CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207986Orig1s000

MICROBIOLOGY/VIROLOGY REVIEW(S)

Division of Anti-Infective Products
Clinical Microbiology Review

TYPE OF SUBMISSION:
505(b)(2)-New Drug Application (NDA)

NDA:	**207986**
Date Company Submitted:	2/25/2015
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Reviewer:	Jalal Sheikh, Ph.D.

NAME AND ADDRESS OF APPLICANT:
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CONTACT PERSON:
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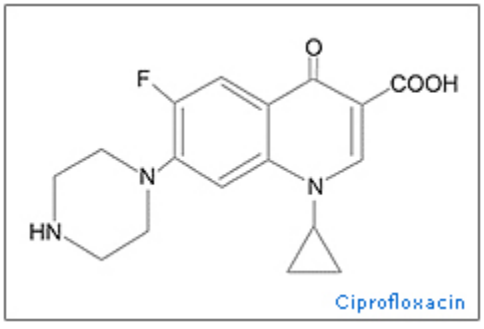
DRUG PRODUCT NAMES:
Proprietary Name: Otiprio is the proposed name approved by FDA but OTO-201 was used during the drug development process. Therefore Otiprio and OTO-201has been used interchangeably throughout this review.
Established Name: Ciprofloxacin otic solution, 6%
Chemical Name: Ciprofloxacin**:** 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride, monohydrate combined in a suspension with 12% Poloxamer 407
Molecular Formula: Ciprofloxacin: $C_{28}H_{39}N_3O_7$, (b) (4)
Molecular Weight: (b) (4)
Structural Formula: Ciprofloxacin



DRUG CATEGORY: Anti-bacterial

PROPOSED INDICATION:
Otiprio is indicated for the treatment of middle ear effusion in pediatric patients with otitis media who are undergoing tympanostomy tube (TT) placement.

PROPOSED DOSAGE FORM AND STRENGTH:
The drug product of this NDA, Otiprio, is an (b) (4) otic suspension of 6% (60 mg/mL, w/v) ciprofloxacin in a neutral pH buffered, isotonic solution containing a mucoadhesive glycol polymer, poloxamer 407. Poloxamer has thermo-sensitive properties. It exists as a liquid at room temperature or below, and transforms into a gel when exposed to body temperature in the middle ear.
Otiprio has been designed as a single administration and the recommended dose is 0.1 mL (6 mg) in each ear at the time of surgery. It is available in a single-patient use glass vial containing 1 mL of the otic suspension.

ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
Otiprio is an otic suspension intended to be used as a single intratympanic administration of 0.1 mL into each affected ear following suctioning of the middle ear effusion.

DISPENSED:
Otiprio is a Prescription Product.

RELATED DOCUMENTS:
PIND/IND 110244 - submitted on 10/18/2010; IND (b) (4) - submitted 6/12/2009

REMARKS/ PURPOSE OF SUBMISSION:
The applicant in this submission is seeking approval to market Otiprio, a 6% Ciprofloxacin otic suspension for the treatment of middle ear effusion in pediatric patients with otitis media those are undergoing with TT placement. The applicant has conducted two pivotal Phase 3 efficacy studies in support of the safety and efficacy of Otiprio otic suspension in pediatric subjects of both sexes aged from 6 months to 17 years.

SUMMARY AND RECOMMENDATIONS:
From a clinical microbiology perspective, the information provided by the Applicant supports the efficacy of Otiprio, a 6% ciprofloxacin otic suspension for the treatment of middle ear effusion in pediatric patients with otitis media due to *H influenzae, S pneumoniae,* and *M catarrhalis*. In addition, the applicant stated that the Otiprio is also active against otic infections caused by *P. aeruginosa* and *S. aureus*. However, clinical efficacy for both organisms was studied in fewer than 10 infections. Please refer to the Section 6 of this review for the proposed labeling of the clinical microbiology subsection of the Otiprio package insert.

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EXECUTIVE SUMMARY

Indications

The applicant for this NDA submission, Otonomy Inc. is seeking approval to market Otiprio, a ciprofloxacin otic suspension for the treatment of middle ear effusion in pediatric patients with otitis media those are undergoing with tympanostomy tube (TT) placement.

Otiprio is a suspension of 6% ciprofloxacin in buffered solution containing a glycol polymer, poloxamer 407. At the concentration used in the Otiprio formulation, the (b)(4)% poloxamer 407 exhibits thermoreversible properties. It exists as a liquid at room temperature and converts to a gel polymer after administration into the middle ear due to exposure to the body temperature.

Antimicrobial Spectrum of Activity

Otiprio has been proposed to use to treat the most commonly found bacterial pathogens in otitis media e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*.[1] However, the applicant proposed to treat additional otic pathogens, *P aeruginosa* and *S aureus* where clinical efficacies for these organisms were studied in fewer than 10 infections.

Mechanism of action

This NDA is a 505(b)(2) submission and relies on prior nonclinical safety information of FDA-approved ciprofloxacin tablets, NDA019537 (Cipro® Tablets Prescribing Information). [2]

Ciprofloxacin is a broad-spectrum anti-bacterial agent of the fluoroquinolone class. The bactericidal action of ciprofloxacin involves with the inhibition of the enzymes topoisomerase II, also known as DNA gyrase and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling, and recombination. Ciprofloxacin has *in vitro* activity against a wide range of both gram-positive and gram-negative microorganisms. Cross-resistance has been observed between ciprofloxacin and other fluoroquinolones drugs but generally no cross-resistance has been observed between ciprofloxacin and other classes of antibacterial agents.

Surveillance Data

A surveillance study was conducted to understand the trends regarding bacterial susceptibility/resistance of relevant pathogens to ciprofloxacin and other flouroquinolone drugs. The applicant provided antimicrobial susceptibility testing (AST) surveillance data for ciprofloxacin and levofloxacin from 2008 to 2010. Resistance to either ciprofloxacin or levofloxacin was rare among *S. pneumoniae* and *H. influenzae*. Little data was available for *M. catarrhalis* to evaluate the resistance profile to either ciprofloxacin or levofloxacin. However, *M. catarrhalis* isolates were 100% susceptible to both ciprofloxacin and levofloxacin based on published literature.[3] Increased resistance to both ciprofloxacin and levofloxacin was observed for *P. aeruginosa* and *S. aureus* isolates.

Bactericidal Activity
Bactericidal activity of Ciprofloxacin was conducted by measuring time-kill assessments of recent bacterial isolates (collected 2010-2012) recovered from otic infections. The applicant conducted studies to determine the bactericidal activity of ciprofloxacin against the key otic pathogens *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* by using different concentrations that were multiples of the ciprofloxacin minimal inhibitory concentration (MIC) for each strain tested. Bactericidal activity was defined as a ≥ 3-log reduction in cfu/ml relative to the initial inoculum size. Based on time kill analysis, Ciprofloxacin demonstrated bactericidal activity with concentrations of 4 and 8X of the MICs. However, bactericidal activity was only achieved with higher MIC concentrations for some isolates. Based on these findings, the applicant demonstrated that ciprofloxacin perform as a bactericidal agent against target otic pathogens.

Animal Studies
The applicant demonstrated the efficacy of Otiprio in a Chinchilla animal model. Otiprio was shown to be effective in reducing middle ear effusion volume and also eradicated middle ear bacterial counts in a Chinchila animal model of acute OME. The applicant demonstrated that following middle ear drainage and ventilation tube placement, recurrence of otitis media at Day 6 was significantly reduced by a single intratympanic administration of various doses of Otiprio compared to vehicle-treated chinchillas. The applicant also demonstrated that a single intratympanic administration of Otiprio at and above 0.6% (doses ranged from 0.06% to 6.0%) was as effective in reducing bacterial load and effusion of the middle ear effusion following treatment regimen of the comparator drugs, Cetraxal and Ciprodex.

Clinical Trials
The applicant, Otonomy completed two randomized, prospective, double-blind, sham-controlled Phase 3 clinical trials with identical protocols among pediatric subjects male or female aged from 6 months to 17 years at approximately 55 centers in the United States and Canada. Both trials enrolled a total of 532 randomized subjects comprising 357 randomized to Otiprio 6 mg group and 175 randomized to the sham group. The primary efficacy endpoint for both studies was the cumulative proportion of subjects designated as study treatment failures through the Day 15 Visit.

Results from both Phase 3 clinical trials demonstrated that Otiprio achieved the primary efficacy endpoint, a significant association between treatment and treatment failure, $p < 0.001$. The data from these 2 independent Phase 3 studies support the efficacy of Otiprio in the treatment of middle ear effusion in pediatric subjects with otitis media requiring TT placement.

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

12.4 Microbiology

Mechanism of Action
The bactericidal action of ciprofloxacin results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA.

Resistance
Bacterial resistance to fluoroquinolones can develop through chromosomally- or plasmid-mediated (b) (4) [redacted] *In vitro* studies demonstrated cross-resistance between ciprofloxacin and some fluoroquinolones. There is generally no cross-resistance between ciprofloxacin and other classes of antibacterial agents, such as beta-lactams or aminoglycosides.

Antimicrobial Activity
Ciprofloxacin has been shown to be active against most isolates of the following bacteria (b) (4) [redacted]

Gram-positive Bacteria
- *Staphylococcus aureus*
- *Streptococcus pneumonia*

Gram-negative Bacteria
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Pseudomonas aeruginosa*

(b) (4) [redacted]

1. INTRODUCTION

The subject of this NDA is Otiprio, a 6% ciprofloxacin otic suspension intended for the treatment of middle ear effusion in pediatric patients with otitis media who are undergoing with tympanostomy tube (TT) placement.

Otitis media is one of the most common infections in children in the United States.[3] Standard of care for acute otitis media generally involves the administration of oral antibiotics.[3] However, 30-40% of children will progress to chronic or recurrent otitis media with effusion (OME).[4] The most commonly found bacterial species in otitis media are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis.*[1] If OME is not appropriately treated, the disease can progress to more complications e.g., irreversible hearing loss, delays in speech, hindering language and learning development and can lead to severe conditions including mastoiditis and meningitis.

Children with chronic or recurrent OME who do not respond to oral antibiotics commonly require surgical intervention with TT placement. This procedure is applied to clear the infection and to avoid further complications. About one million surgeries are performed each year for the insertion of TT placement in pediatric patients in the United States.[5]

There are limited approved drug therapies available in the United States for the treatment of OME at the time of TT placement. It has been found that topical quinolone antibiotics are more effective than topical non-quinolone antibiotics in treating aural discharge.[6] The applicant, Otonomy Inc. has developed the drug product, Otiprio, for the treatment of pediatric patients with otitis media who are undergoing TT placement.

The applicant conducted two Phase 3 clinical trials among 532 subjects comprising 357 subjects randomized to the Otiprio 6 mg group and 175 subjects randomized to the sham group. In both Phase 3 studies, Otiprio was found to be both clinically and statistically superior compared to sham with regard to the primary endpoint of study treatment failure through Day 15.

1.1 Regulatory History

PIND/IND 110244 - submitted on 10/18/2010; IND (b) (4) - submitted 6/12/2009

2. *IN VITRO* ACTIVITY

2.1 Mechanism of Action

The drug product Otiprio is an otic suspension of ciprofloxacin which is a broad-spectrum anti-infective agent of the fluoroquinolone class. The bactericidal action of ciprofloxacin results from the inhibition of enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling, and

recombination. DNA gyrase is a heterotetrameric enzyme (A2B2) consisting of two subunits, GyrA and GyrB encoded by the *gryA* and *gyrB* genes. Like DNA gyrase, topoisomerase IV enzymes is also composed of two subunits, the *parC* and *parE* genes, respectively. Because of this bactericidal mechanism of action, ciprofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms.

2.2 In Vitro Susceptibility Analysis: Surveillance Study (b) (4) Report: 2008-2010)

The applicant submitted the *in vitro* susceptibility profile of ciprofloxacin and levofloxacin, against major otic pathogens. These data were extracted from (b) (4) electronic database known as the (b) (4) The surveillance data are collected from 150 clinical laboratories in the United States. The applicant submitted summarized susceptibility data of Ciprofloxacin and Levofloxacin from 2008 to 2010 against target otic pathogens of *H. influenzae, M. catarrhalis, S. pneumoniae, P. aeruginosa*, and *S. aureus*. Based on (b) (4) data, susceptibility of bacterial isolates from the United States to either ciprofloxacin or levofloxacin has been stable during the period 2008 to 2010.

Resistance to either ciprofloxacin or levofloxacin was rare among *S. pneumoniae* and *H. influenzae*. Little data was presented for *M. catarrhalis* to evaluate the resistance profile to either ciprofloxacin or levofloxacin through (b) (4). However, *M. catarrhalis* isolates were 100% susceptible to both ciprofloxacin and levofloxacin based on published literature.[6] Increased resistance was observed for *P. aeruginosa* and *S. aureus* isolates to both ciprofloxacin and levofloxacin. However, both pathogens are usually recovered from otitis externa (OE).

The sources of the isolates recovered from different specimens are listed in Appendix1.

Appendix 1. Distribution (%) of Ciprofloxacin Results by (b) (4) Specimen Sources, 2008 - 2010

TSN Specimen Source	*H. influenzae* %	*M. catarrhalis* %	*S. pneumoniae* %	*P. aeruginosa* %	*S. aureus* %
Bronch. Brush Biopsy	3.2			0.6	0.4
Bronchial Alveolar Lavage	18.1	14.3	14.8	5.1	4.2
Bronchial Washing	3.3	14.3	1.8	3.5	3.8
CF Sputum / Bronchial	0.1			7.6	2.3
CF Upper Respiratory				0.1	0.2
Chest Tube				0.1	0.1
Ear, External	12.6		1.8	3.7	5.3
Ear, Internal	1.0		0.6	0.3	0.4
Lung	1.0			0.3	0.4
Mandible					0.1
Maxilla				0.3	0.4
Nasopharynx	6.8	7.1	0.6	0.2	1.9
Nose	2.3	28.6		0.4	12.0
Oral				0.1	1.2
Pleural Biopsy	0.1		4.1		
Pleural Fluid	0.3		0.6	0.3	0.8
Sinus	2.9	7.1	4.1	2.9	4.8
Sputum	34.0	28.6		53.8	41.7
Thoracentesis Fluid	0.1				0.1
Throat	4.1		5.3	5.5	9.8
Tonsil	0.1				0.1
Tracheal Aspirate	9.0		66.3	13.7	8.4
Tracheostomy				0.8	0.7
Transtracheal Aspirate	1.0			0.6	0.9

Interpretive criteria for ciprofloxacin and levofloxacin were defined based on FDA breakpoints with the exception of *M. catarrhalis* where CLSI M100 S25[7] breakpoints were applied. These breakpoints are established based on systemic administration of the drugs. Bacterial isolates were classified as susceptible, intermediate or resistant based on interpretive criteria, or breakpoints. Breakpoints utilized for analysis are listed in Table 1.

Table 1. Interpretive Criteria (µg/mL) Applied to (b) (4) Data

	Ciprofloxacin			Levofloxacin		
	S	I	R	S	I	R
H. influenzae	≤1	NA	NA	≤2	NA	NA
M. catarrhalis	≤1	NA	NA	≤2	NA	NA
S. pneumoniae	≤1	2	≥4	≤2	4	≥8
S. aureus	≤1	2	≥4	≤2	4	≥8
P. aeruginosa	≤1	2	≥4	≤2	4	≥8

NA: not applicable; no breakpoints available for interpretation
S: susceptible, I: intermediate, R: resistant

The susceptibility profiles of ciprofloxacin and levofloxacin against target pathogens associated with ear and other respiratory infections were compiled by covering three years of data, 2008-2010 and presented in Table 2.

Table 2. Categorical Interpretation for Ciprofloxacin and Levofloxacin from Ear and Other Respiratory Specimens, 2008 - 2010

		Ciprofloxacin			
			%S/I/R		
Organism	**Specimen Sources**	**Totals**	**S**	**I**	**R**
H. influenzae	Ear	107	100.0	--[a]	--[a]
	Other Respiratory	682	100.0	--[a]	--[a]
M. catarrhalis	Other Respiratory	14	100.0	--[a]	--[a]
S. pneumoniae	Ear	4	75.0	25.0	0.0
	Other Respiratory	165	42.4	55.2	2.4
P. aeruginosa	Ear	3009	87.9	4.0	8.1
	Other Respiratory	71649	63.1	11.1	25.7
S. aureus	Ear	2683	67.1	1.6	31.3
	Other Respiratory	44438	55.6	2.3	42.1

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant

S: susceptible, I: intermediate, R: resistant

		Levofloxacin			
			%S/I/R		
Organism	**Specimen Sources**	**Totals**	**S**	**I**	**R**
H. influenzae	Ear	32	100.0	--[a]	--[a]
	Other Respiratory	2263	99.9	--[a]	--[a]
M. catarrhalis	Other Respiratory	19	100.0	--[a]	--[a]
S. pneumoniae	Ear	894	99.9	0.1	0.0
	Other Respiratory	11754	97.8	0.5	1.8
P. aeruginosa	Ear	2394	84.7	6.2	9.1
	Other Respiratory	52454	58.6	10.2	31.2
S. aureus	Ear	4142	68.8	10.0	21.1
	Other Respiratory	76743	56.5	8.8	34.6

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant

S: susceptible, I: intermediate, R: resistant

The categorical interpretation of ciprofloxacin and levofloxacin susceptibility profiles against isolates recovered from ear and other respiratory specimens are presented yearly from 2008, 2009, and 2010 in Table 3 and Table 4.

Table 3. Categorical Interpretation by Year for Ciprofloxacin from Ear and Other Respiratory Specimens, 2008 - 2010

		Ciprofloxacin 2008			
			%S/I/R		
Organism	**Specimen Sources**	**Totals**	**S**	**I**	**R**
H. influenzae	Ear	45	100.0	–[a]	–[a]
	Other Respiratory	308	100.0	–[a]	–[a]
M. catarrhalis	Other Respiratory	10	100.0	–[a]	–[a]
S. pneumoniae	Ear	2	100.0	0.0	0.0
	Other Respiratory	77	31.2	64.9	3.9
P. aeruginosa	Ear	1126	87.8	3.5	8.7
	Other Respiratory	26800	63.1	10.7	26.2
S. aureus	Ear	823	66.2	2.2	31.6
	Other Respiratory	15801	52.8	1.7	45.5

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant

S: susceptible, I: intermediate, R: resistant

		Ciprofloxacin 2009			
			%S/I/R		
Organism	**Specimen Sources**	**Totals**	**S**	**I**	**R**
H. influenzae	Ear	30	100.0	–[a]	–[a]
	Other Respiratory	224	100.0	–[a]	–[a]
M. catarrhalis	Other Respiratory	2	100.0	–[a]	–[a]
S. pneumoniae	Ear	2	50.0	50.0	0.0
	Other Respiratory	68	45.6	54.4	0.0
P. aeruginosa	Ear	940	87.7	4.1	8.2
	Other Respiratory	22804	63.1	11.2	25.7
S. aureus	Ear	755	67.4	1.9	30.7
	Other Respiratory	13041	56.5	2.8	40.6

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant

S: susceptible, I: intermediate, R: resistant

		Ciprofloxacin 2010			
			%S/I/R		
Organism	**Specimen Sources**	**Totals**	**S**	**I**	**R**
H. influenzae	Ear	32	100.0	–[a]	–[a]
	Other Respiratory	150	100.0	–[a]	–[a]
M. catarrhalis	Other Respiratory	2	100.0	–[a]	–[a]
S. pneumoniae	Ear	0	0.0	0.0	0.0
	Other Respiratory	20	75.0	20.0	5.0
P. aeruginosa	Ear	943	88.1	4.6	7.3
	Other Respiratory	22045	63.2	11.6	25.2
S. aureus	Ear	1105	67.4	1.0	31.6
	Other Respiratory	15596	57.7	2.5	39.8

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant

S: susceptible, I: intermediate, R: resistant

Table 4. Categorical Interpretation by Year for Levofloxacin from Ear and Other Respiratory Specimens, 2008 - 2010

		Levofloxacin 2008			
			%S/I/R		
Organism	Specimen Sources	Totals	S	I	R
H. influenzae	Ear	16	100.0	--[a]	--[a]
	Other Respiratory	982	99.9	--[a]	--[a]
M. catarrhalis	Other Respiratory	12	100.0	--[a]	--[a]
S. pneumoniae	Ear	363	100.0	0.0	0.0
	Other Respiratory	4307	98.0	0.5	1.5
P. aeruginosa	Ear	904	87.8	3.3	8.8
	Other Respiratory	20973	61.0	9.3	29.7
S. aureus	Ear	1398	68.6	9.2	22.2
	Other Respiratory	28588	54.8	8.6	36.5

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant
S: susceptible, I: intermediate, R: resistant

		Levofloxacin 2009			
			%S/I/R		
Organism	Specimen Sources	Totals	S	I	R
H. influenzae	Ear	10	100.0	--[a]	--[a]
	Other Respiratory	707	99.9	--[a]	--[a]
M. catarrhalis	Other Respiratory	4	100.0	--[a]	--[a]
S. pneumoniae	Ear	234	100.0	0.0	0.0
	Other Respiratory	3787	98.1	0.5	1.3
P. aeruginosa	Ear	709	86.0	4.7	9.3
	Other Respiratory	16750	57.8	10.4	31.8
S. aureus	Ear	1328	68.1	9.9	22.1
	Other Respiratory	24443	57.2	8.8	34.0

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant
S: susceptible, I: intermediate, R: resistant

		Levofloxacin 2010			
			%S/I/R		
Organism	Specimen Sources	Totals	S	I	R
H. influenzae	Ear	6	100.0	--[a]	--[a]
	Other Respiratory	574	99.8	--[a]	--[a]
M. catarrhalis	Other Respiratory	3	100.0	--[a]	--[a]
S. pneumoniae	Ear	297	99.7	0.3	0.0
	Other Respiratory	3660	97.1	0.4	2.5
P. aeruginosa	Ear	781	79.9	11.0	9.1
	Other Respiratory	14731	56.1	11.1	32.7
S. aureus	Ear	1416	69.8	10.9	19.3
	Other Respiratory	23712	57.9	9.1	33.0

[a] dashed line indicates CLSI/FDA criteria not available for interpretation of intermediate or resistant
S: susceptible, I: intermediate, R: resistant

2.3 In Vitro Activity and MIC Distributions of Fluoroquinolones (APPLICANT REPORT: 2010-2012)

Otonomy conducted microbiological studies to demonstrate the *in vitro* activity and MIC distributions of ciprofloxacin, and other fluoroquinolone comparator antibiotics, against isolates of targeted otic pathogens namely *S. pneumoniae, H. influenzae, M. catarrhalis, P. aeruginosa,* and *S. aureus*.

The purpose of this study was to profile the *in vitro* activity of ciprofloxacin and other commonly used antibacterial drugs against at least 100 isolates of each target otic pathogens, including those with important resistance phenotypes. Minimum inhibitory concentrations (MICs) were determined by standard microbiological techniques and investigator's SOPs.[7, 8, 9] In addition, susceptibility was also assessed against ampicillin, oxacillin and penicillin those are frequently used for the treatment of ear infections. The test panel consisted of recent clinical isolates from otic or respiratory sources of varied geographical locations (US, EU, Asia), preferably isolated in recent 3 years. Isolates were recovered from specimens collected between 2010 and 2012. Table 5 summarizes the geographical distribution of the organisms tested.

Table 5: Geographic Distribution of the Clinical Isolates Tested

Organism	Total N	N by Geographic Region			
		US	Europe	Asia	ROW
S. aureus	150	106	22	22	0
S. pneumoniae	148	86	27	28	7
P. aeruginosa	150	100	21	29	0
M. catarrhalis	104	0	74	26	4
H. influenzae	149	45	47	57	0
TOTALS:	**701**	**337**	**191**	**162**	**11**

Table 6 summarizes the MIC ranges of all antibiotics tested against the targeted pathogens.

Table 6: Antibiotics Tested Against the Various Targeted Otic Pathogens

Antibiotic	Organism / MIC Range Tested (µg/mL)				
	S.aureus	*S.pneumoniae*	*P.aeruginosa*	*M.catarrhalis*	*H.influenzae*
Ciprofloxacin	0.015-128	0.002-8	0.008-128	0.015-128	0.002-8
Levofloxacin	0.015-128	0.002-8	0.008-128	0.015-128	0.002-8
Ofloxacin	0.015-128	0.002-8	0.008-128	0.015-128	0.002-8
Azithromycin	0.12-4	0.03-4	-	0.12-4	0.03-4
Amoxicillin	0.12-1	0.015-4	-	0.12-1	0.015-4
Amoxicillin-Clav	0.06-4	0.015-16	-	0.06-4	0.015-16
Cefuroxime	0.25-16	0.12-8	-	0.25-16	0.12-8
Oxacillin	0.06-4	-	-	-	-
Trimeth/Sulfa	0.25-2	0.015-2	0.25-32	0.25-2	0.015-2
Gentamicin	0.06-8	-	0.06-8	-	-
Penicillin	0.12-2	0.06-4	-	0.12-2	-
Ampicillin	-	0.5-8	-	-	0.5-8

The applicant also determined the MIC_{90} (Minimal Inhibitory Concentration required to inhibit the growth of 90% of the isolates) of tested antibacterial drugs against target otic pathogens. The susceptibility patterns of all target otic pathogens to ciprofloxacin, levofloxacin, ofloxacin, macrolides and β-lactams are presented in a tabular format in Tables 7-16.

2.3.1 Susceptibility of *S. aureus* (Tables 7 and 8)

The ciprofloxacin MIC_{90} was 128 µg/ml for all MRSA isolates. The majority of MRSA (83.8%) isolates were non-susceptible to ciprofloxacin. Similarly, high MICs were observed for other quinolones, e.g., levofloxacin and ofloxacin. This high rate of fluoroquinolone resistance among MRSA has been well described in published literature and is not unexpected. In contrast, MIC range was low (0.06 - 1 µg/ml) for MSSA isolates against all three quinolones tested.

Table 7 Activity of (MIC in µg/mL) Ciprofloxacin and Comparators against *S. aureus* According to the Phenotypes

Organism	Drug	Phenotype	Total N	Range	Mode	MIC 50	MIC 80	nS	%S	nI	%I	nR	%R
S. aureus	CIPROFLOXACIN	All	150	0.06->128	0.25	0.25	128	82	54.7	0	0	68	45.3
		CIP NS	68	4->128	128	64	>128	0	0	0	0	68	100
		CIP S	82	0.06-1	0.25	0.25	0.25	82	100	0	0	0	0
		MRSA	74	0.12->128	128	64	>128	12	16.2	0	0	62	83.8
		MSSA	76	0.06-64	0.25	0.25	1	70	92.1	0	0	6	7.9
	LEVOFLOXACIN	All	150	0.06->128	0.12	0.25	32	83	55.3	1	0.7	66	44
		CIP NS	68	0.5->128	32	16	>128	1	1.5	1	1.5	66	97.1
		CIP S	82	0.06-0.5	0.12	0.12	0.12	82	100	0	0	0	0
		MRSA	74	0.06->128	32	16	>128	12	16.2	0	0	62	83.8
		MSSA	76	0.06-32	0.12	0.12	0.5	71	93.4	1	1.3	4	5.3
	OFLOXACIN	All	150	0.12->128	0.25	0.5	64	83	55.3	0	0	67	44.7
		CIP NS	68	1->128	64	32	>128	1	1.5	0	0	67	98.5
		CIP S	82	0.12-1	0.25	0.25	0.5	82	100	0	0	0	0
		MRSA	74	0.12->128	64	32	>128	12	16.2	0	0	62	83.8
		MSSA	76	0.12-64	0.25	0.25	1	71	93.4	0	0	5	6.6
	AMOXICILLIN	All	150	≤0.12->1	>1	>1	>1	0	0	0	0	0	0
		CIP NS	68	≤0.12->1	>1	>1	>1	0	0	0	0	0	0
		CIP S	82	≤0.12->1	>1	>1	>1	0	0	0	0	0	0
		MRSA	74	0.5->1	>1	>1	>1	0	0	0	0	0	0
		MSSA	76	≤0.12->1	>1	>1	>1	0	0	0	0	0	0
	AMOXICILLIN/ CLAVULANATE	All	150	0.12->4	>4	1	>4	102	68	0	0	48	32
		CIP NS	68	0.12->4	>4	>4	>4	27	39.7	0	0	41	60.3
		CIP S	82	0.12->4	1	0.5	4	75	91.5	0	0	7	8.5
		MRSA	74	0.25->4	>4	>4	>4	26	35.1	0	0	48	64.9
		MSSA	76	0.12-1	1	0.5	1	76	100	0	0	0	0
Organism	Drug	Phenotype	Total N	Range	Mode	MIC 50	MIC 80	nS	%S	nI	%I	nR	%R
S. aureus	AZITHROMYCIN	All	150	0.5->4	>4	>4	>4	64	42.7	0	0	86	57.3
		CIP NS	68	1->4	>4	>4	>4	6	8.8	0	0	62	91.2
		CIP S	82	0.5->4	1	1	>4	58	70.7	0	0	24	29.3
		MRSA	74	1->4	>4	>4	>4	6	8.1	0	0	68	91.9
		MSSA	76	0.5->4	1	1	>4	58	76.3	0	0	18	23.7
	CEFUROXIME/ AXETIL	All	150	0.5->16	1	2	>16	108	72	8	5.3	34	22.7
		CIP NS	68	0.5->16	>16	8	>16	32	47.1	3	4.4	33	48.5
		CIP S	82	0.5->16	1	1	4	76	92.7	5	6.1	1	1.2
		MRSA	74	1->16	>16	8	>16	32	43.2	8	10.8	34	45.9
		MSSA	76	0.5-2	1	1	1	76	100	0	0	0	0
	GENTAMICIN	All	150	≤0.06->8	0.25	0.25	>8	124	82.7	0	0	26	17.3
		CIP NS	68	≤0.06->8	0.25	0.25	>8	47	69.1	0	0	21	30.9
		CIP S	82	≤0.06->8	0.25	0.25	0.5	77	93.9	0	0	5	6.1
		MRSA	74	≤0.06->8	0.25	0.25	>8	49	66.2	0	0	25	33.8
		MSSA	76	≤0.06->8	0.25	0.25	0.5	75	98.7	0	0	1	1.3
	OXACILLIN	All	150	0.12->4	>4	2	>4	76	50.7	0	0	74	49.3
		CIP NS	68	0.25->4	>4	>4	>4	6	8.8	0	0	62	91.2
		CIP S	82	0.12->4	0.25	0.5	>4	70	85.4	0	0	12	14.6
		MRSA	74	4->4	>4	>4	>4	0	0	0	0	74	100
		MSSA	76	0.12-2	0.25	0.25	1	76	100	0	0	0	0
	PENICILLIN	All	150	≤0.12->2	>2	>2	>2	25	16.7	0	0	125	83.3
		CIP NS	68	≤0.12->2	>2	>2	>2	5	7.4	0	0	63	92.6
		CIP S	82	≤0.12->2	>2	>2	>2	20	24.4	0	0	62	75.6
		MRSA	74	≤0.12->2	>2	>2	>2	1	1.4	0	0	73	98.6
		MSSA	76	≤0.12->2	>2	1	>2	24	31.6	0	0	52	68.4
	TRIMETH/ SULFA	All	150	≤0.25->2	≤0.25	≤0.25	≤0.25	148	98.7	0	0	2	1.3
		CIP NS	68	≤0.25->2	≤0.25	≤0.25	0.5	66	97.1	0	0	2	2.9
		CIP S	82	≤0.25-0.5	≤0.25	≤0.25	≤0.25	82	100	0	0	0	0
		MRSA	74	≤0.25->2	≤0.25	≤0.25	0.5	72	97.3	0	0	2	2.7
		MSSA	76	≤0.25-0.5	≤0.25	≤0.25	≤0.25	76	100	0	0	0	0

Table 8 MIC Distribution (µg/mL) of Ciprofloxacin and Fluoroquinolone Comparators Against *S. aureus* According to the Phenotypes

Organism	Drug	Phenotype		Total	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
S. aureus	CIPROFLOXACIN	All	Total N	150			2	27	46	4	3		1	9	6	5	18	20	9
			%	100			1.3	18	30.7	2.7	2		0.7	6	4	3.3	12	13.3	6
			Cumulative %	100			1.3	19.3	50	52.7	54.7		55.3	61.3	65.3	68.7	80.7	94	100
		CIP NS	Total N	68									1	9	6	5	18	20	9
			%	100									1.5	13.2	8.8	7.4	26.5	29.4	13.2
			Cumulative %	100									1.5	14.7	23.5	30.9	57.4	86.8	100
		CIP S	Total N	82			2	27	46	4	3								
			%	100			2.4	32.9	56.1	4.9	3.7								
			Cumulative %	100			2.4	35.4	91.5	96.3	100								
		MRSA	Total N	68									1	9	6	5	18	20	9
			%	100									1.5	13.2	8.8	7.4	26.5	29.4	13.2
			Cumulative %	100									1.5	14.7	23.5	30.9	57.4	86.8	100
		MSSA	Total N	82			2	27	46	4	3								
			%	100			2.4	32.9	56.1	4.9	3.7								
			Cumulative %	100			2.4	35.4	91.5	96.3	100								

Organism	Drug	Phenotype		Total	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
S. aureus	LEVOFLOXACIN	All	Total N	150			8	66	3	6		1	7	12	15	20	2	2	8
			%	100			5.3	44	2	4		0.7	4.7	8	10	13.3	1.3	1.3	5.3
			Cumulative %	100			5.3	49.3	51.3	55.3		56	60.7	68.7	78.7	92	93.3	94.7	100
		CIP NS	Total N	68						1		1	7	12	15	20	2	2	8
			%	100						1.5		1.5	10.3	17.6	22.1	29.4	2.9	2.9	11.8
			Cumulative %	100						1.5		2.9	13.2	30.9	52.9	82.4	85.3	88.2	100
		CIP S	Total N	82			8	66	3	5									
			%	100			9.8	80.5	3.7	6.1									
			Cumulative %	100			9.8	90.2	93.9	100									
		MRSA	Total N	68						1		1	7	12	15	20	2	2	8
			%	100						1.5		1.5	10.3	17.6	22.1	29.4	2.9	2.9	11.8
			Cumulative %	100						1.5		2.9	13.2	30.9	52.9	82.4	85.3	88.2	100
		MSSA	Total N	82			8	66	3	5									
			%	100			9.8	80.5	3.7	6.1									
			Cumulative %	100			9.8	90.2	93.9	100									

Organism	Drug	Phenotype		Total	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
S. aureus	OFLOXACIN	All	Total N	150				5	67	6	5		1	8	12	14	20	3	9
			%	100				3.3	44.7	4	3.3		0.7	5.3	8	9.3	13.3	2	6
			Cumulative %	100				3.3	48	52	55.3		56	61.3	69.3	78.7	92	94	100
		CIP NS	Total N	68							1		1	8	12	14	20	3	9
			%	100							1.5		1.5	11.8	17.6	20.6	29.4	4.4	13.2
			Cumulative %	100							1.5		2.9	14.7	32.4	52.9	82.4	86.8	100
		CIP S	Total N	82				5	67	6	4								
			%	100				6.1	81.7	7.3	4.9								
			Cumulative %	100				6.1	87.8	95.1	100								
		MRSA	Total N	68							1		1	8	12	14	20	3	9
			%	100							1.5		1.5	11.8	17.6	20.6	29.4	4.4	13.2
			Cumulative %	100							1.5		2.9	14.7	32.4	52.9	82.4	86.8	100
		MSSA	Total N	82				5	67	6	4								
			%	100				6.1	81.7	7.3	4.9								
			Cumulative %	100				6.1	87.8	95.1	100								

2.3.2 Susceptibility of *S. pneumoniae* (Tables 9 and 10)

The ciprofloxacin MIC_{90} was 1 µg/ml for all *S pneumoniae* isolates. The overall ciprofloxacin MIC range for all strains was 0.12 - > 8 µg/ml. Ciprofloxacin was highly active against *S. pneumoniae*.

Table 9 Activity of (MIC in µg/mL) Ciprofloxacin and Comparators Against *S. pneumoniae* According to the Phenotypes

Organism	Drug	Phenotype	Total N	Range	Mode	MIC 60	MIC 90	nS	%S	nI	%I	nR	%R
S. pneumoniae	CIPROFLOXACIN	All	148	0.12->8	0.5	0.5	1	–[1]	–	–	–	–	–
		LVX NS	7	>8->8	>8	NA	NA	0	0	0	0	0	0
		LVX S	141	0.12-4	0.5	0.5	1	0	0	0	0	0	0
		PEN NS	8	0.5->8	0.5	NA	NA	0	0	0	0	0	0
		PEN S	140	0.12->8	0.5	0.5	1	0	0	0	0	0	0
	LEVOFLOXACIN	All	148	0.25->8	0.5	0.5	1	141	95.3	1	0.7	6	4.1
		LVX NS	7	4->8	>8	NA	NA	0	0	1	14.3	6	85.7
		LVX S	141	0.25-2	0.5	0.5	0.5	141	100	0	0	0	0
		PEN NS	8	0.5->8	0.5	NA	NA	7	87.5	0	0	1	12.5
		PEN S	140	0.25->8	0.5	0.5	1	134	95.7	1	0.7	5	3.6
	OFLOXACIN	All	148	0.5->8	1	1	2	140	94.6	1	0.7	7	4.7
		LVX NS	7	8->8	>8	NA	NA	0	0	0	0	7	100
		LVX S	141	0.5-4	1	1	2	140	99.3	1	0.7	0	0
		PEN NS	8	1->8	1	NA	NA	7	87.5	0	0	1	12.5
		PEN S	140	0.5->8	1	1	2	133	95	1	0.7	6	4.3
	AMOXICILLIN	All	148	≤0.015->4	≤0.015	0.03	4	0	0	0	0	0	0
		LVX NS	7	0.25->4	1	NA	NA	0	0	0	0	0	0
		LVX S	141	≤0.015->4	≤0.015	0.03	4	0	0	0	0	0	0
		PEN NS	8	>4->4	>4	NA	NA	0	0	0	0	0	0
		PEN S	140	≤0.015->4	≤0.015	0.03	2	0	0	0	0	0	0
	AMOXICILLIN/ CLAVULANATE	All	148	≤0.015-8	≤0.015	0.03	4	129	87.2	9	6.1	10	6.8
		LVX NS	7	0.25-4	1	NA	NA	5	71.4	2	28.6	0	0
		LVX S	141	≤0.015-8	≤0.015	0.03	4	124	87.9	7	5	10	7.1
		PEN NS	8	4-8	8	NA	NA	0	0	1	12.5	7	87.5
		PEN S	140	≤0.015-8	≤0.015	0.03	2	129	92.1	8	5.7	3	2.1
	AMPICILLIN	All	148	≤0.5-8	≤0.5	≤0.5	4	0	0	0	0	0	0
		LVX NS	7	≤0.5-4	1	NA	NA	0	0	0	0	0	0
		LVX S	141	≤0.5-8	≤0.5	≤0.5	2	0	0	0	0	0	0
		PEN NS	8	4-8	8	NA	NA	0	0	0	0	0	0
		PEN S	140	≤0.5-8	≤0.5	≤0.5	2	0	0	0	0	0	0

Organism	Drug	Phenotype	Total N	Range	Mode	MIC 60	MIC 90	nS	%S	nI	%I	nR	%R
	AZITHROMYCIN	All	148	0.06->4	>4	0.12	>4	83	56.1	2	1.4	63	42.6
		LVX NS	7	1->4	>4	NA	NA	0	0	1	14.3	6	85.7
		LVX S	141	0.06->4	>4	0.12	>4	83	58.9	1	0.7	57	40.4
		PEN NS	8	>4->4	>4	NA	NA	0	0	0	0	8	100
		PEN S	140	0.06->4	>4	0.12	>4	83	59.3	2	1.4	55	39.3
	CEFUROXIME/ AXETIL	All	148	≤0.12->8	≤0.12	≤0.12	8	106	71.6	12	8.1	30	20.3
		LVX NS	7	0.5->8	4	NA	NA	2	28.6	1	14.3	4	57.1
		LVX S	141	≤0.12->8	≤0.12	≤0.12	8	104	73.8	11	7.8	26	18.4
		PEN NS	8	8->8	8	NA	NA	0	0	0	0	8	100
		PEN S	140	≤0.12->8	≤0.12	≤0.12	4	106	75.7	12	8.6	22	15.7
	PENICILLIN	All	148	≤0.06-4	≤0.06	≤0.06	2	140	94.6	8	5.4	0	0
		LVX NS	7	0.25-4	1	NA	NA	6	85.7	1	14.3	0	0
		LVX S	141	≤0.06-4	≤0.06	≤0.06	2	134	95	7	5	0	0
		PEN NS	8	1-4	4	NA	NA	0	0	8	100	0	0
		PEN S	140	≤0.06-2	≤0.06	≤0.06	1	140	100	0	0	0	0
	TRIMETH/ SULFA	All	148	0.06->2	0.12	0.25	>2	92	62.2	16	10.8	40	27
		LVX NS	7	0.25->2	>2	NA	NA	2	28.6	1	14.3	4	57.1
		LVX S	141	0.06->2	0.12	0.25	>2	90	63.8	15	10.6	36	25.5
		PEN NS	8	>2->2	>2	NA	NA	0	0	0	0	8	100
		PEN S	140	0.06->2	0.12	0.25	>2	92	65.7	16	11.4	32	22.9

[1] Dashes indicate that no interpretive CLSI breakpoints are available
NA: Not applicable

Table 10 MIC Distribution (µg/mL) of Ciprofloxacin and Fluoroquinolone Comparators Against *S. pneumoniae* According to the Phenotypes

Organism	Drug	Phenotype		Total	≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
S. pneumoniae	CIPROFLOXACIN	All	Total N	148							2	27	88	21	2	1		7
			% at MIC	100							1.4	18.2	59.5	14.2	1.4	0.7		4.7
			Cumulative %	100							1.4	19.6	79.1	93.2	94.6	95.3		100
		LVX NS	Total N	7														7
			% at MIC	100														100
			Cumulative %	100														100
		LVX S	Total N	141							2	27	88	21	2	1		
			% at MIC	100							1.4	19.1	62.4	14.9	1.4	0.7		
			Cumulative %	100							1.4	20.6	83	97.9	99.3	100		
		PEN NS	Total N	8									4	3				1
			% at MIC	100									50	37.5				12.5
			Cumulative %	100									50	87.5				100
		PEN S	Total N	140							2	27	84	18	2	1		6
			% at MIC	100							1.4	19.3	60	12.9	1.4	0.7		4.3
			Cumulative %	100							1.4	20.7	80.7	93.6	95	95.7		100
	LEVOFLOXACIN	All	Total N	148								3	124	13	1	1	1	5
			% at MIC	100								2	83.8	8.8	0.7	0.7	0.7	3.4
			Cumulative %	100								2	85.8	94.6	95.3	95.9	96.6	100
		LVX NS	Total N	7												1	1	5
			% at MIC	100												14.3	14.3	71.4
			Cumulative %	100												14.3	28.6	100
		LVX S	Total N	141								3	124	13	1			
			% at MIC	100								2.1	87.9	9.2	0.7			
			Cumulative %	100								2.1	90.1	99.3	100			
		PEN NS	Total N	8									7					1
			% at MIC	100									87.5					12.5
			Cumulative %	100									87.5					100
		PEN S	Total N	140								3	117	13	1	1	1	4
			% at MIC	100								2.1	83.6	9.3	0.7	0.7	0.7	2.9
			Cumulative %	100								2.1	85.7	95	95.7	96.4	97.1	100

Organism	Drug	Phenotype		Total	≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
S. pneumoniae	OFLOXACIN	All	Total N	148									2	116	22	1	1	6
			% at MIC	100									1.4	78.4	14.9	0.7	0.7	4.1
			Cumulative %	100									1.4	79.7	94.6	95.3	95.9	100
		LVX NS	Total N	7													1	6
			% at MIC	100													14.3	85.7
			Cumulative %	100													14.3	100
		LVX S	Total N	141									2	116	22	1		
			% at MIC	100									1.4	82.3	15.6	0.7		
			Cumulative %	100									1.4	83.7	99.3	100		
		PEN NS	Total N	8										4	3			1
			% at MIC	100										50	37.5			12.5
			Cumulative %	100										50	87.5			100
		PEN S	Total N	140									2	112	19	1	1	5
			% at MIC	100									1.4	80	13.6	0.7	0.7	3.6
			Cumulative %	100									1.4	81.4	95	95.7	96.4	100

2.3.3 Susceptibility of *P. aeruginosa* (Tables 11 and 12)

The ciprofloxacin MIC_{90} was 64 µg/ml for all *P aeruginosa* isolates tested. Of them, 25.3% of the isolates were non-susceptible to ciprofloxacin. The overall ciprofloxacin MIC range for all strains was 0.015 - 64 µg/ml. Among the ciprofloxacin-susceptible population, the MIC_{90} was 0.5 µg/ml. The MIC level was very similar to the level of activity obtained with levofloxacin and ofloxacin.

Table 11 Activity (MIC in µg/mL) of Ciprofloxacin and Comparators against *P. aeruginosa* According to the Phenotypes

Organism	Drug	Phenotype	Total N	Range	Mode	MIC_{50}	MIC_{90}	nS	%S	nI	%I	nR	%R
P. aeruginosa	CIPROFLOXACIN	All	150	0.015-64	0.06	0.12	16	106	70.7	6	4	38	25.3
		CIP NS	44	1-64	16	16	64	0	0	6	13.6	38	86.4
		CIP S	106	0.015-1	0.06	0.06	0.5	106	100	0	0	0	0
		MDR	22	0.03-64	32	16	32	3	13.6	1	4.5	18	81.8
		non-MDR	128	0.015-64	0.06	0.12	8	103	80.5	5	3.9	20	15.6
	LEVOFLOXACIN	All	150	0.03-128	0.25	0.5	32	103	68.7	7	4.7	40	26.7
		CIP NS	44	4-128	32	16	64	0	0	4	9.1	40	90.9
		CIP S	106	0.03-4	0.25	0.25	2	103	97.2	3	2.8	0	0
		MDR	22	0.06-64	32	32	32	3	13.6	1	4.5	18	81.8
		non-MDR	128	0.03-128	0.25	0.25	16	100	78.1	6	4.7	22	17.2
	OFLOXACIN	All	150	0.06->128	0.5	1	64	98	65.3	11	7.3	41	27.3
		CIP NS	44	1->128	64	32	128	1	2.3	2	4.5	41	93.2
		CIP S	106	0.06-4	0.5	0.5	2	97	91.5	9	8.5	0	0
		MDR	22	0.06-128	64	64	64	3	13.6	0	0	19	86.4
		non-MDR	128	0.06->128	0.5	1	32	95	74.2	11	8.6	22	17.2
	GENTAMICIN	All	150	0.12->8	1	1	>8	127	84.7	4	2.7	19	12.7
		CIP NS	44	0.12->8	>8	4	>8	24	54.5	2	4.5	18	40.9
		CIP S	106	0.12->8	1	1	2	103	97.2	2	1.9	1	0.9
		MDR	22	0.12->8	>8	4	>8	11	50	1	4.5	10	45.5
		non-MDR	128	0.12->8	1	1	4	116	90.6	3	2.3	9	7
	TRIMETH/ SULFA	All	150	≤0.25->32	4	4	32	0	0	0	0	0	0
		CIP NS	44	≤0.25->32	>32	8	>32	0	0	0	0	0	0
		CIP S	106	≤0.25->32	4	2	16	0	0	0	0	0	0
		MDR	22	≤0.25->32	>32	8	>32	0	0	0	0	0	0
		non-MDR	128	≤0.25->32	4	4	16	0	0	0	0	0	0

Table 12 MIC Distribution (µg/mL) of Ciprofloxacin and Fluoroquinolone Comparators Against *P. aeruginosa* According to the Phenotypes

Organism	Drug	Phenotype		Total	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
P. aeruginosa	CIPROFLOXACIN	All	Total N	150		4	12	38	27	11	7	7	6	6	8	10	9	5		
			%	100		2.7	8	25.3	18	7.3	4.7	4.7	4	4	5.3	6.7	6	3.3		
			Cumulative %	100		2.7	10.7	36	54	61.3	66	70.7	74.7	78.7	84	90.7	96.7	100		
		CIP NS	Total N	44									6	6	8	10	9	5		
			%	100									13.6	13.6	18.2	22.7	20.5	11.4		
			Cumulative %	100									13.6	27.3	45.5	68.2	88.6	100		
		CIP S	Total N	106		4	12	38	27	11	7	7								
			%	100		3.8	11.3	35.8	25.5	10.4	6.6	6.6								
			Cumulative %	100		3.8	15.1	50.9	76.4	86.8	93.4	100								
		MDR	Total N	22			1	1	1				1	2	2	5	8	1		
			%	100			4.5	4.5	4.5				4.5	9.1	9.1	22.7	36.4	4.5		
			Cumulative %	100			4.5	9.1	13.6				18.2	27.3	36.4	59.1	95.5	100		
		non-MDR	Total N	128		4	11	37	26	11	7	7	5	4	6	5	1	4		
			%	100		3.1	8.6	28.9	20.3	8.6	5.5	5.5	3.9	3.1	4.7	3.9	0.8	3.1		
			Cumulative %	100		3.1	11.7	40.6	60.9	69.5	75	80.5	84.4	87.5	92.2	96.1	96.9	100		
Organism	**Drug**	**Phenotype**		**Total**	**≤0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**
P. aeruginosa	LEVOFLOXACIN	All	Total N	150			3	8	24	36	11	13	8	7	11	10	13	5	1	
			%	100			2	5.3	16	24	7.3	8.7	5.3	4.7	7.3	6.7	8.7	3.3	0.7	
			Cumulative %	100			2	7.3	23.3	47.3	54.7	63.3	68.7	73.3	80.7	87.3	96	99.3	100	
		CIP NS	Total N	44										4	11	10	13	5	1	
			%	100										9.1	25	22.7	29.5	11.4	2.3	
			Cumulative %	100										9.1	34.1	56.8	86.4	97.7	100	
		CIP S	Total N	106			3	8	24	36	11	13	8	3						
			%	100			2.8	7.5	22.6	34	10.4	12.3	7.5	2.8						
			Cumulative %	100			2.8	10.4	33	67	77.4	89.6	97.2	100						
		MDR	Total N	22				1	1	1				1	4	2	10	2		
			%	100				4.5	4.5	4.5				4.5	18.2	9.1	45.5	9.1		
			Cumulative %	100				4.5	9.1	13.6				18.2	36.4	45.5	90.9	100		
		non-MDR	Total N	128			3	7	23	35	11	13	8	6	7	8	3	3	1	
			%	100			2.3	5.5	18	27.3	8.6	10.2	6.3	4.7	5.5	6.3	2.3	2.3	0.8	
			Cumulative %	100			2.3	7.8	25.8	53.1	61.7	71.9	78.1	82.8	88.3	94.5	96.9	99.2	100	

Organism	Drug	Phenotype		Total	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
P. aeruginosa	OFLOXACIN	All	Total N	150				3	8	14	37	24	12	11	5	10	7	13	5	1
			%	100				2	5.3	9.3	24.7	16	8	7.3	3.3	6.7	4.7	8.7	3.3	0.7
			Cumulative %	100				2	7.3	16.7	41.3	57.3	65.3	72.7	76	82.7	87.3	96	99.3	100
		CIP NS	Total N	44								1		2	5	10	7	13	5	1
			%	100								2.3		4.5	11.4	22.7	15.9	29.5	11.4	2.3
			Cumulative %	100								2.3		6.8	18.2	40.9	56.8	86.4	97.7	100
		CIP S	Total N	106				3	8	14	37	23	12	9						
			%	100				2.8	7.5	13.2	34.9	21.7	11.3	8.5						
			Cumulative %	100				2.8	10.4	23.6	58.5	80.2	91.5	100						
		MDR	Total N	22				1		1	1				1	5	1	11	1	
			%	100				4.5		4.5	4.5				4.5	22.7	4.5	50	4.5	
			Cumulative %	100				4.5		9.1	13.6				18.2	40.9	45.5	95.5	100	
		non-MDR	Total N	128				2	8	13	36	24	12	11	4	5	6	2	4	1
			%	100				1.6	6.3	10.2	28.1	18.8	9.4	8.6	3.1	3.9	4.7	1.6	3.1	0.8
			Cumulative %	100				1.6	7.8	18	46.1	64.8	74.2	82.8	85.9	89.8	94.5	96.1	99.2	100

2.3.4 Susceptibility of *M. catarrhalis* (Tables 13 and 14)

The ciprofloxacin MIC_{90} was 0.03 µg/ml for all *M. catarrhalis* isolates. The overall ciprofloxacin MIC range for all strains was < 0.015 - 1 µg/ml. This level of activity was very similar to the level of activity obtained with levofloxacin and ofloxacin.

Table 13 Activity (MIC in µg/mL) of Ciprofloxacin and Comparators Against *M. catarrhalis* According to the Phenotype

Organism	Drug	Phenotype[1]	Total N	Range	Mode	MIC 50	MIC 90	nS	%S	nI	%I	nR	%R
M. catarrhalis	CIPROFLOXACIN	CIP S	104	≤0.015-0.12	≤0.015	≤0.015	0.03	104	100	0	0	0	0
	LEVOFLOXACIN	CIP S	104	≤0.015-1	0.03	0.03	0.03	104	100	0	0	0	0
	OFLOXACIN	CIP S	104	0.03-1	0.06	0.06	0.06	0	0	0	0	0	0
	AMOXICILLIN	CIP S	104	≤0.12->1	>1	1	>1	0	0	0	0	0	0
	AMOXICILLIN/ CLAVULANATE	CIP S	104	≤0.06-0.25	0.12	0.12	0.25	104	100	0	0	0	0
	AZITHROMYCIN	CIP S	104	≤0.12-≤0.12	≤0.12	≤0.12	≤0.12	104	100	0	0	0	0
	CEFUROXIME/ AXETIL	CIP S	104	≤0.25-4	1	1	1	0	0	0	0	0	0
	GENTAMICIN	CIP S	104	≤0.06-0.25	0.12	0.12	0.12	0	0	0	0	0	0
	TRIMETH/ SULFA	CIP S	104	≤0.25-1	≤0.25	≤0.25	≤0.25	100	96.2	4	3.8	0	0

[1] All *M. catarrhalis* evaluated in this study were susceptible to ciprofloxacin, levofloxacin, and ofloxacin.

Table 14 MIC Distribution (µg/mL) of Ciprofloxacin and Fluoroquinolone Comparators Against *M. catarrhalis* According to the Phenotype

Organism	Drug	Phenotype[1]		Total	≤0.015	0.03	0.06	0.12	0.25	0.5	1	>1
M. catarrhalis	CIPROFLOXACIN	CIP S	Total N	104	88	15		1				
			%	100	84.6	14.4		1				
			Cumulative %	100	84.6	99		100				
Organism	**Drug**	**Phenotype[1]**		**Total**	**≤0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**>1**
M. catarrhalis	LEVOFLOXACIN	CIP S	Total N	104	9	93	1				1	
			%	100	8.7	89.4	1				1	
			Cumulative %	100	8.7	98.1	99				100	
Organism	**Drug**	**Phenotype[1]**		**Total**	**≤0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**>1**
M. catarrhalis	OFLOXACIN	CIP S	Total N	104		26	77				1	
			%	100		25	74				1	
			Cumulative %	100		25	99				100	

[1] All *M. catarrhalis* evaluated in this study were susceptible to ciprofloxacin, levofloxacin, and ofloxacin.

2.3.5 Susceptibility of *H. influenzae* (Tables 15 and 16)

The ciprofloxacin MIC_{90} was 0.03 µg/ml for all *H. influenzae* isolates tested. The overall ciprofloxacin MIC range for all strains was < 0.004 - > 8 µg/ml. This level of activity was very similar to the level of activity obtained with levofloxacin and ofloxacin.

Table 15 Activity (MIC in µg/mL) of Ciprofloxacin and Comparators Against *H. influenzae* According to Phenotype

Organism	Drug	Phenotype	Total N	Range	Mode	MIC 50	MIC 90	nS	%S	nI	%I	nR	%R
H. influenzae	CIPROFLOXACIN	All	149	0.004->8	0.008	0.008	0.5	136	91.3	0	0	0	0
		CIP NS	13	2->8	>8	>8	>8	0	0	0	0	0	0
		CIP S	136	0.004-0.5	0.008	0.008	0.015	136	100	0	0	0	0
		AMP NS	42	0.004->8	0.008	0.008	4	36	85.7	0	0	0	0
		AMP S	107	0.004->8	0.008	0.008	0.015	100	93.5	0	0	0	0
	LEVOFLOXACIN	All	149	0.008->8	0.015	0.015	0.5	137	91.9	0	0	0	0
		CIP NS	13	2->8	8	8	>8	1	7.7	0	0	0	0
		CIP S	136	0.008-0.5	0.015	0.015	0.015	136	100	0	0	0	0
		AMP NS	42	0.008->8	0.015	0.015	8	36	85.7	0	0	0	0
		AMP S	107	0.008->8	0.015	0.015	0.015	101	94.4	0	0	0	0
	OFLOXACIN	All	149	0.015->8	0.03	0.03	1	136	91.3	0	0	0	0
		CIP NS	13	4->8	>8	>8	>8	0	0	0	0	0	0
		CIP S	136	0.015-1	0.03	0.03	0.03	136	100	0	0	0	0
		AMP NS	42	0.015->8	0.03	0.03	>8	36	85.7	0	0	0	0
		AMP S	107	0.015->8	0.03	0.03	0.03	100	93.5	0	0	0	0
	AMOXICILLIN	All	149	0.12->4	0.5	0.5	>4	102	68.5	3	2	44	29.5
		CIP NS	13	0.12->4	>4	>4	>4	4	30.8	1	7.7	8	61.5
		CIP S	136	0.25->4	0.5	0.5	>4	98	72.1	2	1.5	36	26.5
		AMP NS	42	0.25->4	>4	>4	>4	2	4.8	0	0	40	95.2
		AMP S	107	0.12->4	0.5	0.5	1	100	93.5	3	2.8	4	3.7
	AMOXICILLIN/ CLAVULANATE	All	149	0.12-4	0.5	0.5	2	149	100	0	0	0	0
		CIP NS	13	0.25-4	4	2	4	13	100	0	0	0	0
		CIP S	136	0.12-4	0.5	0.5	2	136	100	0	0	0	0
		AMP NS	42	0.5-4	1	1	4	42	100	0	0	0	0
		AMP S	107	0.12-4	0.5	0.5	2	107	100	0	0	0	0
	AMPICILLIN	All	149	≤0.5->8	≤0.5	≤0.5	>8	107	71.8	2	1.3	40	26.8
		CIP NS	13	≤0.5->8	≤0.5	1	>8	7	53.8	0	0	6	46.2
		CIP S	136	≤0.5->8	≤0.5	≤0.5	>8	100	73.5	2	1.5	34	25
		AMP NS	42	2->8	>8	>8	>8	0	0	2	4.8	40	95.2
		AMP S	107	≤0.5-1	≤0.5	≤0.5	≤0.5	107	100	0	0	0	0
H. influenzae	AZITHROMYCIN	All	149	≤0.03->4	1	1	2	146	98	0	0	0	0
		CIP NS	13	0.5->4	2	2	2	12	92.3	0	0	0	0
		CIP S	136	≤0.03->4	1	1	2	134	98.5	0	0	0	0
		AMP NS	42	≤0.03->4	1	1	2	40	95.2	0	0	0	0
		AMP S	107	0.25->4	1	1	2	106	99.1	0	0	0	0
	CEFUROXIME/ AXETIL	All	149	≤0.12-4	0.5	0.5	2	149	100	0	0	0	0
		CIP NS	13	0.5-4	2	2	2	13	100	0	0	0	0
		CIP S	136	≤0.12-4	0.5	0.5	2	136	100	0	0	0	0
		AMP NS	42	0.25-4	0.5	0.5	2	42	100	0	0	0	0
		AMP S	107	≤0.12-4	0.5	0.5	2	107	100	0	0	0	0
	TRIMETH/ SULFA	All	149	≤0.015->2	>2	0.12	>2	100	67.1	5	3.4	44	29.5
		CIP NS	13	0.06->2	>2	>2	>2	1	7.7	0	0	12	92.3
		CIP S	136	≤0.015->2	>2	0.12	>2	99	72.8	5	3.7	32	23.5
		AMP NS	42	0.03->2	>2	0.25	>2	24	57.1	0	0	18	42.9
		AMP S	107	≤0.015->2	>2	0.12	>2	76	71	5	4.7	26	24.3

Table 16 MIC Distribution (µg/mL) of Ciprofloxacin and Fluoroquinolone Comparators Against *H. influenzae* According to Phenotype

Organism	Drug	Phenotype		Total	≤0.002	0.004	0.008	0.015	0.03	0.06	0.5	1	2	4	8	>8
H. influenzae	CIPROFLOXACIN	All	Total N	149		3	114	16	1		2		2	2	1	8
			% at MIC	100		2	76.5	10.7	0.7		1.3		1.3	1.3	0.7	5.4
			Cumulative %	100		2	78.5	89.3	89.9		91.3		92.6	94	94.6	100
		CIP NS	Total N	13									2	2	1	8
			% at MIC	100									15.4	15.4	7.7	61.5
			Cumulative %	100									15.4	30.8	38.5	100
		CIP S	Total N	136		3	114	16	1		2					
			% at MIC	100		2.2	83.8	11.8	0.7		1.5					
			Cumulative %	100		2.2	86	97.8	98.5		100					
		AMP NS	Total N	42		1	30	3			2			2	1	3
			% at MIC	100		2.4	71.4	7.1			4.8			4.8	2.4	7.1
			Cumulative %	100		2.4	73.8	81			85.7			90.5	92.9	100
		AMP S	Total N	107		2	84	13	1				2			5
			% at MIC	100		1.9	78.5	12.1	0.9				1.9			4.7
			Cumulative %	100		1.9	80.4	92.5	93.5				95.3			100
Organism	**Drug**	**Phenotype**		**Total**	**≤0.002**	**0.004**	**0.008**	**0.015**	**0.03**	**0.06**	**0.5**	**1**	**2**	**4**	**8**	**>8**
H. influenzae	LEVOFLOXACIN	All	Total N	149			17	114	2	1	2		1	1	6	5
			% at MIC	100			11.4	76.5	1.3	0.7	1.3		0.7	0.7	4	3.4
			Cumulative %	100			11.4	87.9	89.3	89.9	91.3		91.9	92.6	96.6	100
		CIP NS	Total N	13									1	1	6	5
			% at MIC	100									7.7	7.7	46.2	38.5
			Cumulative %	100									7.7	15.4	61.5	100
		CIP S	Total N	136			17	114	2	1	2					
			% at MIC	100			12.5	83.8	1.5	0.7	1.5					
			Cumulative %	100			12.5	96.3	97.8	98.5	100					
		AMP NS	Total N	42			5	29			2				4	2
			% at MIC	100			11.9	69			4.8				9.5	4.8
			Cumulative %	100			11.9	81			85.7				95.2	100
		AMP S	Total N	107			12	85	2	1			1	1	2	3
			% at MIC	100			11.2	79.4	1.9	0.9			0.9	0.9	1.9	2.8
			Cumulative %	100			11.2	90.7	92.5	93.5			94.4	95.3	97.2	100

Organism	Drug	Phenotype		Total	≤0.002	0.004	0.008	0.015	0.03	0.06	0.5	1	2	4	8	>8
H. influenzae	OFLOXACIN	All	Total N	149				42	90	2		2		1	1	11
			% at MIC	100				28.2	60.4	1.3		1.3		0.7	0.7	7.4
			Cumulative %	100				28.2	88.6	89.9		91.3		91.9	92.6	100
		CIP NS	Total N	13										1	1	11
			% at MIC	100										7.7	7.7	84.6
			Cumulative %	100										7.7	15.4	100
		CIP S	Total N	136				42	90	2		2				
			% at MIC	100				30.9	66.2	1.5		1.5				
			Cumulative %	100				30.9	97.1	98.5		100				
		AMP NS	Total N	42				11	23			2				6
			% at MIC	100				26.2	54.8			4.8				14.3
			Cumulative %	100				26.2	81			85.7				100
		AMP S	Total N	107				31	67	2				1	1	5
			% at MIC	100				29	62.6	1.9				0.9	0.9	4.7
			Cumulative %	100				29	91.6	93.5				94.4	95.3	100

2.4 In Vitro Bactericidal Activity - Time Kill Kinetics Analysis

The applicant conducted in vitro microbiological study to demonstrate the bactericidal activity of ciprofloxacin, by time kill kinetics, against susceptible, intermediate and resistant isolates of targeted key otic pathogens *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa*, and *Staphylococcus aureus.* Bactericidal activity was defined as a ≥ 3-log reduction in cfu/ml relative to the initial inoculum size.

According to the applicant, the organisms selected for testing were clinical isolates either from otic or respiratory sources that were collected from the US, Europe, and Asia between 2010-2012. Three isolates of each species were selected and tested, each with a low, medium, and high ciprofloxacin MIC. The bactericidal activity was performed by using different concentrations of ciprofloxacin that were multiples of MIC for each pathogen tested. The ciprofloxacin MICs were established for each strain following CLSI M100-S25[7], M7-A10[8] guidelines and the investigator's SOPs.[9] Bactericidal activity was measured following CLSI M26-A[10] guidelines and investigators SOPs. The table below shows the year, source and country of origin of the isolates tested.

Eurofins ID	Isolation Year	Organism	Specimen Source	Country of Origin
2896396	2012	*S. aureus*	Ear	US
2797343	2011	*S. aureus*	Tracheal Aspirate	Italy
2879223	2011	*S. aureus*	Ear	US
2802733	2011	*P. aeruginosa*	Ear	US
2899751	2012	*P. aeruginosa*	Tracheal Aspirate	US
2382177	2010	*P. aeruginosa*	Sputum	United Kingdom
2400357	2010	*M. catarrhalis*	Bronchial Washings/BAL	Taiwan
2751545	2010	*M. catarrhalis*	Tracheal Aspirate	Spain
2975500	2012	*M. catarrhalis*	Sputum	Hong Kong
2399874	2010	*H. influenzae*	Sputum	South Korea
2975254	2012	*H. influenzae*	Sputum	Hong Kong
2324550	2010	*H. influenzae*	Nasopharynx/Throat/Nose	Germany
2963031	2012	*S. pneumoniae*	Bronchial Washings/BAL	Singapore
2328015	2010	*S. pneumoniae*	Ear	Spain
2692497	2010	*S. pneumoniae*	Sputum	US

Based on the provided data, ciprofloxacin demonstrated bactericidal activity against all organisms tested with different concentrations of antibiotics. However, in some cases bactericidal activity was shown to be achieved only with higher concentrations of the antibiotics used.

The following table summarizes the tested isolates of targeted pathogens with their susceptibility to ciprofloxacin.

Table 17: Characteristics of the Target Otic Pathogens Tested

Organism	MIC (µg/ml)	Specimen Source	Country of Origin
S. aureus			
susceptible	0.06	Ear	US
intermediate	0.25	Ear	US
resistant	2.0	Tracheal Aspirate	Italy
S. pneumoniae			
susceptible	0.5	Sputum	US
intermediate	1.0	Ear	Spain
resistant	16.0	Bronchial Washings/BAL	Singapore
P. aeruginosa			
susceptible	0.03	Sputum	United Kingdom
intermediate	0.5	Tracheal Aspirate	US
resistant	4.0	Ear	US
M. catarrhalis			
susceptible	0.015	Tracheal Aspirate	Spain
intermediate	0.03	Sputum	Hong Kong
resistant	0.5	Bronchial Washings/BAL	Taiwan
H. influenzae			
susceptible	0.008	Nasopharynx/Throat/Nose	Germany
intermediate	1.0	Sputum	Hong Kong
resistant	8.0	Sputum	South Korea

2.4.1 Time Kill Kinetics with *S. aureus*

The bactericidal activity of ciprofloxacin was shown at 8X the MIC with the susceptible isolate (MIC of 0.06 µg/ml), at 8X the MIC with the intermediate isolate (MIC of 0.25 µg/ml), and at 4X the MIC with the resistant isolate (MIC of 2.0 µg/ml).

***S. aureus* CID 2896396, a sensitive isolate to ciprofloxacin, MIC ≥0.06 µg/ml (Table 18; Figure 1)**

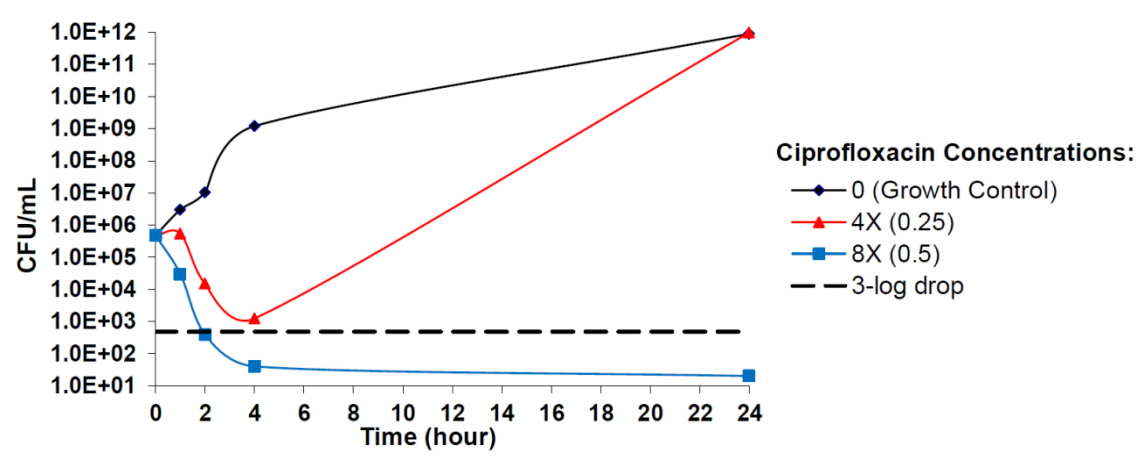
Bactericidal activity was achieved at 4X (0.25 µg/ml) of the MIC after 4 hr, but the organism regrew to a concentration above the bactericidal threshold after 24 hr. Bactericidal activity was achieved at 8X (0.5 µg/ml) of the MIC.

Table 18. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2896396 (CIP MIC = 0.06 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	0.25 µg/mL	0.5 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	4.80E+05	4.80E+05	4.80E+05
1	3.00E+06	5.40E+05	3.00E+04
2	1.04E+07	1.52E+04	4.00E+02
4	1.20E+09	1.24E+03	4.00E+01
24	9.00E+11	9.80E+11	2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 1. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2896396 (CIP MIC = 0.06 µg/mL)



***S. aureus* CID 2879223, an intermediate isolate to ciprofloxacin, MIC ≥0.25 µg/ml (Table 19; Figure 2)**

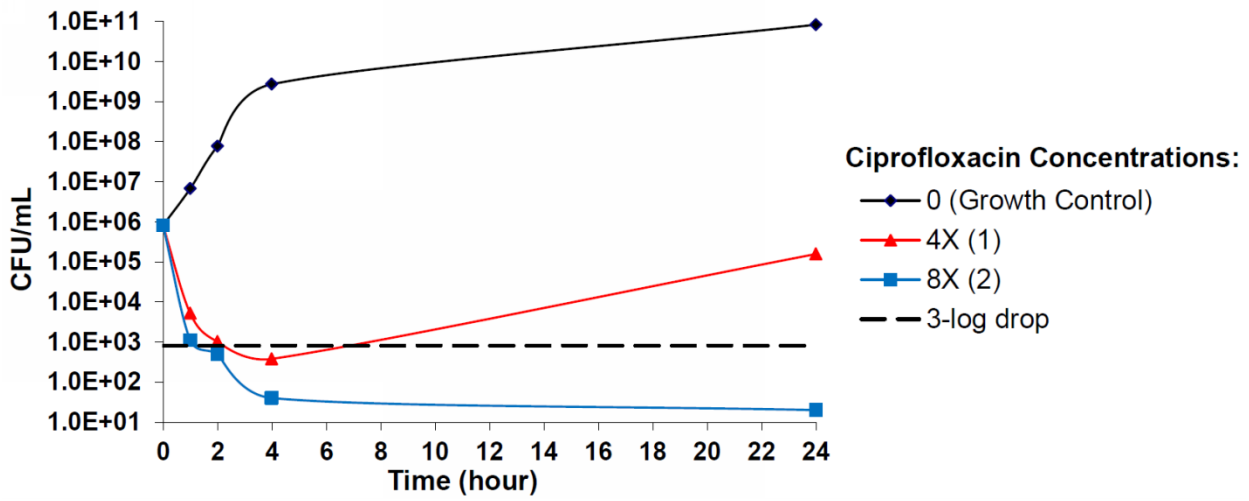
Bactericidal activity was achieved at 4X (1 µg/ml) of the MIC after 4 hr, but the organism regrew to a concentration above the bactericidal threshold after 24 hr. Bactericidal activity was achieved at 8X (2 µg/ml) of the MIC.

Table 19. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2879223 (CIP MIC = 0.25 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	1 µg/mL	2 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	8.20E+05	8.20E+05	8.20E+05
1	6.80E+06	5.20E+03	1.12E+03
2	7.80E+07	1.02E+03	5.00E+02
4	2.74E+09	3.80E+02	4.00E+01
24	8.40E+10	1.58E+05	2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 2. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2879223 (CIP MIC = 0.25 µg/mL)



***S. aureus* CID 2797343, a resistant isolate to ciprofloxacin, MIC ≥2 µg/ml (Table 20; Figure 3)**

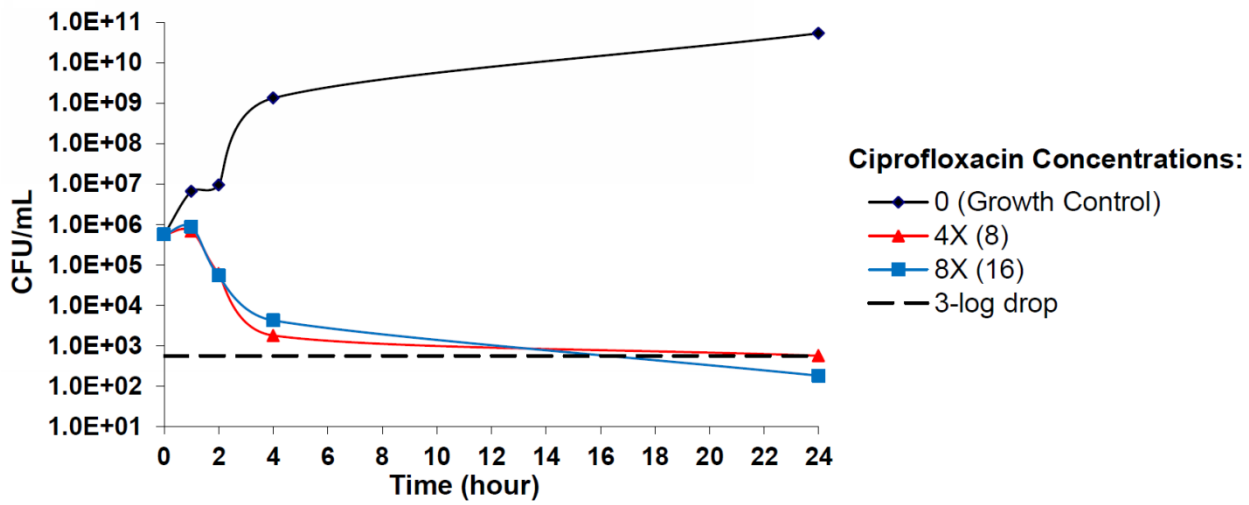
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 8 and 16 µg/ml, respectively) after 24 hr of exposure.

Table 20. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2797343 (CIP MIC = 2 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	8 µg/mL	16 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	5.60E+05	5.60E+05	5.60E+05
1	6.60E+06	6.80E+05	8.60E+05
2	9.40E+06	6.00E+04	5.40E+04
4	1.34E+09	1.78E+03	4.20E+03
24	5.40E+10	5.60E+02	1.80E+02

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 3. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. aureus* CID 2797343 (CIP MIC = 2 µg/mL)



2.4.2 Time Kill Kinetics with *S. pneumoniae*

The bactericidal activity of ciprofloxacin was shown at 4X the MIC with the susceptible isolate (MIC of 0.5 µg/ml), at 4X the MIC with the intermediate isolate (MIC of 1.0 µg/ml), and at 4X the MIC with the resistant isolate (MIC of 16 µg/ml).

***S. pneumoniae* CID 2692497, a sensitive isolate to ciprofloxacin, MIC ≥0.5 µg/ml (Table 21; Figure 4)**

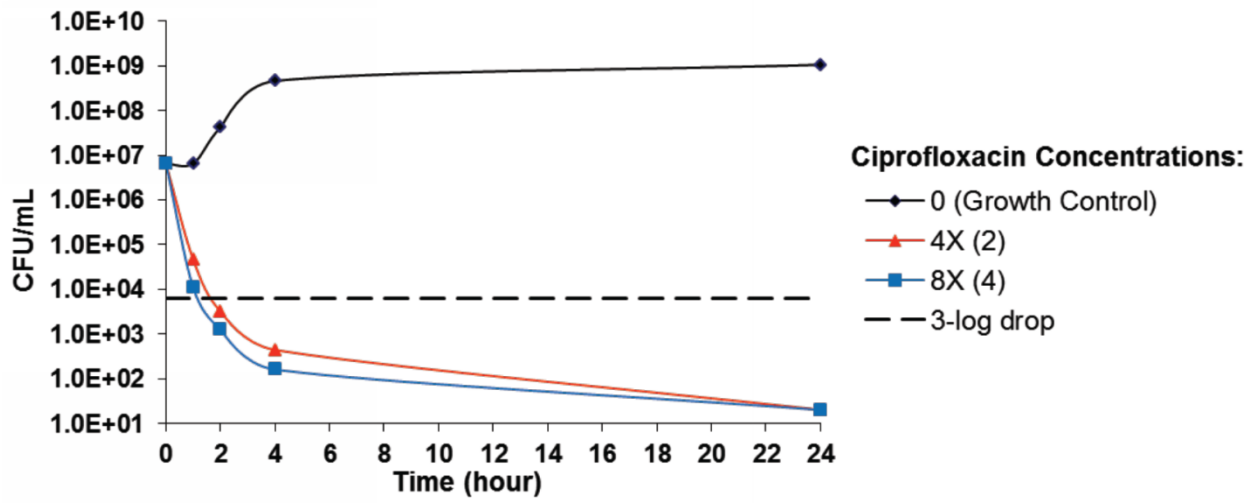
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 2 and 4 µg/ml, respectively) after 2 hr and continued throughout 24 hr of exposure.

Table 21. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2692497 (CIP MIC = 0.5 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	2 µg/mL	4 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	6.40E+06	6.40E+06	6.40E+06
1	6.60E+06	4.60E+04	1.08E+04
2	4.20E+07	3.20E+03	1.24E+03
4	4.60E+08	4.40E+02	1.60E+02
24	1.06E+09	< 2.00E+01	< 2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 4. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2692497 (CIP MIC = 0.5 µg/mL)



***S. pneumoniae* CID 2328015, an intermediate isolate to ciprofloxacin, MIC ≥1.0 µg/ml (Table 22; Figure 5)**

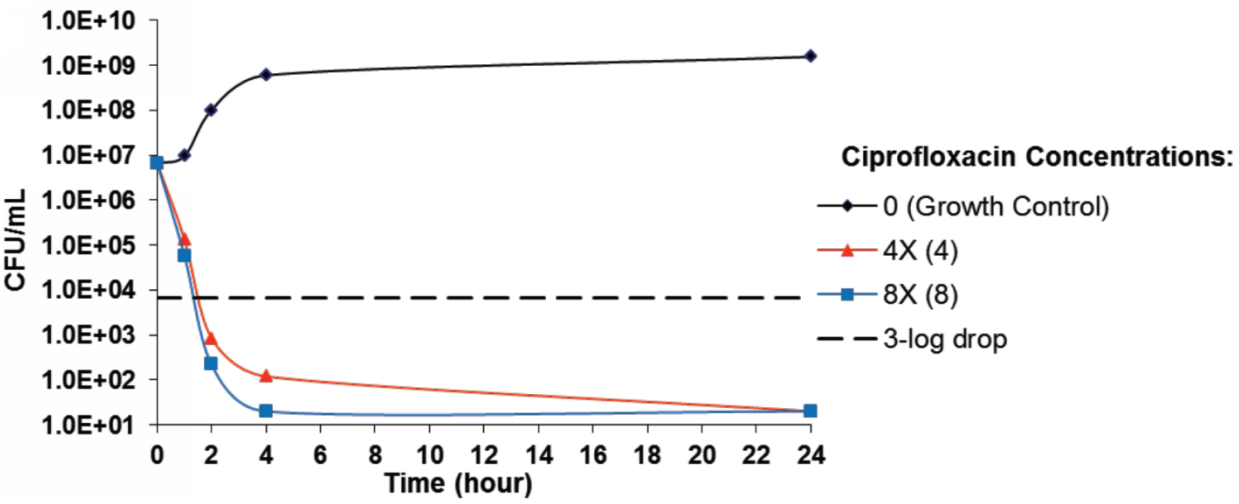
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 4 and 8 µg/ml, respectively) after 2 hr and continued throughout 24 hr of exposure.

Table 22. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2328015 (CIP MIC = 1 µg/mL)

		4X MIC		8X MIC
	0 µg/mL[1]	4 µg/mL		8 µg/mL
Time (hours):		Concentration (CFU/mL)		
0	6.60E+06	6.60E+06		6.60E+06
1	9.60E+06	1.32E+05		5.80E+04
2	1.00E+08	8.40E+02		2.20E+02
4	6.00E+08	1.20E+02	<	2.00E+01
24	1.54E+09	< 2.00E+01	<	2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 5. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2328015 (CIP MIC = 1 µg/mL)



***S. pneumoniae* CID 2963031, a resistant isolate to ciprofloxacin, MIC ≥16 µg/ml (Table 23; Figure 6)**

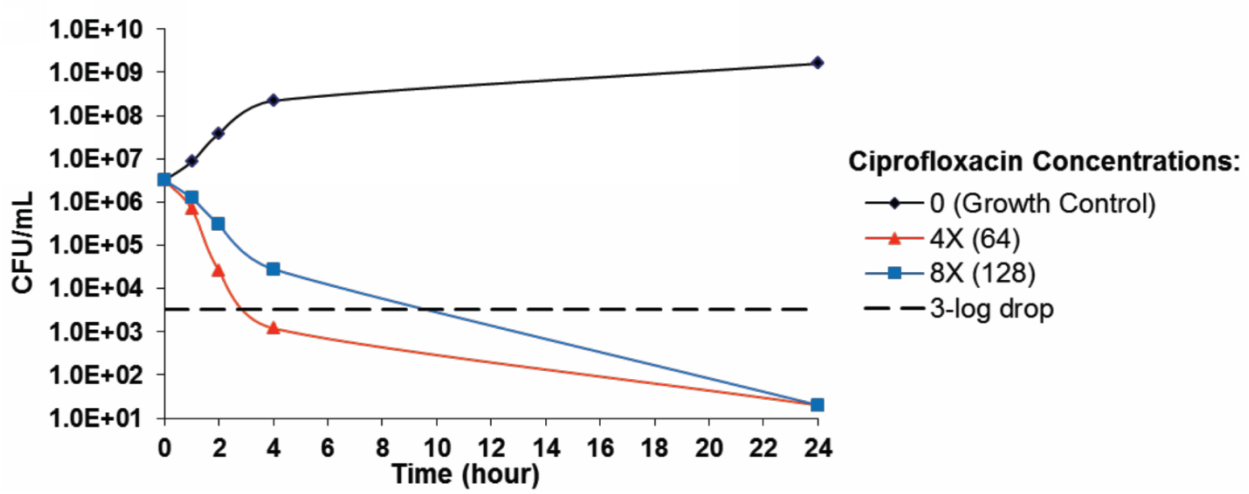
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 64 and 128 µg/ml, respectively) after 24 hr of exposure.

Table 23. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2963031 (CIP MIC = 16 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	64 µg/mL	128 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	3.20E+06	3.20E+06	3.20E+06
1	8.60E+06	1.22E+06	6.80E+05
2	3.80E+07	3.00E+05	2.60E+04
4	2.20E+08	2.80E+04	1.20E+03
24	1.60E+09	< 2.00E+01	< 2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 6. Time Kill Kinetics of Ciprofloxacin (CIP) Against *S. pneumoniae* CID 2963031 (CIP MIC = 16 µg/mL)



2.4.3 Time Kill Kinetics with *P. aeruginosa*

The bactericidal activity of ciprofloxacin was shown at 64X the MIC with the susceptible strain of *P. aeruginosa* (MIC of 0.03 µg/ml), at 8X the MIC with the intermediate strain (MIC of 0.5 µg/ml), and at 4X the MIC with the resistant strain (MIC of 4.0 µg/ml).

***P. aeruginosa* CID 2382177, a susceptible isolate to ciprofloxacin, MIC ≥0.03 µg/ml (Table 24A and 24B; Figure 7A and 7B)**

The applicant initially started with 4X (0.12 µg/ml), and 8X (0.25 µg/ml) the MIC and the bactericidal activity was achieved at 4 hr, but the organism regrew after 24 hr. The test was repeated with similar results. The ciprofloxacin concentration was increased to 64X (2 µg/ml) and the bactericidal activity was achieved at 2 hr and maintained throughout the full exposure time. The applicant made an assumption from these findings that the multiple of the MIC may not be as critical for bactericidal activity but a minimum concentration (at least 2 µg/ml) is required for demonstrating the bactericidal activity against *P aeruginosa* isolates.

Table 24A. Time Kill Kinetics of Ciprofloxacin (CIP) Against *P. aeruginosa* CID 2382177 (CIP MIC = 0.03 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	0.12 µg/mL	0.25 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	1.34E+06	1.34E+06	1.34E+06
1	3.60E+06	4.00E+05	4.00E+03
2	1.54E+07	5.20E+04	2.80E+02
4	2.40E+08	8.40E+04	1.02E+03
24	1.12E+09	1.04E+08	7.80E+07

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 7A. Time Kill Kinetics of Ciprofloxacin (CIP) Against *P. aeruginosa* CID 2382177 (CIP MIC = 0.03 µg/mL)

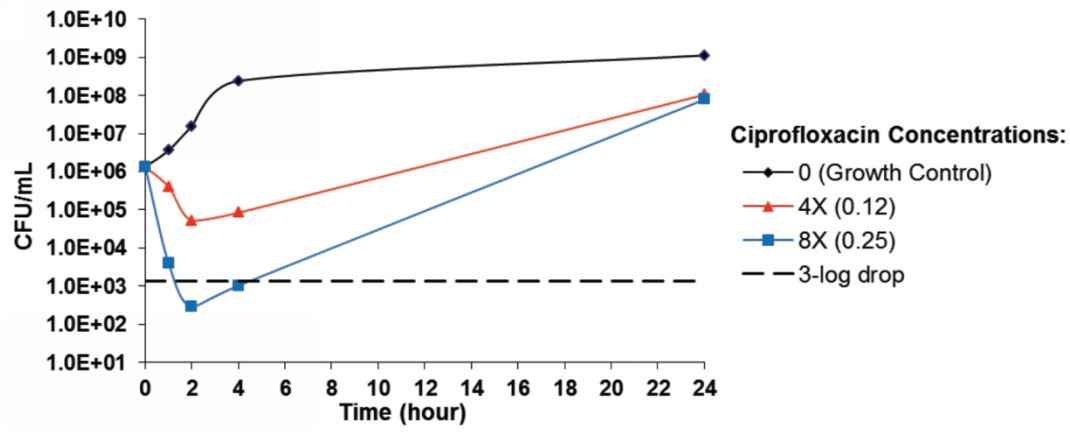
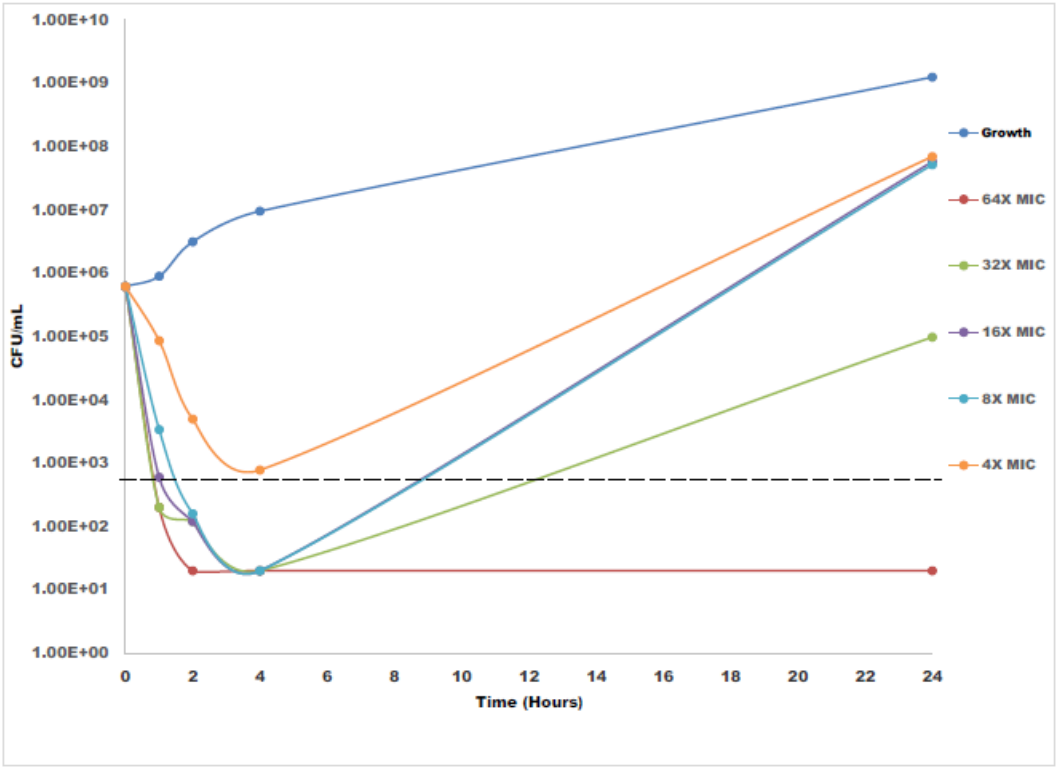


Table 24B. Timekill kinetics of Ciprofloxacin (CIP) against CIP Susceptible *P. aeruginosa* CID 2382177 (CIP MIC = 0.03 mg/mL)

		64X MIC	32X MIC	16X MIC	8X MIC	4X MIC
	0 µg/mL*	2 µg/mL	1.0 µg/mL	0.5 µg/mL	0.25 µg/mL	0.12 µg/mL
Time (Hours):	Concentration (CFU/mL)					
0	6.20E+05	6.20E+05	6.20E+05	6.20E+05	6.20E+05	6.20E+05
1	9.00E+05	2.00E+02	2.00E+02	6.00E+02	3.40E+03	8.60E+04
2	3.14E+06	2.00E+01	1.20E+02	1.20E+02	1.60E+02	5.00E+03
4	9.60E+06	2.00E+01	2.00E+01	2.00E+01	2.00E+01	7.80E+02
24	1.26E+09	2.00E+01	9.80E+04	5.80E+07	5.20E+07	7.00E+07

Figure 7B. Timekill kinetics of Ciprofloxacin (CIP) against CIP Susceptible *P. aeruginosa* CID 2382177 (CIP MIC = 0.03 mg/mL)



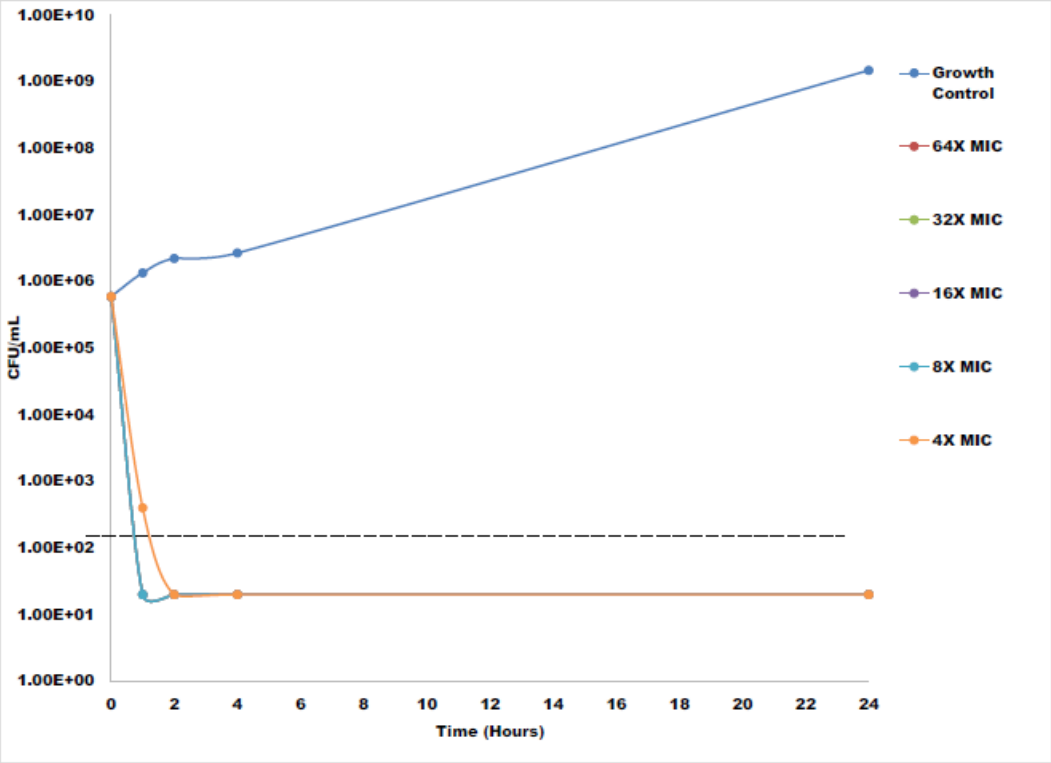
***P. aeruginosa* CID 2899751, an intermediate isolate to ciprofloxacin, MIC ≥0.5 µg/ml (Table 25; Figure 8)**

Bactericidal activity was achieved at all concentrations tested (4X, 8X, 16X, 32X, and 64X of the MIC, 2, 4, 16 and 32 µg/ml, respectively) after 2 hr and continued throughout 24 hr of exposure.

Table 25. Time kill kinetics of Ciprofloxacin (CIP) against CIP intermediate *P.aeruginosa* CID 2899751 (CIP MIC = 0.5 mg/mL)

		64X MIC)	32X MIC	16X MIC	8X MIC	4X MIC
	0 µg/mL*	32 µg/mL	16.0 µg/mL	8 µg/mL	4.0 µg/mL	2 µg/mL
Time (hours):	Concentration (CFU/ml)					
0	6.00E+05	6.00E+05	6.00E+05	6.00E+05	6.00E+05	6.00E+05
1	1.36E+06	2.00E+01	2.00E+01	2.00E+01	2.00E+01	4.00E+02
2	2.24E+06	2.00E+01	2.00E+01	2.00E+01	2.00E+01	2.00E+01
4	2.70E+06	2.00E+01	2.00E+01	2.00E+01	2.00E+01	2.00E+01
24	1.50E+09	2.00E+01	2.00E+01	2.00E+01	2.00E+01	2.00E+01

Figure 8. Time kill kinetics of Ciprofloxacin (CIP) against CIP intermediate *P.aeruginosa* CID 2899751 (CIP MIC = 0.5 mg/mL)



***P. aeruginosa* CID 2802733, a resistant isolate to ciprofloxacin, MIC ≥4.0 µg/ml (Table 26; Figure 9)**

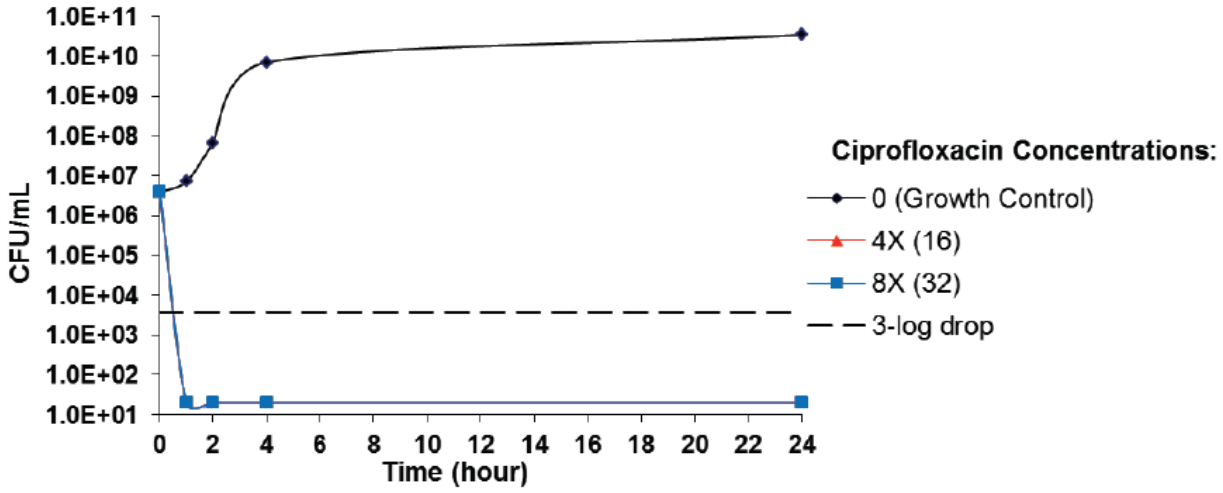
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 16 and 32 µg/ml, respectively) after 2 hr and continued throughout 24 hr of exposure.

Table 26. Time Kill Kinetics of Ciprofloxacin (CIP) Against *P. aeruginosa* CID 2802733 (CIP MIC = 4 µg/mL)

			4X MIC		8X MIC
	0 µg/mL[1]		16 µg/mL		32 µg/mL
Time (hours):	Concentration (CFU/mL)				
0	3.80E+06		3.80E+06		3.80E+06
1	7.20E+06	<	2.00E+01	<	2.00E+01
2	6.60E+07	<	2.00E+01	<	2.00E+01
4	7.00E+09	<	2.00E+01	<	2.00E+01
24	3.40E+10	<	2.00E+01	<	2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 9. Time Kill Kinetics of Ciprofloxacin (CIP) Against *P. aeruginosa* CID 2802733 (CIP MIC = 4 µg/mL)



(Note: Visibility of the 4X curve [red line] is obscured because the 4X and 8X curves share the same data points.)

2.4.4 Time Kill Kinetics with *M. catarrhalis*

The bactericidal activity of ciprofloxacin was shown at 8X the MIC with the susceptible strain of *M. catarrhalis* (MIC of 0.015 µg/ml), at 4X the MIC with the intermediate strain (MIC of 0.03 µg/ml), and at 4X the MIC with the resistant strain (MIC of 0.5 µg/ml).

***M. catarrhalis* CID 2751545, a susceptible isolate to ciprofloxacin, MIC ≥0.015 µg/ml (Table 27; Figure 10)**

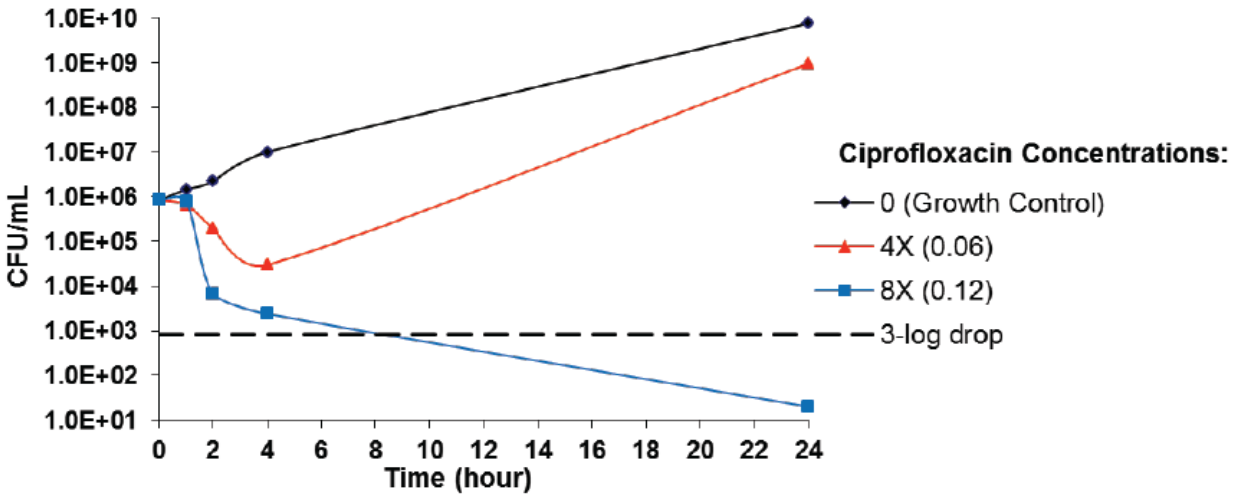
Bactericidal activity was achieved at 8X (0.12 µg /ml) the MIC after 24 hr of exposure.

Table 27. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2751545 (CIP MIC = 0.015 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	0.06 µg/mL	0.12 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	8.40E+05	8.40E+05	8.40E+05
1	1.44E+06	6.60E+05	7.60E+05
2	2.20E+06	2.00E+05	6.60E+03
4	1.00E+07	3.00E+04	2.40E+03
24	7.60E+09	9.40E+08	< 2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 10. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2751545 (CIP MIC = 0.015 µg/mL)



***M. catarrhalis* CID 2975500, an intermediate isolate to ciprofloxacin, MIC ≥0.03 µg/ml (Table 28; Figure 11)**

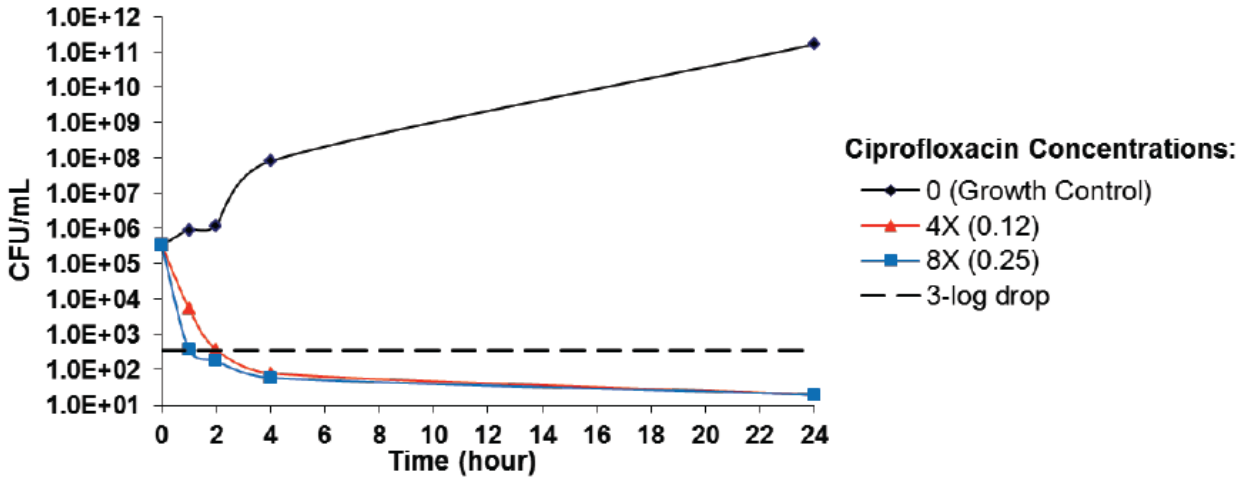
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 0.12 and 0.25 µg/ml, respectively) after 2 hr and continued throughout 24 hr of exposure.

Table 28. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2975500 (CIP MIC = 0.03 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	0.12 µg/mL	0.25 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	3.40E+05	3.40E+05	3.40E+05
1	9.20E+05	5.60E+03	3.80E+02
2	1.20E+06	3.80E+02	1.80E+02
4	8.20E+07	8.00E+01	6.00E+01
24	1.66E+11	< 2.00E+01	< 2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 11. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2975500 (CIP MIC = 0.03 µg/mL)



***M. catarrhalis* CID 2400357, a resistant isolate to ciprofloxacin, MIC ≥0.5 µg/ml (Table 29; Figure 12)**

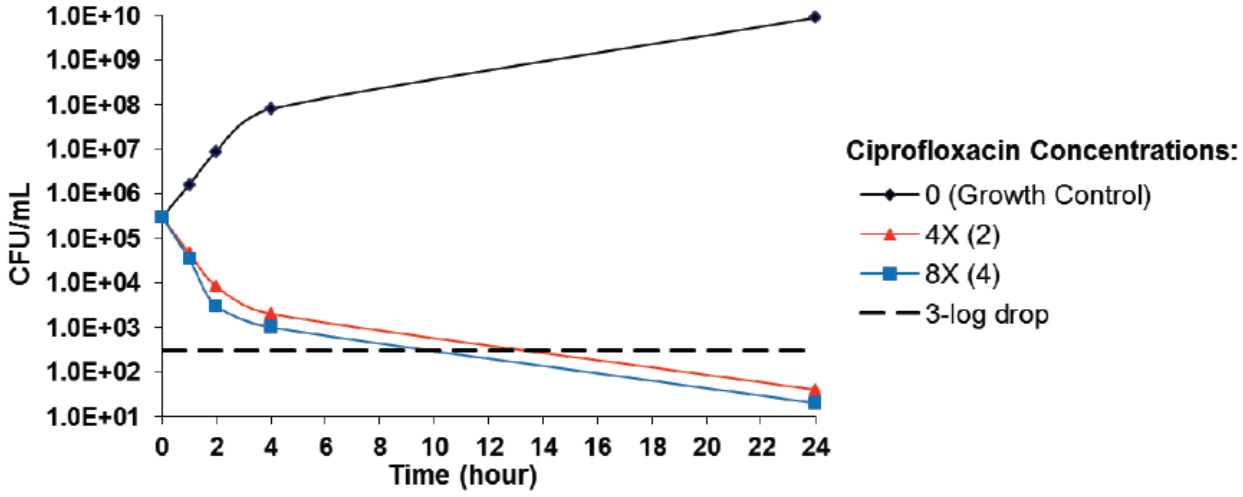
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 2 and 4 µg/ml, respectively) after 24 hr of exposure.

Table 29. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2400357 (CIP MIC = 0.5 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	2 µg/mL	4 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	3.00E+05	3.00E+05	3.00E+05
1	1.60E+06	4.60E+04	3.40E+04
2	8.80E+06	8.40E+03	3.00E+03
4	8.00E+07	2.00E+03	1.00E+03
24	9.00E+09	4.00E+01 <	2.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 12. Time Kill Kinetics of Ciprofloxacin (CIP) Against *M. catarrhalis* CID 2400357 (CIP MIC = 0.5 µg/mL)



2.4.5 Time Kill Kinetics with *H. influenzae*

The bactericidal activity of ciprofloxacin was shown at 4X the MIC with the susceptible strain of *H. influenzae* (MIC of 0.008 µg/ml), at 4X the MIC with the intermediate strain (MIC of 1.0 µg/ml), and at 4X the MIC with the resistant strain (MIC of 8.0 µg/ml).

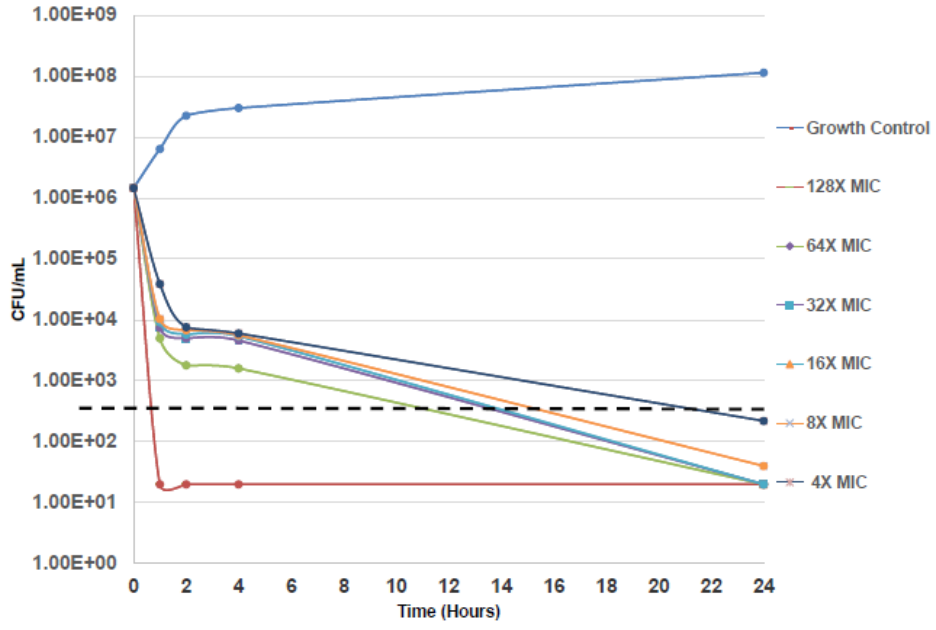
***H. influenzae* CID 2324550, a susceptible isolate to ciprofloxacin, MIC ≥0.008 µg/ml (Table 30; Figure 13)**

Bactericidal activity was achieved at all concentrations tested (4X, 8X, 16X, 32X, and 64X of the MIC, 0.03, 0.06, 0.12, 0.25, and 0.5 µg/ml, respectively) after 24 hr of exposure period.

Table 30. Timekill kinetics of Ciprofloxacin (CIP) against CIP Susceptible *H.influenzae* CID 2324550 (CIP MIC = 0.008 mg/mL)

		128X MIC	64X MIC	32X MIC	16X MIC	8X MIC	4X MIC
	0 µg/mL*	1 µg/mL	0.5 µg/mL	0.25 µg/mL	0.12 µg/mL	0.06µg/mL	0.03 µg/mL
Time (hours):	Ciprofloxacin (CFU/mL)						
0	1.46E+06	1.46E+06	1.46E+06	1.46E+06	1.46E+06	1.46E+06	1.46E+06
1	6.40E+06	2.00E+01	5.00E+03	7.40E+03	9.20E+03	1.04E+04	3.90E+04
2	2.24E+07	2.00E+01	1.80E+03	5.00E+03	5.80E+03	6.80E+03	7.60E+03
4	3.00E+07	2.00E+01	1.60E+03	4.60E+03	5.40E+03	5.60E+03	6.00E+03
24	1.14E+08	2.00E+01	2.00E+01	2.00E+01	2.00E+01	4.00E+01	2.20E+02

Figure 13. Timekill kinetics of Ciprofloxacin (CIP) against CIP Susceptible *H.influenzae* CID 2324550 (CIP MIC = 0.008 mg/mL)



***H. influenzae* CID 2975254, an intermediate isolate to ciprofloxacin, MIC ≥1.0 µg/ml (Table 31; Figure 14)**

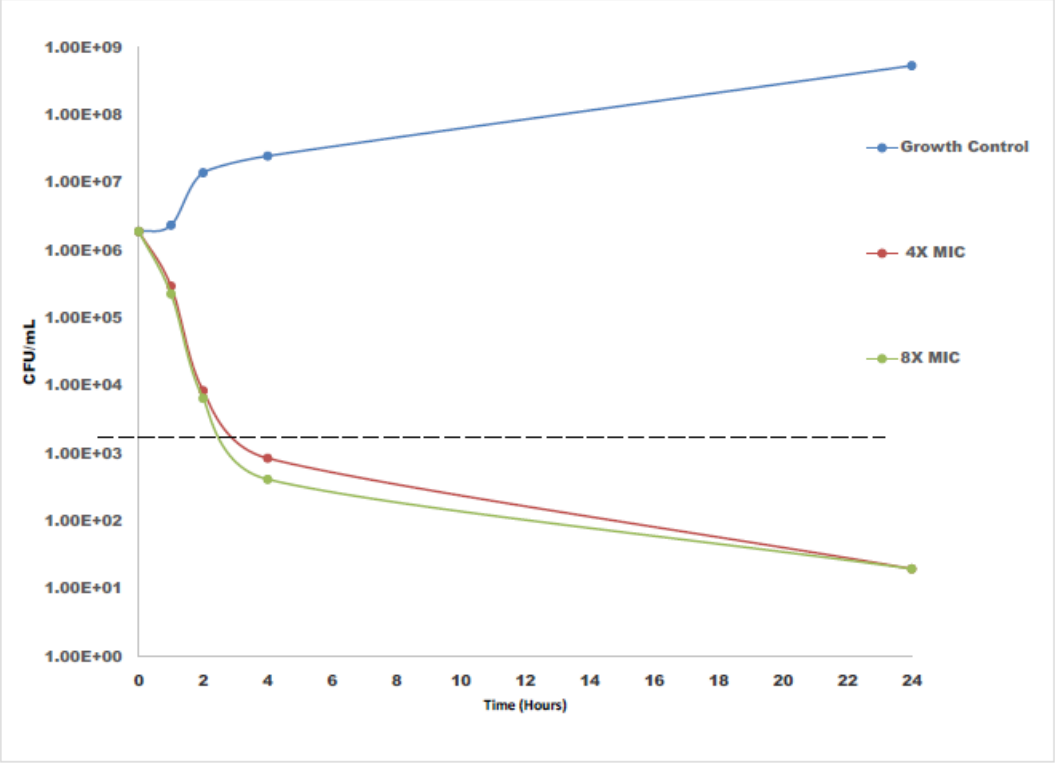
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 4 and 8 µg/ml, respectively) after 24 hr of exposure.

Table 31. Timekill kinetics of Ciprofloxacin (CIP) against CIP intermediate *H.influenzae* CID 2975254 (CIP MIC = 1.0 mg/mL)

		8X MIC	4X MIC
	0 µg/mL*	8 µg/mL	4 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	1.92E+06	1.92E+06	1.92E+06
1	2.38E+06	2.30E+05	3.00E+05
2	1.42E+07	6.60E+03	8.60E+03
4	2.50E+07	4.20E+02	8.60E+02
24	5.40E+08	2.00E+01	2.00E+01

* Growth Control

Figure 14. Timekill kinetics of Ciprofloxacin (CIP) against CIP intermediate *H.influenzae* CID 2975254 (CIP MIC = 1.0 mg/mL)



***H. influenzae* CID 2399874, a resistant isolate to ciprofloxacin, MIC ≥8.0 µg/ml (Table 32; Figure 15)**

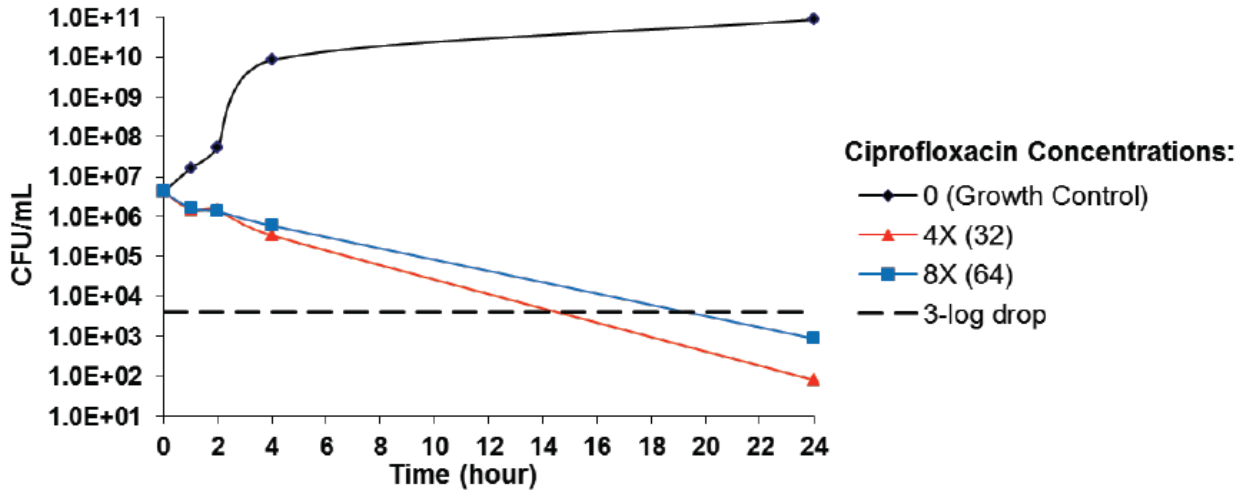
Bactericidal activity was achieved at both concentrations tested (4X and 8X of the MIC, 32 and 64 µg/ml, respectively) after 24 hr of exposure.

Table 32. Time Kill Kinetics of Ciprofloxacin (CIP) Against *H. influenzae* CID 2399874 (CIP MIC = 8 µg/mL)

		4X MIC	8X MIC
	0 µg/mL[1]	32 µg/mL	64 µg/mL
Time (hours):	Concentration (CFU/mL)		
0	4.20E+06	4.20E+06	4.20E+06
1	1.64E+07	1.66E+06	1.50E+06
2	5.40E+07	1.36E+06	1.46E+06
4	8.40E+09	6.00E+05	3.40E+05
24	8.80E+10	8.80E+02	8.00E+01

[1] Growth control
Highlighting denotes time point where 3-log kill relative to initial inoculum was achieved.

Figure 15. Time Kill Kinetics of Ciprofloxacin (CIP) Against *H. influenzae* CID 2399874 (CIP MIC = 8 µg/mL)



3. ANIMAL DISEASE MODELS OF OME

Animal models of OME have been studied in several species including mouse, rat, and chinchillas. According to the published literature, the chinchilla preclinical efficacy model most closely resembles the course of the disease observed in humans.[11, 12] The *in vivo* pharmacodynamic evaluation of Otiprio and the comparator CETRAXAL® was conducted in a chinchilla model of OME. The applicant conducted a total of 4 pharmacodynamics studies of Otiprio in chinchillas which are listed in Table 33.

Table 33: Pharmacodynamics studies for Otiprio

Study	Study Description	Formulation	Study number
1	Otic administration of CETRAXAL® in chinchillas with OME induced with *S. pneumoniae*	CETRAXAL®	OTO-201-RSP-006
2	Intratympanic administration of OTO-201 in chinchillas with OME induced with *S. pneumoniae*	OTO-201	OTO-201-RSP-003
3	Intratympanic administration of low dose OTO-201 in chinchillas with OME induced with *S. pneumoniae*	OTO-201	OTO-201-RSP-013
4	Co-treatment of poloxamer 407 and CIPRODEX® in chinchillas with OME induced with *S. pneumoniae*	Poloxamer 407, CIPRODEX®	OTO-201-RSP-021

According to the submitted reports generated by all studies, the middle ears of chinchillas were inoculated with *S. pneumoniae* serotype 6C variant 10AR0004 (200 CFUs per ear).This *S. pneumoniae* isolate was chosen because it is a commonly isolated pathogen in acute OM.[4]

Briefly, the OM with effusion was established in chinchilla after three days of bacterial inoculation with 200 CFUs per ear. The middle ear was suctioned to remove any middle ear fluid exudates and administration of either a single intratympanic of Otiprio or vehicle control was done immediately before placement of a ventilation tube. Both CETRAXAL and CIPRODEX were also used as active controls in some experiments. The CETRAXAL® and CIPRODEX® groups received topical treatment given as ear drops bid for 3 days via the tympanostomy tube in the amount of 15 µL and 10 µL AU, respectively. Clinical cure was assessed by quantitatively determining the degree of bacterial eradication and disappearance of effusion in the middle ear at 6 days post-infection. At termination, the effusion from each middle ear was collected, the volume was quantified and the bacterial count determined via plating and quantification of serial dilutions of the middle ear exudates.

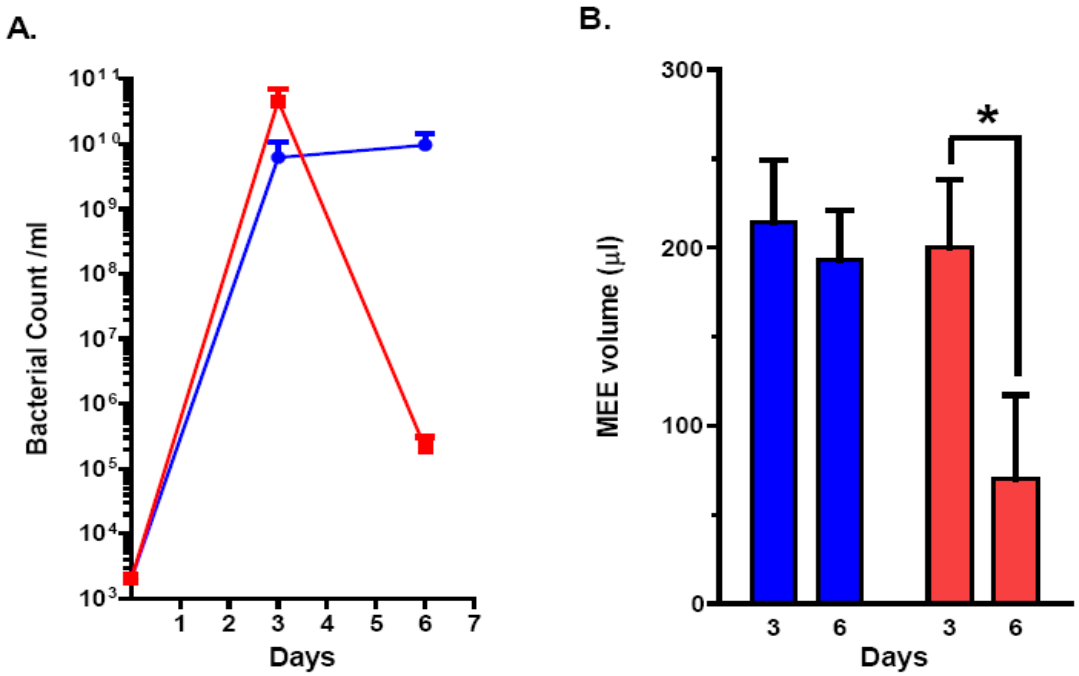
3.1 Otic Administration of Cetraxal in Chinchillas

The applicant demonstrated in Study OTO-201-RSP-006 that significant reduction (P value ≤ 0.05) in bacterial load and effusion volume was achieved in chinchillas treated with Cetraxal (Figure 16). Briefly, chinchillas (6-12 ears) with established *S. pneumoniae*-induced OME (Day 3 post inoculation) underwent drainage of their middle ear effusion, followed by tympanostomy

tube placement and initiation of a 3-day twice daily treatment course of CETRAXAL® ear drops.

CETRAXAL® treatment reduced the middle ear bacterial load by approximately 5 orders of magnitude, and significantly decreased the middle ear effusion volume. In contrast, in untreated group, chinchillas that only underwent middle ear effusion drainage and tympanostomy had persistently elevated bacterial counts and effusion volumes at Day 6.

Figure 16: Middle ear bacterial load and effusion volume in chinchillas treated with comparator, Cetraxal.

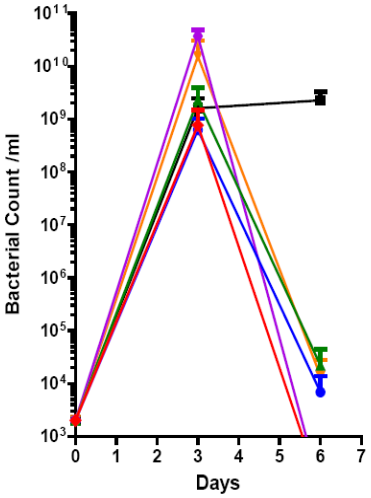


Middle ear bacterial count (A) and effusion volume (B) from untreated (blue circles, bars) or CETRAXAL-treated (red squares, bars) chinchillas. Data are presented as mean ± SEM (n=6-12 ears per group).
MEE: middle ear effusion.
* P value ≤ 0.05.

3.2 Intratympanic Administration of Otiprio in Chinchillas

The effectiveness of Otiprio was demonstrated in chinchillas (3-13 ears) with established *S. pneumoniae*-induced OME underwent middle ear drainage, followed by a single intratympanic injection of Otiprio immediately prior to tympanostomy tube placement. Five dose levels of Otiprio were evaluated, spanning a 100-fold dosage range: 0.06, 0.2, 0.6, 2.0 and 6.0%. Otiprio reduced the middle ear bacterial load by greater than 5 orders of magnitude at all doses tested (Figure 17).

Figure 17: Middle ear bacterial load in chinchillas with OME treated with a single intratympanic administration of Otiprio.

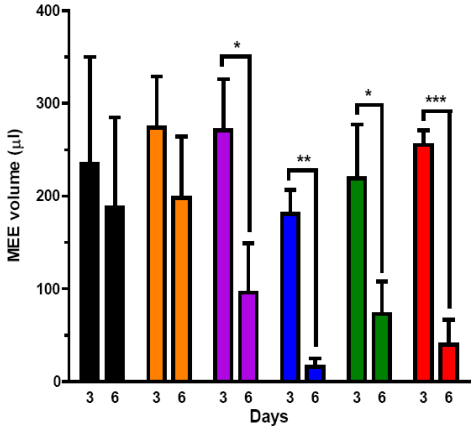


Middle ear bacterial count in chinchillas treated with poloxamer 407 vehicle (black squares) or with various doses of OTO-201: 0.06% (orange inverted triangles), 0.2% (violet hexagons), 0.6% (blue circles), 2.0% (green triangles) and 6.0% (red diamonds). Data are presented as mean ± standard error of mean (SEM) (n=3-6 ears per group).

3.3 Lowest Effective Dose of Otiprio and Middle Ear Ciprofloxacin Levels

The reduction of middle ear bacterial load was not dose-dependent; however, middle ear effusion was significantly reduced at OTO-201 doses of 0.2% and higher (Figure 18).

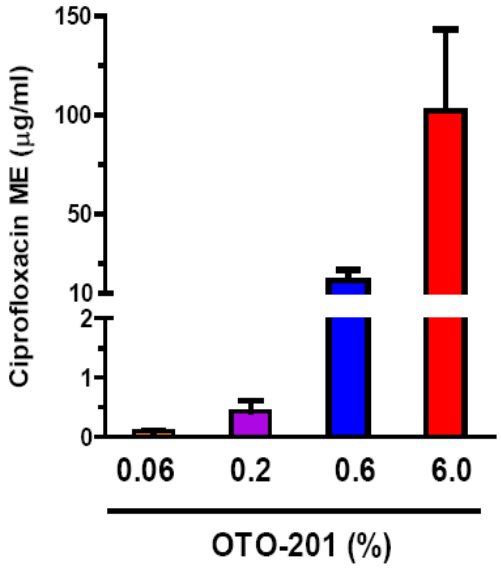
Figure 18: Middle ear effusion volume in chinchillas with OME treated with a single intratympanic administration of OTO-201.



Middle ear effusion volume in chinchillas treated with poloxamer 407 vehicle (black bars) or with various doses of OTO-201: 0.06% (orange bars), 0.2% (violet bars), 0.6% (blue bars), 2.0% (green bars) and 6.0% (red bars). Data are presented as mean ± SEM (n=6-13 ears per group).
MEE: middle ear effusion.
P values: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.

The applicant provided the middle ear levels of ciprofloxacin concentrations at termination. Concentrations varied from 0.10 µg/mL at the 0.06% dose to 102.2 µg/mL in the ears of chinchillas treated with 6.0% OTO-201(Figure 19).

Figure 19: Middle ear ciprofloxacin levels in chinchillas with OME treated with a single intratympanic administration of OTO-201

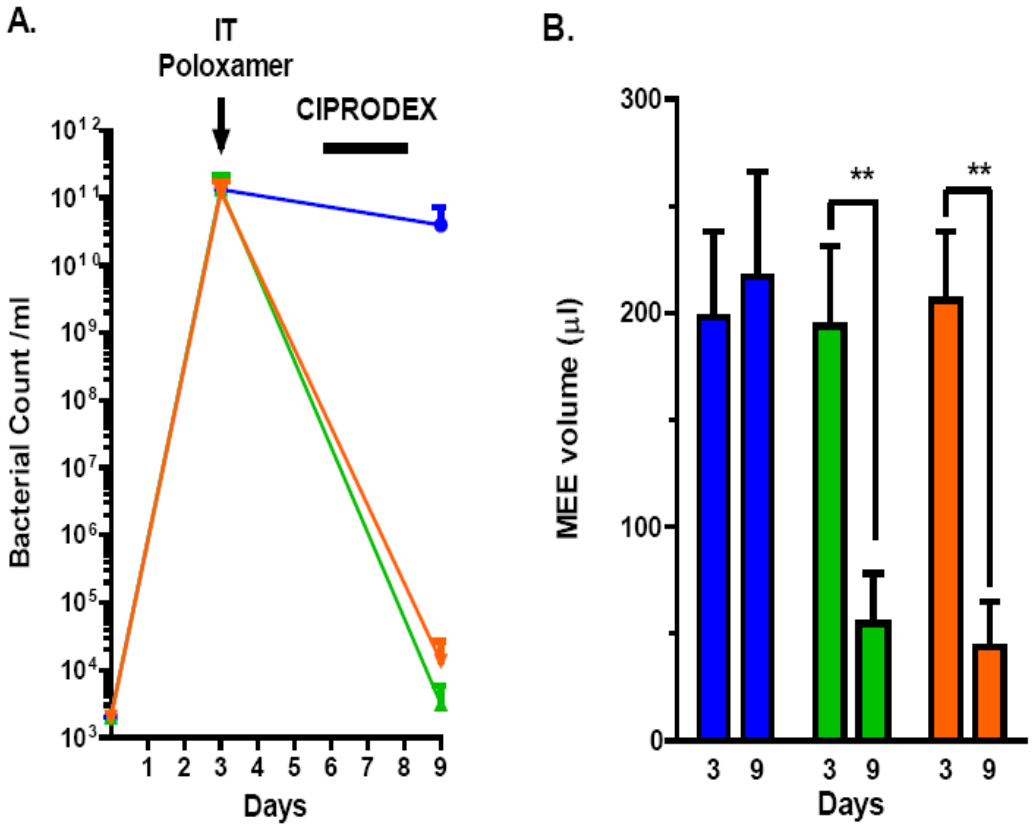


Middle ear ciprofloxacin levels in chinchillas treated with various doses of OTO-201: 0.06% (orange bars), 0.2% (violet bars), 0.6% (blue bars) and 6.0% (red bars). Data are presented as mean ± SEM (n=6-10 ears per group). No ciprofloxacin was present in vehicle (poloxamer 407) treated chinchillas.

3.4 No Impact on Antimicrobial Activity using Poloxamer

The applicant demonstrated that the presence of poloxamer in the middle did not reduce the antimicrobial activity of CIPRODEX®. Briefly, chinchillas with established *S. pneumoniae* induced OME (Day 3 post inoculation) underwent drainage of their middle ear effusion, followed by a single intratympanic injection of poloxamer 407 vehicle immediately prior to tympanostomy tube placement. Three days later on Day 6, a 3- day twice daily treatment course of CIPRODEX® ear drops was initiated. There were no differences in the degree of bacterial eradication and reduction of middle ear effusion volume in chinchillas receiving poloxamer 407 + CIPRODEX® compared to those receiving only CIPRODEX® (Figure 20).

Figure 20: Middle ear bacterial load and effusion volume in chinchillas with poloxamer 407 and CIPRODEX® Otic suspension.



Middle ear bacterial count (A) and effusion volume (B) from untreated chinchillas (blue circles, bars), and chinchillas treated with CIPRODEX® (green triangles, bars) or co-treated with poloxamer 407 and CIPRODEX® (orange inverted triangles, bars). Data are presented as mean ± SEM (n=5-13 ears per group).
MEE: middle ear effusion.
** P value ≤ 0.01.

In summary, the applicant demonstrated that all tested doses of Otiprio were effective at reducing the middle ear bacterial load. With the exception of the 0.06% dose of Otiprio, all decreased the middle ear effusion volume. A single intratympanic administration of Otiprio at and above 0.6% was effective in reducing bacterial load and effusion volume of the middle ear as compared to a 3-day, twice daily treatment regimen of the comparator agent CETRAXAL® and CIPRODEX® in animal models of OME. The different dosing regimens yielded ciprofloxacin levels at study termination ranging from values at the *S. pneumoniae* MIC to values that were 500-fold above the MIC. The presence of the Otiprio vehicle, poloxamer 407, did not appear to have any significant effect on the therapeutic efficacy of CIPRODEX® to alleviate OME when both were administered.

4. PHARMACOKINETIC / PHARMACODYNAMIC STUDIES

There were no clinical pharmacokinetic or clinical pharmacology studies conducted with Otiprio in human subjects. However, the applicant conducted nonclinical pharmacokinetic studies in animal models.

The applicant demonstrated that the middle ear drug concentration of Otiprio at (b) (4) 6.0% achieved T>MIC values of 413 hours and 715 hours, respectively. These values were comparable to those of comparators, Cetraxal (15 µL AU bid for 7 days) and Ciprodex (10 µL AU bid for 7 days), 611 hours and 601 hours, respectively. Systemic exposure (plasma) following Otiprio was also comparable to that of Cetraxal or Ciprodex.

Toxicology studies conducted in guinea pigs showed that macroscopic and microscopic otic tissue assessments following (b) (4) 6.0% Otiprio were indistinguishable from those following treatment with Cetraxal or Ciprodex administered BID for 7 days.

The applicant demonstrated that no systemic toxicity was observed due to Otiprio administration, and due to the local administration which results in low plasma levels, the risk of systemic toxicity was low.

5. CLINICAL EFFICACY TRIALS

5.1 Study Design and Patient Population

The applicant demonstrated the efficacy of Otiprio based on data from two pivotal Phase 3 studies (201-201302 and 201-201303). Both studies were similar in design, and consisted of randomized, double-blinded, sham-controlled and enrolled a total of 532 pediatric subjects, age ranged from 6 months to 17 years. Three hundred and fifty seven subjects randomized to the Otiprio 6 mg group and 175 subjects randomized to the sham group for the treatment of middle ear effusion in pediatric subjects with otitis media requiring TT placement. The two Phase 3 studies were conducted in 55 centers in the United States (U.S.) and Canada and were considered pivotal.

Of the 532 subjects enrolled in both Phase 3 studies, 302 (57.0%) were male, and 228 (43.0%) were female. The mean age of the subjects was 2.4 (2.12) years, with a range of 6 months to 12.6 years. The majority of subjects (326 subjects, 61.5%) were 6 months through 2 years of age, and the remaining subjects (204 subjects, 38.5%) were >2 years of age. The number of subjects <4 years of age was 435. The subjects enrolled in these studies were primarily white (80.6%) and black or African American (12.5%); the remaining 7.0% of subjects were Asian, Native American/Canadian, Native Hawaiian or other Pacific Islander, or other. In both Phase 3 studies, the majority of enrolled subjects (98.9%) completed the studies.

An overview of both studies is presented in Table 34.

Table 34: Overview of Clinical Efficacy Program

Protocol Number	Study Design	Treatment Groups	Treatment Duration	Randomized Subjects	Treated Subjects	Median Age (range)	Age Stratum 6 M to 2 Y (%)/ >2 Y (%)
Phase 3 Adequate and Well-Controlled Studies							
201-201302	Phase 3, randomized, DB, sham-controlled	6 mg OTO-201, Sham	Single bilateral administration	N = 266 6 mg: 179 Sham: 87	N = 265 6 mg: 179 Sham: 86	1.585 (0.50 to 12.60)	162 (60.9%) 104 (39.1%)
201-201303	Phase 3, randomized, DB, sham-controlled	6 mg OTO-201, Sham	Single bilateral administration	N = 266 6 mg: 178 Sham: 88	N = 265 6 mg: 178 Sham: 87	1.535 (0.51 to 11.63)	164 (61.7%) 102 (38.3%)

Randomization stratified by age 6 months through 2 years or >2 years for all studies.
Note: The counts for Randomized Subjects are presented by randomized treatment group, while the counts for Treated Subjects are presented by actual treatment received. In study 201-201302, Subject 042-208-7 was randomized to 6 mg OTO-201 but was never treated. In the same study, Subject 051-203-9 was randomized to sham but received 6 mg OTO-201. In study 201-201303, Subject 083-640-1 was randomized to 6 mg OTO-201 but never treated. In the same study, Subjects 002-603-5 and 007-609-6 were randomized to sham but received 6 mg OTO-201, while Subject 038-606-6 was randomized to 6 mg OTO-201 but received sham.
DB = double-blind; M = months; PBO = placebo; Y = years.
Source: Clinical study reports for 201-201101, 201-201302, and 201-201303

The results from both Phase 3 clinical studies, 201-201302 and 201-201303 demonstrated superiority of Otiprio treatment group over sham group and clinically significant across a number of different endpoints and similarity of results across different subpopulations. The applicant demonstrated that treatment with Otiprio in the pediatric population was overall safe and well-tolerated through 28 days post-treatment in both Phase 3 studies.

Overall, Otiprio was found to be both clinically and statistically superior to sham with regard to the primary endpoint of study treatment failure through Day 15. The data from these 2 independent Phase 3 studies support the efficacy of Otiprio in the treatment of middle ear effusion in pediatric subjects with otitis media requiring TT placement.

5.2 Efficacy Endpoints

The applicant described the following efficacy endpoints in the submission:

5.2.1 Primary Efficacy Endpoint:

The primary efficacy endpoint for both Phase 3 studies assessed the cumulative proportion of study treatment failures through the Day 15 Visit. The study treatment failure was defined as the presence of visible otorrhea or the study subject was treated with an otic or systemic antibiotic within the defined study visit of Day 15. The applicant elaborated treatment failure as the occurrence of any one or combinations of the following events: otorrhea treatment failure, otic antibiotic treatment failure, systemic antibiotic treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
The applicant provided the definitions of all events as follows:

- Otorrhea treatment failure: Subject with otorrhea in 1 or both ears through the Day 15 Visit that was observed by the blinded assessor on or after the third day postsurgery.
- Otic antibiotic treatment failure: Subject received an otic antibiotic drop any time postsurgery through the Day 15 Visit prior to confirmation of otorrhea by the blinded assessor.
- Systemic antibiotic treatment failure: Subject received a systemic antibiotic any time postsurgery through the Day 15 Visit prior to confirmation of otorrhea by the blinded assessor.
- Lost-to-follow-up treatment failure: Subject whose study treatment failure status was unknown at the scheduled Day 15 Visit due to him/her being lost to follow-up.
- Missed visit treatment failure: Subject, not lost to follow-up, who had a missing treatment failure status at a particular visit through the Day 15 Visit because they did not return to the clinic for a blinded assessment within the analytic time window and who had not yet been identified as a study treatment failure.

5.2.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints for this integrated analysis were followings:

- Cumulative proportion of study treatment failures through the Day 4, Day 8, and Day 29 Visits.
- Cumulative proportion of otorrhea-only treatment failures through the Day 15 Visit.
- Time to study treatment failure through the Day 15 Visit, censoring lost-to-follow-up treatment failures.
- Microbiological response by the Day 15 and Day 29 Visits.

The applicant further classified the microbiological responses into two categories:

1. Microbiological response without presumption: For secondary endpoint analysis of the Phase 3 studies, microbiological response was defined as the documented eradication of target otic pathogens (without presumption) and microbiologic nonresponse via documented persistence of target otic pathogens (without presumption).
2. Presumed microbiological response: For presumed microbiologic response, eradication was presumed if the subject was not declared to be a treatment failure due to otorrhea or presumed otorrhea. For presumed microbiologic nonresponse, persistence was presumed if the subject was declared a treatment failure due to otorrhea or presumed otorrhea.

Microbiological processing of otorrhea specimens:

According to the protocol submitted by the applicant, effusion specimens from each ear were obtained prior to Otiprio or sham injection during surgery. A total of four samples per subject were collected if both ears presented otorrhea: two from the right ear and two from the left ear. One sample per ear was used for culture and susceptibility testing and one sample per ear was used for PCR testing.

Caregivers were instructed to bring the subject to the study site for examination if otorrhea in one or both ears was observed on or after 3 days of post-surgery (Day 4). A blinded assessor who

was not involved in the administration of study drug assessed the presence of otorrhea in each study visit from visit 3-6 until Day 29, and if present, an effusion specimen was collected for culture. The effusion specimen was not collected for culture if the subject had no otorrhea reported by the blinded assessor. All samples were shipped to a central microbiology laboratory for analysis. All specimens were analyzed for microbiological culture, sensitivity and exploratory microbiology testing (polymerase chain reaction for middle ear pathogens) conducted by the central microbiology laboratory. The Microbiological Central Laboratory processed otic specimens to recover and identify all relevant organisms. Susceptibility testing for ciprofloxacin was done against all targeted otic pathogens (e.g., *S. aureus*, *S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, and *M. catarrhalis*). The culture and susceptibility testing results for each subject was provided to the investigator.

5.3 Efficacy Analysis

The primary and sensitivity analyses for both Phase 3 studies are summarized in Table 35.

Table 35: Summary of Primary Clinical Efficacy Results from Phase 3 clinical studies, 201-201302 and 201-201303

	201-201302		201-201303	
Statistic	OTO-201 N = 179	Sham N = 87	OTO-201 N = 178	Sham N = 88
Primary – Cumulative proportion of study treatment failures through Day 15				
n (%)	44 (24.6%)	39 (44.8%)	38 (21.3%)	40 (45.5%)
Relative risk (95% CI)	0.548 (0.3901, 0.7709)		0.463 (0.3258, 0.6590)	
Odds ratio (95% CI)	0.388 (0.2232, 0.6758)		0.299 (0.1689, 0.5287)	
Risk difference (95% CI)	-0.202 (-0.3245, -0.0804)		-0.241 (-0.3613, -0.1209)	
p-value	<0.001		<0.001	
Sensitivity - Excludes systemic antibiotic treatment failure from the definition of treatment failure				
n (%)	42 (23.5%)	35 (40.2%)	32 (18.0%)	37 (42.0%)
Relative risk (95% CI)	0.583 (0.4054, 0.8393)		0.421 (0.2861, 0.6193)	
Odds ratio (95% CI)	0.447 (0.2558, 0.7819)		0.273 (0.1512, 0.4946)	
Risk difference (95% CI)	-0.168 (-0.2880, -0.0474)		-0.241 (-0.3582, -0.1231)	
p-value	0.004		<0.001	
Sensitivity - Excludes lost-to-follow-up treatment failure from the definition of treatment failure				
n (%)	43 (24.0%)	39 (44.8%)	37 (20.8%)	40 (45.5%)
Relative risk (95% CI)	0.536 (0.3804, 0.7551)		0.451 (0.3161, 0.6424)	
Odds ratio (95% CI)	0.375 (0.2147, 0.6546)		0.285 (0.1599, 0.5065)	
Risk difference	-0.208		-0.247	

Statistic	201-201302 OTO-201 N = 179	201-201302 Sham N = 87	201-201303 OTO-201 N = 178	201-201303 Sham N = 88
(95% CI)	(-0.3299, -0.0862)		(-0.3666, -0.1268)	
p-value	<0.001		<0.001	
Sensitivity - Excludes missed visit treatment failure from the definition of treatment failure				
n (%)	29 (16.2%)	30 (34.5%)	29 (16.3%)	35 (39.8%)
Relative risk (95% CI)	0.470 (0.3055, 0.7228)		0.403 (0.2682, 0.6062)	
Odds ratio (95% CI)	0.349 (0.1892, 0.6448)		0.268 (0.1465, 0.4890)	
Risk difference (95% CI)	-0.183 (-0.2963, -0.0693)		-0.235 (-0.3506, -0.1190)	
p-value	<0.001		<0.001	
Sensitivity – Per-Protocol Analysis Set				
N[a]	148	70	159	74
n (%)	18 (12.2%)	27 (38.6%)	27 (17.0%)	29 (39.2%)
Relative risk (95% CI)	0.321 (0.1919, 0.5355)		0.428 (0.2787, 0.6565)	
Odds ratio (95% CI)	0.211 (0.1040, 0.4293)		0.284 (0.1483, 0.5455)	
Risk difference (95% CI)	-0.264 (-0.3897, -0.1385)		-0.222 (-0.3477, -0.0965)	
p-value	<0.001		<0.001	
Sensitivity - Multiple imputation[b]				
% (95% CI)	16.0% (10.6%, 21.4%)	35.0% (24.8%, 45.1%)	15.7% (10.3%, 21.1%)	40.7% (30.2%, 51.1%)
Relative risk (95% CI)	0.458 (0.2965, 0.7065)		0.380 (0.2501, 0.5764)	
Odds ratio (95% CI)	0.334 (0.1787, 0.6228)		0.244 (0.1312, 0.4524)	
Risk difference (95% CI)	-0.190 (-0.3045, -0.0748)		-0.250 (-0.3670, -0.1323)	
p-value	<0.001		<0.001	

a In this display, N = Per-Protocol Analysis Set which differs from the other analyses in this table in which N = Full Analysis Set defined as the intent-to-treat population, including all randomized subjects.
b As a sensitivity analysis, data for subjects who either missed a visit or were lost to follow-up were multiply imputed. For study 201-201302, the data for the OTO-201 group were imputed using a model with Day 15 failure status and age strata. The data for the sham group were imputed using a model with Day 15 failure status. For study 201-201303, the data for the OTO-201 group were imputed using a model with Day 15 failure status, Day 8 failure status, and age strata. The data for the sham group were imputed using a model with Day 15 failure status and Day 4 failure status.
Note: A study treatment failure was defined as the occurrence of any of the following events: otic treatment failure, systemic antibiotic treatment failure, otorrhea treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
Note: The p-values were derived from a Cochran-Mantel-Haenszel test stratified by age strata.
Note: The relative risk, odds ratio, and corresponding 95% CIs for OTO-201 versus sham are adjusted for age strata.
Note: All risk differences and the corresponding 95% CIs are not adjusted for age strata. Risk difference is estimated by the proportion of subjects with treatment failure in the OTO-201 group - the proportion of subjects with treatment failure in the sham group.
CI = confidence interval.
Source: Clinical study reports for studies 201-201302 and 201-201303

5.4 Primary Efficacy Results

The primary efficacy endpoint in the integrated analysis was the cumulative proportion of study treatment failures through the Day 15 Visit. Table 36 summarizes the comparison of the primary efficacy endpoint between the Otiprio and sham treatment groups for the Full Analysis Set (FAS).

Table 36: Cumulative Proportion of Subjects with Study Treatment Failure through Day 15 by Treatment Group (Full Analysis Set)

	Study 201-201302		Study 201-201303		Pooled	
	OTO-201 6 mg N = 179	Sham N = 87	OTO-201 6 mg N = 178	Sham N = 88	OTO-201 6 mg N = 357	Sham N = 175
Cumulative proportion of study treatment failures[a] through Day 15						
n (%)	44 (24.6%)	39 (44.8%)	38 (21.3%)	40 (45.5%)	82 (23.0%)	79 (45.1%)
RR (95% CI)[b]	0.548 (0.3901, 0.7709)		0.463 (0.3258, 0.6590)		0.506 (0.3960, 0.6457)	
OR (95% CI [b]	0.388 (0.2232, 0.6758)		0.299 (0.1689, 0.5287)		0.341 (0.2294, 0.5082)	
Risk Difference (95% CI)[c]	-0.202 (-0.3245, -0.0804)		-0.241 (-0.3613, -0.1209)		-0.222 (-0.3074, -0.1361)	
p-value[d]	<0.001		<0.001		<0.001	

a A study treatment failure was defined as the occurrence of any of the following events: otic treatment failure, systemic antibiotic treatment failure, otorrhea treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
b The relative risk, odds ratio, and corresponding 95% CIs for OTO-201 6 mg versus sham were adjusted for age strata.
c All risk differences and the corresponding 95% CIs were not adjusted for age strata. Risk differences were estimated by the proportion of subjects with treatment failure in the OTO-201 6 mg group – the proportion of subjects with treatment failure in the sham group.
CI = confidence interval; OR = odds ratio; RR = relative risk.
d P-values were derived from a Cochran-Mantel-Haenszel test stratified by age strata (6 months through 2 years and >2 years).
Source: ISE Post-text Table 3.1

5.5 Baseline Disease Characteristics and Baseline Microbiology Culture

The most common type of middle ear effusion at baseline was mucoid effusion, which was reported in at least 1 ear in 57.3% of subjects. A serous effusion was reported in at least 1 ear in 39.8% of subjects, and a purulent effusion was reported in at least 1 ear in 13.2% of subjects. The frequencies of these 2 types of effusions were similar between treatment groups. Only 5 subjects (0.9%) reported a sanguineous effusion.

Overall, 119 subjects (22.4%) had a positive culture result at baseline of at least 1 of the 5 targeted otic pathogens in at least 1 ear, with the proportion being smaller in the Otiprio treatment group than in the sham control group (19.6% versus 28.0%). The most common microorganism cultured was *H. influenzae*, being reported in at least 1 ear in 12.4% of subjects and the proportion was smaller in the Otiprio treatment group than in the sham control group (10.9% versus 15.4%). At least 1 ear was positive for *S. pneumoniae* in 6.0% of subjects, and for

M. catarrhalis in 4.1% of subjects. *S. aureus* and *P. aeruginosa* were reported in at least 1 ear in 10 (1.9%) and 3 (0.6%) subjects, respectively.

Baseline disease characteristics and baseline microbiology culture are summarized and presented in Table 37.

Table 37: Disease Baseline Characteristics by Treatment Group (Full Analysis Set)

Characteristics	OTO-201 6 mg N = 357	Sham N = 175	Total N = 532
Baseline effusion type – n (%)[a]			
Unknown[b]			
Neither Ear	352 (98.6%)	174 (99.4%)	526 (98.9%)
One Ear	0	0	0
Both Ears	5 (1.4%)	1 (0.6%)	6 (1.1%)
At Least One Ear[c]	5 (1.4%)	1 (0.6%)	6 (1.1%)
Serous			
Neither Ear	212 (59.4%)	108 (61.7%)	320 (60.2%)
One Ear	32 (9.0%)	16 (9.1%)	48 (9.0%)
Both Ears	113 (31.7%)	51 (29.1%)	164 (30.8%)
At Least One Ear	145 (40.6%)	67 (38.3%)	212 (39.8%)
Purulent			
Neither Ear	308 (86.3%)	154 (88.0%)	462 (86.8%)
One Ear	16 (4.5%)	7 (4.0%)	23 (4.3%)
Both Ears	33 (9.2%)	14 (8.0%)	47 (8.8%)
At Least One Ear	49 (13.7%)	21 (12.0%)	70 (13.2%)
Sanguineous			
Neither Ear	356 (99.7%)	171 (97.7%)	527 (99.1%)
One Ear	1 (0.3%)	1 (0.6%)	2 (0.4%)
Both Ears	0	3 (1.7%)	3 (0.6%)
At Least One Ear	1 (0.3%)	4 (2.3%	5 (0.9%)
Mucoid			
Neither Ear	155 (43.4%)	72 (41.1%)	227 (42.7%)
One Ear	41 (11.5%)	18 (10.3%)	59 (11.1%)
Both Ears	161 (45.1%)	85 (48.6%)	246 (46.2%)
At Least One Ear	202 (56.6%)	103 (58.9%)	305 (57.3%)
Baseline microbiology culture – n (%)			
Overall			
Unknown[d]			
Neither Ear	346 (96.9%)	170 (97.1%)	516 (97.0%)
One Ear	6 (1.7%)	3 (1.7%)	9 (1.7%)
Both Ears	5 (1.4%)	2 (1.1%)	7 (1.3%)
At Least One Ear	11 (3.1%)	5 (2.9%)	16 (3.0%)
Positive[e]			
Neither Ear	287 (80.4%)	126 (72.0%)	413 (77.6%)
One Ear	46 (12.9%)	32 (18.3%)	78 (14.7%)
Both Ears	24 (6.7%)	17 (9.7%)	41 (7.7%)
At Least One Ear	70 (19.6%)	49 (28.0%)	119 (22.4%)

Characteristics	OTO-201 6 mg N = 357	Sham N = 175	Total N = 532
***P. aeruginosa* - positive**			
Neither Ear	356 (99.7%)	173 (98.9%)	529 (99.4%)
One Ear	1 (0.3%)	2 (1.1%)	3 (0.6%)
Both Ears	0	0	0
At Least One Ear	1 (0.3%)	2 (1.1%)	3 (0.6%)
***S. aureus* - positive**			
Neither Ear	351 (98.3%)	171 (97.7%)	522 (98.1%)
One Ear	2 (0.6%)	3 (1.7%)	5 (0.9%)
Both Ears	4 (1.1%)	1 (0.6%)	5 (0.9%)
At Least One Ear	6 (1.7%)	4 (2.3%)	10 (1.9%)
***S. pneumoniae* - positive**			
Neither Ear	337 (94.4%)	163 (93.1%)	500 (94.0%)
One Ear	12 (3.4%)	9 (5.1%)	21 (3.9%)
Both Ears	8 (2.2%)	3 (1.7%)	11 (2.1%)
At Least One Ear	20 (5.6%)	12 (6.9%)	32 (6.0%)
***H. influenzae* - positive**			
Neither Ear	318 (89.1%)	148 (84.6%)	466 (87.6%)
One Ear	28 (7.8%)	14 (8.0%)	42 (7.9%)
Both Ears	11 (3.1%)	13 (7.4%)	24 (4.5%)
At Least One Ear	39 (10.9%)	27 (15.4%)	66 (12.4%)
***M. catarrhalis* - positive**			
Neither Ear	343 (96.1%)	167 (95.4%)	510 (95.9%)
One Ear	14 (3.9%)	7 (4.0%)	21 (3.9%)
Both Ears	0	1 (0.6%)	1 (0.2%)
At Least One Ear	14 (3.9%)	8 (4.6%)	22 (4.1%)

a Baseline was defined as the last measurement taken on or prior to the day of study drug administration.
b Effusion type unknown indicates that the type of effusion was not recorded. Subjects with missing effusion type for both ears are included in the "Neither Ear" category for each of the other effusion types.
c "At Least one ear" includes "One ear" and "Both ears".
d Microbiology culture unknown indicates that the microbiology culture results were not recorded. Subjects with missing microbiology culture for both ears are included in the "Neither Ear" category for "Positive" and for each individual organism.
e This category represents subjects for which the baseline microbiology culture was positive for at least 1 of the following 5 organisms: *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*.
Haemophilus influenzae = *H. influenzae*; *Moraxella catarrhalis* = *M. catarrhalis*; *Pseudomonas aeruginosa* = *P. aeruginosa*; *Staphylococcus aureus* = *S. aureus*; *Streptococcus pneumoniae* = *S. pneumoniae*.
Source: ISE Post-text Table 2.1

5.6 Microbiological Response from Phase 3 Clinical Study, 201-201302

At both defined efficacy time points, the total microbiological response was higher in the Otiprio treatment group than in the sham group (80.5% versus 40.9% at Day 15; and 75.6% versus 31.8% at Day 29). This pattern was also observed for presumed microbiological response. The results for microbiological response without presumption were comparable between the Otiprio and sham groups at both time points.

Microbiological response in the age stratum of 6 months to 2 years was higher in the Otiprio group than in the sham group (78.6% versus 37.5% at Day 15;and 78.6% versus 31.3% at Day 29). In the age stratum of >2 years, the total microbiological response rate was higher in the Otiprio group than in the sham treatment group (84.6% versus 50.0% at Day 15; and 69.2% versus 33.3% at Day 29).

5.7 Microbiological Response from Phase 3 Clinical Study, 201-201303

At both defined efficacy time points analyzed, the total microbiological response was higher in the Otiprio Treatment group than in the sham control group (82.8% versus 48.1% at Day 15; and 72.4% versus 37.0% at Day 29). This pattern was also observed for microbiological response based on post-baseline cultures and for presumed microbiological response.
Microbiological response in the age stratum of 6 months to 2 years was higher in the Otiprio group than in the sham group (82.6% versus 23.5% at Day 15; and 69.6% versus 17.6% at Day 29).
In the age stratum of >2 years, the respective total microbiological response rates for the Otiprio and sham treatment groups were 83.3% and 90.0% at Day 15, and 83.3% and 70.0% at Day 29. However, the numbers of subjects >2 years in the Otiprio and sham groups belonging to the Microbiologically Evaluable Set (MES) were very small (6 and 10 subjects, respectively).

5.8 Microbiological Response by the Day 15 and Day 29 Visits

Table 38 summarizes microbiological response through the Day 15 and Day 29 Visits (Visit 5 and Visit 6, respectively), including microbiological response based on post baseline cultures (without presumption) and presumed microbiologic responses based on treatment failure outcome for those subjects without post baseline cultures. At each time point, the total microbiological response was higher in the Otiprio group than in the sham group (81.4% versus 44.9% at Day 15; and 74.3% versus 34.7% at Day 29). This pattern was also observed for microbiological response with and without presumed microbiological response.

Table 38: Microbiological Response by Day 15 and Day 29 by Treatment Group (Microbiologically Evaluable Set)

Endpoint Timepoint	OTO-201 6 mg N = 70 n (%)	Sham N = 49 n (%)
Microbiological response (total)		
Through Day 15	57 (81.4%)	22 (44.9%)
Through Day 29	52 (74.3%)	17 (34.7%)
Microbiological response without presumption only[a]		
Through Day 15	8 (11.4%)	3 (6.1%)
Through Day 29	9 (12.9%)	3 (6.1%)
Microbiological response with presumption only[b]		
Through Day 15	49 (70.0%)	19 (38.8%)
Through Day 29	43 (61.4%)	14 (28.6%)

a The "microbiological response without presumption" included subjects with a positive baseline culture and negative postbaseline cultures.
b The "microbiological response with presumption" included subjects with a positive baseline culture but no postbaseline culture who were not study treatment failures. A study treatment failure was defined as the occurrence of any of the following events: otic treatment failure, systemic antibiotic treatment failure, otorrhea treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
Source: ISE Post-text Table 3.6.1

5.9 Microbiological Response by the Day 15 and Day 29 Visits Using Observed/Presumed Otorrhea Treatment Failure when Evaluating Presumed Response

For presumed microbiological response, eradication was presumed if the subject was not declared to be a treatment failure due to otorrhea or presumed otorrhea. For presumed microbiological nonresponse, persistence was presumed if the subject was declared a treatment failure due to otorrhea or presumed otorrhea.

Table 39 summarizes the microbiological response using observed/presumed otorrhea treatment failure when evaluating presumed response through the Day 15 and Day 29 Visits, including microbiological response based on post baseline cultures (without presumption) and presumed microbiological responses based on treatment failure outcome for those subjects without post baseline cultures. At each time point, the total microbiological response was higher in the Otiprio group than in the sham group (95.7% versus 51.0% through Day 15; and 91.4% versus 44.9% at Day 29). This pattern was also observed for microbiological response with and without presumed microbiological response.

Table 39: Microbiological Response by Day 15 and Day 29 by Treatment Group, Otorrhea Defined as Observed/Presumed Otorrhea (Microbiologically Evaluable Set)

Endpoint Timepoint	OTO-201 6 mg N = 70 n (%)	Sham N = 49 n (%)
Microbiological response (total)		
Through Day 15	67 (95.7%)	25 (51.0%)
Through Day 29	64 (91.4%)	22 (44.9%)
Microbiological response without presumption only[a]		
Through Day 15	8 (11.4%)	3 (6.1%)
Through Day 29	9 (12.9%)	3 (6.1%)
Microbiological response with presumption only[b]		
Through Day 15	59 (84.3%)	22 (44.9%)
Through Day 29	55 (78.6%)	19 (38.8%)

a The "microbiological response without presumption" included subjects with a positive baseline culture and negative postbaseline cultures.
b The "microbiological response with presumption" included subjects with a positive baseline culture but no postbaseline culture who were not treatment failures due to observed/presumed otorrhea. A treatment failure due to observed/presumed otorrhea was defined as either: 1) observed otorrhea – study treatment failure due to observed otorrhea by the blinded assessor, or 2) presumed otorrhea – study treatment failure via antibiotic treatment (either otic or systemic antibiotics) if the antibiotic was prescribed for otorrhea (defined as otorrhea, ear drainage, ear infection, effusion, otitis externa, otitis media).
Source: ISE Post-text Table 3.6.2

5.10 Primary Efficacy Analysis with Baseline Microbiology Culture

The primary efficacy endpoint was evaluated for Otiprio and sham groups by baseline microbiology culture status category (positive or negative) and by baseline microbiologic pathogen type (*P. aeruginosa*, *S. aureus*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*). The results are summarized in the following Integrated Summary of Efficacy (ISE) Post-text Table 3.7.1.5 and ISE Post-text Table 3.7.1.6, respectively.

Integrated Summary of Efficacy
Table 3.7.1.5
Summary of Study Treatment Failure through Day 15 by Baseline Microbiology Culture Status and Treatment Group
Population: Full Analysis Set
Studies: 201-201302 and 201-201303

Baseline Microbiology Culture Status: Positive	Study 201-201302		Study 201-201303		Pooled	
	OTO-201 6 mg N=41	Sham N=22	OTO-201 6 mg N=29	Sham N=27	OTO-201 6 mg N=70	Sham N=49
Cumulative proportion of study treatment failures [1]						
Through Day 15						
n (%)	11 (26.8%)	15 (68.2%)	10 (34.5%)	15 (55.6%)	21 (30.0%)	30 (61.2%)
RR (95% CI) [2]	0.403 (0.2285, 0.7106)		0.552 (0.3165, 0.9625)		0.474 (0.3160, 0.7103)	
OR (95% CI) [2]	0.164 (0.0809, 0.5278)		0.314 (0.0986, 0.9975)		0.228 (0.1002, 0.5182)	
Risk Difference (95% CI) [3]	-0.414 (-0.6507, -0.1763)		-0.211 (-0.4658, 0.0443)		-0.312 (-0.4858, -0.1386)	
p-value [4]	0.002**		0.082*		<0.001***	
Exact test p-value [5]	0.003**		0.045*		<0.001***	

[1] A study treatment failure was defined as the occurrence of any of the following events: otic treatment failure, systemic antibiotic treatment failure, otorrhea treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
[2] The RR, OR, and corresponding 95% CIs for OTO-201 6 mg versus Sham were adjusted for age strata.
[3] All risk differences and the corresponding 95% CIs were not adjusted for age strata. Risk differences were estimated by the proportion of subjects with treatment failure in the OTO-201 6 mg group - the proportion of subjects with treatment failure in the Sham group.
[4] P-values were derived from a Cochran-Mantel-Haenszel test stratified by age strata (6 months through 2 years and >2 years).
[5] P-values were derived from a Cochran-Mantel-Haenszel exact test stratified by age strata (6 months through 2 years and >2 years).
Note: Baseline microbiology culture status was based on whether cultures were positive for at least 1 of the following 5 organisms: Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenza, or Moraxella catarrhalis. Subjects with a positive baseline microbiology culture status for at least 1 ear were included in "Positive". Subjects with baseline microbiology culture result for at least 1 ear but neither ear positive were included in "Negative". Subjects with missing baseline microbiology cultures for both ears were included in "Unknown".
*p-value < 0.05; **p-value < 0.01; ***p-value < 0.001
CI = confidence interval; NE = not estimable; OR = odds ratio; RR = relative risk.

Integrated Summary of Efficacy
Table 3.7.1.6
Summary of Study Treatment Failure through Day 15 by Baseline Microbiology Culture Status by Organism and Treatment Group
Population: Full Analysis Set
Studies: 201-201302 and 201-201303

Subgroup	OTO-201 6 mg N=357	Sham N=175
Baseline microbiology culture - n (%) [1]		
Overall		
Unknown [2]		
Neither Ear	79/346 (22.8%)	78/170 (45.9%)
One Ear	1/ 6 (16.7%)	1/ 3 (33.3%)
Both Ears	2/ 5 (40.0%)	0/ 2 (0.0%)
At Least One Ear [3]	3/ 11 (27.3%)	1/ 5 (20.0%)
Positive [4]		
Neither Ear	61/287 (21.3%)	49/126 (38.9%)
One Ear	13/ 46 (28.3%)	18/ 32 (56.3%)
Both Ears	8/ 24 (33.3%)	12/ 17 (70.6%)
At Least One Ear	21/ 70 (30.0%)	30/ 49 (61.2%)
P. aeruginosa		
Positive		
Neither Ear	81/356 (22.8%)	79/173 (45.7%)
One Ear	1/ 1(100.0%)	0/ 2 (0.0%)
Both Ears	0/ 0 (0.0%)	0/ 0 (0.0%)
At Least One Ear	1/ 1(100.0%)	0/ 2 (0.0%)
S. aureus		
Positive		
Neither Ear	80/351 (22.8%)	77/171 (45.0%)
One Ear	0/ 2 (0.0%)	1/ 3 (33.3%)
Both Ears	2/ 4 (50.0%)	1/ 1(100.0%)
At Least One Ear	2/ 6 (33.3%)	2/ 4 (50.0%)

[1] Baseline was defined as the last measurement taken on or prior to the day of injection.
[2] Microbiology culture unknown indicates that the microbiology culture results were not recorded. Subjects with missing microbiology culture for both ears are included in the "Neither Ear" category for "Positive" and for each individual organism.
[3] "At Least one Ear" includes "One Ear" and "Both Ears".
[4] This category represents subjects for which the baseline microbiology culture was positive for at least 1 of the following 5 organisms: P. aeruginosa, S. aureus, S. pneumoniae, H. influenzae, or M. catarrhalis.
Note: A study treatment failure was defined as the occurrence of any of the following events: otic treatment failure, systemic antibiotic treatment failure, otorrhea treatment failure, lost-to-follow-up treatment failure, or missed visit treatment failure.
H. influenzae = Haemophilus influenzae; M. catarrhalis = Moraxella catarrhalis; P. aeruginosa = Pseudomonas aeruginosa; S. aureus = Staphylococcus aureus; S. pneumoniae = Streptococcus pneumoniae.

The applicant acknowledged the limitations of making any meaningful comparisons for baseline microbiology pathogens with few subjects comprising culture positive for *P. aeruginosa* (n = 3; 1 in Otiprio treatment group and 2 in sham group) and for *S. aureus* (n = 10; 7 in Otiprio treatment group and 3 in sham group).

For subjects with *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in at least 1 ear at baseline, study treatment failure through Day 15 was greater for the sham group than for the Otiprio group (83.3% [10/12 subjects] versus 45.0% [9/20 subjects], 59.3% [16/27 subjects] versus 20.5% [8/39 subjects], and 50.0% [4/8 subjects] versus 42.9% [6/14 subjects], respectively). Baseline microbiology culture status/pathogen type did not show any meaningful difference in treatment effect.

6. LABELING

6.1 Applicant's Proposed Subsection of the Package Insert for Clinical Microbiology (Section 12 through 12.4)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ciprofloxacin is a fluoroquinolone antimicrobial *[see 12.4* (b) (4) *MICROBIOLOGY]*.

12.3 Pharmacokinetics

The plasma concentration of ciprofloxacin following bilateral administration of 0.1 mL BRAND NAME was not measured.

12.4 Microbiology

The bactericidal action of ciprofloxacin results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA. Bacterial resistance to quinolones can develop through chromosomally- or plasmid-mediated mechanisms.

(b) (4)

(b) (4) *In vitro* studies demonstrated cross-resistance between ciprofloxacin and some fluoroquinolones. There is generally no cross-resistance between ciprofloxacin and other classes of antibacterial agents, such as beta-lactams or aminoglycosides.

(b) (4)

6.2 Agency's proposed subsection of the package insert

The agency is proposing the following modifications in labeling under 12.4 Microbiology sub-section (see tracked changes version in Executive Summary section of this review):

- Headings were added for Mechanism of Action, Resistance, and Antimicrobial Activity discussions.
- (b) (4)
- (b) (4)

12.4 Microbiology

Mechanism of Action
The bactericidal action of ciprofloxacin results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA.

Resistance
Bacterial resistance to fluoroquinolones can develop through chromosomally- or plasmid-mediated mechanisms. *In vitro* studies demonstrated cross-resistance between ciprofloxacin and some fluoroquinolones. There is generally no cross-resistance between ciprofloxacin and other classes of antibacterial agents, such as beta-lactams or aminoglycosides.

Antimicrobial Activity
Ciprofloxacin has been shown to be active against most isolates of the following bacteria:

Gram-positive Bacteria
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*

Gram-negative Bacteria
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Pseudomonas aeruginosa*

7. RECOMMENDATIONS

From a clinical microbiology perspective, the application is approvable pending an accepted version of the labeling.

8. REFERENCES

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