 - >256	**Broth Microdilution (CLSI)[D]**
	Ciprofloxacin	>256	>256	>256	128	>256	8 - >256	
	Levofloxacin	128	>256	4 - >256	32	64	8 - 64	
Escherichia coli **(N=8)**	Finafloxacin	0 25	8	0 06 - 8	1	16	0 25 - 16	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	2	>32	0 5 - >32	0 5	8	0 125 - 8	
	Levofloxacin	2	32	1 - 32	0 5	8	0 25 - 8	
	Moxifloxacin	2	32	1 - 32	0 5	16	0 25 - 16	
Klebsiella pneumoniae **(N=10)**	Finafloxacin	0 25	1	0 008 – 1	1	4	0 125 – 4	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	2	8	0 125 – 8	0 25	1	0 016 – 1	
	Ofloxacin	--	--	--	--	--	--	
	Moxifloxacin	8	16	0 5 – 16	1	1	0 03 - 1	
Proteus mirabilis **(N=10)**	Finafloxacin	0 25	8	0 25 – 8	1	16	0 5 – 16	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	0 125	8	0 03 – 8	0 03	1	0 016 – 1	
	Ofloxacin	0 5	32	0 125 – 32	0 125	4	0 03 – 4	
	Moxifloxacin	4	64	2 – 64	0 5	16	0 25 - 16	
Providencia **spp. (N=11)**	Finafloxacin	1	8	≤0 03 - 8	8	16	0 06 - 16	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	16	>16	0 125 - >16	1	4	≤0 03 - 16	
	Levofloxacin	8	>16	0 125 - >16	1	2	≤0 03 - 17	
	Moxifloxacin	8	>16	0 125 - >16	0 5	2	≤0 03 - 18	
Salmonella **spp. (N=8)**	Finafloxacin	0 5	4	0 06 - 4	2	16	0 5 - 16	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	8	>32	1 - >32	1	32	0 125 - 32	
	Moxifloxacin	4	>32	1 - >32	2	16	0 25 - 16	
	Levofloxacin	4	32	0 5 - 32	2	16	0 25 - 16	
Serratia marcescens **(N=12)**	Finafloxacin	0 25	2	0 06 - 4	1	8	0 125 - 8	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin	1	16	0 125 - >16	≤0 03	1	≤0 03 - 1	
	Levofloxacin	1	16	0 25 - >16	0 125	1	≤0 03 - 4	
	Moxifloxacin	4	>16	1 - >16	0 125	2	0 06 - 4	

References: A = TDOC0011241; B = TDOC0017294; C = PH32656; D = RR-2009-004

2.2.2.3. *Non-fermentative Gram-negative bacteria*

Against ciprofloxacin-susceptible *P. aeruginosa* the finafloxacin MIC_{90} values were 4 µg/mL; though the finafloxacin MIC values were higher (>512 µg/mL) against fluoroquinolone-resistant *P. aeruginosa* (Table 7). Against other non-fermentative Gram-negative bacilli such as *P. otitidis* and *S. maltophilia*, finafloxacin MIC_{90} values were 0.25 µg/mL and 4µg/mL, respectively.

Table 7: Activity of finafloxacin and comparators against non-fermentative gram-negative clinical isolates

Organism	Compound	Minimum Inhibitory concentration (µg/mL)						Method
		pH 5.8			pH 7.3			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
P. aeruginosa **(N = 20)**	Finafloxacin				4	32	1 - 64	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin				0.03	0.5	0.008 - 2	
	Moxifloxacin				1	4	0.25 - 8	
Ciprofloxacin-resistant *P. aeruginosa* (N=11)	Finafloxacin	16	512	2 – 512	512	>512	8 - >512	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	64	>512	8 - >512	16	512	4 – 512	
	Ofloxacin	256	>512	64 - >512	64	>512	8 - >512	
	Moxifloxacin	256	>512	32 - >512	128	>512	16 - >512	
Ciprofloxacin-susceptible *P. aeruginosa* (N=14)	Finafloxacin	1	4	0.25 – 4	4	16	2 – 16	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	1	8	0.125 – 8	0.5	2	0.06 – 2	
	Ofloxacin	4	32	1 – 32	2	4	1 – 4	
	Moxifloxacin	4	32	2 – 32	2	8	1 - 8	
Ciprofloxacin-susceptible *P. aeruginosa* (N=100)	Finafloxacin	0.5	1	0.125 – 2	4	8	1 – 16	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	0.25	1	0.125 – 16	0.125	0.5	0.06 – 0.5	
	Ofloxacin	2	4	1 – 8	1	2	0.5 – 4	
	Moxifloxacin	--	--	--	--	--	--	
Pseudomonas otitidis **(N=10)**	Finafloxacin	0.125	0.25	0.03 – 0.25	0.5	2	0.5 – 2	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	0.25	0.5	0.06 – 0.5	0.06	0.125	0.03 – 0.125	
	Ofloxacin	1	2	0.25 – 2	0.25	0.5	0.125 – 0.5	
	Moxifloxacin	4	4	0.5 – 4	1	8	0.25 - 8	
Stenotrophomonas maltophilia **(N = 10)**	Finafloxacin	0.25	4	0.125 = 4	2	8	0.5 – 8	**Broth Microdilution (CLSI)[A]**
	Ciprofloxacin	8	64	2 – 64	2	32	0.5 – 32	
	Ofloxacin	--	--	--	--	--	--	
	Moxifloxacin	2	64	0.25 – 64	0.5	8	0.031 - 8	
Acinetobacter **spp. (N = 12)**	Finafloxacin				0.06	0.25	0.004 - 8	**Broth Microdilution (CLSI)[C]**
	Ciprofloxacin				0.06	0.5	0.004 - 8	
	Levofloxacin				0.06	0.5	0.016 - 2	
	Moxifloxacin				0.016	0.25	0.001 - 1	
References: A = TDOC0011241; B = TDOC0017294; C = PH32656								

2.2.3. Anaerobes

Finafloxacin showed variable activity against select anaerobic bacteria. The MIC_{90} values against *P. acnes* were 4 µg/mL whereas against *C. amycolatum* were 256 µg/mL (Table 8). The MIC values were <0.5 µg/mL against *B. fragilis, C. perfringens* and *P.*

anaerobius (Table 9). The finafloxacin MIC values were comparable (within one dilution) to moxifloxacin. However, there were <10 isolates tested against most isolates.

Table 8: Activity of Finafloxacin (AL-60371) and comparators against anaerobic isolates

Organism	Minimum Inhibitory concentration (µg/mL)							
	pH 5.8				pH 7.3			
	Finafloxacin	Ciprofloxacin	Ofloxacin	Moxifloxacin	Finafloxacin	Ciprofloxacin	Ofloxacin	Moxifloxacin
***Corynebacterium amycolatum* (N=10)**								
MIC_{50}	32	32	--	64	128	32	--	16
MIC_{90}	256	128	--	256	512	64	--	32
Range	0.008 – 256	0.016 – 128	--	0.03 – 256	0.03 – 512	0.03 – 64	--	0.016 - 32
***Propionibacterium acnes* (N = 10)**								
MIC_{50}	0.125	0.5	--	0.25	0.25	0 25	--	0.25
MIC_{90}	4	4	--	8	4	4	--	4
Range	0.06 – 4	0.125 – 4	--	0.25 – 8	0.125 – 4	0.25 – 4	--	0.125 - 4

Source: Alcon Study Report# TDOC0011241

Table 9: Activity of Finafloxacin (AL-60371) and comparators against anaerobic isolates

Species	BAY 35-3377	Cipro	Moxi	Levo
B. fragilis ES 25	0.5	4	0.25	1
B. fragilis ES 27	0.5	4	0.25	1
B. fragilis 11654	0.5	4	0.25	1
B. fragilis 06688	0.5	8	0.25	1
B. fragilis 09052	2	8	2	8
B. fragilis 010848	0.5	8	0.5	1
B. fragilis 011303	0.5	4	0.25	1
B. fragilis 012998	0.5	4	0.25	1
B. fragilis 7667	0.5	4	0.25	1
B. fragilis 10267	0.5	4	0.5	1
C. perfringens 1024027	0.125	1	0.5	0.25
C. perfringens 24072	0.125	0.5	0.5	0.5
C. perfringens 24057	0.125	0.5	0.5	0.25
C. perfringens 24053	0.125	1	1	0.25
C. perfringens 24047	0.5	2	2	0.5
P. anaerobius II	0.125	1	0.125	0.5
P. anaerobius DSM 20357	0.125	1	0.125	0.5
P. anaerobius DSM 2221/82	0.125	2	0.5	2

Source: (b) (4) Study Report# PH32656

2.2.4. Atypical Microorganisms

The activity of finafloxacin was evaluated against several isolates of *C. pneumoniae, Mycoplasma pneumoniae* and *Mycobacterium tuberculosis.* Against *Mycobacterium*

tuberculosis, the MICs were measured using the radiometric method BACTEC 460 TB though the details of the method were not provided. Susceptibility testing of *Chlamydia* isolates were cultivated on Hep-2 cells and the MICs were determined by photometric measurement of chlamydial antigen ((b) (4)). The results were confirmed by microscopic examination of inclusion bodies. The MICs against *Mycoplasma pneumoniae* were determined by the microdilution method using PPLO medium supplemented with 10 mg/mL glucose and 0.002% phenol red and were read after 6 days of incubation at 37°C. Against *M. tuberculosis* ATCC 27294 finafloxacin MIC was 8 µg/mL; in comparison the moxifloxacin MIC was ≤ 0.5 µg/mL (Table 10). Against *Chlamydia pneumoniae* ATCC VR1310 and ATCC VR-2282 showed similar susceptibilities with MIC values of 1 µg/mL for finafloxacin and 0.1 µg/mL for moxifloxacin. Against *M. pneumoniae* finafloxacin MIC values ranged from 0.25 to 1 µg/mL, which were 2 – 3 –fold higher than moxifloxacin.

Table 10: Activity of Finafloxacin (AL-60371) and comparators against atypical isolates

Organism	Finafloxacin	Moxifloxacin
Mycobacterium tuberculosis ATCC 27294	8	0.5
Chlamydia pneumoniae ATCC VR-1310	1	0.1
Chlamydia pneumoniae ATCC VR-22822	1	0.1
Mycoplasma pneumoniae ATCC 15531	0.5 – 1	0.06 – 0.125
Mycoplasma pneumoniae ATCC 15293	0.5 – 1	0.125 – 0.25
Mycoplasma pneumoniae ATCC 29342	0.25 – 0.5	0.125 – 0.25

Source: (b) (4) Study Report# PH32656

Reviewer's comments:
Unlike other fluoroquinolones which tend to lose activity at an acidic pH, finafloxacin antibacterial activity is enhanced at lower (pH 5.8) than normal pH (7.2). Under acidic conditions (pH 5.8) at least 50% of the isolates tested exhibited finafloxacin MICs (MIC_{50}) that ranged from 0.008 µg/mL to 16 µg/mL. Against Staphylococcus spp., finafloxacin MICs was higher in ciprofloxacin resistant (2 – 4 µg/mL) compared to ciprofloxacin susceptible isolates (0.016 µg/mL). Overall, finafloxacin MIC values were 4- to 64- fold lower than ciprofloxacin or ofloxacin against gram positive organisms. For gram-negative organisms, finafloxacin MICs were equivalent or higher (0.008 – 512 µg/mL) than ciprofloxacin or ofloxacin under acidic conditions. The highest finafloxacin MICs of 512 µg/mL were observed for P. aeruginosa isolates. At neutral pH (7.2 – 7.4) finafloxacin exhibited a susceptibility profile comparable with other fluoroquinolones.

2.3. Bactericidal Activity

2.3.1. Minimal Bactericidal Concentration

The minimal bactericidal concentrations (MBC) were determined for several bacterial pathogens. The MBC was defined as the lowest concentration of drug which killed ≥ 99.9% equivalent to 3 $\log_{10}$ reductions in CFU of the initial inoculum of 5 x 10^5 CFU/mL within 24 hours. The MBC was determined in parallel at pH 7.2 and pH 5.8. The results showed that MBC/MIC relationship was not altered at lower pH and both finafloxacin and ciprofloxacin were 1- 2 fold greater than the MIC (Table 11).

Table 11: MBC, MIC and MBC/MIC ratios of finafloxacin and ciprofloxacin against gram-positive and gram-negative bacteria at pH 7.2 and pH 5.8

Strain	MIC [mg/L]				MBC[mg/L]				MBC/MIC [mg/L]			
	pH 5.8		pH 7.2		pH 5.8		pH 7.2		pH 5.8		pH 7.2	
	FIN	CIP	FIN	CIP	FIN	FIN	CIP	CIP	FIN	CIP	FIN	CIP
Staphylococcus aureus ATCC 29213	0.06	1	0.25	0.5	0.125	1	2	1	2	1	1	2
Staphylococcus aureus NRS 384	0.06	4	0.25	2	0.125	2	2	4	2	2	2	2
Staphylococcus saprophyticus ATCC 15305	0.06	1	0.25	0.5	0.06	2	2	1	1	2	2	2
Staphylococcus epidermidis ATCC 12228	0.06	0.5	0.125	0.125	0.06	2	2	0.25	1	1	2	2
Enterococcus faecalis ATCC 29212	0.25	2	1	1	0.5	2	2	2	2	4	2	2
Streptococcus pneumoniae ATCC 49619	n.d.	n.d.	0.5	0.5	n.d.	2	2	1	n.d	n.d	2	2
Escherichia coli ATCC 700928	0.016	0.125	0.125	0.016	0.03	1	1	0.016	2	1	1	1
Pseudomonas aeruginosa ATCC 27853	1	1	8	0.25	2	2	2	0.5	2	4	2	2
Enterobacter cloacae ATCC 13047	0.06	0.25	0.25	0.03	0.06	1	1	0.03	1	1	1	1
Proteus mirabilis ATCC 14153	0.25	0.125	1	0.004	0.5	2	1	0.004	2	4	2	1
Acinetobacter baumannii ATCC 19606	0.06	4	0.5	1	0.125	2	1	1	2	2	2	1
Escherichia coli 2C35_06 (FQ-R)	0.5	8	2	0.5	0.5	1	1	0.5	1	1	1	1

Abbreviations: CIP, ciprofloxacin, FIN; finafloxacin, FQ-R; fluoroquinolone resistant.

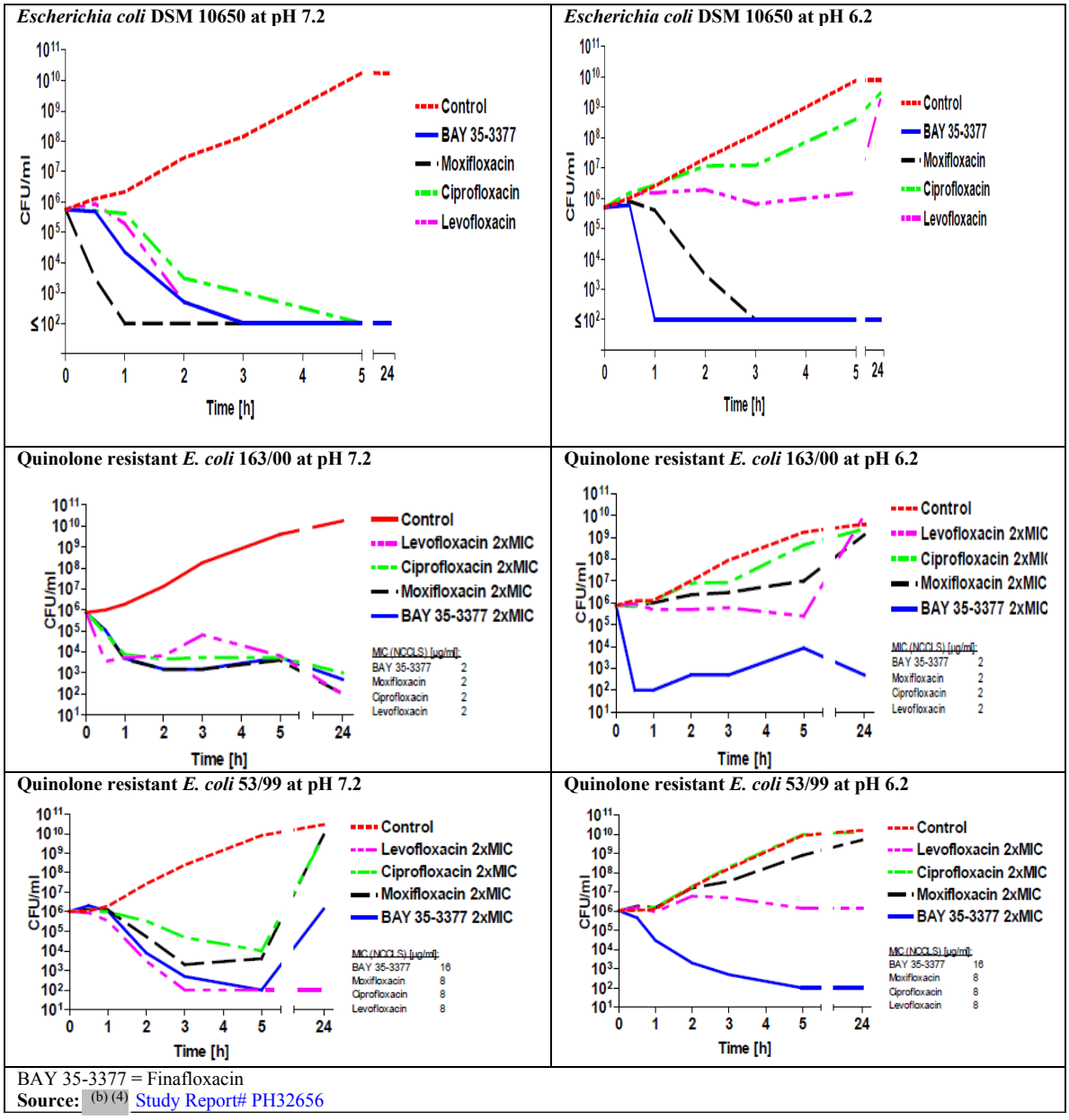
Source: (b) (4) Study Report# RR-2009-004

2.3.2. Time Kill Studies

Time kill kinetic studies were performed to evaluate the bactericidal activity of finafloxacin and comparators against quinolone-sensitive and quinolone-resistant of the *E. coli* DSM 10650 strain. The MICs of finafloxacin and comparators were determined for each of the organisms tested using standard CLSI methods. Bacterial isolates were grown in brain heart infusion (BHI) broth with antibiotic at concentrations of 1x, 4x and 16x MIC as well as at pH 5.2, 6.2 and 7.2. Viable bacteria were counted at hourly intervals up to 5 hours and after 24 hours of incubation at 37°C. A bactericidal effect was defined as a 3 $\log_{10}$ reduction compared with the initial inoculum over a 24 hour incubation. Under acidic conditions (at pH 6.2) finafloxacin showed to be most active compound showing a maximal bactericidal effect (4 $\log_{10}$ reduction) within 1 hour while moxifloxacin showed a similar bactericidal effect within 3 hours; whereas ciprofloxacin and levofloxacin showed no bactericidal activity and only inhibited bacterial growth (Figure 3). In comparison under neutral conditions, moxifloxacin was the most rapid

bactericidal compound which reduced the bacterial cell count by approximately 4 $\log_{10}$ within 1 hour whereas finafloxacin were comparable to other comparators and showed a maximal bactericidal effects within 3 to 5 hours.

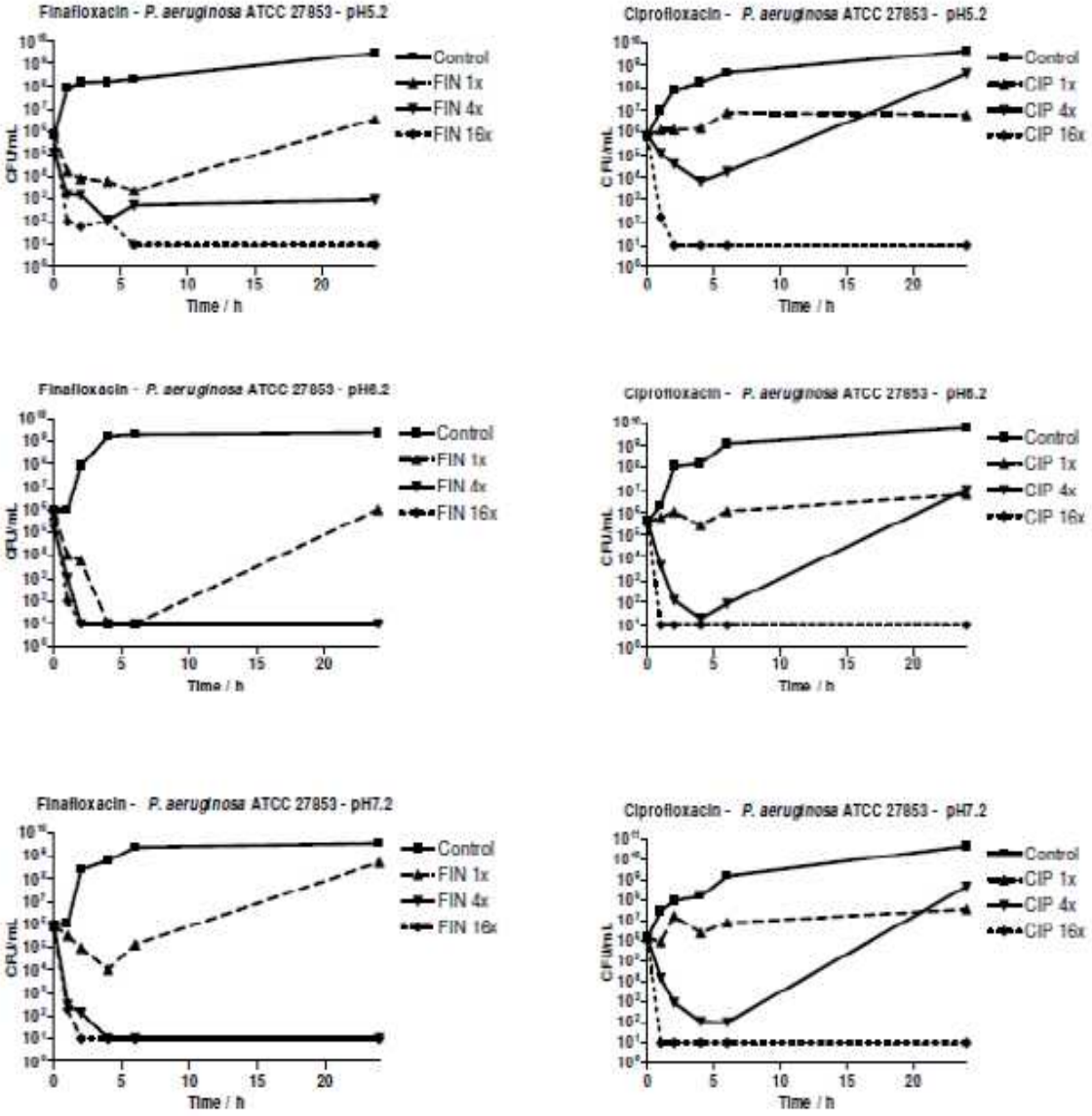
Figure 3: Time Kill kinetic profiles of finafloxacin and ciprofloxacin against *E. coli* DSM 10650 strain determined at pH 7.2 and 6.2.



BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

Against *P. aeruginosa* strains, finafloxacin showed a trend of decreasing activity as the pH increased from 5.2 to 7.2, the best results when the testing conditions were at pH 6.2, which is near the optimal pH of 5.8 (Figure 4). The converse was seen for ciprofloxacin, where the activity increased as the pH increased.

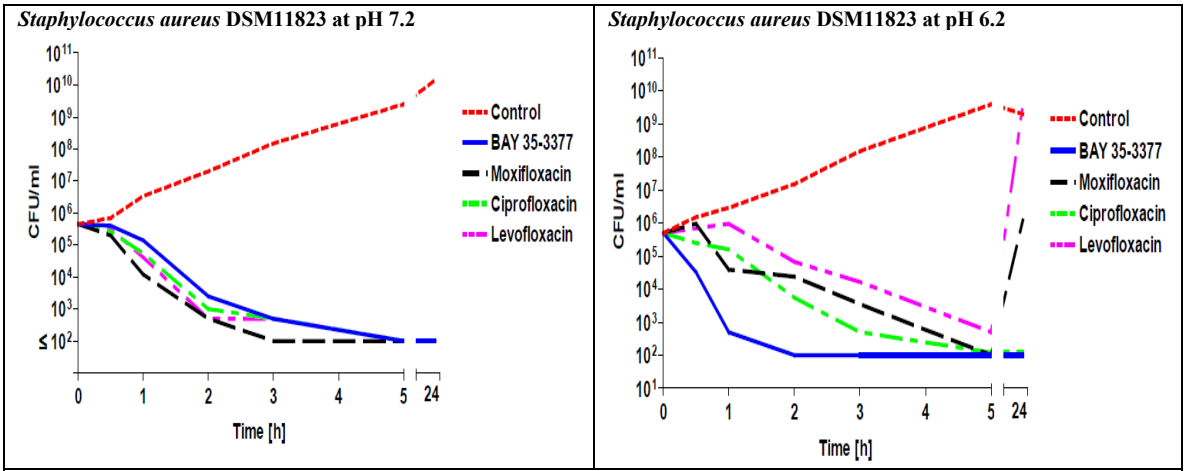
Figure 4 : Time Kill kinetic profiles of finafloxacin and ciprofloxacin against P. aeruginosa ATCC 27853 determined at pH 7.2, 6.2 and 5.2



Source: Pharmacology Written Summary Figure 2.6.2.2-1

Against *S. aureus* DSM 11823 strain, all test compounds showed similar bactericidal effects within 2 to 3 hours at pH 7.2 (Figure 5). Under acidic conditions, finafloxacin showed to be more rapidly bactericidal within 1 hour compared to moxifloxacin, ciprofloxacin and levofloxacin which were bactericidal within 3 to 5 hours.

Figure 5: Time Kill kinetic profiles of finafloxacin and ciprofloxacin against *S. aureus* DSM 1823 strain determined at pH 7.2 and 6.2.



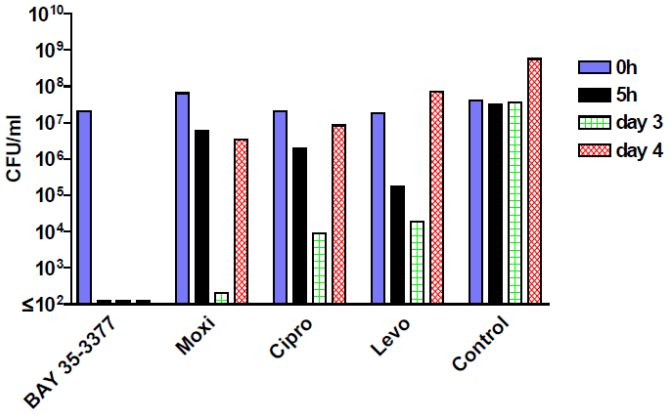
BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

2.3.3. Biofilm formation

The bactericidal activity of finafloxacin and comparator fluoroquinolones against adherent bacteria was evaluated using several systems.

In one study, *E. coli* C600 cells were grown on membrane filter disks (b) (4) under a continuous flow of brain heart infusion (BHI) broth at pH 6. Once steady state had been established (10^7 – 10^8 CFU/mL of perfusate), finafloxacin , ciprofloxacin, moxifloxacin or levofloxacin were perfused at 5 µg/mL for 3 days followed by drug free media for a further 24 hours. The adherent population was determined by sampling the perfusate and viable counts were determined at T_0 (steady state), 5 h, 3d and 4d as shown in Figure 6. Finafloxacin had a rapid effect on the viability of the adherent *E. coli* populations, causing a 5 $\log_{10}$ reduction to below the limit of detection (<10^2 CFU/mL) within 5 hours of exposure. In comparison, levofloxacin caused a 2-$\log_{10}$ reduction. Overall, all drugs had significantly reduced viability by 3 days, however rapid re-growth of the adherent population was observed following perfusion with drug-free media in the ciprofloxacin, levofloxacin or moxifloxacin treated populations; however, no viable cells were recovered from the regrowth sample (day 4) following exposure to finafloxacin.

Figure 6: Effect of finafloxacin and comparators on adherent *E. coli* to membrane filters

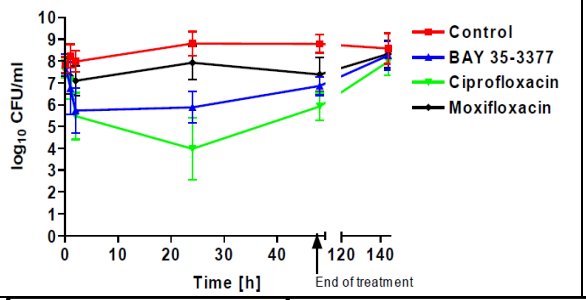


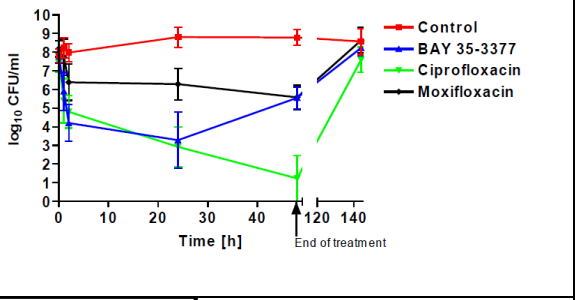
Shown is the shed-off of planktonic daughter cells from a layer of adherent mother cells. The filters were perfused constantly with BHI broth. For antibiotic treatment of the adherent cultures, the filters were perfused between t = 0 h and day 3 with BHI broth containing 5 µg/mL of drug.
BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

The activity of finafloxacin and comparators was evaluated against adherent *P. aeruginosa* using Nealton® gynecological disposable plastic catheters ((b) (4)). The disposable plastic catheters were inoculated with a suspension of *P. aeruginosa* DSM12055 in urine (2 x 10^5 CFU/mL) and incubated at 37°C for 1 hour. The pH values for native urine were in the range of pH 6.2 to pH 6.5. The catheters were then connected to a multi-channel peristaltic pump and perfused with 20 mL of urine (10 mL/min). Over a period of 24 hours, the catheters were perfused at regular intervals of 4 hours with 6.6 mL of urine (3.3 mL/min). The catheters remained filled with urine (last portion of the perfusion volume) during the incubation intervals. It has been shown by microscopic examination that this procedure leads to a dense confluent layer of *P. aeruginosa* covering the entire interior surface of the catheters. At the end of the 24 hour pre-incubation period (t = 0 hours), urine containing 1 µg/mL and 5 µg/mL of test substances were used for perfusion of the catheters. The catheters were perfused at t=0h, t= 1h and t = 2h (2 min, 3,3 µL/min each time), t = 4 h and thereafter at regular 4-hours intervals up to t = 48h. At 48 hours, the antibiotic treatment was terminated and antibiotic free urine was used for the further perfusion of the catheters (2 min, 3.3 µL/min each) at regular 4 hour-intervals up the 144th hour. Viable colony counts were determined at t=0, t=4, t= 24, t = 48h and t = 144 h. The results showed that ciprofloxacin was the most effective drug under the test conditions (Figure 7). At a concentration of 1 µg/mL, ciprofloxacin and finafloxacin showed similar initial effects, however, ciprofloxacin had a more pronounced effect at t = 24 hours; whereas moxifloxacin showed only a marginal effect under the same testing conditions. At a concentration of 5µg/mL, ciprofloxacin and

finafloxacin were similar in activity showing a 5 log_{10} reduction in bacterial shedding. At t= 48 h, ciprofloxacin showed a further 2 log_{10} reduction whereas there was a regrowth of organisms after 48h in the presence of 5 µg/mL of finafloxacin and at144h the biofilm had returned to control level in all samples after 4 days of incubation in antibiotic free urine. Overall, the results indicated that none of the tested fluoroquinolones including finafloxacin was capable of killing or inhibiting bacteria in a biofilm.

Figure 7: Effect of finafloxacin and comparators in a catheter colonized with biofilms of *P. aeruginosa* DSM 12055.





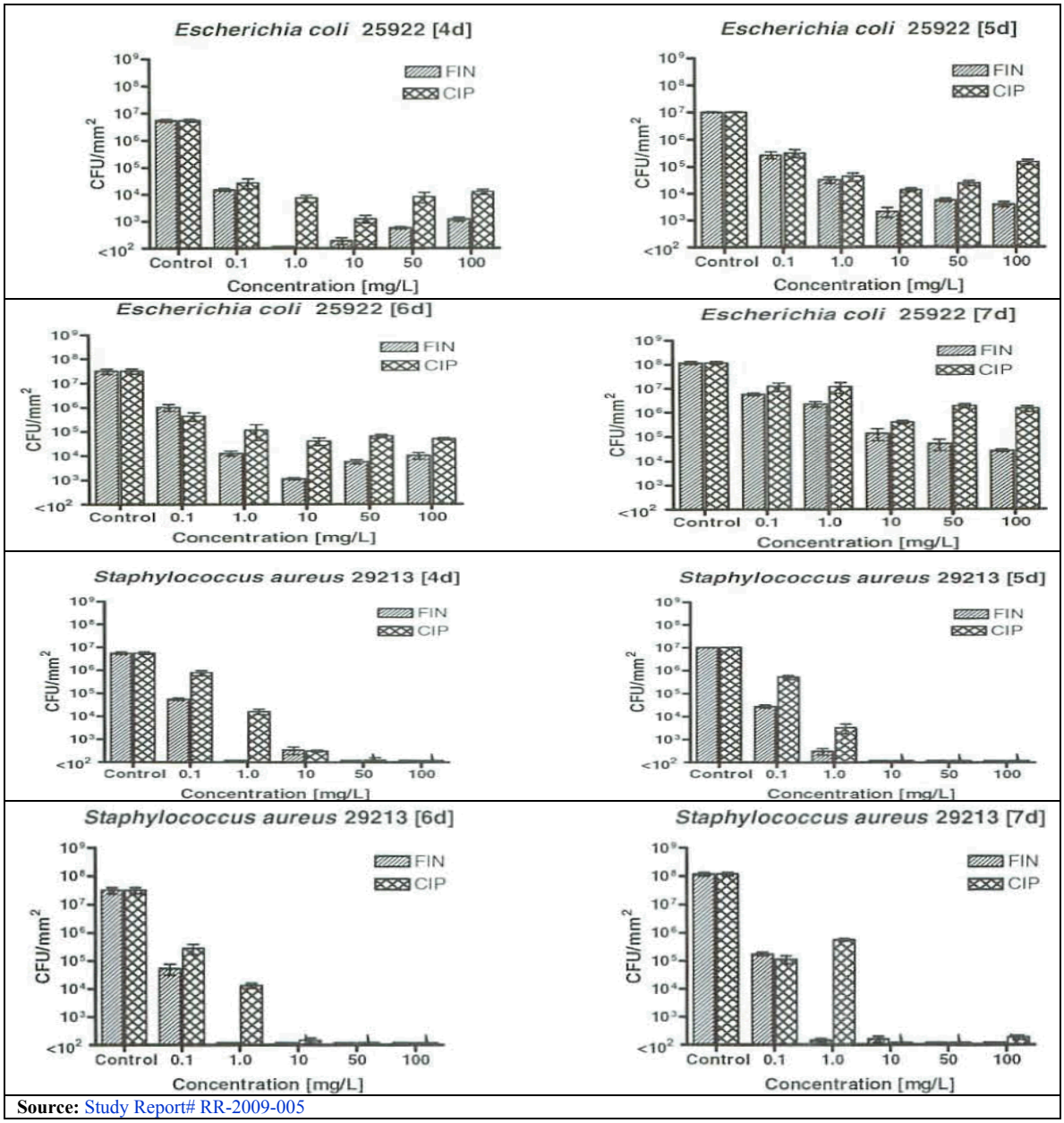
Test substance	MIC [µg/ml]	
	MH broth	Urine
Bay 35-3377	8	0.5
Ciprofloxacin	0.25	0.25
Moxifloxacin	2	4

BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

The activity of finafloxacin and ciprofloxacin was also investigated against *E. coli* and *S. aureus* adherent population to urinary catheter material. Briefly, *E. coli* ATCC 25922 or *S. aureus* ATCC 29213 were grown on segments (1 cm^2) of silicone coated adult 2-way Foley urinary catheters (Procare™, USA) suspended in artificial urine medium (pH 5.8) for 24 hours to 6 days. Catheter adherent cultures of different ages were washed and exposed to varying concentrations of finafloxacin or ciprofloxacin for 24 hours. The surviving catheter-adherent cells were recovered in PBS by sonication and vortexing and plated out for CFUs. The antibacterial effect was quantified in terms of the number of viable cells per cm^2 of catheter that were recovered following exposure. Preliminary experiments had demonstrated that after 24 hour incubation was sufficient to achieve an adherent population of a constant size (10^6 – 10^7CFU/mL). Catheter-adherent populations of *E. coli* and *S. aureus* showed age-dependent susceptibilities to antibiotics. For example, when adherent bacteria were allowed to form for 72 hours (3 days), adherent populations of both species were completely eradicated from the catheter following exposure to 0.1 µg/mL of finafloxacin or ciprofloxacin (data no shown). Older populations showed phenotypic resistance to the drugs and hence were more difficult to treat. However, finafloxacin showed greater bactericidal activity than ciprofloxacin at concentrations of 1 mg/L and above against 4- and 6-day old catheter adherent

populations of *E. coli* and *S. aureus* (Figure 8). On average, finafloxacin reduced the adherent populations to 1 – 2 $\log_{10}$ lower than equivalent ciprofloxacin treated populations.

Figure 8: Eradication of catheter adherent *E. coli* and *S. aureus* populations following exposure to finafloxacin or ciprofloxacin in artificial urine (pH 5.8)

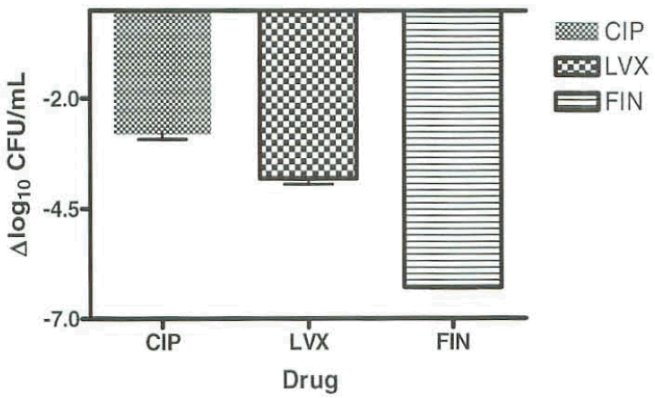


Source: Study Report# RR-2009-005

2.3.4. Stationary Phase Killing

The activity of finafloxacin and comparators was evaluated against stationary phase (non-dividing) cultures of *E. coli* ATCC 25922. The isolate was grown in CAMHB for 24 hours to the point of saturation (~2 x 10^9 CFU/mL). Finafloxacin, ciprofloxacin or levofloxacin at 10 mg/L were added to these stationary-phase broths for a further 24 hours. Viable counts were determined before and after drug exposure. The recovered (persistent) bacteria were expressed as a fraction of the starting cell number. Finafloxacin reduced the viable cell count by greater than 6 $\log_{10}$ reduction compared to ciprofloxacin which reduced the viable cell count by 3 $\log_{10}$ and levofloxacin by 4 $\log_{10}$ units (Figure 9).

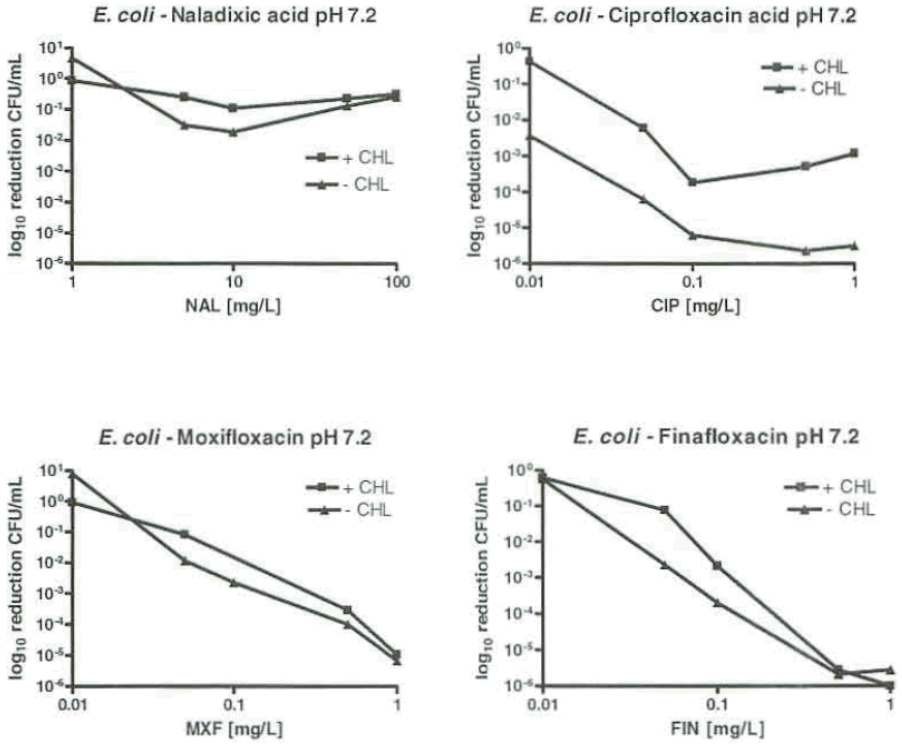
Figure 9: Magnitude of activity of finafloxacin and comparators against stationary phase (non-dividing) cultures of *E. coli* ATCC 25922 strain



Source: Study Report# RR-2009-005

The extent of killing of finafloxacin and comparators ($\Delta\log_{10}$ CFU/mL) was determined against dividing and non-dividing cells over a range of drug concentrations of each drug at pH 7.2. The *E. coli* ATCC 25922 was exposed to chloramphenicol to halt growth through inhibition of protein synthesis (non-dividing cells) as well as in the absence of chloramphenicol (dividing cells). Overall, finafloxacin and moxifloxacin showed similar concentration dependent killing profiles against both dividing and non-dividing cells (Figure 10). In comparison, ciprofloxacin showed a concentration dependent bactericidal activity against dividing cells but was less effective against non-dividing cells in which an apparent killing occurred up to a concentration of 0.1 mg/L and then a plateau effect was observed at which no further killing was observed at greater concentration. Nalidixic acid showed a weak killing action that was concentration dependent up to 10 mg/L where greater concentrations caused a lesser degree of killing; no significant killing action was observed for nalidixic acid against non-dividing cells.

Figure 10: Extent of killing activity of finafloxacin and comparator fluoroquinolones against dividing and non-dividing E. coli ATCC 25922 cells at pH 7.2

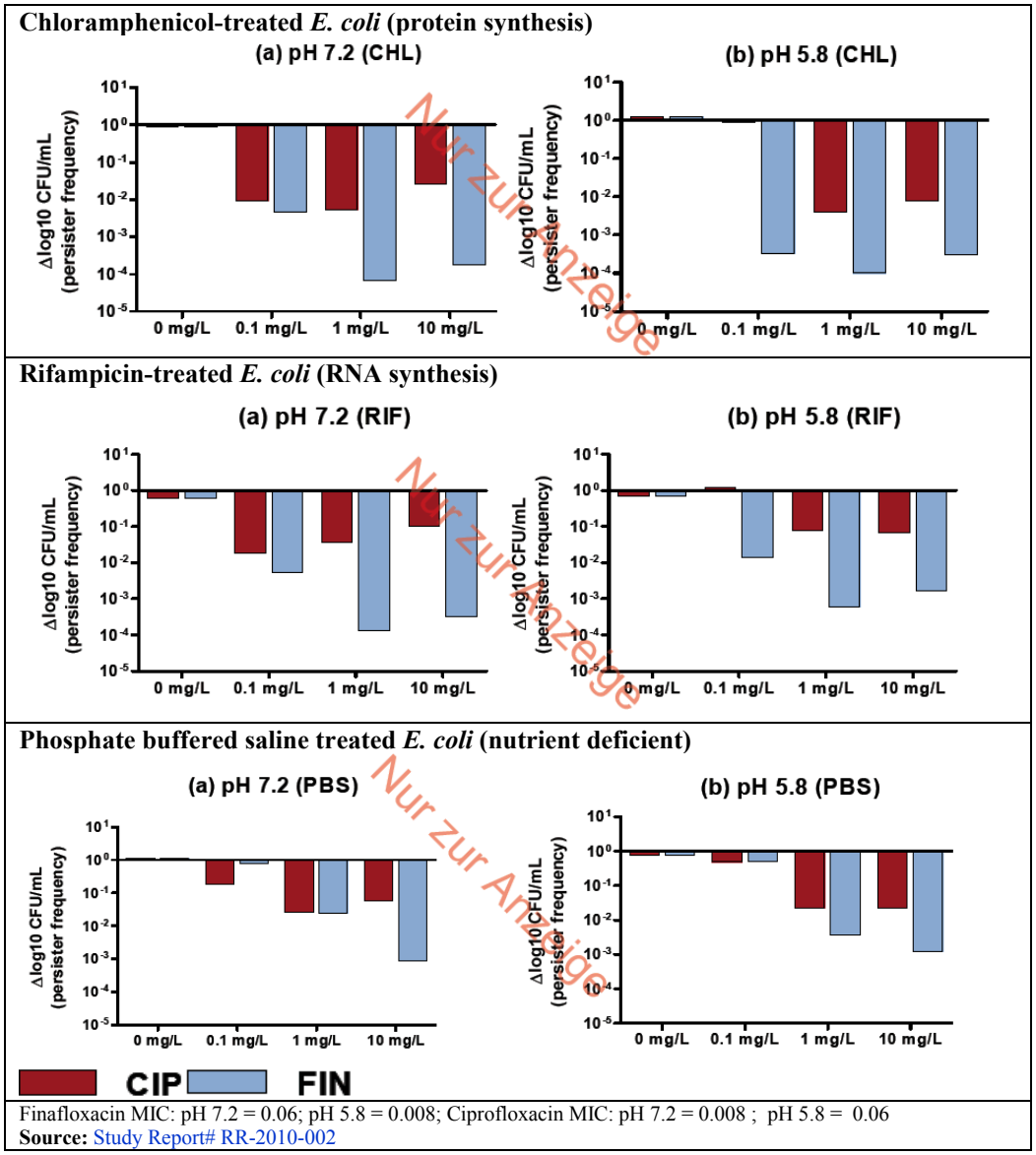


Source: Study Report# RR-2009-005

In another experiment, the activity of finafloxacin and comparators were evaluated against *E. coli* cells in which growth had been arrested by exposure to chloramphenicol, rifampicin or suspended in PBS to halt growth through inhibition of protein synthesis, RNA synthesis or nutrient starvation. Experiments were performed at pH 7.2 and pH 5.8. Briefly, a cell density of 3 x 10^8 CFU/mL was prepared from stationary phase cultures to ensure a significant population of persister cells were exposed to the drug. Overall, finafloxacin showed enhanced activity in terms of MIC, under slightly acidic activity in comparison ciprofloxacin showed reduced activity and vice versa (Figure 11). The most prominent activity was observed at the lowest quinolone concentrations tested (0.1 mg/L) whereas at higher concentrations (1 and 10 mg/L) little distinction could be made between the killing effect of either drug at pH 7.2 and pH 5.8. This is likely due to the fact that at the higher concentrations are equivalent to 20 to >100x MIC of the drugs, thereby the pH changes has minimal effect. The minimal effect of pH on the activity of finafloxacin or ciprofloxacin at concentrations of 1 – 10 mg/L may also demonstrate that the killing effect is saturated, though at distinct levels for the two drugs. The bactericidal activity from three experiments with three forms of growth-arrested *E. coli* demonstrates

that finafloxacin showed greater bactericidal activity to ciprofloxacin. In all three experiments, the persister subpopulation that remained following exposure to finafloxacin was 1 – 3 $\log_{10}$ units smaller than that left after exposure to equivalent concentrations of ciprofloxacin.

Figure 11: Bactericidal activity of finafloxacin and ciprofloxacin against chloramphenicol, rifampicin and PBS treated *E. coli*



Finafloxacin MIC: pH 7.2 = 0.06; pH 5.8 = 0.008; Ciprofloxacin MIC: pH 7.2 = 0.008 ; pH 5.8 = 0.06
Source: Study Report# RR-2010-002

Reviewer's comments:
Time kill studies and minimum bactericidal concentration (MBC) determinations of finafloxacin showed bactericidal activity against E. coli (including quinolone-sensitive and quinolone-resistant), S. aureus and P. aeruginosa strains. Most of the tested organisms were bactericidal at or above 4x the MIC. In vitro time kill studies showed that under acidic conditions, finafloxacin had a bactericidal effect after 1 hour incubation whereas under neutral conditions finafloxacin was comparable to other fluoroquinolones showing a maximal bactericidal (>3log$_{10}$ reduction) within 3 to 5 hours.

Finafloxacin showed variable bactericidal activity against forms of quiescent growth. In E. coli and S. aureus biofilms, finafloxacin showed a rapid decrease on the viability of adherent cells showing no re-growth of sample by day 4; whereas against P. aeruginosa biofilms, finafloxacin was similar in activity to ciprofloxacin by reducing the bacterial growth by 24 hours, however regrowth of organisms were observed by day 4. Finafloxacin showed similar concentration dependent killing profiles as comparator fluoroquinolones against both dividing and non-dividing cells including persisters.

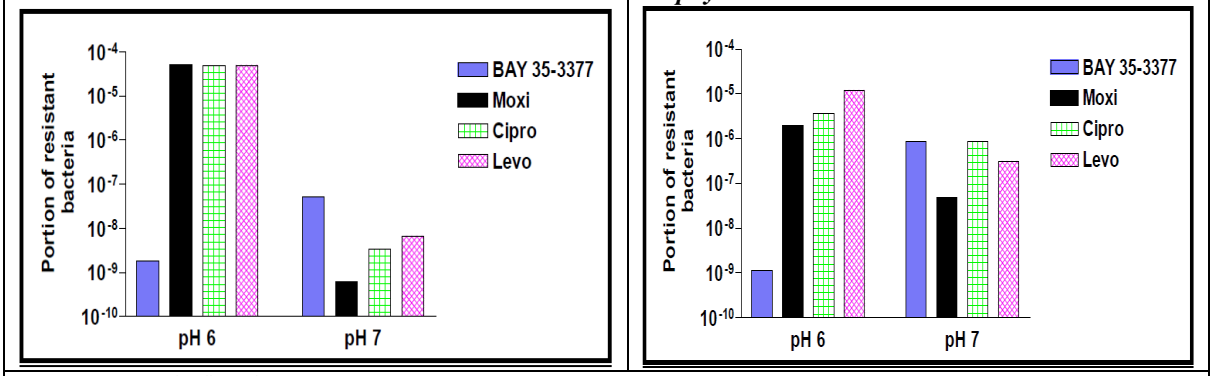
2.4. Intracellular antimicrobial concentration assessment
There were no studies that evaluated the activity of finafloxacin inside the cell against target microorganisms.

2.5. Development of Resistance and Resistance Mechanisms
Several studies were conducted that evaluated the development of resistance *in vitro* and the mechanisms associated with finafloxacin resistance.

2.5.1. Selection of Spontaneous mutants
The frequency of spontaneous mutations *in vitro* was evaluated against gram-positive and gram-negative isolates. Briefly, overnight cultures grown in Brain Heart Infusion or Mueller Hinton broth (~10^{9-11} CFU/mL) were inoculated on agar plates containing 2x, 4x or 8x MIC of antibiotic. The pH of the agar was adjusted with varying concentrations of finafloxacin or comparators at pH 7.2 and pH 5.8. After incubation for 24 to 48 hours, the numbers of colonies growing on the plates were enumerated. The spontaneous mutation frequencies were calculated by dividing the number of bacteria growing on the antibiotic plate by the total number of colonies in the original inoculum. In cultures where no mutants were observed, the mutational frequency was taken as less than the reciprocal of the total number of viable organisms.

- Under neutral conditions (pH 7), the spontaneous resistance frequencies against *E. coli* DSM 10650 and *S. aureus* DSM 11823 strains at 2X MIC were $<10^{-9}$ for the comparator fluoroquinolones whereas finafloxacin spontaneous mutation frequencies were higher at a frequency of 2.4×10^{-8} for *E. coli* DSM 10650 and $> 10^{-6}$ for *S. aureus* DSM 11823 strains (Figure 12). Moxifloxacin showed the lowest resistance frequencies observed.
- Under acidic conditions (pH=6), finafloxacin spontaneous mutation frequencies were reduced by 2 to 3 $\log_{10}$ units in comparison to rates observed at pH 7, whereby the spontaneous mutation frequencies were $<10^{-9}$ against the *S. aureus* DSM 11823 and *E. coli* DSM 10650 strains. In comparison, moxifloxacin, levofloxacin and ciprofloxacin increased slightly for *S. aureus* (1 to 2 $\log_{10}$ units) and greater that 4 to 5$\log_{10}$ unit-change or confluent bacterial growth was observed for the three fluoroquinolones against the *E. coli* DSM 10650 strain.
- The applicant states that much lower resistance rates were obtained when selective drug concentrations of 8X MIC were used (data not shown).

Figure 12: Spontaneous Frequencies as determined at pH 6 and pH 7 using *E. coli* DSM 10650 and *S. aureus* DSM 11823 at 2X MIC



BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

The propensity to select spontaneous resistant mutants were evaluated for finafloxacin and comparator fluoroquinolones against *S. aureus* NRS384 (USA300, CA-MRSA), *S. aureus* ATCC 29213 and *E. coli* ATCC25922. Resistance selection was performed on agar at pH 7.2 and pH 5.8 against the isolates, as previously described. From these data, the mutation prevention concentrations (MPC) were also determined. The mutation prevention concentration (MPC) was defined as the concentration of drug at which no spontaneous mutants could be selected from a population of 10^{10} CFU.

- Spontaneous mutation frequencies for finafloxacin against *S. aureus* strains ranged from 6.7×10^{-11} to $\leq 4.4 \times 10^{-11}$ at 2x – 4x MIC and $\leq 8.2 \times 10^{-11}$ against *E.*

coli ATCC25922 strain at 4x the MIC (Table 12). These results were similar to resistance frequencies for other molecules in the fluoroquinolone class of antibiotics.

- Finafloxacin MPCs varied by strain, however was not higher than 4x the MIC against *S. aureus* isolates and 8x to 16x the MIC against *E. coli* strain. Under acidic conditions, finafloxacin MPC against the *E. coli* ATCC 25922 strain was 4-fold lower than ciprofloxacin and 8-fold lower than levofloxacin. Similarly against *S. aureus* ATCC 29213 and NRS 384 strains, finafloxacin MPC was 2-fold lower than moxifloxacin, 8-fold lower than levofloxacin and 16-fold lower than ciprofloxacin at pH 5.8. Overall, the finafloxacin MPC were lower at the acidic pH; conversely, ciprofloxacin, levofloxacin and moxifloxacin MPCs were lower at neutral pH.

Table 12: Spontaneous resistance frequencies and mutation prevention concentration to finafloxacin and ciprofloxacin against gram-positive and gram-negative strains

***S. aureus* ATCC 29213**

Drug	pH	Resistance frequency at drug concentration [mg/L]						
		0.125	0.25	0.5	1	2	4	8
FIN	7.2	CG	2.7×10^{-8}	**$\leq 6.7 \times 10^{-11}$**				
CIP					CG	1.1×10^{-8}	**$\leq 6.7 \times 10^{-11}$**	
LVX				CG	1.1×10^{-9}	**$\leq 6.7 \times 10^{-11}$**		
MXF		CG	4×10^{-9}	**$\leq 6.7 \times 10^{-11}$**				
FIN	5.8	CG	1.25×10^{-8}	**$\leq 1.3 \times 10^{-10}$**				
CIP						CG	4.9×10^{-8}	**$\leq 1.3 \times 10^{-10}$**
LVX					CG	5.5×10^{-8}	**$\leq 1.3 \times 10^{-10}$**	
MXF			CG	CG	**$\leq 1.3 \times 10^{-10}$**			

***E. coli* ATCC 25922**

Drug	pH	Resistance frequency at drug concentration [mg/L]						
		0.03	0.06	0.125	0.25	0.5	1	2
FIN	7.2			CG	4.2×10^{-9}	4.1×10^{-10}	**$\leq 8.2 \times 10^{-11}$**	
CIP		CG	2.2×10^{-9}	**$\leq 8.2 \times 10^{-11}$**				
LVX			CG	1.3×10^{-9}	**$\leq 8.2 \times 10^{-11}$**			
FIN	5.8	CG	1.1×10^{-9}	1.2×10^{-10}	**$\leq 1.2 \times 10^{-10}$**			
CIP					CG	3.2×10^{-9}	**$\leq 1.2 \times 10^{-10}$**	
LVX					CG	4.8×10^{-9}	4.9×10^{-10}	**$\leq 1.2 \times 10^{-10}$**

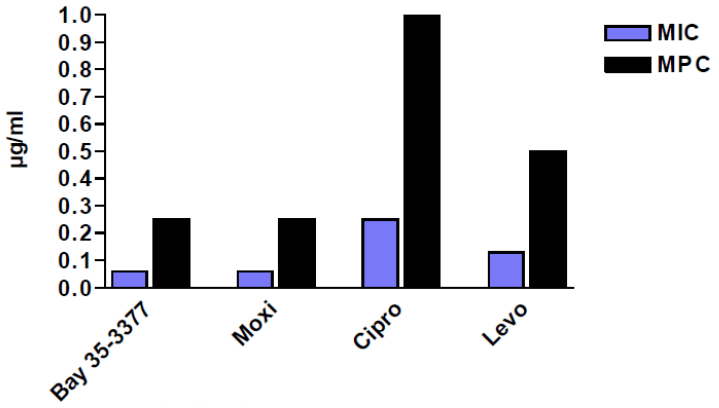
***S. aureus* NRS-384**

Drug conc. [mg/L]	Resistance frequency					
	pH 7.2			pH 5.8		
	MXF	CIP	FIN	MXF	CIP	FIN
0.125	CG		2.4×10^{-9}			2.7×10^{-9}
0.25	8.04×10^{-9}		5.2×10^{-10}	CG		4.3×10^{-10}
0.5	**$\leq 4.4 \times 10^{-11}$**		**$\leq 4.4 \times 10^{-11}$**	2.8×10^{-9}		**$\leq 4.4 \times 10^{-11}$**
1				3.5×10^{-10}		
2		CG		**$\leq 4.4 \times 10^{-11}$**		
4		3.5×10^{-10}				
8		5.6×10^{-10}			CG	
16		4.3×10^{-10}			6.0×10^{-10}	
32		**$\leq 3.7 \times 10^{-11}$**			4.5×10^{-10}	
64					**$\leq 3.7 \times 10^{-11}$**	

Abbreviations: CG: Confluent growth; CIP, ciprofloxacin; FIN, Finafloxacin, LVX, levofloxacin; MXF, moxifloxacin
Mutation prevention concentration (MPC) is shown in **bold**
Source: Study Report# RR-2009-006, RR-2009 -007

Against *S. aureus* DSM 11823, finafloxacin MPC were similar to comparator and not higher than 4X the respective MICs (Figure 13). With regard to the absolute MICs, finafloxacin and moxifloxacin were 2-fold lower than levofloxacin and 4-fold lower than ciprofloxacin.

Figure 13: Mutation prevention concentration (MPC) and minimum inhibitory concentration (MIC) of finafloxacin and comparators against *S. aureus* DSM 11823

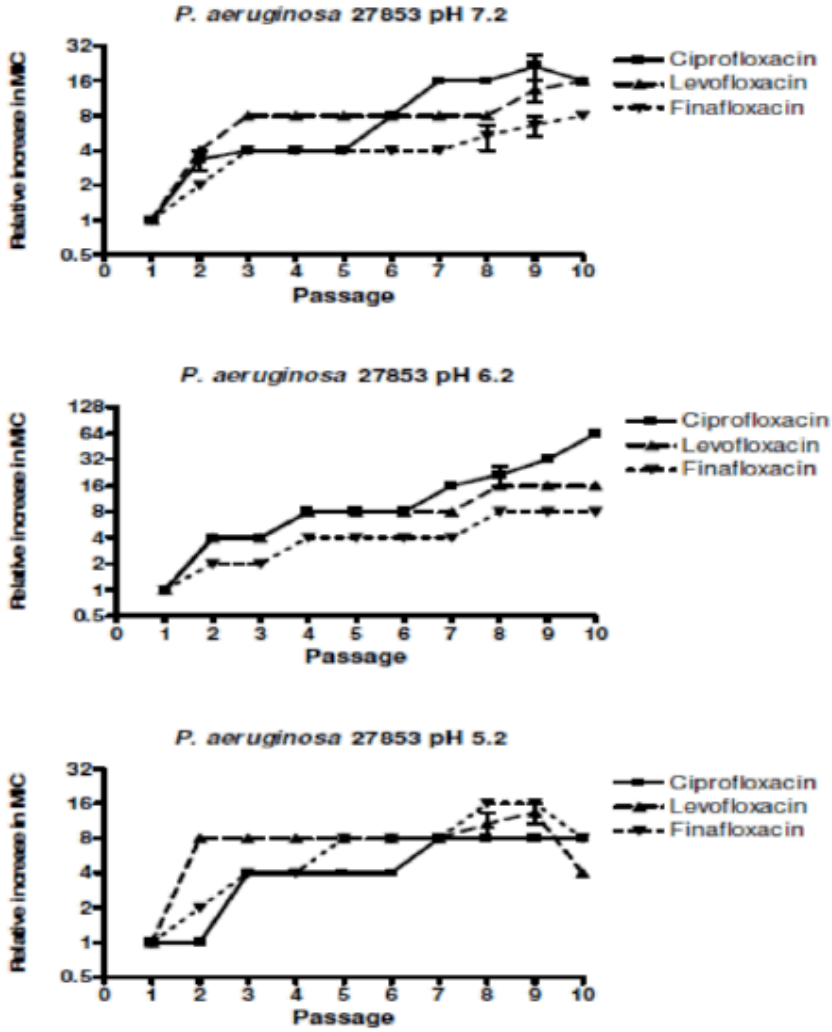


BAY 35-3377 = Finafloxacin
Source: (b) (4) Study Report# PH32656

2.5.2. Multiple Passage Resistance Studies

The development of resistance to finafloxacin and comparators was evaluated against *P. aeruginosa* ATCC 27853 strain using multiple passage resistance studies. The parent strain was serially passaged 10 times and the MIC was tested and determined between each passage. The MIC was defined as the lowest drug concentration to inhibit bacterial growth and was expressed as a multiple of the MIC determined prior to passaging. The results showed that susceptibility to finafloxacin was reduced at a similar rate and by similar magnitude during the 10 passages as compared to ciprofloxacin and levofloxacin (Figure 14). The observed reduction in susceptibility was not affected by pH.

Figure 14: Serial passage of *P. aeruginosa* ATCC 27853 with sub-inhibitory concentrations of finafloxacin, ciprofloxacin and levofloxacin at pH 7.2, 6.2 and 5.2



Source: Pharmacology Written Summary Figure 2.6.2.2-2

2.5.3. Alteration of Target Sites

The general mechanisms of acquired resistance to fluoroquinolones have been associated to mutations in the quinolone-resistance determining region (QRDR) of the target enzymes, DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *ParE*). Mutations in one or more of the target enzymes were evaluated against the laboratory-induced finafloxacin or other fluoroquinolones resistant mutants selected from *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. DNA sequencing of QRDRs was performed

using standard techniques. Single step mutants selected from finafloxacin, moxifloxacin, ciprofloxacin and levofloxacin are shown in Table 13:

- Single step mutants of *E. coli* ATCC 25922 strain showed a 8 – 32 fold increase in MIC to both the selective and comparator fluoroquinolones. Mutants to each drug harbored single point mutations conferring one of the following amino acid substitutions within the QRDR of *gyrA*: G81D, S83L or D87N. These substitutions most often occurred within the QRDR of *gyrA* at nucleotide positions: 242, 248 or 259, respectively. Similar to ciprofloxacin and levofloxacin, no mutations were detected within QRDR of *parC* suggesting that finafloxacin shares a common target.
- Single step mutants of *S. aureus* ATCC29213 or NRS384 strains to finafloxacin showed either S80F or E84K substitutions within *grlA*; in comparison moxifloxacin or ciprofloxacin mutants resulted in only S80F substitution within *grlA*. Overall, these mutations conferred an 8 to 16 fold reduction in susceptibility than those exhibited by the parent.

Table 13: Characteristics of first step mutants of E. coli ATCC 25922 and S. aureus ATCC 29213 selected by finafloxacin and comparator fluoroquinolones

Selective Drug	Selective pH	Substitution		Minimum inhibitory concentration (µg/mL)							
				pH 5.8				pH 7.2			
		gyrA	grlA/ parC	FIN	CIP	LVX	MXF	FIN	CIP	LVX	MXF
***E. coli* ATCC 25922 and single step mutants**											
None	WT	--	--	0.008	0.125	0.25	ND	0.03	0.008	0.015	ND
Finafloxacin	7.2	G81D	--	0.125	1	2	ND	0.5 – 1	0.125	0.25	ND
	7.2	D87N	--	0.125	2	2	ND	0.25	0.125	0.125	ND
	5.8	G81D	--	0.125	1	2	ND	0.5	0.125	0.25	ND
	5.8	S83L	--	0.125	2	4	ND	0.5	0.25	0.25	ND
	5.8	D87N	--	0.125	1 – 2	2	ND	0.25	0.125	0.25	ND
Ciprofloxacin	7.2	G81D	--	0.125	1	2	ND	0.5	0.125	0.25	ND
	7.2	S83L	--	0.125	2	2	ND	0.5	0.25	0.5	ND
	5.8	S83L	--	0.125	2	4	ND	0.5	0.25	0.25	ND
	5.8	D87N	--	0.06	2	4	ND	0.5	0.125	0.25	ND
Levofloxacin	7.2	G81D	--	0.125	1	2	ND	0.5	0.125	0.25	ND
	7.2	S83L	--	0.125	2	2	ND	0.5	0.25	0.25	ND
	7.2	D87N	--	0.06	1	1	ND	0.25	0.06	0.125	ND
	5.8	G81D	--	0.125 – 0.25	1 – 2	2 – 4	ND	0.5 – 1	0.125	0.25 – 0.6	ND
	5.8	S83L	--	0.125	2	4	ND	0.5	0.25	0.5	ND
***S. aureus* ATCC 29213 and single step mutants**											
None	WT	--	--	0.03	0.5	0.25	0.125	0.25	0.5	0.5	0.06
Finafloxacin	7.2	--	E84K	0.125	4	2	0.5	0.5	2	1	0.25
	5.8	--	S80F	0.125	4	2	0.5	0.5	2	1	0.25
	5.8	--	E84K	0.125	4	2	0.5	0.5	2	1	0.25
Ciprofloxacin	5.8	--	S80F	0.125	4	2	0.5	0.5	2	1	0.25
Levofloxacin	5.8	--	S80F	0.125	4	2	0.5	0.5	2	1	0.25
Moxifloxacin	7.2	--	S80F	0.125	4	2	0.5	0.5	2	1	0.25
	5.8	--	S80F	0.125	4	2	0.5	0.5	2	1	0.25
***S. aureus* NRS 384 and single step mutants**											
None	WT	--	--	0.125	4	ND	0.5	0.5	2	ND	0.125
Finafloxacin	7.2	--	E84K	1	32	ND	8	4	16	ND	2
	7.2	--	E84K	1	32	ND	8	4	16	ND	2
	5.8	--	S80F	2	32	ND	16	4	32	ND	4
	5.8	--	S80F	2	32	ND	16	4	32	ND	4
Moxifloxacin	7.2	--	S80F	2	32	ND	8	4	32	ND	2
	7.2	--	S80F	2	32	ND	8	4	32	ND	1
	5.8	--	S80F	2	32	ND	8	4	32	ND	2
	5.8	--	S80F	2	32	ND	8	4	32	ND	2
Ciprofloxacin	7.2	--	S80F	2	32	ND	16	4	32	ND	4
	7.2	E88K	S80F	1	32	ND	8	4	32	ND	2
	5.8	S84L	S80F	2	32	ND	16	4	32	ND	4
	5.8	E88K	S80F	1	32	ND	8	4	16	ND	2

Abbreviations: CIP, ciprofloxacin; FIN, Finafloxacin, LVX, levofloxacin; MXF, moxifloxacin; WT, Wild Type; ND, Not Determined

Source: Study Report# RR-2009-006; RR-2009-007

Dual target mutants (i.e., a combination of single mutations in *gyrA* and *parC*) of *E. coli* and *Salmonella enterica* showed a 16 – 64 increase in MIC to finafloxacin and comparator fluoroquinolones (Table 14). A third mutation resulted in a further 4 – 16 fold increase in MIC. All mutants showed relative increases in MIC to finafloxacin and comparator fluoroquinolones when compared under the prevailing pH conditions.

Table 14: Activity of finafloxacin and comparator quinolones against genetically defined mutants in the quinolone resistance defining region (QRDR) of *E. coli* and *S. enterica* serovar *typhimurium*

Strain	Substitution		Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
	gyrA	parC	pH6.2	pH 7.2	pH6.2	pH 7.2	pH6.2	pH 7.2	pH6.2	pH 7.2
***E. coli* 4/99 (WT)**	--	--	**0.008**	**0.06**	**0.125**	**0.06**	**0.06**	**0.0156**	**0.06**	**0.03**
E. coli 155/00	S83A	--	0.06	0.25	1	0.25	0.5	0.125	1	0.25
E. coli 84/00	S83L	--	0.25	0.5	2	0.5	2	0.5	2	0.5
E. coli 44/99	S83L, D87N	--	0.25	1	2	0.5	2	0.5	2	0.5
E. coli 172/00	--	--	0.125	0.5	2	0.5	2	0.5	2	0.5
E. coli 163/00	S83L	S80I	4	16	32	8	32	4	16	4
E. coli 22/99	S83L, D87N	S80R	4	16	32	8	32	4	16	4
E. coli 82/00	S83L D87N	S80I	8	16	32	16	>32	8	32	8
E. coli 53/99	S83L D87Y	S80I	4	16	32	8	>32	8	16	8
***S. enterica* 04329/99 (WT)**	--	--	**0.25**	**0.5**	**2**	**1**	**1**	**0.25**	**1**	**0.5**
S. enterica 00680/99	S87Y	--	0.06	0.5	1	0.25	1	0.125	0.5	0.25
S. enterica 02116/99	S83Y N87D	--	0.125	1	1	0.5	2	0.5	2	0.5
S. enterica 000277/99	S83F, N87D	--	0.5	2	8	2	8	1	4	2
S. enterica 01592/01	S83F, N87D	--	1	2	4	2	8	1	4	2
S. enterica 00281/99	S83F, N87D	--	1	4	8	4	8	2	8	2
S. enterica 00608/93	ND	ND	4	16	>32	16	>32	32	32	16
S. enterica 00154/94	ND	ND	4	16	>32	16	>32	32	32	16

ND = not determined
Source: Study Report# PH32655

Similarly, double and triple fluoroquinolone mutants of *S. aureus* mutants showed a 16-64 increase in MIC to finafloxacin and moxifloxacin; however, these changes were less affected than the MICs of ciprofloxacin and levofloxacin for the various target mutations tested (Table 15). In the presence of reserpine, a known inhibitor of gram-positive efflux pumps, had little effect on the activity of finafloxacin, suggesting the finafloxacin is not a substrate of gram-positive efflux pumps.

Table 15: Activity of finafloxacin and comparator quinolones against genetically defined mutants in the quinolone resistance defining region (QRDR) of *S. aureus*

Strain	Substitution		Finafloxacin			Ciprofloxacin	Levofloxacin	Moxifloxacin
	gyrA	grlA	pH 6	pH 7.2	Reserpine			
WT	--	--	**≤0.03**	**0.06**	**0.06**	**0.25**	**0.125**	**0.06**
	--	E84K	2	2	4	32	16	2
	S84A	S80F	0.5	1	1	8	4	0.5
	S84L	S80Y	1	4	4	32	16	2
	S84L	S80Y, E84K	4	4	4	64	32	8
	S84L	E84K	4	8	8	128	64	4
	S84L	S80Y, E84K	16	32	32	512	128	32
WT2	--	--	**≤0.03**	**0.06**	**0.06**	**0.25**	**0.125**	**0.06**
	S84L	S80Y, E84K	2	2	1	256	32	2
	S84L	E84K	8	16	16	128	64	32
	S84L	S80Y	4	8	8	32	16	4
	S84L	S80Y	32	32	32	512	128	32
	--	S80F	1	2	2	16	8	2
MRSA	--	--	**≤0.03**	**0.06**	**0.06**	**0.25**	**0.25**	**0.06**
	--	--	1	2	2	64	16	2
	--	S80F	0.125	0.25	0.25	0.5	0.25	0.06
	--	--	0.125	0.25	0.25	1	0.5	1
	--	S80F	0.25	1	1	16	8	0.25
	--	--	0.125	0.25	0.25	1	0.5	0.06
	--	--	0.125	0.25	0.125	16	4	1
BB 27	--	--	**≤0.03**	**0.06**	**0.06**	**0.5**	**0.25**	**0.06**
	--	E84K	0.25	0.5	0.5	8	4	0.125
	S84L	S80F	1	1	1	32	16	0.5
	S84L	S80Y	0.5	1	1	4	8	1
	S84L	S80Y, E84K	1	1	1	16	8	1
	S84L	S80F	2	8	8	16	16	2
	S84L	S80F	2	4	2	128	32	4
BB 25	--	--	**≤0.03**	**0.06**	**0.06**	**0.5**	**0.25**	**0.06**
	S84L	E84K	4	4	2	64	32	2
	S84L	E84K	0.5	2	2	8	8	1
	S84L, E88K	S80F	1	1	1	32	16	1
	S84L	S80F	0.5	2	2	8	4	0.5
	E88K	E84K	1	2	2	32	16	1

Source: Study Report# PH32655

Single step mutants of *S. pneumoniae* strains showed either S81F or S81Y mutations within *gyrA*, resulting in 4 to 8 fold reduction in susceptibility than those exhibited by the parent and a second or third mutation resulted in further 4 to 16 fold increase in MIC (Table 16). Overall, the MICs of finafloxacin against the mutants were similar to moxifloxacin and generally lower than ciprofloxacin and levofloxacin. There was little or no effect on the activity of finafloxacin in the presence of reserpine against *S. pneumoniae.*

Table 16: Activity of finafloxacin and comparator quinolones against genetically defined mutants in the quinolone resistance defining region (QRDR) of *S. pneumoniae*

Strain	Substitution		Finafloxacin			Ciprofloxacin	Levofloxacin	Moxifloxacin
	gyrA	GrlA/parC	pH 6	pH 7.2	Reserpine			
WT3	--	--	0.06	0.125	0.125	0.5	0.25	0.06
	S81F	S79Y	1	2	2	64	32	2
	S81F	S79Y	2	4	4	64	32	16
	S81F	S79Y	2	4	4	64	32	4
71B	--	--	0.03	0.06	0.06	1	1	0.03
	--	--	1	2	0.5	8	4	0.25
	S81F	--	0.5	0.5	0.5	4	4	0.5
	--	--	0.5	0.5	1	8	4	0.5
	S81F	--	1	1	1	16	16	1
	S81Y	--	1	1	1	16	8	0.5
	--	D78N	2	2	4	32	32	2
3C172	--	--	0.125	0.125	0.125	4	2	0.125
	S81F	S79Y, N83G	2	2	2	64	16	2
	S81F	--	2	4	4	0.25	32	4
4B2	--	--	≤0.03	0.125	0.125	1	0.5	0.125
	S81F	K137N, S79Y, D83G	2	2	2	64	32	2
	S81F	K137N	0.25	0.25	0.25	8	8	1
	S81F	K137N	0.5	0.5	0.5	8	4	1
4A245	--	--	0.125	0.125	0.06	1	0.5	0.125
	--	--	0.25	0.25	0.25	4	4	0.25
	S81F	S79Y	4	4	4	64	32	4
	S81Y	S79F	4	4	4	128	64	4
	S81F		1	2	2	32	8	2
	S81Y	S79F	4	8	8	64	32	4
85B	--	--	0.06	0.06	0.06	0.25	0.5	0.03
	S81F	S79Y	8	8	4	64	32	2

Source: Study Report# PH32655

Against *S. pyogenes* isolates, mutations in *gyrA* and *grlA/parC* also conferred 8- 16 fold reduction in MICs to finafloxacin and by a similar magnitude to moxifloxacin, ciprofloxacin and levofloxacin in (Table 17). Overall, the MICs of finafloxacin against the isolates were similar to moxifloxacin and generally lower than that of ciprofloxacin and levofloxacin.

Table 17: Activity of finafloxacin and comparator quinolones against genetically defined mutants in the quinolone resistance defining region (QRDR) of *S. pyogenes*

Strain	Substitution		Finafloxacin			Ciprofloxacin	Levofloxacin	Moxifloxacin
	gyrA	GrlA/parC	pH 6	pH 7.2	Reserpine			
8D134	--	--	**0.06**	**0.12**	**0.12**	**1**	**0.5**	**0.125**
	--	--	1	1	1	64	32	0.5
	S81Y	S74A	0.5	0.5	1	4	2	0.5
	G85K	--	0.25	0.5	0.5	8	4	1
	S81F	--	1	2	2	32	8	1
	--	D78E, E149K	0.06	0.12	0.12	2	1	0.5
	--	--	0.25	0.25	0.25	4	2	0.5
30A21	--	--	**0.06**	**0.06**	**0.06**	**1**	**0.5**	**0.06**
	E85K	S79Y	2	4	4	32	16	2
	S82F	D78N	8	8	8	64	32	8
	S81F	S79Y	2	2	2	64	32	4
	E85K	S79V	16	32	16	128	64	16
	--	--	2	4	2	32	16	2
	--	--	1	1	1	8	8	2
30A118	--	**S79A**	**0.125**	**0.25**	**0.125**	**2**	**0.5**	**0.25**
	--	S79A	2	2	2	8	8	2
	--	S79F	2	2	2	32	16	2
	S81F	S79A	2	2	2	32	16	2
	E85K	S79V	16	16	32	64	32	16
	S81F	N83Y	16	16	32	128	64	16
	--	S79A	0.5	2	2	32	16	2
5A111	--	--	**0.125**	**0.125**	**0.125**	**0.5**	**0.5**	**0.125**
	S81F	S79Y	4	4	4	64	32	4
	S81Y	S79A, D83Y	2	2	2	64	32	4
	--	--	1	1	1	8	4	1
	--	--	0.5	0.5	0.5	4	2	1
	S81F	--	0.25	0.5	0.5	2	2	0.5
	--	S79Y	1	0.5	0.5	16	4	8
6A47	--	--	**≤0.03**	**0.125**	**0.125**	**0.5**	**0.25**	**0.125**
	--	S79F	0.25	1	1	4	2	1
	--	S79F	0.5	2	2	4	2	1
	S81F	--	0.25	1	0.5	4	4	1
	E85K	D84N	1	4	4	64	8	2
	S81Y	G58D, S79A	0.25	0.5	0.5	4	2	0.25
	--	S80F	1	1	1	4	4	1
7A2	--	--	**0.5**	**0.25**	**0.25**	**2**	**1**	**0.25**
	--	--	1	1	1	8	8	0.5
	--	S80F	2	2	2	8	8	1
	--	D78N	1	2	2	16	8	1
	--	D84N	1	2	2	8	4	2
	--	S79V	0.5	1	1	8	64	1
	E85K	S79F	8	16	8	16	16	8

Source: Study Report# PH32655

The activity of finafloxacin and ciprofloxacin was determined against 68 ciprofloxacin-sensitive and –resistant *A. baumannii* clinical isolates with characterized *gyrA* and *parC* genes under standard testing conditions and acidic pH. The results are shown in Table 18 and summarized as follows:

- At normal pH (pH 7.2), finafloxacin showed similar activity to ciprofloxacin against 18 wild type isolates (with no mutations in the *gyrA* and *parC* genes) with both having MIC_{50} and MIC_{90} values of 0.25µg/mL and 1 µg/mL, respectively. At lower pH, finafloxacin MIC_{50} and MIC_{90} values were 0.12 µg/mL compared to ciprofloxacin which was 2 µg/mL and 4 µg/mL, respectively.
- Single step mutants of *A. baumannii* isolates resulted in a 2 – 64 fold reduction in finafloxacin MIC showing mutations within *gyrA* at positions Ser83Leu or Glu87Gly. The finafloxacin MICs were similar or within one dilution of ciprofloxacin MIC under normal testing conditions. However, at the lower pH, finafloxacin MIC_{90} values were lowered from16 µg/mL to 2 µg/mL whereas ciprofloxacin MIC_{90} values increased from 16 µg/mL to > 128 µg/mL, respectively.
- Finafloxacin were less affected against *A. baumannii* isolates with double mutations within the QRDR of the *gyrA*/*parC* genes. Under normal testing conditions, finafloxacin MIC_{50} and MIC_{90} values were 32 µg/mL and 64 µg/mL, respectively; compared to ciprofloxacin in which the MIC_{50} and MIC_{90} were 128 µg/mL and >128 µg/mL, respectively. Under acidic testing conditions (pH 5.8), finafloxacin MICs reduced by 16-fold with MIC_{50} and MIC_{90} values of 4 µg/mL and 8 µg/mL, respectively. In contrast, all isolates showed ciprofloxacin MICs of >128 µg/mL.
- In the two isolates which showed to overexpress the *AdeB* efflux pump also showed a 16-fold reduction in finafloxacin MICs under acidic conditions.

Table 18: Activity of finafloxacin and ciprofloxacin against characterized *Acinetobacter baumannii* isolates under normal pH (7.2) and acidic (pH5.8) testing conditions

Strain no.	Amino acid substitutions		MIC (µg/ml)			
	GyrA	ParC	CIP normal	FIN normal	CIP acidic	FIN acidic
1			0.06	0.12	2	0.06
2			0.06	0.5	2	0.12
3			0.12	0.12	2	0.06
4			0.12	0.25	1	0.03
5–7			0.12	0.25	2	0.06
8–10			0.25	0.25	2	0.12
11			0.25	0.25	4	0.06
12			0.5	1	2	0.12
13			0.5	1	4	0.12
14			0.5	1	4	0.25
15–17			1	1	4	0.12
18			1	1	8	0.12
19	Ser83-Leu		1	0.5	4	0.12
20	Ser83-Leu		2	1	4	0.06
21	Ser83-Leu		4	1	32	0.25
22	Ser83-Leu		4	8	32	1
23	Glu87-Gly		4	8	32	1
24–26	Ser83-Leu		4	16	32	2
27, 28	Ser83-Leu		4	16	32	1
29	Ser83-Leu		4	32	32	4
30	Ser83-Leu		8	8	32	0.5
31	Ser83-Leu		8	16	32	1
32	Ser83-Leu		16	16	32	1
33	Ser83-Leu		8	16	128	1
34, 35	Ser83-Leu		8	16	128	2
36, 37	Ser83-Leu		16	16	128	2
38	Ser83-Leu		32	16	128	2
39–42	Ser83-Leu		16	16	>128	2
43	Ser83-Leu		32	16	>128	2
44	Ser83-Leu	Glu84-Lys	32	16	>128	4
45, 46	Ser83-Leu	Ser80-Leu	64	16	>128	2
47	Ser83-Leu	Ser80-Phe	64	32	>128	4
48, 49	Ser83-Leu	Ser80-Leu	128	16	>128	2
50	Ser83-Leu	Ser80-Leu	128	16	>128	4
51–53	Ser83-Leu	Ser80-Leu	128	32	>128	2
54–56	Ser83-Leu	Ser80-Leu	128	32	>128	4
57	Ser83-Leu	Ser80-Phe	128	32	>128	4
58	Ser83-Leu	Ser80-Leu	128	64	>128	4
59	Ser83-Leu	Glu84-Lys	128	64	>128	8
60–62	Ser83-Leu	Ser80-Leu	128	64	>128	8
63	Ser83-Leu	Glu84-Lys	>128	64	>128	2
64	Ser83-Leu	Ser80-Leu	>128	64	>128	4
65	Ser83-Leu	Ser80-Leu	>128	64	>128	8
66	Ser83-Leu		32	16	128	2
67,[a] 68[a]	Ser83-Leu	Ser80-Leu	>128	64	>128	4

[a] *adeB* overexpressing; isolates 67 and 68 are clinical isolates that are otherwise identical to isolate 66 (based on pulsed-field gel electrophoresis pattern).

Source: Higgins *et al., Antimicrob Agents Chemother* 2010; 54(4) 1613 - 1615

2.5.4. Efflux Pumps

The effect of efflux pumps on the activity of finafloxacin and comparator quinolones were evaluated against *S. aureus* parent strains (wild type strain *S. aureus* 8325 v. *S. aureus* 11758) as well as mutations within the QRDR. Disruption by the *norA* gene in *S. aureus* resulted in a two-fold increase in finafloxacin MICs and four-fold increase in ciprofloxacin MIC, while the sensitivity to moxifloxacin was not affected (Table 19). The overexpression of the *norA S. aureus* MT 23142 mutant showed no change in the finafloxacin MIC, whereas moxifloxacin and levofloxacin showed reduced activity (two-fold increase in MIC) as well as ciprofloxacin (four-fold increase in MIC). Overall, the results suggest that finafloxacin is a poor substrate of the common *S. aureus* efflux pumps.

Table 19: Activity of finafloxacin and comparator quinolones against various efflux mutants in *S. aureus*

Strain	Efflux	Plasmid	Finafloxacin	Moxifloxacin	Ciprofloxacin	Levofloxacin
S. aureus 8235 (WT)	--	--	0.25	0.12	1	ND
S. aureus 1758 (ermC cassette)	norA disruption	--	0.12	0.12	0.25	ND
S. aureus ISP 794 (WT)	--	--	0.06	0.015	0.06	0.06
S. aureus MT 23142	norA overexpression	--	0.06	0.03	0.25	0.125

Source: Study Report# PH32655

The effect of known chromosomal mutations and multiple drug efflux (MDE) pumps alone as well as in combination was evaluated on the *in vitro* activity of finafloxacin and comparators against *P. aeruginosa* strains. Mutants were constructed containing MDE resistance markers conferring hyper-expression and overexpression of *mexCD* (the most prevalent efflux pump in *Pseudomonas* spp.) and overexpression of *mexAB* efflux pumps. Overexpression of *MexAB* and *MexCD* efflux pumps increased the finafloxacin MICs by 16 – 128 fold and to a similar degree to other fluoroquinolones, suggesting that efflux pumps played a role in the reduced susceptibility of the *P. aeruginosa* MDE mutants (Table 20).

Table 20: MICs of isogenic strains harboring MDR mutations in the presence and absence of efflux pumps in *P. aeruginosa* strains.

Strain	Efflux	Plasmid	Finafloxacin	Moxifloxacin	Ciprofloxacin	Levofloxacin
P. aeruginosa PAO 200	ΔmexAB	--	≤0.125	≤0.125	≤0.125	≤0.125
P. aeruginosa PAO 200-2	ΔmexAB	mexCD overexpression	8	64	2	4
P. aeruginosa PAO 200-3	ΔmexAB	mexCD hyperexpression	16	64	4	8
P. aeruginosa K 1121	ΔmexAB	--	0.5	2	≤0.125	1
P. aeruginosa K 1121	--	mexAB overexpression	4	8	1	2

Source: Study Report# PH32655

Table 21: Activity of finafloxacin and comparator fluoroquinolones against fluoroquinolone resistant *E. coli* at pH 7.2 and pH 5.8

E. coli Strain	Substitution			Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
	gyrA	parC	marR	pH5.8	pH 7.2	pH5.8	pH 7.2	pH5.8	pH 7.2	pH5.8	pH 7.2
WT	--	--	--	**0.015**	**0.125**	**0.5**	**0.06**	**0.25**	**0.015**	**0.25**	**0.06**
WT-4	--	S80I	--	0.03	0.25	1	0.06	0.25	≤0.008	0.25	0.03
WT-4M101	D87N	S80I	--	0.25	2	16	1	4	0.125	4	0.25
WT-4M37	G81C	S80I	--	0.25	1	4	0.5	2	0.25	2	0.25
WT-4M35	D87G	S80I	--	0.25	1	8	0.5	2	0.125	2	0.125
WT-3.2	D87G	--	--	0.5	4	8	2	8	0.5	8	1
WT-3-1	D87G, S83L	--	--	0.5	2	8	1	4	0.25	8	0.5
WT-3-M21	D87G, S83L	E84K	--	16	128	>256	32	>256	128	256	32
MI	S83L	--	--	0.5	2	8	1	4	0.5	4	1
MII	S83L	--	+	1	4	64	2	32	0.5	32	1
MII-3.2	D87G	--	+	1	4	32	2	8	0.5	8	1
MII-4T2	S83L	S80I	+	2	16	128	8	>256	4	64	8
MIII	D87G, S83L	S80I	+	32	256	>256	128	>256	>256	>256	128

Source: (b) (4) Study Report# RR-2009-004

2.5.5. Other Resistance Mechanisms

Recently, three novel transferable plasmid-mediated quinolone resistance (PMQR) mechanisms have been described[24 - 26]. These include MDR efflux pumps (*qepA1* and *qepA2*), five different classes of topoisomerase protection proteins (*qnrA, qnrB, qnrC, qnrD* and *qnrS*) and a new variant of the aminoglycoside acetyltransferase AAC(6')-1b capable of modifying C7-piperazinyl-fluoroquinolones like ciprofloxacin and norfloxacin (*aac(6')-lb-cr*). The effect of known chromosomal mutations (*gyrA, parC* and *marR*) and PMQR mechanisms alone as well as in combination was evaluated on the *in vitro* activity of finafloxacin at different pH values. The strains were all derived from *E. coli* wild type isolated and the PMQR genes were placed under the control of the p*lac* promoter of plasmid vectors pUC18 or pUC19 except *qepA* which was cloned into pSTV28. The MICs for the fluoroquinolones were determined at pH 5.8 and 7.2 for all strains. The results are shown in Table 22.

- Finafloxacin MICs were 2 to 8 fold lower at pH 5.8 compared with pH 7.2 for all strains tested. In contrast, the MICs of comparator fluoroquinolones were higher at pH 5.8 compared with pH 7.8 by 8 to 16-fold for ciprofloxacin, 8-fold for levofloxacin and 4 to 16-fold for moxifloxacin. Overall, finafloxacin MICs were 2 to 16-fold lower than comparator fluoroquinolones, irrespective of the presence of chromosomally encoded or plasmid-encoded factors mediating fluoroquinolone resistance
- For mutants carrying a *gyrA* mutation (i.e., mutant M1), finafloxacin MICs showed a similar increase by a factor of 8 – 16 compared to ciprofloxacin, levofloxacin and moxifloxacin. A similar increase (4 to 16 fold) in finafloxacin MICs was observed against the double mutant (*gyrA- parC*) isolate WT-4-M-35. The acquisition of a *marR* deletion mutation in the absence (WT-III) or presence (MII) of an additional *gyrA* mutation resulted in a 4-fold increase in the MICs of finafloxacin, ciprofloxacin and levofloxacin and by 8-fold for moxifloxacin.
- In the presence of *qepA* plasmid, the finafloxacin MICs were unaffected at pH 5.8 and pH 7.2 (1 – 2 fold increase) while the MICs of ciprofloxacin were increased 8 – 64 fold, 2 to 4-fold for levofloxacin and 2 – 4-fold for moxifloxacin.
- In the presence of topoisomerase protection proteins QnrA1, QnrB1 and QnrS1 all four fluoroquinolones had increased MICs by 2 – 64-fold at pH 5.8 and pH 7.2. However, QnrS seemed to have a slightly higher impact on the MICs for all four fluoroquinolones.
- In the presence of the *aac(6')-1b-cr* variant gene resulted in an increase in the MICs 1 – 4 fold higher for ciprofloxacin at pH 5.8 and pH 7.2, while finafloxacin, levofloxacin and moxifloxacin showed no change in MICs. This may be due to the fact that finafloxacin, levofloxacin and moxifloxacin do not have a piperazinyl substitutent at position C-7. In contrast, kanamycin MICs were increase by 2 – 4-fold at pH5.8 and 32-fold at pH 7.2 compared with the parent strains,

demonstrating the specific acetylating activity of AAC(6')-Ib-cr promoter.

Table 22: Activity of finafloxacin and ciprofloxacin for *E. coli* parent strains (WT) and isogenic fluoroquinolone-resistant derivatives carrying combinations of chromosomal and plasmid-mediated fluoroquinolone resistance gene mutations

	MIC (mg/L)									
	finafloxacin		ciprofloxacin		levofloxacin		moxifloxacin		kanamycin	
E. coli strain	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
WT	0.015	0.06	0.125	0.015	0.25	0.03	0.25	0.03	4	2
WT+*qepA*	0.015	0.06	2	0.125	0.5	0.06	1	0.06	ND	ND
WT+*qnrA1*	0.125	1	1	0.06	2	0.125	2	0.5	ND	ND
WT+*qnrB1*[a]	0.5	2	2	0.25	2	0.25	4	0.5	ND	ND
WT+*qnrS1*[a]	0.25	0.5	2	0.06	2	0.125	2	0.125	ND	ND
WT+*aac(6')-Ib-cr*[a]	≤0.0075	0.03	0.5	0.015	0.125	0.015	0.125	0.015	16	64
MI (*gyrA*^{S83L})	0.25	1	2	0.25	4	0.5	4	0.25	8	4
MI+*qepA*	0.25	1	64	4	8	1	8	1	ND	ND
MI+*qnrA1*	2	4	16	1	8	1	16	2	ND	ND
MI+*qnrB1*	2	8	8	1	8	1	16	2	ND	ND
MI+*qnrS1*	8	16	32	4	32	4	64	4	ND	ND
MI+*aac(6')-Ib-cr*	0.25	1	4	0.5	4	0.25	4	0.25	16	>128
MII (*gyrA*^{S83L}+*marRΔ175bp*)	1	4	16	1	16	2	16	2	8	4
MII+*qepA*	1	4	≥256	64	64	4	32	8	ND	ND
MII+*qnrA1*	8	32	128	4	64	4	64	8	ND	ND
MII+*qnrB1*	8	32	32	4	32	4	64	4	ND	ND
MII+*qnrS1*	32	64	256	16	128	32	256	32	ND	ND
MII+*aac(6')-Ib-cr*	1	4	≥16	4	8	1	8	2	32	>128
WT-III (*marRΔ74bp*)	0.06	0.25	0.5	0.06	1	0.125	1	0.25	4	2
WT-III+*qepA*	0.03	0.25	8	1	2	0.25	2	0.5	ND	ND
WT-III+*qnrA1*	1	4	8	1	8	1	16	2	ND	ND
WT-III+*qnrB1*	2	8	4	0.5	8	1	16	2	ND	ND
WT-III+*qnrS1*	2	16	32	2	16	2	32	2	ND	ND
WT-III+*aac(6')-Ib-cr*	0.03	0.125	2	0.06	1	0.06	1	0.06	8	64
WT-4-M35 (*gyrA*^{D87G}+*parC*^{S80I})	0.125	0.5	1	0.06	1	0.125	2	0.5	4	2
WT-4-M35+*qepA*	0.125	0.5	32	1	4	0.25	8	1	ND	ND
WT-4-M35+*qnrA1*	4	16	64	2	32	2	64	8	ND	ND
WT-4-M35+*qnrB1*	2	8	4	0.5	8	1	16	2	ND	ND
WT-4-M35+*qnrS1*	4	16	32	2	16	2	64	4	ND	ND
WT-4-M35+*aac(6')-Ib-cr*	0.125	0.25	4	0.125	1	0.125	2	0.25	8	64

Source: Emrich *et al., Journal of Antimicrob Chemother* 2010; 65: 2530 - 2533

Reviewer's comments:
In vitro studies suggest the potential for the development of resistance to finafloxacin was variable depending on testing conditions. Under normal testing conditions (pH 7.2), the spontaneous frequencies against gram-positive organisms ranged from ≤6.7 x 10-^{11} to 10^{-6} (at 2x MIC) and against gram-negative organisms ranging from 4.1 x 10^{-10} to 2.4 x 10^{-8} (at 2x MIC). Under acidic testing conditions (pH 6), the spontaneous frequencies against gram-positive organisms ranged from ≤1.3 x 10^{-10} to <10^{-9} (at 2x MIC) and against gram-negative organisms ranging from 1.2 x 10^{-10} to <10^{-9} (at 2x MIC). The concentration at which no spontaneous mutants was selected (i.e., MPCs) was similar in

magnitude to comparator fluoroquinolones and no higher than 4x the MIC against S. aureus and 8x to 16x the MIC against E. coli strains.

The predominant mechanisms of resistance to fluoroquinolones are the alteration of chromosomal target genes gyrA/B and parC/E encoding DNA gyrase and topoisomerase IV, respectively. Single step mutants showed a 2 – 32 fold increase in MIC in finafloxacin against gram-negative and gram-positive organisms. These single step mutants often occurred within the QRDR of gyrA genes for gram-negative organisms and streptococcal isolates whereas in the parC/grlA genes against S. aureus organisms. Double and triple mutations in the chromosomal genes resulted in an additional 4 – 16 fold increase in finafloxacin MICs. There was little or no effect on the activity of finafloxacin against known gram-positive efflux pumps (e.g., reserpine and NorA). However, efflux pumps played a role in the reduced susceptibility to some gram negative organisms (e.g., due to inactivation of the repressor MarR), P. aeruginosa (e.g., MDE efflux pumps such as mexCD and mexAB) as well as Acinetobacter spp. (e.g., AdeB). Resistance due to transferable plasmid-mediated quinolone resistance (PMQR) genes including MDR efflux pumps (such as qepA1) and topoisomerase protection proteins (qnrA1, qnrB1 and qnrS1)which resulted in 2 – 64-fold increase in finafloxacin MICs at pH 5.8 and pH 7.2. However, in the presence of the PMQR aac(6')-lb-cr variant gene (a new variant of the aminoglycoside acetyltransferase capable of modifying C7-piperazinyl-fluoroquinolones) showed no changes in finafloxacin MIC similar to moxifloxacin and levofloxacin; which may be due to the fact that finafloxacin do not have piperazinyl motifs.

In general, against gram-positive mutants, finafloxacin MICs were similar to moxifloxacin and lower than ciprofloxacin and levofloxacin under normal conditions (at pH 7.2), whereas under slight acidic conditions finafloxacin MICs showed greater activity than comparator fluoroquinolones. For gram-negative organisms, finafloxacin MICs were equivalent or lower than ciprofloxacin, levofloxacin or ofloxacin under acidic conditions. Overall, against highly resistant gram-positive and gram-negative mutants with several accumulated target mutations, the pH effect of finafloxacin showed no specific advantage over comparator fluoroquinolones.

2.6. Antimicrobial interactions and fixed combination studies

There were no studies that evaluated the activity of finafloxacin in combination with other antimicrobial agents.

2.7. Other effects of Antibacterial Drug Product

The post antibiotic effect (PAE) of finafloxacin and comparator fluoroquinolones was tested against *E. coli* ATCC 25922 strain. Briefly, drugs were added to logarithmic phase

bacterial suspensions to achieve a final concentration of 5x MIC. The tubes were incubated for an hour and washed three times with pre-warmed media to remove extracellular antibiotic and incubation was continued. Viable counts were performed before drug was added (T_{-1}), after drug treatment and washing (T_0) and at subsequent hourly intervals. The PAE was defined as the time required for an increase in CFU in the absence of drug by 1 $\log_{10}$ increase in cell number compared to the time required for a similar increase in drug free controls. A 1-hour exposure to finafloxacin at 5x the MIC resulted in suppression of growth for approximately 2.1 hours at pH 5.8 compared to 1.05 hours at pH 7.2 (Table 23). All three fluoroquinolones showed a slightly longer PAE at pH 5.8 than at pH 7.2.

Table 23: Post-antibiotic effect of finafloxacin and comparator fluoroquinolones against *E. coli* ATCC 25922 at pH 7.2 and pH 5.8

	PAE [pH 7.2] (h)	PAE [pH 5.8] (h)
CIP	2.4 (1.9 - 2.9)	2.9 (2.4 - 3.4)
LVX	1.27 (1.2 - 1.3)	1.85 (1.8 - 1.9)
FIN	1.05 (0.5 - 1.6)	2.1 (1.8 - 2.4)

Abbreviations: CIP, ciprofloxacin, FIN; finafloxacin, LVX; levofloxacin.
Source: (b) (4) Study Report# RR-2009-004

3. SUSCEPTIBILITY TESTING METHODS

No susceptibility interpretive criteria were provided for finafloxacin against potential pathogens. There were no studies that evaluated the activity of finafloxacin against standard quality control strains.

4. ANIMAL MODELS OF INFECTION

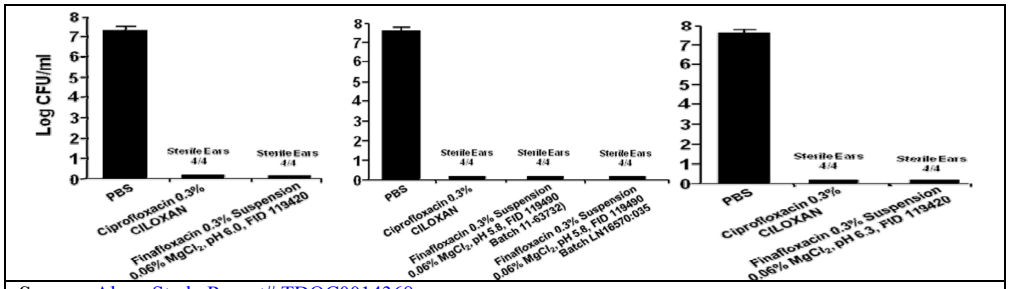
Several studies were conducted that evaluated the activity of finafloxacin in animal models of infections.

4.1. Acute Otitis Externa

In a guinea pig experimental model of acute otitis externa (AOE), the activity of finafloxacin and comparators was evaluated against infections caused by ciprofloxacin-susceptible *P. aeruginosa* LM1018 strain. The finafloxacin MIC at pH 5.8 was 0.125 µg/mL and at pH 7.2 was 1 µg/mL. Ciprofloxacin MICs were 0.25 µg/mL and 0.125 µg/mL, under similar testing conditions. Briefly, guinea pig ears were infected with approximately 10^7 CFU of the *P. aeruginosa* strain. At 16 hours post-infection, animals were administered a single dose of 100 µL of the test article (finafloxacin, ciprofloxacin or saline). At 24 hours post-infection, animal's ears were cultured by saline lavage to determine CFU of *P. aeruginosa* remaining after treatment. The results showed a

reduction in *P. aeruginosa* CFU below the limit of detection (1.7 log CFU/mL) in finafloxacin treated animals in comparison to animals treated with saline alone at 24-hours post-infection (Figure 15). Formulations of 0.3% finafloxacin at pH 5.8 and at pH 6.3 showed similar activity as the 0.3% finafloxacin formulation at pH 6.0. Overall, finafloxacin 0.3% formulation was similar in activity to that of Ciloxan® (Ciprofloxacin 0.3% suspension).

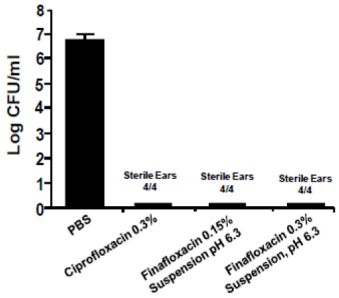
Figure 15: Activity of 0.3% finafloxacin or ciprofloxacin formulations against *P. aeruginosa* LM1018 strain in an experimental AOE guinea pig model at 24 hours post-infection



Source: Alcon Study Report# TDOC0014368

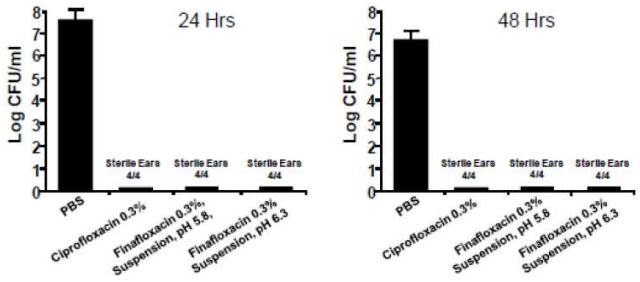
In another experiment, the activity of finafloxacin was evaluated at varying concentrations. The results showed that at concentrations of 0.15% or greater at pH 6.3 resulted in complete eradication of *P. aeruginosa* strain (Figure 16).

Figure 16: Activity of 0.15% or 0.3% suspension of finafloxacin at pH 6.3 or 0.3% ciprofloxacin at 24-hours post-infection against P. aeruginosal strain in an experimental AOE guinea pig model



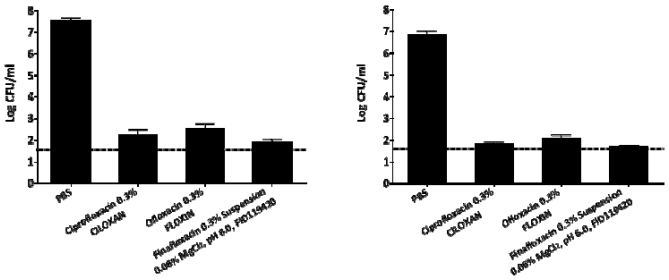
In another experiment, animals were treated with one dose of 0.3% suspension of ciprofloxacin or finafloxacin in animals infected with *P. aeruginosa* LM1018 strain. At 24 and 48 hours post-infection, animal's ears were cultured by saline lavage to determine CFU of *P. aeruginosa* remaining after treatment. There was no *P. aeruginosa* isolates recovered from guinea pig ears at 24 hours (Figure 17). No re-growth was observed in finafloxacin or ciprofloxacin treated animals at 48 hours.

Figure 17: Activity of 0.3% suspension of finafloxacin or ciprofloxacin at 24 and 48-hours post infection against *P. aeruginosa* LM1018 strain in an experimental AOE guinea pig model



When the model was evaluated using test article concentrations at 0.03% (i.e., 10-fold less concentrated), differences between the test article groups were observed (Figure 18). There was a statistical significance between the ofloxacin treated group and all other treatment groups, but no significant difference between finafloxacin and ciprofloxacin. This study also demonstrated that ofloxacin did not completely eradicated *P. aeruginosa* as rapidly as finafloxacin or ciprofloxacin at 24 hours post infection.

Figure 18: Therapeutic activity of 0.3% finafloxacin, 0.3% ofloxacin or 0.3% ciprofloxacin against *P. aeruginosa* strain in an experimental AOE guinea pig model at 24 hours (left) and 48-hours (right) post infection



The activity of finafloxacin and comparators were evaluated against infections caused by different *P. aeruginosa* strains (LM1018, A82039, A83210 and A82358). At 16 hours post-infection, animals were administered a single dose of 100µL of the test article (finafloxacin, ciprofloxacin, ofloxacin or saline) at 0.03% or 0.3% suspension. At 24-hours and 48-hours, animal's ears were cultured by saline lavage to determine CFU of *P. aeruginosa* remaining after treatment. The results showed that at 0.3% finafloxacin showed a 4 to 6-fold reduction at 24 hours and an additional 0.5 – 1.5 fold reduction in *P. aeruginosa* CFUs at 48 hours (Table 24). Finafloxacin showed similar activity as ciprofloxacin, however was superior to ofloxacin at 24 and 48-hours post infection.

Table 24: Effect of finafloxacin 0.3% solution and comparators in treatment of acute otitis externa due to *P. aeruginosa* infection in guinea pigs

Species and Strain	Route and frequency	Endpoint	Dose	Finafloxacin ΔLog_{10} CFU/mL	Ofloxacin ΔLog_{10} CFU/mL	Ciprofloxacin ΔLog_{10} CFU/mL
P. aeruginosa LM1018	Drops, 16 p.i.	24 h p.i.	0	7.21	7.08	7.21
			0.03	--	3.31	--
			0.3%	2.43	0.73	0.17
P. aeruginosa A82039	Drops, 16 p.i.	24 h p.i.	0	7.82	7.38	7.43
			0.03%	--	3.62	3.46
			0.3%	2.58	0.68	1.67
P. aeruginosa A82310	Drops, 16 p.i.	24 h p.i.	0	7.44	7.41	7.44
			0.03%	--	3.69	--
			0.3%	3.37	1.51	0.17
P. aeruginosa A82358	Drops, 16 p.i.	24 h p.i.	0	7.69	7.41	7.69
			0.03%	--	3.43	--
			0.3%	0.62	2.03	1.29
P. aeruginosa LM1018	Drops, 16 p.i.	48 h p.i.	0	6.36	6.66	6.36
			0.03%	--	2.68	--
			0.3%	1.84	0.62	0.17
P. aeruginosa A82310	Drops, 16 p.i.	48 h p.i.	0	6.72	6.80	6.72
			0.03%	--	2.91	--
			0.3%	2.79	1.23	0.17
P. aeruginosa A82358	Drops, 16 p.i.	48 h p.i.	0	6.77	6.96	6.77
			0.03%	--	2.63	--
			0.3%	0.58	1.75	1.32
P. aeruginosa A82039	Drops, 16 p.i.	48 h p.i.	0	6.96	7.20	6.48
			0.03	--	2.95	2.93
			0.3%	2.58	1.19	1.15

Source: Study Report# TDOC-0017323

4.2. Sepsis Models

The activity of finafloxacin and comparators were evaluated in a sepsis animal model of infection. Briefly, female CFW-1 mice (18 – 20g) or Wistar rats (150-200 g) were rendered neutropenic by two intraperitoneal injections of cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) prior to infection. Animals were infected with log-phase cultures of organisms by intraperitoneal injections. Treatment was administered either orally or intravenously at 30 minutes post-infection twice daily for 3 days. Oral treatment was evaluated in immunocompetent animals; while intravenous treatment was evaluated in neutropenic animals prior to infection. Efficacy was assessed as the lowest dose that resulted in 100% survival of infected animals at 3 or 5 days post-infection. The results are shown in Table 25 and summarized as follows:

- Against *S. aureus,* MRSA, streptococcal and enterococcal sepsis models, finafloxacin showed similar *in vitro* activity to moxifloxacin and lower than comparator fluoroquinolones. Finafloxacin was similar in activity as moxifloxacin, however more effective than ciprofloxacin or levofloxacin.
- Against *P. aeruginosa, Serratia marcescens*, ciprofloxacin resistant *E. coli* and *K. pneumoniae* sepsis models, finafloxacin was the least effective compared to

ciprofloxacin and levofloxacin.

Table 25: Effective dose of finafloxacin and comparator fluoroquinolones in various murine sepsis models

Species and strain	Inoculum	Model	Dose [mg/kg]	Route of Administration	Endpoint	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	ED [mg/kg]	MIC [mg/L]	ED [mg/kg]	MIC [mg/L]	ED [mg/kg]	MIC [mg/L]	ED [mg/kg]
Staphylococcus aureus DSM 11823	1.2×10^6	Mouse	1, 10, 25	p.o. (s.d. 30 min p.i.)	Survival at 5d	0.125	10	0.125	25	0.5	25	0.25	>25
	1.5×10^6	Mouse	0.1, 0.5, 1, 10, 25	i.v. (s.d. 30 min p.i.)	Survival at 5d	0.125	1	0.125	1	0.5	>25	0.25	10
	4×10^6	Neutropenic mouse	6.25, 12.5, 25, 50	i.v. (s.d. 30 min p.i.)	Survival at 3d	0.125	50	0.125	>50	nd	nd	nd	nd
Staphylococcus aureus DSM 11822 MRSA (CIP^{RES})	9×10^6	Mouse	10, 25, 50	p.o. (s.d. 30 min p.i.)	Survival at 5d	4	50	4	50	32	>50	16	>50
	6×10^6	Mouse	10, 25, 50	i.v. (s.d. 30 min p.i.)	Survival at 5d	4	50	4	25	32	>50	16	>50
Enterococcus faecalis 27159	2.4×10^8	Mouse	1, 10, 25	p.o. (s.d. 30 min p.i.)	Survival at 5d	0.5	1	0.5	>25	2	>25	1	25
	1.8×10^8	Mouse	1, 10, 25	i.v. (s.d. 30 min p.i.)	Survival at 5d	0.5	10	0.5	10	2	25	1	25
Enterococcus faecium L4001 VRE	4.5×10^7	Mouse	10, 25, 50	p.o. (s.d. 30 min p.i.)	Survival at 5d	1	10	0.5	10	2	25	1	25
Streptococcus pneumoniae L3TV	7.5×10^7	Mouse	1, 10, 25	p.o. (b.i.d. 2 d)	Survival at 3d	2	25	0.25	25	nd	nd	nd	nd
	9×10^8	Mouse	10, 25, 50	i.v. (b.i.d. 2 d)	Survival at 3d	2	50	0.25	50	4	>50	2	>50
	8×10^6	Rat	1, 10, 25	p.o. (b.i.d. 2 d)	Survival at 3d	2	25	0.25	>25	4	>25	2	>25
Streptococcus pneumoniae SP665 (PEN^{RES})	1.3×10^8	Mouse	10, 25, 50	p.o. (b.i.d. 2 d)	Survival at 3d	1	25	0.125	50	2	>50	1	50
Streptococcus pyogenes strain Wacker	1×10^3	Mouse	10, 25, 50	p.o. (b.i.d. 2 d)	Survival at 5d	0.5	50	0.25	50	1	>50	0.5	>50
Escherichia coli DSM 10650	3.2×10^7	Mouse	0.1, 0.5, 1, 10	p.o. (s.d. 30 min p.i.)	Survival at 5d	0.06	0.5	0.125	10	≤0.015	1	0.03	10
	2.4×10^7	Mouse	0.1, 0.5, 1, 10, 25	i.v. (s.d. 30 min p.i.)	Survival at 5d	0.06	0.5	0.125	0.5	≤0.015	0.5	0.03	10
	1.3×10^7	Neutropenic mouse	0.1, 0.5, 1, 10, 25	i.v. (s.d. 30 min p.i.)	Survival at 5d	0.06	1	0.125	10	≤0.015	1	0.03	10
Escherichia coli EC53/99 (CIP^{RES})	2×10^6	Mouse	10, 25, 50	p.o. (s.d. 30 min p.i.)	Survival at 3d	16	>50	8	>50	8	>50	8	>50
Pseudomonas aeruginosa DSM 12055	1.5×10^7	Mouse	2.5, 10, 25	p.o. (s.d. 30 min p.i.)	Survival at 5d	2	>25	nd	nd	0.06	25	nd	nd
	1.5×10^7	Mouse	2.5, 10, 25	i.v. (s.d. 30 min p.i.)	Survival at 5d	2	>25	nd	nd	0.06	10	nd	nd
Serratia marcescens	1×10^6	Mouse	0.2, 1, 5	i.v. (s.d. 30 min p.i.)	Survival at 5d	0.125	5	0.125	5	≤0.03	0.2	≤0.03	1
Klebsiella pneumoniae	1×10^6	Mouse	0.25, 1, 5	p.o. (s.d. 30 min p.i.)	Survival at 5d	0.5	>2.5	0.25	1	0.125	2.5	0.125	2.5

b.i.d.: twice daily; MIC: Minimal Inhibitory Concentration; ED: Effective dose (protecting 100% of infected animals); i.v.: intravenous; p.o.: oral; s.d.: single dose; p.i.: post infection

Source: Study Report# PH-32521

4.3. Respiratory Tract Infection

Female CFW-1 mice (18- 20 g) or Wistar rats (150 – 200g) were inoculated intranasally (mouse) or by intratracheal injection (rat). Treatment was administered to groups of 4 – 6 animals either at twice daily intervals (b.i.d.) or at 1h and 4h post infection by oral, intravenous or intraperitoneal routes, except in a semi-chronic rat model of *P. aeruginosa* pneumonia treatment was started 7 days after infection. Lungs were removed aseptically and homogenized in sterile saline. Viable bacterial counts in lungs and blood were determined at different time points post-infection. The results are shown in Table 26 and summarized as follows:

- In the mouse model of pneumococcal pneumonia, moxifloxacin was the most active *in vitro* (MIC; 0.25 μg/mL) and effective at reducing the bacterial count in the lung ($\Delta\log_{10}$ -2.9 CFU for MXF at 25 mg i.v.). Finafloxacin (MIC, 2 μg/mL) showed similar activity to both ciprofloxacin (MIC, 4 μg/mL) and levofloxacin (MIC; 2 μg/mL), which showed a bacteriostatic effect when administered either orally or intravenously at doses up to 25 mg/kg. In contrast to the mouse pneumococcal pneumonia model, finafloxacin (MIC, 2 μg/mL) showed an equivalent degree of activity in the rat model to moxifloxacin (MIC, 0.5 μg/mL) resulting in greater than 4 $\log_{10}$ reduction in CFU in the lungs at the highest dose of 100 mg/kg.
- Intratracheal instillation of *H. influenzae* in rats leads to a colonization of the lung which is self-limiting. Treatment with finafloxacin (MIC, 2 μg/mL) resulted in a $\geq$ 5 $\log_{10}$ reduction in CFU at doses of 2.5 mg/kg while moxifloxacin (MIC; 0.008 μg/mL) resulted in ~ 2$\log_{10}$ reduction in CFU. Overall, finafloxacin was more effective than moxifloxacin.
- Two rat models of *P. aeruginosa* pneumonia were investigated.
  - In the acute model, treatment was started 30 minutes post infection b.i.d for a total of 7 days. Both finafloxacin (MIC, 2 μg/mL) and ciprofloxacin (MIC, 0.06 μg/mL) showed a bacteriostatic effect (<0.5 $\log_{10}$ reduction in CFU) at doses of 1 and 10 mg/kg. At a higher dose of 25 mg/kg, ciprofloxacin was more effective in reducing the bacterial load more than 2$\log_{10}$ CFU whereas the same regimen in finafloxacin resulted in a -1.45$\log_{10}$ reduction in CFU.
  - In the chronic *P. aeruginosa* rat pneumonia model in which treatment was commenced 7 days after infection, ciprofloxacin was the most effective resulting a 2.37 $\log_{10}$ reduction in CFU at the highest dose of 25 mg/kg whereas finafloxacin showed only weak activity in the chronic model at the same dose level.
- A systemic challenge in mice with *M. catarrhalis* leads to a colonization of various organs due to spread of the bacteria. Since *M. catarrhalis* is not pathogenic for rodents, the colonization is self-limiting. The bacterial loads in lung were measured on day 1 after intraperitoneal challenge. Finafloxacin (MIC,

0.03 µg/mL) was more effective in reducing the bacterial load in lung 1-2 $\log_{10}$ units more than the comparator fluoroquinolones.

Table 26: Effect (reduction in CFU in lung homogenate) of finafloxacin and comparator fluoroquinolones in various models of respiratory tract infections

Species and strain	Inoculum	Model	Dose [mg/kg]	Route of Administration	Endpoint	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$
Streptococcus pneumoniae L3TV	4×10^6 (i.n.)	Mouse pneumonia	25	oral (b.i.d.)	Day 2 p.i.	2	-0.22	0.25	-1.66	4	0.06	2	-0.27
			10				-0.21		-1.37		0.22		-0.03
Streptococcus pneumoniae L3TV	6×10^5 (i.n.)	Mouse pneumonia	25	i.v. (b.i.d.)	Day 2 p.i.	2	-0.25	0.25	-2.9	4	-0.19	2	-0.05
			10				-0.06		-1.86		-0.61		0.05
Streptococcus pneumoniae 170/4	1×10^5 (i.t.)	Rat pneumonia	100	oral (1 h & 4 h p.i.)	Day 1 p.i.	2	-4.49	0.5	-5.11	Nd		nd	
			25				-0.93		-0.48				
			5				-0.6		-0.48				
Haemophilus influenzae strain Spain 7	(i.t.)	Rat pneumonia	5	oral (1 h & 4 h p.i.)	Day 1 p.i.	≤0.004	≤ -5	0.008	≤ -5	Nd		nd	
			2.5				≤ -5		-1.81				
			1				-0.72		-0.72				
Pseudomonas aeruginosa DSM 12055	(i.t.)	Rat pneumonia (acute)	25	i.p. (b.i.d.)	Day 7 p.i.	2	-1.45	nd		0.06	-2.37	nd	
			10				-0.5				-0.31		
			1				-0.35				-0.55		
		Rat pneumonia (semi-chronic)	25	i.p. (starting on day 7 p.i.)	Day 10 p.i.	2	-0.71			0.06	-2.7		
			10				-0.84				-1.57		
Moraxella catarrhalis 488	(i.p.)	Systemic colonisation (lung)	100	oral (30 min p.i.)	Day 1 p.i.	0.03	-3.84	0.125	-2.34	0.06	-1.9	0.06	-2.34
			25				-3.55		-1.62		-1.96		-2.64
			5				-1.94		-0.66		-1.43		-1.44

$\Delta\log_{10}$: Δlog10 CFU/mL of lung homogenate, change in number of colony forming units; b.i.d.: twice daily; i.n.: intranasal; i.p.: intraperitoneal; i.t.: intratracheal; i.v.: intravenous; p.i.: post infection; nd: not done

Source: Study Report# PH-32521

4.4. Gastrointestinal and Intra-abdominal Infections

The activity of finafloxacin and comparator fluoroquinolones were evaluated in mouse infection models (female CFW-1 mice; 18 – 20g) of gastrointestinal infection, peritonitis, post-surgical sepsis and lipopolysaccharide (LPS)-induced shock. Treatment was administered to groups of 4 – 6 mice by oral and intravenous routes and survival was assessed at defined time points post infection. The results are shown in Table 27 and summarized as follows:

- The peritonitis model involved intraperitoneal injection of *Listeria monocytogenes.* Survival from groups of 6 mice was assessed at 3 days post-infection. Finafloxacin was the most effective resulting in complete survival at 25 mg/kg dose. Moxifloxacin was less effective (83% survival), while ciprofloxacin and levofloxacin showed a week effect (30% survival) at the highest dose.
- The enteritis model involved an oral challenge with *Salmonella typhimurium.* All fluoroquinolones were effective at doses between 0.5 – 10 mg/kg. Levofloxacin was found to be less effective at the lowest dose of 0.1 mg/kg.
- For the post-operative or post-surgical infection of the peritoneum (cecal ligation model), anesthetized mice were cut at the peritoneum, the cecum was taken out and a ligation was set at the proximal end of the cecum. The ligation was punctured with a needle and the ligated part of the intestine was placed back within the peritoneum and the wound closed. As control, mice were sham operated without ligation and puncture of the cecum. In this model, polymicrobial peritonitis resulting from the intestinal trauma causes subsequent sepsis 1 – 2 days after this procedure and multiple organ failure from which untreated animals died within 4 days. The effect of both oral and intravenous administration of finafloxacin was 100% survival after 7 days.
- In the LPS-induced shock model, mice were infected intraperitoneally with 20 mg/kg LPS fro *S. typhimurium*. Previous studies show that LPS induces irreversible shock symptoms leading to death of the animals. Finafloxacin showed a slightly higher survival (83%) than moxifloxacin and ciprofloxacin (67%).

Table 27: Effect of finafloxacin and comparator fluoroquinolones on survival in models of gastrointestinal and intra-abdominal infections

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
					MIC [mg/L]	% survival	MIC [mg/L]	% survival	MIC [mg/L]	% survival	MIC [mg/L]	% survival
Peritonitis - mouse	*Listeria monocytogenes* EGD / 1 x 10^6	25	oral, 2 h, 18 h, 24 h p.i.	Day 3 p.i.	1	100	0.5	83	1	33	1	33
		10				50		50		0		0
		1.0				33		0		0		0
Oral *Salmonella typhimurium* infection - mouse	*Salmonella typhimurium* 25268 / 5 x 10^5	10	oral, 2 h, 18 h, 24 h p.i.	Day 10 p.i.	0.06	100	0.03	100	0.125	100	0.25	100
		1.0				100		100		83		67
		0.5				83		100		83		83
		0.1				83		67		83		50
Cecal ligation and puncture) Post-operative polymicrobial sepsis	na	10	oral, 4 h, 18 h, 24 h	Day 4	na	100	na	70	na	90	na	60
				Day 7		100		60		90		50
			i.v., 4 h, 18 h, 24 h p.s.	Day 4		100		90		100		90
				Day 7 p.s.		100		90		100		60
LPS-induced shock -	na / 20 mg/kg	10	i.v. 15 min p.c.	Day 5 p.i.	na	83	na	67	na	67		

na: not applicable; i.v.: intravenous; p.c.: post challenge; p.i.: post infection; p.s.: post surgery
Source: Study Report# PH-32521

4.5. Skin and Soft Tissue Infections

The activity of finafloxacin and comparator fluoroquinolones were evaluated in mouse models (CFW-1 mice, 18 – 20g) including subcutaneous infections that modeled abscess formation (Gelfoam® Model), foreign body infection (with implanted catheter) and tissue infection (granuloma pouch and thigh infection). In each of the models, the endpoint was determined as the reduction of the bacterial load in infected tissues compared to an untreated control animal. The results are summarized below.

In the Gelfoam® model, Gelfoam® pieces were implanted subcutaneously on the back of mice. This induced formation of an abscess-like capsule surrounding the material after 3 to 4 days. Mice were infected with bacterial suspensions of *Staphylococcus aureus* or *Pseudomonas aeruginosa* by injection into the implanted Gelfoam®. Previous experiments showed colonization of the implanted material and spread of the bacteria into different organs, leading to continuous bacterial load in different organs of the infected mice. Treatment was administered orally at 10 mg/kg b.i.d. at 2h post-infection of the implant. Bacterial counts were determined in the implant and in the different organs which were harvested on day 4 and day 7 post-infection. The results are summarized in Table 28.

- In the *S. aureus* Gelfoam® model, finafloxacin (MIC, 0.125 µg/mL) was similarly effective as moxifloxacin (MIC, 0.125 µg/mL) in reducing the bacterial load in multiple organs and the Gelfoam® implant at 10 mg/kg dose. Finafloxacin treatment had a more rapid effect in the Gelfoam implant and spleen as shown on day 4 post-infection and resulted in a greater overall reduction of bacterial load in the lung at day 7 post-infection.
- In the *P. aeruginosa* Gelfoam® model involved assessment of the bacterial load in the Gelfoam® implant only. Only marginal effects (Δlog_{10} -0.5) were observed at day 4 post-infection for the fluoroquinolones tested. On day 7 post-infection, finafloxacin showed a $1log_{10}$ reduction in the implant which was greater than the effect observed for levofloxacin and ciprofloxacin. Moxifloxacin was the least effective.

Table 28: Effect (change in CFU in tissue) of finafloxacin and comparators in SSTI that model abscess formation (Gelfoam®)

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Tissue	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}
Infected abscess (mouse Gelfoam® implant)	*Staphylococcus aureus* DSM 18823 / 1×10^5	10	oral (b.i.d.), 2 h p.i.	4 days p.i.	Gelfoam®	0.125	-2	0.125	-1	nd		nd	
					Spleen		-2		-0.9				
					Liver		-1.7		-0.8				
					Lung		-1.1		-1.6				
					Heart		-1.1		-1.1				
				7days p.i.	Gelfoam®	0.125	-1.89	0.125	-2.03				
					Spleen		-3.93		-3.54				
					Liver		-1.74		-1.35				
					Lung		-2.95		-1.71				
					Heart		-2.75		-3.68				
	Pseudomonas aeruginosa DSM 12055 / 1×10^4	10		4 days p.i.	Gelfoam®	2	-0.54	0.25	-0.43	0.06	-0.48	0.25	-0.41
				7days p.i.			-1.12		-0.28		-0.71		-0.86

Δlog10 CFU/mL: Δlog10 CFU/mL of lung homogenate, change in number of colony forming units; change in number of colony forming units; b.i.d.: twice daily; p.i.: post infection; s.c.: subcutaneous; nd: not done

Source: Study Report# PH-32521

In the foreign body infection model (infected catheter model), pieces of Nealton® gynecological plastic catheters ([redacted] (b) (4)) were incubated overnight in cultures of *S. aureus* or *P. aeruginosa* and then implanted subcutaneously onto the back of mice. Previous control experiments have shown that the catheter pieces were densely covered with adherent bacterial cells after an overnight incubation and subcutaneous implantation of a contaminated catheter resulted in a massive dermatitis with bacterial colonization of the surrounding tissue areas. Treatment was administered at 3h after surgical procedure. The results are shown in Table 29:

- In the *S. aureus* model of infection at day 4 post-infection, moxifloxacin and levofloxacin was slightly more effective than finafloxacin in reducing the bacterial biofilms adherent to the catheter material by 2log_{10} units. On day 7 posit infection, finafloxacin was most effective compound in terms of overall reduction in CFU, followed by moxifloxacin and levofloxacin.
- In the *P. aeruginosa* model of infection, marginal effects (Δlog_{10} -0.5) were observed at day 4 post-infection for the fluoroquinolones tested. At day 7 post-infection, finafloxacin showed a similar degree of effectiveness as levofloxacin and ciprofloxacin in reducing the bacterial count, whereas moxifloxacin did not show any antibacterial effect under the tested conditions.

Table 29: Effect (change in CFU in tissue) of finafloxacin and comparators in SSTI that model tissue infection using an implanted infected catheter model

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Tissue	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}	MIC [mg/L]	Δlog_{10}
Infection with colonised catheter material	*Staphylococcus aureus* DSM 18823 / nd	10	oral (b.i.d.), 3 h p.i.	4 days p.i.	Implant homogenate	0.125	-1.55	0.125	-2.71	0.5	-0.05	0.25	-2.18
				7days p.i.			≤ -6		-5.05		-0.62		-3.56
	Pseudomonas aeruginosa DSM 12055 / nd			4 days p.i.		2	-0.65	0.25	-0.35	0.06	0.19	0.25	0.09
				7days p.i.			-2.73		-0.32		-2.62		-2.61

Δlog_{10} CFU/mL: Δlog_{10} CFU/mL of lung homogenate, change in number of colony forming units; change in number of colony forming units; b.i.d.: twice daily; p.i.: post infection; s.c.: subcutaneous; nd: not done

Source: Study Report# PH-32521

In the granuloma pouch model, a pouch was formed by injecting 5 mL air and 0.5 mL 0.1% croton oil (in olive oil) into the loose subcutaneous connective tissue on the backs of mice. At 72 hours, most of the air was withdrawn and replaced with 1 mL of 0.25% agar in saline. An infection was initiated 48h later by injection of a suspension of *Staphylococcus aureus* A12 (5 x 10^5 CFU/mL in Columbia broth) directly into the pouch. Treatment was administered at 0.5h post-infection with further dosing at 4h, 24h and 32h post-infection. At 48h post-infection, the bacterial load in the pouch exudates were determined. Finafloxacin was similarly effective as the comparator fluoroquinolones in reducing the bacterial load at doses of 12.5 mg/kg and 50 mg/kg (Table 30). However, at the lowest dose of 3.1 mg/kg which showed a bacteriostatic effect, finafloxacin was the more effective than moxifloxacin and levofloxacin in reducing the bacterial load.

Table 30: Effect (change in CFU in tissue) of finafloxacin and comparators in SSTI that model tissue infection using the granuloma pouch exudate model

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Tissue	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$
Mouse model of *Staphylococcus aureus* infected granuloma pouch	*Staphylococcus aureus* A12 / 2.5×10^5	50	oral at 0.5 h, 4 h, 24 h and 32 h p.i.	48 h p.i.	Granuloma pouch exudate	0.25	-3.65	≤0.125	-3.26	nd		0.25	-3.31
		12.5					-3.28		-3.19				-2.7
		3.1					-2		-1.11				-1.02

Δlog10 CFU/mL: Δlog10 CFU/mL of lung homogenate, change in number of colony forming units; change in number of colony forming units; b.i.d.: twice daily; p.i.: post infection; s.c.: subcutaneous; nd: not done
Source: Study Report# PH-32521

In a thigh infection model, CFW-1 mice were rendered neutropenic by 150 mg/kg and 100 mg/kg of cyclophosphamide injected intraperitoneally on days -4 and -1, respectively. Mice were infected with bacterial suspension of *S. aureus* DSM 11823 (3×10^7 CFU/mL) into the thigh muscle of the right hind leg on day 0. Treatment was administered at 0.5h post-infection with a further dosing at 4h post-infection. Viable bacterial counts in the thigh homogenates were determined at 24h post-infection. Finafloxacin was equally effective as moxifloxacin compared to ciprofloxacin and levofloxacin. However, finafloxacin resulted in a greater reduction in CFU in the tissue compared to moxifloxacin at the 10 mg/kg and 50 mg/kg dose (Table 31).

Table 31: Effect (change in CFU in tissue) of finafloxacin and comparators in SSTI that model tissue infection using a neutropenic thigh infection model

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Tissue	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
						MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$	MIC [mg/L]	$\Delta\log_{10}$
Staphylococcus aureus thigh muscle infection in neutropenic	*Staphylococcus aureus* DSM 18823 / 3×10^6	50	s.c. at 0.5 h and 4 h p.i.	24 h p.i.	Thigh	0.125	-5.75	0.125	-4.29	0.5	-2.64	0.25	-2.62
		10					-4.91		-2.67		-0.71		-1.87
		2					-0.38		-0.89		0.22		-0.45

Δlog10 CFU/mL: Δlog10 CFU/mL of lung homogenate, change in number of colony forming units; change in number of colony forming units; b.i.d.: twice daily; p.i.: post infection; s.c.: subcutaneous; nd: not done
Source: Study Report# PH-32521

4.6. Urinary Tract Infections

The activity of finafloxacin and comparator fluoroquinolones was evaluated in a mouse model (CFW-1 mice 18 – 20g) of severe pyelonephritis against two strains of *E. coli*. Pyelonephritis was induced by direct injection of bacterial suspension into the right kidney of anaesthetized mice. Treatment was at 2 hours post-infection and the kidney was removed aseptically and homogenized in saline. Viable bacterial counts in the kidney were determined at different time points post-infection. Against the sensitive strain *E. coli* DSM 10650, finafloxacin was similarly effective as comparator fluoroquinolones resulting in > $3\log_{10}$ reduction in CFU in the kidney homogenate when administered at 10

mg/kg (Table 32). Finafloxacin, levofloxacin and ciprofloxacin was more effective than moxifloxacin at the lower dose of 1 mg/kg showing a similar reduction in bacterial counts in the kidneys. Against the ciprofloxacin-resistant isolate EC 53/99 (MICs, 8 – 16 µg/mL), none of the four fluoroquinolones showed a therapeutic effect even at 50 mg/kg.

Table 32: Effect of (change in CFU in kidney homogenates) of finafloxacin and comparator fluoroquinolones in urinary tract infection models

Model	Strain / Inoculum [CFU/mL]	Dose [mg/kg]	Route and frequency	Endpoint	Finafloxacin		Moxifloxacin		Ciprofloxacin		Levofloxacin	
					MIC [mg/L]	Δlog10 CFU/mL of tissue homogenate	MIC [mg/L]	Δlog10	MIC [mg/L]	Δlog10	MIC [mg/L]	Δlog10
Severe pyelonephritis - mouse	*Escherichia coli* DSM 10650 / 1 x 10^5	10	oral, 2 h p.i.	Day 2 p.i.	0.06	-4.15	0.125	-3.2	≤0.015	-3.12	0.03	-3.9
		1.0				-4.02		-1		-3.04		-3.52
	Escherichia coli EC 53/99 / 1 x 10^5	10			16	<-1	8	<-1	8	<-1	8	<-1
		1.0				<-1		<-1		<-1		<-1
Ascending UTI - mouse	*Proteus mirabilis* 8336 / 3 x 10^7	100	oral, 2 h, 5 h, 24 h and 32 h, p.i.		0.5	-4.21	nd		0.06	-4.39	nd	
		10				-2.09				-3.52		
		1.0				-0.32				-2.01		
		0.1				0.2				-0.18		

Δlog_{10}: Δlog10 CFU/mL of lung homogenate, change in number of colony forming units; p.i.: post infection; nd: not done
Source: Study Report# PH-32521

Finafloxacin was also assessed and compared with ciprofloxacin in a mouse model (CFW-1 mice, 18 – 20g) of ascending *Proteus mirabilis* UTI (cystitis). Infection was initiated by direct injection in the bladder of anaesthetized mice. Treatment was administered at 2h post-infection with a further dosing at 5h, 24h and 32h post-infection. Viable bacterial counts in kidneys were determined at 48 h post-infection. Kidneys were removed aseptically and homogenized in sterile saline. Viable counts in the kidney were determined. Finafloxacin (MIC, 0.5 µg/mL) and ciprofloxacin (MIC, 0.06 µg/mL) resulted in greater than 4 log_{10} reduction in CFU in the kidneys at 100 mg/kg (Table 32). Ciprofloxacin was more effective in reducing the bacterial load (>3log_{10}) at lower doses of 10 mg/kg than finafloxacin.

Reviewer's comments:
In guinea pig experimental model of acute otitis externa, finafloxacin demonstrated a dose-dependent response at concentrations tested from 0.03% to 0.3% suspension formulations. At 0.15% suspension concentration of finafloxacin or higher resulted in reduction of P. aeruginosa CFU counts to below the limit of detection. Overall, finafloxacin antibacterial activity was similar to ciprofloxacin but superior to ofloxacin.

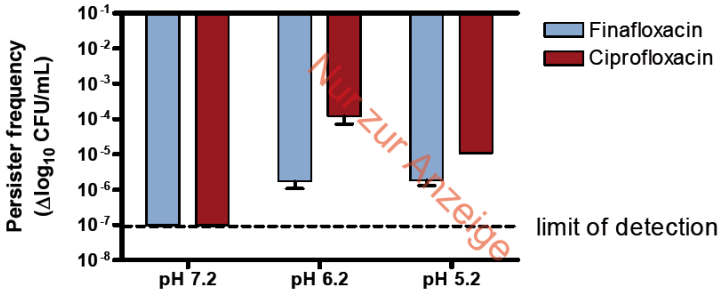
A variety of other animal models of infection were used including sepsis, respiratory tract infections, implanted foreign body infection, skin and soft tissue infections, post-operative polymicrobial sepsis, urinary tract infections including ascending polynephritis and LPS induced shock model. Finafloxacin was shown to be effective in treating these systemic infections caused by gram-positive and gram-negative bacteria. Overall, finafloxacin was shown to be as effective as moxifloxacin, ciprofloxacin and levofloxacin.

5. PHARMACOKINETICS/PHARMACODYNAMICS

5.1. *In vitro* pharmacodynamics

The extent of killing of finafloxacin and ciprofloxacin was evaluated against stationary phase population of *P. aeruginosa* ATCC 27853. An inoculum of 2 x 10^9 CFU/mL was tested and exposed to the test drugs for 24 hours. Finafloxacin was evaluated at a concentration equating to the serum Cmax measured after a single 800 mg oral dose in healthy volunteers and ciprofloxacin was investigated at a concentration equating to the serum C_{max} measured after a single 750 mg oral dose in adults with cystic fibrosis. The concentrations used were finafloxacin; 11.1 mg/L and ciprofloxacin, 3.24 mg/L. There was a pH dependent profile independent of the MIC (Figure 19). At lower pH values of 6.2 and 5.2, finafloxacin showed reduced killing compared to at pH7.2, though still reduced the population to approximately 6 $\log_{10}$ units. In comparison, ciprofloxacin appeared to kill *P. aeruginosa* populations more extensively at pH 5.2 compared at pH 6.2. This is unusual since the MICs of ciprofloxacin are higher at the lower pH. The results may reflect a pH dependent effect on the physiology of *P. aeruginosa* that alters its susceptibility to the activity of ciprofloxacin and finafloxacin.

Figure 19: Bactericidal activity of finafloxacin and ciprofloxacin against stationary phase *P. aeruginosa* ATCC 27853.



Finafloxacin MIC: pH 7.2 = 4; pH 6.2 = 1; pH 5.2 = 1; CiprofloxacinMIC: pH 7.2 = 0.25 ; pH 6.2 = 1; pH 5.2 = 4

Source: Study Report# RR-2010-002

5.2. *In vivo* pharmacodynamics
There were no studies provided that evaluated the activity of finafloxacin in *in vivo* pharmacodynamic models of infection.

5.3. Human pharmacodynamics
Pharmacokinetic studies showed very low systemic plasma levels after topical application of 0.3% finafloxacin otic suspension. Following single topical doses (either 4 drops/ear or 8 drops/ear) in AOE patients, quantifiable finafloxacin (>0.05 ng/mL) were only observed in plasma samples from 2 of 36 AOE subjects. Following repeat topical doses (4 drops/ear for 7 days) in healthy subjects, quantifiable finafloxacin concentration (>0.05 ng/mL) were only observed in plasma samples from 2 of 14 subjects at a total of 3 time points.

The proposed concentration of finafloxacin of 0.3% with a volume of 200 µL for the initial doses was selected in order to provide a high dose delivered to the site of infection while the total daily dose is within the safety margins of the drug. The selection of 4 drops for the subsequent dosing is consistent with the posology of similar otic products. Doses in excess of 4 to 5 drops tend to "flow out" of the canal due to the presence of cerumen and/or otorrhea and swelling of canal. Conversely, doses of fewer than 4 to 5 drops may result in delivery of only 1 – 2 drops due to movement of "missed" drops.

Reviewer's comments:
Systemic exposure to finafloxacin ototopical solution was shown to be extremely low and as a result there was insufficient data to determine pharmacokinetic/pharmacodynamic parameters associated with efficacy. Overall, in healthy and AOE subjects that received 4 drops of 0.3% finafloxacin otic suspension in each ear twice daily for 7.5 days, had very low finafloxacin concentrations (>0.05 ng/mL) in plasma samples which were within the safety margins of the drug.

6. CLINICAL EFFICACY TRIALS

The clinical development program supporting the efficacy of finafloxacin otic suspension, 0.3% for the treatment of acute otitis externa included two pivotal phase 3 studies (C-10-018 and C-10-019) conducted in subjects 6 months of age or older with a clinical diagnosis of AOE of less than 4 weeks duration. A summary of the study designs are presented in Table 33.

Table 33: Summary of Study Designs for Acute Otitis Externa

Study Phase	Design	Population	Endpoints	Treatment Groups	Number of Patients	Dosing Regimen/ Duration
Safety and Efficacy Studies in Patients with acute otitis externa (AOE)						
C-10-018 Phase 3	Multicenter, double-masked, parallel group, vehicle-controlled, randomized	Patients 6 months of age or older with a clinical diagnosis of AOE of less than 4 weeks duration	1° - Clinical cure at Day 11 (TOC) 2° - Microbiological success at Day 11 (TOC); Time to cessation of ear pain as reported by patient diary	AL-60371 Otic Suspension 0.3% Vehicle	347 346	4 drops, twice daily for 7 days
C-10-019 Phase 3	Multicenter, double-masked, parallel group, vehicle-controlled, randomized	Patients 6 months of age or older with a clinical diagnosis of AOE of less than 4 weeks duration	1° - Clinical cure at Day 11 (TOC) 2° - Microbiological success at Day 11 (TOC); Time to cessation of ear pain as reported by patient diary	AL-60371 Otic Suspension 0.3% Vehicle	274 275	4 drops, twice daily for 7 days

6.1. Study Design

The two studies (C-10-018 and C-10-019) were similar in design with respect to the visit schedule, subject population, treatment administered, efficacy endpoints and statistical considerations. The differences between the two studies included the fact that C-10-018 was conducted at different investigational sites by different investigators than C-10-019. C-10-018 enrolled 693 subjects from at least 17 different investigational sites including 13 in the US, 2 in Canada and 2 in Puerto Rico. C-10-019 enrolled 549 subjects from 21 different investigational sites in U.S. and 1 in Canada.

In both studies, all patients underwent otic examinations consisting of an examination of the outer ear and ear canal of both ears, ear cleansing of all fluid and debris along with assessments of tenderness and erythema of the external ear and edema of the external auditory canal. Patients who had a combined numerical score for tenderness, erythema and edema of 4 or greater in at least 1 ear and an otic specimen collected for microbiological evaluation were enrolled in the studies. The definition of the study ear is shown in **Table 34**. Patients who presented with a unilateral ear infection had both ears evaluated but only the affected ear enrolled was cultured and treated. Patients that presented with a bilateral ear infection had both ears evaluated, cultured and treated (both ears were designated as 'enrolled ears").

Table 34: Study Ear Definition

Hierarchical Order for Defining Study Ear	Enrolled Ear(s)	Culture Positive[a] Ear(s)	Pathogen Positive[b] Ear(s)	Study Ear Definition
1	Both[c]	Both	Both	The evaluable ear with the higher value for the sum of the numerical scores of tenderness, erythema and edema at baseline. If both ears were rated equally, the right ear was defined as the study ear.
2	Both[c]	Both	One	The pathogen positive ear
3	Both[c]	One	One	The pathogen positive ear
4	Both[c]	Both	Neither	The evaluable ear with the higher value for the sum of the numerical scores of tenderness, erythema and edema at baseline. If both ears were rated equally, the right ear was defined as the study ear.
5	Both[c]	One	Neither	The culture positive ear
6	Both[c]	Neither	Neither	The evaluable ear with the higher value for the sum of the numerical scores of tenderness, erythema and edema at baseline. If both ears were rated equally, the right ear was defined as the study ear.
7	One	One	One	The enrolled ear
8	One	One	Neither	The enrolled ear
9	One	Neither	Neither	The enrolled ear

[a] Culture Positive: a microbiological specimen from the enrolled ear(s) that contained 1 or more bacterial organism(s); this included all bacteria recovered at baseline
[b] Pathogen Positive: a microbiological specimen that contained at least 1 of the following organisms (considered etiological agents of AOE) in the enrolled ear(s) at baseline: *Pseudomonas aeruginosa* and/or *Staphylococcus aureus*
[c] Only the enrolled ear(s) that also met the baseline requirement for signs and symptoms were considered in the determination of study ear
Note: Patients with a baseline culture that was positive for GAS, pure culture yeast or fungi were exited from the study and were not considered in the subsets of culture -positive patients.

Source: Study Report C-10-18 Table 9.7.1.1-1 (pg86) and Study Report C-10-19

Subjects were randomized in a 1:1 allocation ratio to receive finafloxacin otic suspension, 0.3% or vehicle administered as 4 drops in the affected ear(s) twice daily for 7 days. The first dose of study drug was administered during the study visit by a designated individual at each investigational center and thereafter, the patients (or their parents/legal guardians) were given instructions to administer the assigned drug. If the patient's ear canal was compromised by 50% or more (i.e., due to moderate to severe edema), an otowick was inserted into the affected ear(s).

Subjects were evaluated at baseline and at follow-up visits while on therapy (Day 3), the end of therapy (Day 8) and test of cure (TOC; Day 11). Patients completed a telephone diary twice daily in which they recorded assessments of ear pain, pain medication use and impact of ear pain on their sleep and other daily activities.

Table 35: Study Schedule for Acute Otitis Externa (C-10-018 and C-10-019)

	Visit 1 Screening/ Baseline	Visit 2 On-Therapy	Visit 3 EOT	Visit 4 TOC/Early Exit
Procedure/ Assessment	Day 1	Day 3 ± 2 days	Day 8 ± 2 days	Day 11 ± 2 days
Patient screening	X			
Informed consent	X			
Demographics	X			
Medical history	X			
Concomitant medications	X	X	X	X
Inclusion/Exclusion	X			
Urine pregnancy test [a]	X			X
Clinical assessment	X	X	X	X
Ear cleansing [b]	X	X	X	X
Ear culture	X			X
Otowick insertion [c]	X	X		
Register in IVRS/IWRS	X			
Patient daily diary [d]	X	X	X	X
Dispense study medication/ acetaminophen	X	X[b]		
Demonstrate dosing technique	X	X[b]		
First dose in office	X			
Study coordinator phone call to patient [e]	X	X		
Treatment Satisfaction Questionnaire [f]			X	X[g]
Adverse events [h]	X	X	X	X
Collect study medication			X	X[i]
Exit from IVRS/IWRS				X

EOT = end-of-therapy; IVRS/IWRS = Interactive Voice/Web Response System; TOC = test-of-cure
[a] Performed for women of childbearing potential, before randomization and at exit.
[b] As needed.
[c] Investigators inserted an otowick if the ear canal was compromised by 50% or greater (ie, moderate or severe edema).
[d] Diaries were completed by the patient or parent/legal guardian twice daily throughout the entire study in an electronic diary.
[e] The study coordinator contacted the patient by telephone on Days 2 through 7 (except on the day of Visit 2) to ensure dosing and diary compliance and to evaluate patient progress.
[f] Treatment Satisfaction Questionnaire was completed only by patients > 8 years of age.
[g] Completed only if patient exited prior to Visit 3 (or missed Visit 3).
[h] Monitored for adverse events as described in the study protocol.
[i] Only if the study drug was not collected at Visit 3.

All specimens were sent to a central microbiology laboratory for identification and susceptibility testing, in accordance with the Investigator Laboratory Manual.
Any bacteria, yeast or fungi recovered was identified to the species-level according to the central laboratory standard practices and procedures. Microbiology methods included morphological, phenotypic and genotypic methods. Phenotypic characterization was performed using spot tests, API biochemical test strips or automated phenotypic systems such as Maldi-TOF. Genotypic methods were performed using the RiboPrinter Microbial Characterization System and 16S rRNA gene sequencing. Strain level discrimination within a species was determined by the automated ribotyping using the RiboPrinter Microbial Characterization System. The investigator was contacted if Group A streptococci (*S. pyogenes*) or a positive culture for pure yeast or pure fungi were recovered from the patient specimen, who were then excluded from the study and placed on appropriate therapy. Patients were included in the study analysis if they had a microbiological specimen from the enrolled ear(s) that contained at least 1 of the following organisms considered etiological agents of AOE: *Pseudomonas aeruginosa* and/or *Staphylococcus aureus*.

5.2. Measures of Efficacy

The two phase 3 clinical studies used identical measures of efficacy and at similar assessment time points. **Table 36** shows the number of subjects in each population for studies C-10-018 and C-10-019. For the purposes of this review, efficacy analyses were based on the intent-to-treat which included all patients who were randomized and had a baseline microbiological specimen that is pathogen positive for *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* (i.e., PP-ITT).

Table 36: Summary of subjects with Acute Otitis Externa in studies C-10-018 and C-10-019

Subject Populations	Finafloxacin Otic Solution, 0.3%	Vehicle Control	Total
C-10-018			
Total Randomized	*347*	*346*	*693*
Safety Evaluation	344	342	686
PP-MITT	145	138	283
C-10-019			
Total Randomized	*274*	*275*	*549*
Safety Evaluation	274	274	548
PP-MITT	147	130	277

Safety Evaluation = subjects randomized and received study drug at least once
PP-MITT = Subjects in M ITT that contained a primary pathogen (i.e., study ear specimen was positive for *P. aeruginosa* and/or *Staphylococcus aureus*
Source: Clinical Study Datasets C-10-018 and C-10-019

The majority of patients in the clinical trials had one ear infected, 44.6% in the right ear (250/560) and 45.0% (252/560) in the left ear, 10.4% (58/560) of the patients presented at baseline with bilateral infections (Table 37).

Table 37: Enrolled Ear Status at Baseline by treatment groups

Enrolled Ear	Finafloxacin otic Solution, 0.3% N = 292 [n(%)]	Vehicle Control N = 268 [n(%)]	Total N = 560 [n(%)]
Right	145 (49.7)	105 (39.2)	250 (44.6)
Left	122 (41.8)	130 (48.5)	252 (45.0)
Both	25 (8.6)	33 (12.3)	58 (10.4)

Source: Clinical Study Datasets C-10-018 and C-10-019

The protocol specified primary efficacy endpoint was the proportion of subjects achieving "clinical cures" at day 11 (TOC) visit. A clinical cure was attained if the sum of the numerical scores of the 3 signs and symptoms of AOE (tenderness, erythema and edema) was 0 at Day 11 (TOC). As shown in Table 38, at Day 11 (TOC), "clinical cure"

of acute otitis externa was experienced overall by 70.2% (205/292) of the finafloxacin otic suspension, 0.3% treated subjects compared with 36.9% (99/268) in the vehicle treated arm. Similar results were noted for the secondary outcome of "microbiological success at day 11". The overall proportion of subjects with "microbiological success" (defined as all pre-therapy bacteria absent from the exit specimen) was significantly higher among the Finafloxacin 0.3% treated group (89.7%; 262/292) compared to the vehicle treated group (59.7%; 160/268).

Table 38: Clinical Cure and microbiological success for Acute Otitis Externa (PP-MITT population)

Assessments	Finafloxacin otic Solution, 0.3%	Vehicle Control	P-value
C-10-018			
Clinical Cure (TOC)	104/145 (71.7)	47/138 (34.1)	<0.0001
Microbiological Success (TOC)	129/145 (89.0)	76/138 (55.1)	<0.0001
C-10-019			
Clinical Cure (TOC)	101/147 (68.7)	52/130 (40.0)	<0.0001
Microbiological Success (TOC)	133/147 (90.5)	84/130 (64.6)	<0.0001
Pooled			
Clinical Cure (TOC)	205/292 (70.2)	99/268 (36.9)	<0.0001
Microbiological Success (TOC)	262/292 (89.7)	160/268 (59.7)	<0.0001

Source: **Clinical Study Datasets C-10-018 and C-10-019**

5.3. Microbiological Response

In the combined AOE studies (C-10-018 and C-10-019), there were a total of 2746 pre-therapy isolates recovered from the affected ear(s) of randomized patients, 71.05% (1951) of the pre-therapy isolates were gram-positive, 26.7% (732) were gram-negative and 2.3% (63) were yeast or fungi. Of the 2379 pre-therapy isolates, 560 were considered to be the etiological AOE pathogens of which 405 (72.3%) were *Pseudomonas aeruginosa* and 155 (27.7%) were *Staphylococcus aureus* isolates. Table 39 shows the clinical cure and microbiological response rates by pathogen, however, it is important to note that all of the organisms were mixed culture with either a *P. aeruginosa* or *S. aureus* isolate.

Table 39: Clinical Outcomes and Microbiological Outcomes by baseline pathogen (PP-MITT population)

		Clinical Cure at Test-of-Cure Visit (TOC)				Micobiological Outcome at Test of Cure Visit			
		Finafloxacin		Vehicle		Finafloxacin		Vehicle Control	
		N	(%)	N	(%)	N	(%)	N	
Gram-Positive	*Bacillus cereus*	6	(50 0)	3	(66.7)	6	(100)	3	(100)
	Bacillus species	4	(75 0)	1	(0)	3	(100)	2	(100)
	Bacillus flexus	--	--	--	--	--	--	--	--
	Bacillus megaterium	--	--	--	--	--	--	--	--
	Bacillus pumilus	--	--	--	--	--	--	--	--
	Bacillus subtilis	--	--	--	--	1	(100)	0	(0)
	Brevibacterium mcbrellneri	--	--	--	--	--	--	--	--
	Corynebacterium amycolatum	12	(50 0)	10	(60)	9	(77.8)	8	(62.5)
	Corynebacterium auris	16	(81 3)	8	(37.5)	12	(83.3)	5	(60.0)
	Corynebacterium jeikeium	--	--	--	--	2	(100.0)	--	--
	Corynebacterium propinquum	--	--	--	--	1	(100.0)	2	(100.0)
	Corynebacterium pseudodiphtheriticum	--	--	--	--	1	(100.0)	1	(100.0)
	Corynebacterium species	40	(75 0)	46	(45.7)	30	(96.8)	37	(81.0)
	Enterococcus casseliflavus	5	(80 0)	-	-	2	(100.0)	--	--
	Enterococcus faecalis	28	(53 6)	20	(50)	17	(94.1)	15	(66.7)
	Enterococcus gallinarum	--	--	--	--	2	(100.0)	--	--
	Gemella morbillorum	--	--	--	--	1	(100.0)	--	--
	Kocuria rhizophila	--	--	--	--	1	(100.0)	1	(100.0)
	Kocuria varians	--	--	--	--	1	(100.0)	--	--
	Micrococcus luteus	2	(100 0)	5	(60)	1	(100.0)	3	(100.0)
	Micrococcus species	7	(85 7)	2	(0)	3	(100.0)	1	(100.0)
	Staphylococcus aureus	82	(68 3)	71	(40.8)	72	(87.8)	44	(60.3)
	Staphylococcus auricularis	101	(77 2)	92	(68.5)	48	(90.5)	41	(65.9)
	Staphylococcus capitis	60	(71 7)	83	(54.2)	25	(92)	37	(62.2)
	Staphylococcus caprae	27	(92 6)	36	(61.1)	11	(81.8)	27	(55.6)
	Staphylococcus cohnii	3	(66 7)	3	(33.3)	3	(100.0)	2	(50.0)
	Staphylococcus epidermidis	157	(68 8)	155	(48.4)	89	(85.4)	99	(74.7)
	Staphylococcus haemolyticus	13	(61 5)	10	(50)	8	(100.0)	7	(85.7)
	Staphylococcus hominis	8	(75 0)	14	(57.1)	5	(100.0)	10	(100.0)
	Staphylococcus lugdunensis	7	(57 1)	10	(30)	4	(75.0)	8	(100.0)
	Staphylococcus pasteuri	5	(100 0)	3	(66.7)	3	(100.0)	1	(100.0)
	Staphylococcus simulans	5	(60 0)	6	(50)	6	(100.0)	6	(66.7)
	Staphylococcus species	22	(68 2)	20	(35)	10	(100.0)	15	(86.7)
	Staphylococcus warneri	21	(76 2)	9	(77.8)	16	(100.0)	7	(100.0)
	Streptococcus Group G	3	(66 7)	2	(50)	2	(100.0)	2	(50.0)
	Streptococcus agalactiae	6	(33 3)	3	(66.7)	5	(100.0)	3	(66.7)
	Streptococcus mitis	9	(88 9)	12	(55 6)	7	1(100.0)	11	(100.0)
	Streptococcus sanguinis	4	(50 0)	8	(37 5)	3	(100.0)	4	(100.0)
	Streptococcus species	8	(87 5)	7	(16 1)	7	(100.0)	5	(100.0)
	Streptococcus viridans group	--	--	--	--	2	(100.0)	--	--
	Turicella otitidis	119	(72 3)	131	(13 5)	77	(72.7)	92	(60.9)
Gram-Negative	*Achromobacter xylosoxidans*	5	(80 0)	4	(55)	5	(100.0)	4	(100.0)
	Acinteobacter genospecies 3	--	--	--	--	3	(100.0)	1	(0.0)
	Acinetobacter baumannii	4	(100 0)	1	(100)	4	(100.0)	1	(100.0)
	Aeromonas sobria	--	--	--	--	1	(100.0)	--	--
	Aeromonas veronii	--	--	--	--	--	--	--	--
	Alcaligenes faecalis	--	--	--	--	--	--	--	--
	Citrobacter freundii	2	(50 0)	4	(0)	2	(100.0)	2	(100.0)
	Citrobacter koseri	--	--	--	--	1	(100.0)	3	(33.3)
	Enterobacter aerogenes	4	(50 0)	2	(0)	4	(100.0)	2	(100.0)
	Enterobacter cloacae	4	(100 0)	7	(57 1)	4	(100.0)	4	(75.0)
	Enterobacter hormaechei	--	--	--	--	2	(100.0)	--	--
	Enterobacter sakazakii	--	--	--	--	1	(100.0)	--	--
	Enterobacter species	7	(71 4)	10	(21 4)	5	(100.0)	8	(75.0)
	Escherichia coli	9	(66 7)	8	(41 7)	5	(100.0)	8	(75.0)
	Haemophilus influenzae	--	--	--	--	1	(100.0)	--	--
	Klebsiella oxytoca	1	(100 0)	4	(75)	1	(100.0)	4	(75.0)
	Klebsiella pneumoniae	7	(57 1)	11	(39)	6	(100.0)	8	(75.0)
	Moraxella catarrhalis	--	--	--	--	1	(100.0)	--	--
	Proteus mirabilis	11	(72 7)	7	(15 6)	10	(100.0)	6	(66.7)
	Pseudomonas aeruginosa	230	(70 9)	219	(36 2)	210	(90.5)	195	(60.0)
	Pseudomonas alcaligenes	--	--	--	--	1	(100.0)	--	--
	Pseudomonas otitidis	4	(100 0)	5	(100)	2	(100.0)	4	(75.0)
	Pseudomonas stutzeri	--	--	--	--	1	(100.0)	--	--
	Serratia marcescens	4	(75 0)	5	(35)	4	(100.0)	5	(100.0)
	Stenotrophomonas maltophilia	16	(75 0)	4	(25)	13	(92.3)	4	(100.0)

N = number of subjects that had the specific organism; %Clinical Cure = percent cured with given baseline pathogen; % Microbiological outcome = percent eradicated or presumed eradication with given baseline pathogen. Shaded cells organisms included in proposed label where no information was provided in datasets or by applicant in submission documents SDN-013 and SDN-015

Source: Clinical Cure (Applicant's Data provided in SDN-013)
Microbiological Outcome (C-10-018 and C-10-019 datasets)

The microbiological response by aetiological pathogen present at baseline and by patient response across the treatment groups is shown in Table 40.

- Against *Staphylococcus aureus,* microbiological eradication was achieved 72 patients (87.8%) in the finafloxacin otic 0.3% suspension treatment group and 44 patients (60.3%) in the vehicle treatment group. The eradication rate varied based on fluoroquinolone sensitivity in finafloxacin otic suspension 0.3% treated subjects; 61 patients (91.0%) that had a fluoroquinolone sensitive *S. aureus* isolate eradicated their isolate whereas 11 patients (73.3%) that had a fluoroquinolone resistant *S. aureus* isolate achieved eradication.
- Against *Pseudomonas aeruginosa*, microbiological eradication was achieved in 190 patients (90.5%) in the finafloxacin otic 0.3% suspension treatment group and 117 patients (60.0%) in the vehicle treated group. In the finafloxacin otic suspension 0.3% treated group, 173 patients (90.6%) that had a fluoroquinolone-sensitive *P. aeruginosa* isolate and 17 patients (89.5%) that had a fluoroquinolone-resistant *P. aeruginosa* isolate eradicated their isolate at Day 11 (TOC).

Table 40: Microbiological Response for combined AOE Studies C-10-018 and C-10-019 by baseline pathogen and by patient response (PP-ITT population)

Baseline Pathogen	Finafloxacin Otic suspension 0.3%		Vehicle Control	
	Eradicated[1] N (%)[3]	Not Eradicated[2] N (%)	Eradicated[1] N (%)	Not Eradicated[2] N (%)
Staphylococcus aureus	72 (87.8)	10 (12.2)	44 (60.3)	29 (39.7)
Fluoroquinolone sensitive[4]	61 (91.0)	6 (9.0)	39 (60.9)	25 (39.1)
Fluoroquinolone resistant	11 (73.3)	4 (26.7)	5 (55.6)	4 (44.4)
Pseudomonas aeruginosa	190 (90.5)	20 (9.5)	117 (60.0)	78 (40.0)
Fluoroquinolone sensitive	173 (90.6)	18 (9.4)	104 (60.5)	68 (39.5)
Fluoroquinolone resistant	17 (89.5)	2 (10.5)	13 (56.5)	10 (43.5)

[1] Documented Eradication, Presumed Eradication
[2] Documented Persistence, Presumed Persistence
[3] Percentages are based upon Eradication + Non-Eradication = N = number of patients with given baseline pathogens
[4] Fluoroquinolone resistance was defined as having a ciprofloxacin and/or ofloxacin MIC ≥ 4µg/mL for *Staphylococcus aureus* or *Pseudomonas aeruginosa*
Source: Clinical Study Datasets C-10-018 and C-10-019

5.4. Antimicrobial Susceptibility

Antimicrobial susceptibility testing was performed at the central laboratory on all baseline isolates and repeated during the duration of therapy and again at the conclusion of therapy in patients in which isolates were cultured. The MICs were determined by the broth microdilution method on TREK panels in accordance with CLSI M07-A9 guidelines. Quality control of the broth microdilution panels were performed by (b) (4)

before the lots were released to the central laboratory. The laboratory tested each of the five panels prior to use. Quality control strains tested included *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *H. influenzae* ATCC 49247 and *P. aeruginosa* ATCC 27853.Susceptibility testing were performed at acidic (pH 5.8) and neutral (pH 7.2) pH for finafloxacin and ciprofloxacin.

The antibiotic susceptibility profile of the primary pre-therapy pathogens is shown in Table 41:

- For *S. aureus,* the baseline isolates had MICs that ranged from ≤0.002 to 16µg/ml under acidic conditions (pH 5.8) and MICs ranged from 0.008 to 32 µg/ml under standard testing conditions (pH 7.2). Low levels or resistance were reported for the comparator fluoroquinolones which included 7 (15.6%) isolates were resistant to ciprofloxacin, 4 (8.9%) resistant to moxifloxacin and 7 (15.6%) resistant to ofloxacin. The highest finafloxacin MIC value at which microbiological eradication was observed at pH 7.2 was 32 µg/ml and at pH 5.8 was 8 µg/ml.

- For *P. aeruginosa*, the baseline isolates had MICs that ranged from 0.004 to 2 µg/ml under acidic conditions (pH 5.8) and MICs ranged from 0.03 to 32 µg/ml (pH 7.2). Low levels or resistance were reported for the comparator fluoroquinolones which included 7 (15.6%) isolates were resistant to ciprofloxacin, 4 (8.9%) resistant to moxifloxacin and 7 (15.6%) resistant to ofloxacin. The highest finafloxacin MIC value at which microbiological eradication was observed at pH 7.2 was 32 µg/ml and at pH 5.8 was 2 µg/ml.

Table 41: Antibiotic susceptibility profile of primary pre-therapy pathogens (study ear isolates) in Study C10-018

Organisms Isolated at Pre-Therapy		MIC Range (Min, Max)	MIC_{50}	MIC_{90}	CLSI Breakpoint	Resistant Isolates N (%)	Total Isolates N
Finafloxacin Treatment Group							
Staphylococcus aureus	Amoxicillin	(0.12, >128)	8	128	0.5	45 (76.3)	59
	Ciprofloxacin, pH5	(≤0.002, >128)	0.5	16	2	10 (16.9)	59
	Ciprofloxacin, pH7	(0.06, >128)	0.25	8	2	9 (15.3)	59
	Erythromycin	(0.25, >256)	0.5	128	4	20 (33.9)	59
	Finafloxacin, pH5	(≤0.002, 16)	0.03	0.5	2	2 (3.4)	59
	Finafloxacin, pH7	(0.008, 32)	0.06	1	2	2 (3.4)	59
	Gentamicin	(0.06, 2)	0.25	0.5	8	0 (0.0)	59
	Moxifloxacin	(0.015, 32)	0.06	2	1	7 (11.9)	59
	Ofloxacin	(0.12, >64)	0.5	16	2	9 (15.3)	59
	Oxacillin	(0.12, 64)	0.5	32	2	13 (22.0)	59
	Tetracycline	(0.06, 64)	0.5	16	8	6 (10.2)	59
	Trimethoprim	(0.5, 16)	1	2	8	1 (1.7)	59
Pseudomonas aeruginosa	Ciprofloxacin, pH5	(0.06, 8)	0.25	1	2	5 (5.2)	96
	Ciprofloxacin, pH7	(0.03, 2)	0.12	0.25	2	0 (0.0)	96
	Finafloxacin, pH5	(0.015, 2)	0.25	0.25	2	0 (0.0)	96
	Finafloxacin, pH7	(0.25, 32)	2	4	2	18 (18.8)	96
	Gentamicin	(0.25, 4)	2	2	8	0 (0.0)	96
	Moxifloxacin	(0.25, 16)	1	2	4	2 (2.1)	96
	Ofloxacin	(0.25, 8)	1	2	4	1 (1.0)	96
	Piperacillin	(2, 128)	8	16	128	0 (0.0)	96
	Tetracycline	(8, 64)	16	32	8	91 (94.8)	96
	Trimethoprim	(64, >256)	128	256	256	3 (3.1)	96
Vehicle Treatment Group							
Staphylococcus aureus	Amoxicillin	(0.12, 128)	2	128	0.5	32 (71.1)	45
	Ciprofloxacin, pH5	(0.12, >128)	0.5	16	2	7 (15.6)	45
	Ciprofloxacin, pH7	(0.06, >128)	0.25	8	2	7 (15.6)	45
	Erythromycin	(0.25, >256)	0.5	>256	4	14 (31.1)	45
	Finafloxacin, pH5	(0.008, 4)	0.03	0.5	2	2 (4.4)	45
	Finafloxacin, pH7	(0.015, 8)	0.06	1	2	1 (2.2)	45
	Gentamicin	(0.12, 1)	0.25	0.5	8	0 (0.0)	45
	Moxifloxacin	(0.015, 4)	0.06	1	1	4 (8.9)	45
	Ofloxacin	(0.12, 64)	0.5	8	2	7 (15.6)	45
	Oxacillin	(0.12, >64)	0.5	16	2	5 (11.1)	45
	Tetracycline	(0.25, 2)	0.5	0.5	8	0 (0.0)	45
	Trimethoprim	(0.5, >256)	1	2	8	2 (4.4)	45
Pseudomonas aeruginosa	Ciprofloxacin, pH5	(0.06, 4)	0.25	0.5	2	3 (2.8)	108
	Ciprofloxacin, pH7	(0.03, 2)	0.12	0.25	2	0 (0.0)	108
	Finafloxacin, pH5	(0.015, 4)	0.25	0.5	2	1 (0.9)	108
	Finafloxacin, pH7	(0.25, 16)	2	4	2	22 (20.4)	108
	Gentamicin	(0.25, 16)	2	2	8	1 (0.9)	108
	Moxifloxacin	(0.12, 4)	1	4	4	0 (0.0)	108
	Ofloxacin	(0.25, 8)	1	2	4	1 (0.9)	108
	Piperacillin	(2, 128)	8	16	128	0 (0.0)	108
	Tetracycline	(4, 64)	16	32	8	103 (95.4)	108
	Trimethoprim	(16, >256)	128	>256	256	12 (11.1)	108

Source: CSR 10018 – Table 14.4- 5 and Table 14.4 - 6

Table 42: Antibiotic susceptibility profile of primary pre-therapy pathogens (study ear isolates) in Study C10-019

Organisms Isolated at Pre-Therapy		MIC Range (Min, Max)	MIC_{50}	MIC_{90}	CLSI Breakpoint	Resistant Isolates N (%)	Total Isolates N
Finafloxacin Treatment Group							
Staphylococcus aureus	Amoxicillin	(≤0.06, 128)	8	128	0.5	20 (87.0)	23
	Ciprofloxacin, pH5	(0.25, 16)	0.5	16	2	4 (17.4)	23
	Ciprofloxacin, pH7	(0.06, 16)	0.25	4	2	3 (13.0)	23
	Erythromycin	(0.25, >256)	32	>256	4	12 (52.2)	23
	Finafloxacin, pH5	(0.015, 0.5)	0.03	0.5	2*	0 (0.0)	23
	Finafloxacin, pH7	(0.015, 1)	0.06	1	2*	0 (0.0)	23
	Gentamicin	(0.06, 64)	0.5	1	8	2 (8.7)	23
	Moxifloxacin	(0.015, 2)	0.03	1	1	2 (8.7)	23
	Ofloxacin	(0.25, 16)	0.5	8	2	3 (13.0)	23
	Oxacillin	(0.12, 64)	0.5	32	2	9 (39.1)	23
	Tetracycline	(0.25, 32)	0.5	0.5	8	1 (4.3)	23
	Trimethoprim	(0.5, 2)	1	2	8	0 (0.0)	23
Pseudomonas aeruginosa	Ciprofloxacin, pH5	(0.03, 8)	0.25	1	2	2 (1.5)	137
	Ciprofloxacin, pH7	(≤0.002, 4)	0.12	0.25	2	1 (0.7)	137
	Finafloxacin, pH5	(0.004, 4)	0.25	0.25	2*	1 (0.7)	137
	Finafloxacin, pH7	(0.03, 32)	2	4	2*	16 (11.7)	137
	Gentamicin	(0.25, 16)	2	4	8	1 (0.7)	137
	Moxifloxacin	(0.03, 16)	1	4	4	3 (2.2)	137
	Ofloxacin	(0.03, 16)	1	2	4	2 (1.5)	137
	Piperacillin	(2, >128)	8	16	128	1 (0.7)	137
	Tetracycline	(0.5, 64)	16	32	8	131 (95.6)	137
	Trimethoprim	(4, >256)	128	256	256	11 (8.0)	137
Vehicle Treatment Group							
Staphylococcus aureus	Amoxicillin	(≤0.06, 64)	6	32	0.5	22 (84.6)	26
	Ciprofloxacin, pH5	(0.03, 32)	0.5	16	2	5 (19.2)	26
	Ciprofloxacin, pH7	(0.06, 16)	0.25	8	2	3 (11.5)	26
	Erythromycin	(≤0.03, >256)	0.5	>256	4	7 (26.9)	26
	Finafloxacin, pH5	(0.008, 0.5)	0.03	0.5	2*	0 (0.0)	26
	Finafloxacin, pH7	(0.015, 2)	0.06	1	2*	0 (0.0)	26
	Gentamicin	(≤0.015, 32)	0.5	1	8	2 (7.7)	26
	Moxifloxacin	(0.015, 2)	0.06	1	1	2 (7.7)	26
	Ofloxacin	(0.12, 16)	0.5	8	2	3 (11.5)	26
	Oxacillin	(0.25, 32)	0.5	16	2	3 (11.5)	26
	Tetracycline	(0.06, 32)	0.5	1	8	1 (3.8)	26
	Trimethoprim	(0.5, 4)	1	2	8	0 (0.0)	26
Pseudomonas aeruginosa	Ciprofloxacin, pH5	(0.06, 8)	0.25	0.5	2	1 (0.9)	114
	Ciprofloxacin, pH7	(0.03, 2)	0.12	0.25	2	0 (0.0)	114
	Finafloxacin, pH5	(0.015, 2)	0.25	0.25	2*	0 (0.0)	114
	Finafloxacin, pH7	(0.5, 8)	2	4	2*	12 (10.5)	114
	Gentamicin	(0.5, 4)	2	4	8	0 (0.0)	114
	Moxifloxacin	(0.5, 8)	1	2	4	2 (1.8)	114
	Ofloxacin	(0.5, 8)	1	2	4	1 (0.9)	114
	Piperacillin	(2, >128)	8	16	128	1 (0.9)	114
	Tetracycline	(8, 32)	16	32	8	112 (98.2)	114
	Trimethoprim	(32, >256)	128	256	256	7 (6.1)	114

Veh = Finafloxacin Vehicle

* CLSI Breakpoint has not been established for Finafloxacin.

Source: CSR 10019 – Table 14.4- 5 and Table 14.4 - 6

Reviewer's Comments:

The applicant has conducted two pivotal phase 3 clinical studies that evaluated the efficacy and safety of finafloxacin otic suspension 0.3% solution for the treatment of acute otitis externa in subjects (b) (4) *of age or older. In both phase 3 clinical efficacy studies, the primary endpoint was met which showed that a higher proportion of subjects*

in the finafloxacin otic suspension 0.3% arm had a "clinical cure" compared to subjects in the vehicle control arm.

The pre-specified protocol study analysis included only patients if they had a microbiological specimen from the enrolled ear(s) that contained at least 1 of the following organisms considered etiological agents of AOE: Pseudomonas aeruginosa and/or Staphylococcus aureus. However, the applicant has conducted several sensitivity analysis of finafloxacin compared to vehicle control against 66 species of bacteria in the proposed label. The significance of most of these organisms is unclear.

It is important to note that, microbiological reports of ear cultures when performed, should include enumeration of each pathogen as well as normal flora. This is due to the fact that many bacteria capable of producing disease reside in a balance with non-pathogenic organisms and are considered normal flora; it is only when this balance is disturbed that a disease state can ensue. For this reason, it is necessary, to enumerate pathogens as "many", "moderate", "few" or "rare" so that the physician can evaluate the report in light of the patient's condition. However, the culture techniques used in these studies do not permit a distinction to be made between true pathogens and the overgrowth of normal flora or colonizers.

Based on previous AOE NDA studies, the organisms considered pathogenic include Pseudomonas aeruginosa and Staphylococcus aureus. These organisms have been included in the labeling for the AOE indication because of the frequency with which they are recovered, the demonstration of adequate treatment efficacy and their well-characterized associations of AOE and other skin and soft tissue infections (including malignant otitis externa in the case of P. aeruginosa) [see Medical Officer Review of NDA 21-537 – Ciprodex].

In this NDA, as expected, the most commonly isolated organisms were P. aeruginosa (405 isolates) followed by S. aureus (155 isolates). The results showed that for the finafloxacin otic suspension 0.3% treatment group, microbiological eradication was achieved in 72 patients (87.8%) that had a Staphylococcus aureus isolate and in 190 patients (90.5%) that had a Pseudomonas aeruginosa isolate.

This reviewer recommends that labeling for this indication be limited to the two pathogens most conclusively associated with Acute Otitis Externa - P. aeruginosa and S. aureus.

7. INTERPRETIVE CRITERIA

No susceptibility testing interpretive criteria for finafloxacin otic suspension 0.3% are recommended.

8. LABELING

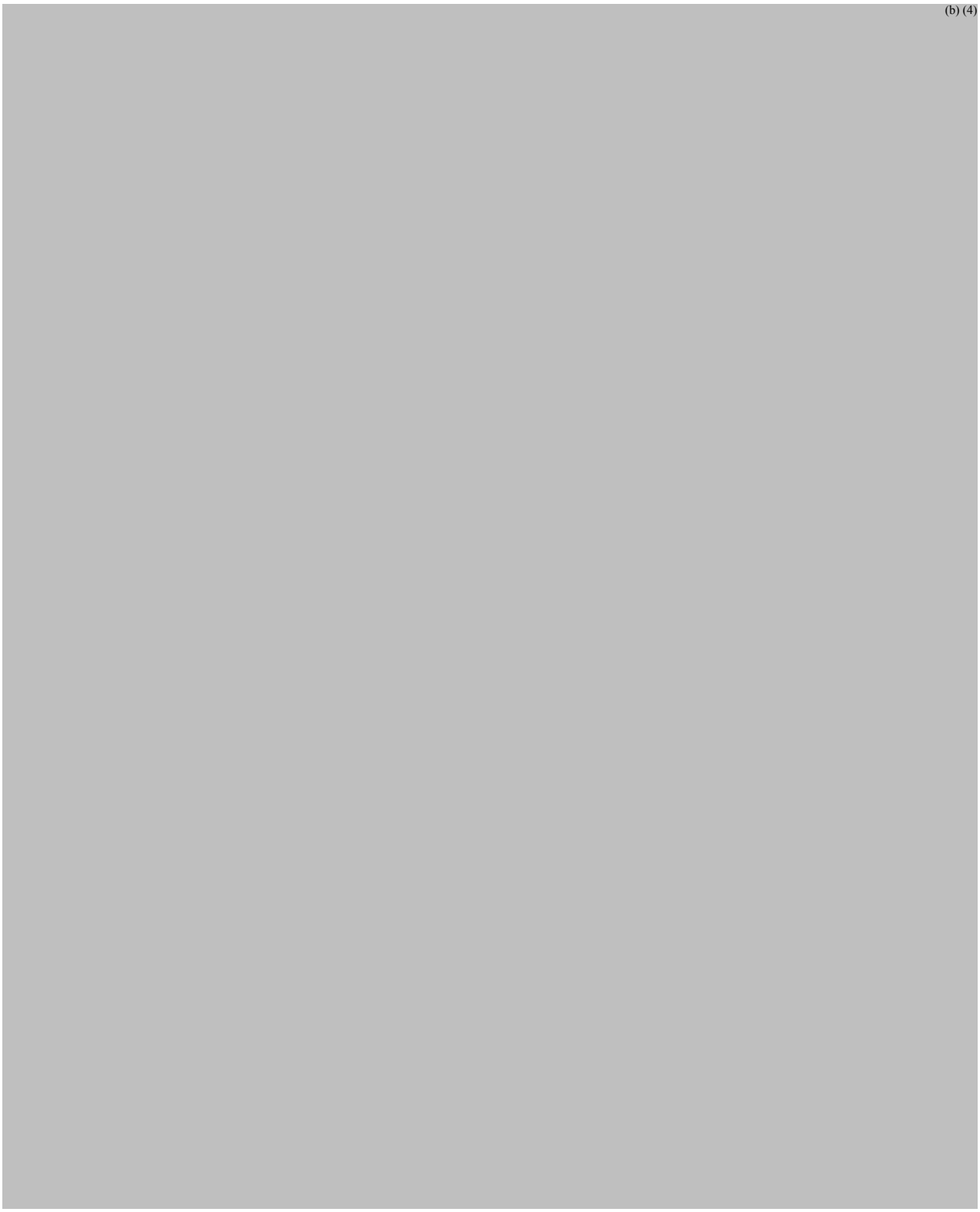
The applicant has provided the proposed labeling (only the microbiology subsection of the labeling is discussed below).

8.1. Applicant's Proposed Labeling

The labeling in the submission dated 4/25/2014 is as follows:

(b) (4)

(b) (4)



(b) (4)

Reviewer's comments

The following changes to the proposed labeling are recommended:

- *The labeling should be consistent with the standard format for Sections "12.1 Mechanism of Action" and "12.4 Microbiology" of the labeling. The applicant should use the standard language and headers as provided in the Physicians Labeling Rule 21 CFR Parts 201, 314 and 601 for "Microbiology" portion of the labeling.*

- *In Section 12.1 Mechanism of Action should read as follows: "Finafloxacin otic suspension 0.3% is an ototopical antibacterial agent [See Microbiology (12.4)]*

- *The applicant claim that* (b) (4) *The clinical significance is unknown.*

- *It is recommended that the sponsor should provide information on the Mechanism of Action of finafloxacin. The following sentence is recommended in the labeling:*

  The mechanism of action of finafloxacin and other fluoroquinolones involves the inhibition of bacterial type II topoisomerase enzymes, DNA gyrase and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination.

- *It is recommended that a Mechanism of Resistance subheading be included in Section 12.4 and should read as follows:*

  "Resistance to fluoroquinolones occurs primarily by mutations in the chromosomal DNA, decreased outer membrane permeability or drug efflux mechanisms. *In vitro* resistance to finafloxacin due to spontaneous mutation is rare."

- *The following are recommendations for the List of Microorganisms in the section*
  - *This reviewer recommends that labeling for this indication be limited to the two pathogens most conclusively associated with Acute Otitis Externa, that is, Pseudomonas aeruginosa and Staphylococcus aureus.*

  - *All other organisms in the proposed list should not be included in the list of organism due to insufficient numbers in the clinical studies and are considered harmless bacteria that can contaminate and/or colonize the skin*

mucosa.

- *In order for the organism to be included in the package insert the applicant should provide*
  - *A discussion of the relevance of each pathogen to the indication sought*
  - *Sufficient scientific evidence demonstrating that the microorganism is a frequent pathogen for an indication and shown to cause disease in the general population.*
  - *In vitro susceptibility information for at least 100 isolates (including range, MIC_{50}, MIC_{90}) of the proposed pathogen. The information provided should support the species included and correlate with the achievable concentrations of the antibacterial using the recommended dosing regimen.*

8.2. FDA's Version of the Labeling

12.1 Mechanism of Action

(b) (4)

12.4. Microbiology

Mechanism of Action

Finafloxacin (b) (4), has broad-spectrum *in vitro* activity against Gram-positive and Gram-negative organisms. (b) (4) The mechanism of action of finafloxacin and other fluoroquinolones involves the inhibition of bacterial type II topoisomerases enzymes, DNA gyrase and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination.

Mechanism of Resistance

Resistance to fluoroquinolones occurs primarily by mutations in the chromosomal DNA, decreased outer membrane permeability or drug efflux mechanisms. *In vitro* resistance to finafloxacin due to spontaneous mutation is rare.

Cross Resistance

Cross-resistance has been observed between finafloxacin and other fluoroquinolones. (b) (4) is (b) (4). No cross-resistance between finafloxacin and other classes of antibacterial agents.

Finafloxacin has been shown to be active against most isolates of the following bacteria, both *in vitro* and clinical studies (b) (4)

(b) (4) as described in the INDICATIONS AND USAGE section of the package insert for Finafloxacin otic suspension, 0.3%.

Pseudomonas aeruginosa
Staphylococcus aureus

(b) (4)

(b) (4)

[redacted]

9. RECOMMENDATIONS

The application is approvable pending an accepted version of the labeling.

10. REFERENCES

1. Rosenfeld RM, Schwartz SR, Cannon CR, Roland RS, Simon GR, Kumar KA, Huang WW, Haskell HW and Robertson PJ. Clinical Practice guideline: acute otitis externa. **Otolaryngology – Head and Neck Surgery** 2014; 150 (1 Suppl) S1 – S24.
2. Rosenfeld RM, Brown L, Cannon CR, Dolor RJ, Ganiats TG, Hannley M, Kokemueller P, Marcy M. Roland PS, Schiffman RN, Stinnett S. and Witsell DL. Clinical practice guideline: Acute otitis externa. **Otolaryngology – Head and Neck Surgery** 2006; 134: S4 – S23.
3. Kim J. and Chin J. Change of external auditory canal in pH in Acute Otitis Externa. **Anals of Otology, Rhinology & Laryngology** 2009; 118(11): 769 – 772.
4. Forbes BA, Sahm DF and Weissfeld. Chapter56. Infections of the Eyes, Ears and Sinuses. In: Bailey & Scott's Diagnostic Microbiology 12th Edition. Mosby 2007.
5. Garcia, Lynne S. (2010). Clinical Microbiology Procedures Handbook, Volumes 1-3 (3rd Edition). American Society for Microbiology (ASM). Online version available

at:
http://app.knovel.com/web/toc.v/cid:kpCMPHVE03/viewerType:toc/root_slug:clinical-microbiology/url_slug:clinical-microbiology/?

6. Alcon Study Report: *In vitro* evaluation of finafloxacin from MerLion Pharmaceuticals. **Study Report# TDOC-0011241**, August 2011.
7. Alcon Study Report TDOC-0014368: Evaluation of clinical finafloxacin (AL-60371) suspension formulations in a guinea pig model of *P. aeruginosa* acute otitis externa. **Study Report# TDOC-0014368**
8. Alcon Study Report: *In vitro* evaluation of finafloxacin against clinical otic pathogens. **Study Report# TDOC0017294**, July 2013.
9. (b) (4) HealthCare Pharma Study Report: Antibacterial *In vitro* evaluation of BAY 35-3377. **Study Report# PH32656,** March 2003.
10. Alcon Study Report: *In vivo* evaluation of AL-60371 (Finafloxacin) clinical formulation. **Study Report# TDOC0017323**. October 2013.
11. Alcon Study Report: *In vivo* evaluation of AL-60371 formulations in a screening model of *P. aeruginosa* acute otitis externa. **Study Report# TD0C-0017324.**
12. (b) (4) Health Care Report: Comparative *In vitro* activity of BAY 35-3377 against defined quinolone resistant strains. **Study Report# PH-32655.**
13. MerLion Research Report: *In vitro* activity of finafloxacin against gram-negative and gram-positive pathogens and fluoroquinolone resistant strains. **Study Report# RR-2009-004** . July 21, 2009.
14. MerLion Research Report: *In vitro* activity of finafloxacin against adherent and difficult to treat populations of *Escherichia coli* and *Staphylococcus aureus*. **Study Report# RR-2009-005.** July 21, 2009.
15. MerLion Research Report: *In vitro* selection and characterization of finafloxacin resistant *Escherichia coli* and *Staphylococcus aureus.* **Study Report# RR-2009-006.** July 21, 2009.
16. MerLion Research Report: *In vitro* activity of finafloxacin against community associated methicillin resistant *Staphylococcus aureus.* **Study Report# RR-2009-007.** July 21, 2009.
17. MerLion Research Report. *In vitro* activity of finafloxacin against stationary phase and growth arrested Gram negative bacteria. **Study Report# RR-2010-002.**
18. (b) (4) Research Report: *In vivo* evaluation of BAY 35-3377 in models of bacterial infection. **Study Report# PH-32521.** December 18, 2002.
19. MerLion Research Report: *In vitro* antibacterial activity of finafloxacin under conditions simulating lower respiratory tract infection. **Study Report# RR-2010-004.** September 24, 2010.
20. Alcon Study Report: Safety and Efficacy Evaluation of Topical AL-60371 Otic suspension 0.3% in the treatment of acute otitis externa. **Study Report# CSR C10-018.**

21. Alcon Study Report: Safety and Efficacy Evaluation of Topical AL-60371 Otic suspension 0.3% in the treatment of acute otitis externa. **Study Report# CSR C10-019 – TDOC-0016451.**
22. Emrich NC., Heisig A, Stubbings W, Labischinski H and Heisig P. Antibacterial activity of finafloxacin under different pH conditions against isogenic strains of *Escherichia coli* expressing combinations of defined mechanisms of fluoroquinolone resistance. **J. Antimicrobial Chemotherapy** 2010; 65: 2530 – 2533.
23. Higgins PG, Stubbings W, Wisplinghoff H and Seifert H. Activity of the investigational fluoroquinolone finafloxacin against ciprofloxacin sensitive and – resistant *Acinetobacter baumannii* isolates. **Antimicrobial Agents and Chemotherapy** 2010; 54(4): 1613 – 1615.
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25. Alekshun MN and Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. **Antimicrobial Agents and Chemotherapy** 1997; 41: 2067–2075.
26. Robicsek A, JStrahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K and Hooper DC. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. **Nature Medicine** 2006; 12: 83-88.
27. Robicsek A, Jacoby GA, and Hooper DC. The worldwide emergence of plasmid mediated quinolone resistance. **Lancet Infectious Diseases.** 2006; 6: 629–40.

Simone M. Shurland
Clinical Microbiology Reviewer
September 26, 2014

Kerry Snow MS, MT(ASCP)
Clinical Microbiology Team Leader
26 September 2014

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/s/

SIMONE SHURLAND
09/26/2014

KERRY SNOW
09/26/2014

Product Quality Microbiology Review

September 11, 2014

NDA: 206307

Drug Product Name
Proprietary: Finafloxacin Otic Suspension
Non-proprietary: finafloxacin [AL-60371] 0.3%

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
April 25, 2014	April 25, 2014	April 25, 2014	April 29, 2014

Submission History (for 2nd Reviews or higher) – N/A

Applicant/Sponsor
Name: Alcon Research, Ltd.
Address: 6201 South Freeway, Fort Worth, TX
Representative: Richard O. Reese, Global Regulatory Manager
Tel: 817-551-4345

Name of Reviewer: Vinayak B. Pawar, Ph.D.

Conclusion: Recommend Approval

Product Quality Microbiology Data Sheet

A. 1. TYPE OF SUBMISSION: Original NDA

2. SUBMISSION PROVIDES FOR: A topical Otic suspension

3. MANUFACTURING SITE: Alcon's ASPEX Manufacturing Facility, Fort Worth, Texas.

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY: Finafloxacin 3 mg/5mL in 8 mLvial.

5. METHOD(S) OF STERILIZATION: (b) (4).

6. PHARMACOLOGICAL CATEGORY: Treatment of Acute Otitis External in pediatric population.

B. SUPPORTING/RELATED DOCUMENTS: None.

C. REMARKS: The subject Original NDA provides for a Topical Otic Suspension which contains 0.3% Finafloxacin. This application is an electronic submission.

filename: **N206307R1**

Executive Summary

I. Recommendations

A. Recommendation on Approvability – Recommend Approval.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N/A

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – After Finafloxacin (b) (4) [redacted]

B. Brief Description of Microbiology Deficiencies – None.

C. Assessment of Risk Due to Microbiology Deficiencies – N/A

D. Contains Potential Precedent Decision(s) - ☐ **Yes** ☒ **No**

III. Administrative

A. Reviewer's Signature _____
Vinayak B. Pawar, Ph.D., Sr. Review Microbiologist, OPS/CDER

B. Endorsement Block _____
Stephen E. Langille, Ph.D., Sr. Review Microbiologist, OPS/CDER

C. CC Block
N/A

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/s/

VINAYAK B PAWAR
09/17/2014

STEPHEN E LANGILLE
09/17/2014

MICROBIOLOGY FILING CHECKLIST

NDA Number:
NDA 206-307

Applicant:
Alcon Research, Ltd

Stamp Date:
4/25/2012

Drug Name:
Finafloxacin Otic Suspension 0.3%

NDA Type:
Original-1 (New NDA)

Supplement Number:
SDN-001

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

	Content Parameter	Yes	No	Comments
1	Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	**X**		
2	Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	**X**		
3	Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin?	**X**		
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?	**X**		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			N/A
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	**X**		
7	Has the applicant submitted the clinical microbiology datasets in a format which intents to correlate baseline pathogen with clinical and microbiologic outcome?	**X**		
8	Has the applicant submitted draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin?			N/A
9	Has the applicant submitted a clinical microbiology dataset in an appropriate/standardized format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome?			N/A

MICROBIOLOGY FILING CHECKLIST

	Content Parameter	Yes	No	Comments
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant included complete 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?	X		
11	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
12	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
13	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
14	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	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? X YES__NO

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

No clinical microbiology comments

Simone M. Shurland, Ph.D. — May 29, 2014
Reviewing Microbiologist — Date

Kerry Snow, MSc (ASCP)
Microbiology Team Leader — Date May 30, 2014

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/s/

SIMONE SHURLAND
06/03/2014

KERRY SNOW
06/03/2014

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 206307 **Applicant:** Alcon Research Ltd **Letter Date:** April 25, 2014

Drug Name: Finafloxacin Otic Suspension, 0.3% **NDA Type:** Original **Stamp Date:** April 25, 2014

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		Section 3.2.P.3.3, Flow Chart Figure 3.2.P.3.3-1.
3	Has the applicant submitted protocols and results of validation studies concerning microbiological control processes used in the manufacture of the drug product?	X		Section 3.2.P.3.5.3 - 3.2.P.3.5.7
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		Validations: (b) (4) - TR TDOC-0014658 (b) (4) - TR TDOC-0003385
6	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		Section 3.2.P.5.1
7	Has the applicant submitted the results of analytical method verification studies?	X		Various Technical Reports.
8	Has the applicant submitted all special/critical studies/data requested during pre-submission meetings and/or discussions?			N/A
9	If sterile, are extended post-constitution and/or post-dilution hold times in the draft labeling supported by microbiological data?			N/A
10	Is this NDA fileable? If not, then describe why.	X		

Additional Comments: The drug product is a sterile otic suspension for topical use in the ear.

Vinayak B. Pawar, Ph.D., Senior Review Microbiologist **Date**

Stephen E. Langille, Ph.D., Senior Review Microbiologist **Date**

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/s/

VINAYAK B PAWAR
05/28/2014

STEPHEN E LANGILLE
05/28/2014