CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206829Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTI-INFECTIVE PRODUCTS
CLINICAL MICROBIOLOGY REVIEW

NDA 206829 DATE REVIEW COMPLETED: 9-26-14
Ceftolozane-Tazobactam

Date Company Submitted: 4-19-14
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Date Assigned: 6-25-14
Reviewer: Kerian Grande Roche

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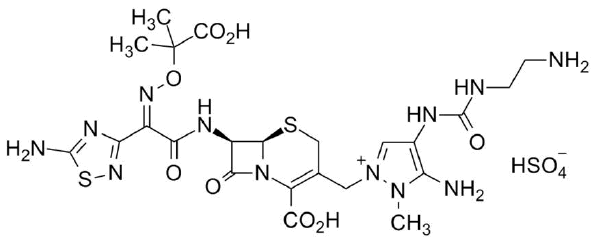
DRUG PRODUCT NAMES:
Proprietary Name: Zerbaxa®
Established Name: Ceftolozane-Tazobactam
Chemical Name:
Ceftolozane sulfate
1H-Pyrazolium,5-amino-4-[[[(2-aminoethyl)amino]carbonyl]amino]-2-[[(6R,7R)-7-[[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-,sulfate (1:1)

Tazobactam sodium
Sodium(2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid-4,4-dioxide

Structural Formula:
Ceftolozane sulfate salt



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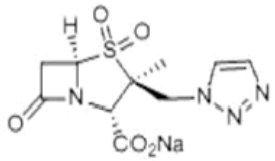
Tazobactam sodium



Molecular Formula:
Ceftolozane sulfate
$C_{23}H_{30}N_{12}O_8S_2 \cdot H_2SO_4$

Tazobactam sodium
$C_{10}H_{11}N_4NaO_5S$

DRUG CATEGORY
Antibacterial

DISPENSED:
Prescription Product

PROPOSED DOSAGE FORMS AND STRENGTHS:
ZERBAXA™ for Injection 1.5 g: powder for reconstitution in single dose vials containing 1.147 g ceftolozane sulfate (equivalent to 1 g of ceftolozane) and 0.537 g tazobactam sodium (equivalent to 0.5 g of tazobactam)

ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
- 1.5 g every 8 hours by IV infusion administered over 1 hour for patients ≥18 years of age with creatinine clearance (CrCL) >50 mL/min.
- Dosage in patients with impaired renal function:

Estimated CrCL (mL/min)	**Recommended Dosage Regimen for ZERBAXA™**
30 to 50	750 mg IV every 8 hours
15 to 29	375 mg IV every 8 hours
End stage renal disease (ESRD) on hemodialysis (HD)	A single loading dose of 750 mg followed by a 150 mg maintenance dose administered IV every 8 hours for the remainder of the treatment period (on hemodialysis days, the dose should be administered at the earliest possible time following completion of dialysis)

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INDICATIONS AND USAGE:
Treatment of the following infections caused by designated susceptible microorganisms:

- Complicated Intra-abdominal Infections
- Complicated Urinary Tract Infections, including Pyelonephritis

RELATED SUBMISSIONS:
(b) (4)
IND 104490

SUMMARY AND RECOMMENDATIONS
From a clinical microbiology perspective the information provided by the Applicant supports the efficacy of ceftolozane-tazobactam for the treatment of susceptible microorganisms for the indications of Complicated Intra-abdominal Infections (cIAI) and Complicated Urinary Tract Infections (cUTI), including Pyelonephritis. Susceptibility testing interpretive criteria for ceftolozane-tazobactam have been proposed by the Applicant and are appropriate from a clinical microbiology perspective, however, discussions with clinical pharmacology within the Agency are still ongoing. See this review for the Applicant's and the FDA's proposed labeling for the clinical microbiology subsection of the ceftolozane-tazobactam package insert (section 12.4) and references (section 15). Major revisions include the following:

REVIEWER'S COMMENTS:

1. Quality control ranges for *Staphylococcus aureus* for ceftolozane-tazobactam were published in the 2014 version of CLSI M100 (S24), but were not proposed in the Applicant's labeling. It is recommended that the labeling include Quality Control strains *S. aureus* ATCC® 25923 and *S. aureus* ATCC® 29213. See Agency's proposed labeling for recommended Quality Control ranges.

2. Each value of the MIC quality control (QC) ranges for ceftolozane-tazobactam is presented by CLSI (M100-S24) in the form of two numbers, one for ceftolozane and one for tazobactam (i.e. *E. coli* ATCC® 25922 QC range for MIC is 0.12/4-0.5/4 mcg/ml). It seems that the MIC quality control ranges for tazobactam were not represented in the Applicant's susceptibility test interpretive criteria or quality control tables (Tables 1 and 2). It is recommended that the concentration of tazobactam is also provided in the MIC tables.

3. Bacteria in the first and second lists should not include information that pertains to resistance, because this information may easily become outdated. Additionally, the resistance mechanisms associated with the bacteria, such as beta-lactamases, may not be the only ones present in the bacterial strains, nor will clinical

microbiology laboratories typically characterize such beta-lactamases in clinical isolates.

4. Bacteria in the list that do not have activity against ceftolozane-tazobactam, (i.e. those that were effectively treated with metronidazole, but not study drug), will not be listed in the label.

5. Footnotes were added to the quality control table for *E. coli* ATCC 35218, *H. influenzae* ATCC 49247, and *K. pneumoniae* ATCC 700603, in order to describe proper handling and storage of these strains.

6. Additional edits to labeling were for clarity, formatting purposes, and to ensure that the claims presented were supported by substantial data from the Applicant.

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EXECUTIVE SUMMARY

In vitro and In vivo Microbiology Studies

The activity of ceftolozane alone and ceftolozane-tazobactam, have been characterized in a series of in vivo and in vitro microbiology studies. Ceftolozane-tazobactam was the most active β-lactam agent tested against *P. aeruginosa* and was 2- to 8-fold more active than ceftazidime or cefepime. Ceftolozane-tazobactam demonstrated greater in vitro activity than piperacillin-tazobactam when tested against Enterobacteriaceae. Furthermore, with the exception of *K. pneumoniae* with an ESBL phenotype, ceftolozane-tazobactam inhibited >90% of Enterobacteriaceae at MIC values ≤8 mcg/mL. Notably, 95.5% of *E. coli* with an ESBL phenotype were inhibited at ≤8 mcg/mL of ceftolozane-tazobactam. Additionally, ceftolozane had activity against MDR and meropenem-resistant isolates of *P. aeruginosa*. The MIC of ceftolozane was ≤8 mcg/mL for approximately 90% of the MDR and 97% of the meropenem-resistant isolates in 2012 United States surveillance data.

Ceftolozane-tazobactam displays time-dependent antibacterial activity against common gram-negative and select gram-positive organisms, including ESBL-producing Enterobacteriaceae and other MDR pathogens. Ceftolozane exerts rapid bactericidal activity by inhibiting essential PBPs, resulting in inhibition of cell-wall synthesis and subsequent cell death . Ceftolozane is an inhibitor of *P. aeruginosa* PBP3, thereby inhibiting pseudomonal cell wall synthesis, cell replication, and viability.

Tazobactam is an inhibitor of common class A and some class C β-lactamases that, by binding to the active site of these enzymes, protects ceftolozane from hydrolysis and broadens coverage to include ESBL-positive Enterobacteriaceae. Tazobactam is not an inhibitor of carbapenemases such as KPC and metallo-β-lactamases such as IMP and VIM.

Single and multiple in vitro passage studies, as well as 10-day hollow-fiber models, indicate a low potential for development of resistance in *P. aeruginosa* and ESBL-positive *E. coli*. Ceftolozane is stable to *P. aeruginosa* AmpC hydrolysis because of its low affinity for that organism's AmpC enzyme. Additionally, ceftolozane is not a substrate for active efflux and is not affected by the loss of outer membrane protein D (OprD) in *P. aeruginosa*, allowing it to remain active against many strains resistant to carbapenems or other cephalosporins.

The spectrum of activity for ceftolozane-tazobactam includes clinically relevant gram-negative pathogens, such as members of the Enterobacteriaceae group (*E. coli* and *K. pneumoniae*), non-fermenters such as *P. aeruginosa*, gram-positive pathogens such as *Streptococcus pneumoniae*, and *S. anginosus* group, and anaerobic pathogens such as *B. fragilis*. Ceftolozane-tazobactam has activity against strains of *P. aeruginosa* that are resistant to carbapenems, cephalosporins, fluoroquinolones, and/or aminoglycosides, including many MDR isolates. Ceftolozane-tazobactam combinations were evaluated in

time-kill studies to characterize in vitro killing kinetics of a range of ceftolozane and tazobactam combinations. Ceftolozane displayed concentration-dependent activity against a wild-type strain and β-lactamase (AmpC, CMY-10, CTX-M-15)-expressing *E. coli* strains in these time-kill assays. As expected, tazobactam had no activity against any of the strains when tested alone. The addition of tazobactam to ceftolozane increased the ceftolozane activity against all evaluated β-lactamase-expressing strains in a concentration-dependent manner.

Ceftolozane alone and ceftolozane-tazobactam have been evaluated in infection models including immunocompetent and neutropenic mouse models, including sepsis, UTI, burn wound, and thigh infection models, and in rabbit models. Ceftolozane was comparable to or better than, comparator antibacterials when evaluated against all pathogens studied, including MDR *P. aeruginosa*.

In summary, ceftolozane-tazobactam is a novel cephalosporin/β-lactamase inhibitor that has activity against *P. aeruginosa,* is active against the majority of Enterobacteriaceae, including most ESBL-producing strains, and has also demonstrated efficacy in animal model studies.

Clinical Microbiology
Microbiological analysis was conducted in the Phase 3 clinical trial program comprising the CXA-cUTI-10-04 and -05 and CXA-cIAI-10-08 and-09 studies; a single central laboratory was used for confirmatory identification and susceptibility testing using validated broth microdilution and disk diffusion assays following Clinical and Laboratory Standards Institute (CLSI) methodology. The microbiological data were evaluated by clinical syndrome, geographic location, and outcomes by study as well as in an integrated analysis. The primary outcomes were correlated with MIC and zone diameter values for the baseline pathogens. These data, in combination with surveillance MIC distributions, animal model efficacy studies, and Pharmacokinetics/Pharmacodynamics (PK/PD) modeling were used to establish susceptibility interpretive criteria for ceftolozane-tazobactam.

Overall, the microbiological success rates in the ceftolozane-tazobactam treatment arm were high and were comparable to the combined comparator arms. In general, the eradication rates were similar between ceftolozane-tazobactam treatment and the comparator in the Phase 3 combined data set. Eradication rates for ceftolozane-tazobactam were higher for the ESBL-positive subgroup of *E. coli* and *K. pneumoniae,* but were lower against enterococci compared with the comparators. Poor eradication of enterococci would be expected as they are intrinsically resistant to cephalosporins.

The ceftolozane-tazobactam MIC50 and MIC90 values for baseline Enterobacteriaceae overall (i.e., including ESBL-producing strains) were 0.25 and 1 mcg/mL, and for baseline *P. aeruginosa* were 1 and 8 mcg/mL. Overall, baseline resistance to ceftolozane-

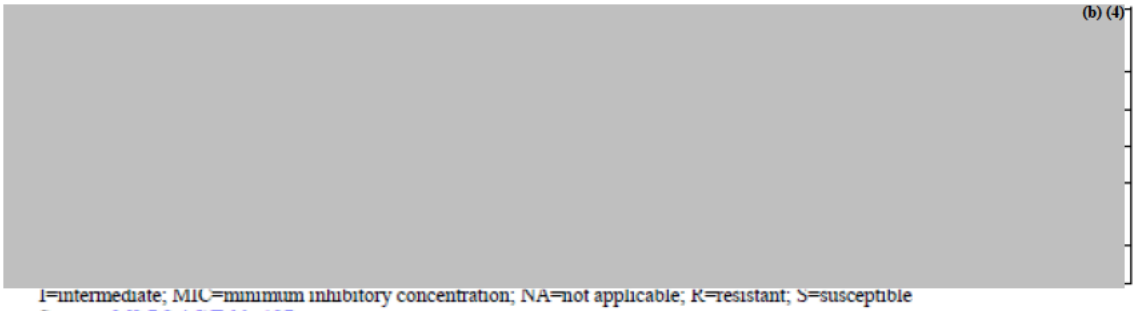
tazobactam was rare among gram-negative aerobic pathogens. The microbiological eradication rates were high in the ceftolozane-tazobactam treatment arm for pathogens with ceftolozane-tazobactam MIC values ≤16 mcg/mL.

Discussions regarding breakpoints are still under discussion within the Agency between clinical microbiology, clinical pharmacology and clinical reviewers. An addendum to this review will follow,. A brief description of breakpoints as proposed by the Applicant is below:

The susceptibility interpretive criteria as proposed by the Applicant are able to differentiate susceptible and resistant isolates, and are supported by PK/PD probability of target attainment (PTA) modeling and by the clinical and microbiological success rates in both the cIAI and cUTI studies. The Applicant's proposed breakpoints were developed based on the following information:

- MIC frequency distributions for surveillance and clinical trial isolates of the target bacterial species. The susceptibilities of the pathogens in the clinical trials were similar to the large scale surveillance studies.
  - Proposed breakpoints are able to differentiate susceptible and resistant populations of pathogens (e.g., KPC-producing *K. pneumoniae*).
- Monte Carlo simulations using human PK data, exposure criteria for efficacy (%T>MIC) observed in animal PD studies, and distribution of isolate MIC values from surveillance and clinical studies.
- Clinical data demonstrating efficacy of ceftolozane-tazobactam against the indicated pathogens across the susceptible MIC and zone diameter ranges. On the basis of these data, the Applicant proposes the ceftolozane-tazobactam interpretive breakpoints presented in the table below. The list of organisms proposed by the applicant is below the table.

Table 1: Proposed Interpretive Criteria for Ceftolozane-Tazobactam



I=intermediate; MIC=minimum inhibitory concentration; NA=not applicable; R=resistant; S=susceptible
Source: M2.7.2.4.8\Table 127

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The Applicant has proposed the following indications for ceftolozane-tazobactam:
Based on the in vitro activity and clinical efficacy data, ceftolozane-tazobactam is proposed as indicated for the treatment of cUTIs, including pyelonephritis caused by susceptible isolates of the following gram-negative bacteria:

- *E. coli*
- (b) (4)
- *K. pneumoniae*
- *P. mirabilis*
- (b) (4)

Based on the in vitro activity and clinical efficacy data, ceftolozane-tazobactam is proposed as indicated for the treatment of complicated intra-abdominal infections caused by susceptible strains of the following gram-negative and gram-positive microorganisms:

- (b) (4)
- *E. coli*
- (b) (4)
- *E. cloacae*
- *K. pneumoniae*
- (b) (4)
- *K. oxytoca*
- *P. mirabilis*
- *P. aeruginosa*
- *S. anginosus*
- *S. constellatus*
- *S. salivarius*
- *B. fragilis*
- (b) (4)

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INTRODUCTION

Ceftolozane is a novel parenteral cephalosporin with activity against *Pseudomonas aeruginosa*. Ceftolozane shares the antibacterial mode of action of other beta-lactams (β-lactams) by targeting penicillin-binding proteins (PBPs) to inhibit the biosynthesis of the bacterial cell wall and to stop bacterial replication. Ceftolozane is a PBP3 inhibitor, and shows affinities at least 2-fold higher than those of ceftazidime for essential PBPs (1b, 1c, 2, and 3) in *P. aeruginosa*. Tazobactam is a "suicide" inhibitor against class A and some class C β-lactamases, with established *in vitro* and *in vivo* efficacy in combination with active β-lactams.

Ceftolozane displays antibacterial activity against common gram-negative and selected gram-positive organisms, including pathogens involved in respiratory and other community- acquired and nosocomial infections, including those caused by streptococci, *Haemophilus influenzae, Moraxella catarrhalis,* the majority of pathogenic enteric bacilli, and selected gram-positive anaerobic species. Ceftolozane also exhibits weak activity against staphylococci. In general, the anti-gram-positive and -negative profile of ceftolozane is similar to that of ceftazidime, but its anti-pseudomonal activity is more than all currently available β-lactams, including the cephalosporins and carbapenems. Most importantly, ceftolozane has been shown to be active against strains of *P. aeruginosa* that are resistant to carbapenems, cephalosporins, fluoroquinolones and/or aminoglycosides, including the majority of multiple-drug-resistant (MDR) isolates. The minimum concentration that inhibits 90% of the microbial strains (MIC90) for *P. aeruginosa* (MIC90 $\leq$ 2 mcg/mL) is the lowest among all systemically administered anti-pseudomonal antibacterials. Like most cephalosporins, it is poorly active against enterococci, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, and gram-negative anaerobes. Tazobactam increases the *in vitro* activity of ceftolozane against the majority of ESBL-producing gram-negative bacilli and some AmpC overexpressed Enterobacteriaceae, as well as against important anaerobic pathogens such as *Bacteroides fragilis*. The addition of tazobactam has no significant impact on the anti-pseudomonal activity of ceftolozane, since *P. aeruginosa* rarely produces ESBLs. The antibacterial activity of ceftolozane - tazobactam was greater than that of piperacillin-tazobactam against Enterobacteriaceae (both ESBL-negative and ESBL-positive isolates) and *P. aeruginosa*. Ceftolozane and ceftolozane-tazobactam show time-dependent bactericidal activity against various gram-negative organisms, including *P. aeruginosa.*

Common drug resistance mechanisms for cephalosporins, including ceftolozane, have been established. They are characterized as: 1) drug degradation by selected β-lactamases, such as ESBLs; 2) altered PBPs (e.g., in *Enterococcus faecium*); and 3) decreased permeability and overexpression of efflux pumps (certain gram-negative species). Drug degradation by ESBLs and AmpC is the predominant resistance mechanism to ceftolozane in gram-negative bacteria. Consequently, ceftolozane is less stable against some AmpC β-lactamase and ESBLs produced by gram-negative pathogens. The addition of a β-lactamase inhibitor (BLI), such as tazobactam, increases its activity against most ESBL-producing pathogens and some AmpC-overproducing

Enterobacteriaceae. Ceftolozane has activity against drug-resistant *P. aeruginosa* because it is relatively stable to hydrolysis by the AmpC enzymes of *P. aeruginosa,* and is also little affected by efflux and decreased porin mechanisms of this organism. Single and multiple *in vitro* passage studies indicate a low predilection for development of resistance in *P. aeruginosa*. Ceftolozane also has anti-biofilm activity in an *in vitro* test system.

The standard Clinical and Laboratory Standards Institute (CLSI) broth or agar dilution, and disk diffusion susceptibility assays are applicable for ceftolozane and ceftolozane-tazobactam. Quality Control (QC) ranges for broth susceptibility assay have been established for ceftolozane for common aerobic American Type Culture Collection (ATCC) strains. Growth conditions and test media have minimal effect on the *in vitro* antibacterial activity of ceftolozane. No significant increase in minimum inhibitory concentrations (MICs) was observed in the presence of human serum; indeed, moderate antimicrobial synergy between ceftolozane and human serum was observed against selected *P. aeruginosa* and *Escherichia coli* isolates. Some synergistic effects were observed for ceftolozane-tazobactam when combined with other β-lactams, aminoglycosides, and tigecycline against selected Enterobacteriaceae and *P. aeruginosa* isolates.

Ceftolozane has proven to be highly effective in various animal models of infection caused by both gram-positive and gram-negative bacteria, including drug-resistant strains of *P. aeruginosa*. The animal models that have been evaluated include peritonitis, uncomplicated and complicated pneumonia, and urinary tract infection (UTI). ceftolozane was also efficacious in neutropenic animal models. Compared to ceftazidime, ceftolozane consistently demonstrated superior activity against ceftazidime-resistant *P. aeruginosa* in these animal models. Furthermore, ceftolozane displayed more rapid killing than ceftazidime in the neutropenic thigh infection model. The efficacy of ceftolozane-tazobactam was shown in two animal models of infection caused by ESBL-producing Enterobacteriaceae. Ceftolozane-tazobactam was superior to piperacillin/ tazobactam against ESBL-producing *E. coli* in a sepsis model.

The results of an *in vivo* pharmacokinetic/pharmacodynamic (PK/PD) relationship study, using a murine thigh infection model with different pathogens, were consistent with the findings for other β-lactam antibacterials. Efficacy was primarily related to time above MIC (T>MIC). The magnitude of the T>MIC required for bacterial growth inhibition varied from species to species. Ceftolozane appeared to require lower T>MIC than other cephalosporins to achieve stasis or 1-log killing. Based on the *in vitro* susceptibility profile of ceftolozane, *in vivo* efficacy, the current epidemiology of resistance, and established PK/PD relationship, susceptibility interpretative criteria (breakpoints) have been proposed by the Applicant for relevant target pathogens.

In summary, ceftolozane is a novel cephalosporin antibiotic that, in combination with a the beta lactamase inhibitor (BLI), tazobactam, has broad-spectrum antibacterial coverage including β-lactam-resistant Enterobacteriaceae and MDR *P. aeruginosa*. Both *in vitro* and *in vivo* efficacy data support its clinical development as a potential human therapeutic agent for the treatment of severe bacterial infections caused by susceptible organisms, including Enterobacteriaceae and *P. aeruginosa.*

Ceftolozane-tazobactam Clinical Development Program
The ceftolozane-tazobactam clinical development program comprised 13 completed studies, all conducted in accordance with International Conference on Harmonization (ICH) and Good Clinical Practice consolidated guidelines and the ethical principles of the Declaration of Helsinki. Ceftolozane was discovered by Fujisawa (now Astellas) and Wakunaga Pharmaceutical Companies in March 2001. Calixa Therapeutics licensed ceftolozane from Astellas in November 2007 and initiated the first human studies, as well as several non-clinical studies, and made the decision to combine ceftolozane with tazobactam. Cubist acquired Calixa in December 2009 while the first Phase 2 study in subjects with cUTI was ongoing.

Nine Phase 1 studies of ceftolozane alone or ceftolozane-tazobactam evaluated a total of 305 subjects and included pharmacokinetic (PK) studies in healthy adults and adults with renal impairment, a drug-drug interaction (DDI) study, and a thorough QT (TQT) study. These studies defined the general PK characteristics of ceftolozane alone or ceftolozane-tazobactam (single and multiple ascending dose studies), effects of intrinsic factors (renal impairment), effects of extrinsic factors (DDI), tissue distribution (epithelial lining fluid [ELF] PK), and pharmacodynamics (PD) (QT/corrected QT [QTc] interval). The doses of ceftolozane and tazobactam evaluated ranged from 250 mg to 3 g and from 250 mg to 1.5 g, respectively.

Two blinded, randomized, controlled Phase 2 studies, designed to assess the safety and efficacy of ceftolozane alone or ceftolozane-tazobactam, were completed in subjects with cUTI or cIAI, respectively. The Phase 2 cUTI study included 129 subjects randomized 2:1 to receive ceftolozane alone or ceftazidime, both 1 g administered as an intravenous (IV) infusion every 8 hours for 7 to 10 days; 127 subjects received study drug. The Phase 2 cIAI study included 122 subjects randomized 2:1 to receive ceftolozane-tazobactam 1.5 g every 8 hours (plus, in most subjects, IV metronidazole 500 mg every 8 hours) or meropenem 1 g every 8 hours, both administered by IV infusion for 4 to 7 days; 121 subjects received study drug. The primary data that support the safety and efficacy of ceftolozane-tazobactam in both the cUTI and cIAI indications were derived from 2 large, identical, multicenter, randomized, double-blind, active-controlled Phase 3 studies per indication, subsequently pooled to form 1 submission dataset for each indication. A total of 2076 subjects were randomized in the Phase 3 studies and 2047 received study drug. The studies were multinational, including sites in North and South America, Eastern and Western Europe, Australasia, and South Africa, thus providing a broad evaluation across

populations and regions. The baseline disease characteristics, including the spectrum of diagnoses and bacteriology, in both the cUTI and cIAI studies, as well as the surgical procedures performed in the cIAI study, were representative of the epidemiology and standard-of-care in the United States.

The Phase 3 comparator agents were levofloxacin 750 mg once daily administered as an IV infusion for the cUTI indication and meropenem 1 g every 8 hours administered as an IV infusion for the cIAI indication. Although ceftolozane-tazobactam has good in vitro activity against some common anaerobes, metronidazole was added for treatment of all patients with cIAI to ensure full anaerobic coverage. Metronidazole is approved and widely used adjunctively (especially with cephalosporins) in the treatment of mixed aerobic and anaerobic infections.

Ongoing studies include a blinded, randomized, controlled study comparing ceftolozane-tazobactam 3 g every 8 hours with meropenem 1 g every 8 hours, both administered by IV infusion in adult subjects with ventilated-associated bacterial pneumonia and ventilated hospital-acquired pneumonia, as well as pediatric and special population PK studies.

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ACTIVITY IN VITRO

A summary of data related to the in vitro activity of ceftolozane-tazobactam is below:

Antimicrobial Spectrum of Activity

Antimicrobial spectrum of activity includes clinically relevant gram-negative pathogens including members of the Enterobacteriaceae such as *E. coli* and *K. pneumoniae*, non-fermenters such as *P. aeruginosa*, gram-positive pathogens such as *S. pneumoniae* and *S. pyogenes* and anaerobic pathogens such as *B. fragilis*. Large scale surveillance studies of ceftolozane and ceftolozane-tazobactam were performed in laboratories in the United States (US), Canada, the United Kingdom/Ireland, and the European Union (EU). More than 33,000 contemporary (2008-2012) clinical isolates were tested for ceftolozane-tazobactam susceptibility using a fixed concentration of tazobactam (4 mcg/mL). These studies included over 4,000 US isolates (2008), over 10,000 US isolates (2011, 2012), over 11,000 EU isolates (2011, 2012). Non-duplicate isolates were collected from patients with serious infections including bloodstream infections, acute bacterial skin and skin structure infections, and respiratory tract infections in hospitalized patients. MIC values were determined using standard broth microdilution or agar dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) documents M7-A8 and CLSI documents M7-A9 for 2011 and 2012 US and EU surveillance data. For United Kingdom surveillance, the British Society for Antimicrobial Chemotherapy utilizes the agar dilution method of Andrews. Manufactured frozen (b) (4) sensititre panels (b) (4) were used in one of these studies while manufactured dried form panels were used in four of these studies. For anaerobic antimicrobial susceptibility testing, agar dilution methodology was used according to the CLSI document M11-A7. Susceptible and resistant breakpoints for comparator antibacterials were based on CLSI criteria in document M100-S17 for studies prior to 2011, document M100-S22 for 2011 US and EU surveillance studies and M100-S23 for 2012 US and EU surveillance. The table below summarizes the spectrum of activity of ceftolozane-tazobactam against over 33,000 contemporary isolates from 2008 (US only) and 2011-2012 (US, Canada, United Kingdom and EU).

The activity of ceftolozane-tazobactam against multi-drug resistant *E. coli*, *K. pneumoniae* and *P. aeruginosa,* was generated as a subset of data from the large scale surveillance data. Activity against anaerobic species, gram-positive organisms, ceftazidime resistant and susceptible organisms, and non-fermentative gram-negative bacilli are included. Finally, ceftolozane-tazobactam MIC distributions generated using combined 2008, 2011 and 2012 surveillance data against the most frequently encountered pathogens in surveillance was provided in a study report. Smaller studies have been completed to further elucidate the activity of ceftolozane-tazobactam against isolates with defined resistance genotypes and phenotypes.

Selection of the Appropriate Concentration of Tazobactam for In Vitro Susceptibility Testing of Ceftolozane-Tazobactam

Susceptibility testing for ceftolozane-tazobactam is performed with a fixed 4 mcg/mL concentration of tazobactam which appears to distinguish tazobactam sensitive ESBL-positive gram-negative bacilli from organisms carrying tazobactam resistant β-lactamases like KPC and IMP enzymes. This testing methodology was verified using ceftolozane and ceftolozane-tazobactam at fixed concentrations or at fixed ratios. In general, the concentration of tazobactam has a direct effect on the activity of ceftolozane only against strains of ESBL-positive gram-negative bacilli and some AmpC overexpressing Enterobacteriaceae.

Table 2: Effect of Different Tazobactam Concentrations on the Ceftolozane MIC Distribution for *Escherichia coli* ESBL-Positive Isolates in the 2008 (b) (4) US Surveillance Study

		Number of Isolates at each MIC (µg/mL)[a]							
Antibiotic	Tazobactam concentration	0.12	0.25	0.5	1	2	4	8	>8
ceftolozane	0	2		2	2	3	6	3	30
ceftolozane	2:1	2	1	4	9	12	15	4	1
ceftolozane	4:1	2	2	2	5	10	12	11	4
ceftolozane	Fixed 4	2	14	16	12		3		1
ceftolozane	Fixed 8	4	15	15	10	2	1		1

[a] ESBL phenotype was determined according to CLSI M100-S19 [18] criteria
Abbreviations: MIC = minimum inhibitory concentration.
Source: M5.3.5.4\CXA201-M-003

The CLSI approved method for susceptibility testing of ceftolozane-tazobactam and Piperacillin-tazobactam uses a fixed 4 mcg/mL concentration of tazobactam. This has become the generally accepted method for in vitro testing of β-lactamase inhibitor compounds.

Summary of Activity against Wild-Type and Resistant Enterobacteriaceae

Ceftolozane-tazobactam has activity against Enterobacteriaceae (see table below). In five large surveillance studies, the activity of ceftolozane-tazobactam was demonstrated against clinical isolates of Enterobacteriaceae including antibiotic resistant isolates. Ceftolozane-tazobactam compares favorably to comparator agents such as ceftazidime, meropenem, piperacillin/tazobactam and levofloxacin against Enterobacteriaceae. Ceftolozane-tazobactam is active against *E. coli* with greater than 99% of strains having an MIC value less than or equal to 8 mcg/mL. The MIC50/90 for *E. coli* is 0.25/0.5 mcg/mL, and for strains with an ESBL phenotype the MIC50/90 is 0.5/4 mcg/mL. No differences in MIC50/90 were detected between 2011 and 2012 EU and US surveillance for this organism.

Table 3: Summary of Activity of Ceftolozane-Tazobactam and Comparators Against *Escherichia coli* and *E. coli* with ESBL Phenotype from 2012 US Surveillance

Genus/species (N)	Antibiotic	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)
Escherichia coli (1447)	ceftolozane/ tazobactam	0.25	0.5	0.06->32
	piperacillin/ tazobactam	2	8	≤0.5->64
	ceftazidime	0.12	2	≤0.015->32
	meropenem	≤0.06	≤0.06	≤0.06-8
E. coli ESBL phenotype (159)[a]	ceftolozane/ tazobactam	0.5	4	0.12->32
	piperacillin/ tazobactam	8	>64	1->64
	ceftazidime	16	>32	0.5->32
	meropenem	≤0.06	≤0.06	≤0.06-8

[a] ESBL defined using CLSI M100-S23 (2013) [17] criteria
Abbreviations: ESBL = extended spectrum beta-lactamase; MIC_{50} = minimum inhibition concentration of 50%; MIC_{90} = minimum inhibition concentration of 90%; N = number.

For multi-drug resistant (MDR) *E. coli* isolates, the ceftolozane MIC50/90 is 0.5/4 mcg/mL while the ceftazidime MIC50/90 is 16/>32 mcg/mL.

Table 4: Summary of Activity of Ceftolozane-Tazobactam and Comparators Against Multi-Drug Resistant (MDR) *Escherichia coli*

Genus/species (N)	Antibiotic	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)
Escherichia coli (974)[ab]	ceftolozane/ tazobactam	0.5	4	0.12->32
	piperacillin/ tazobactam	8	>64	<0.5->64
	ceftazidime	16	>32	0.06->32
	meropenem	≤0.06	≤0.06	≤0.06->8

[a] Multi-drug resistance is defined as resistance to at least 3 different classes of antibiotics
[b] 2011 and 2012 Combined US and EU surveillance (M5.3.5.4\CXA.017.MC, M5.3.5.4\CXA.022.MC, M5.3.5.4\CXA.048.MC, M5.3.5.4\CXA.054.MC)
Abbreviations: N = number; MIC_{50} = minimum inhibitory concentration of 50%; MIC_{90} = minimum inhibitory concentration of 90%.

Ceftolozane-tazobactam has similar activity against isolates of *Citrobacter koseri*, *Morganella morgannii*, *Pantoea agglomerans*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, *Salmonella* spp, *Serratia liquefacians* and *Serratia marcescens*.

The MIC90 for all of these species is ≤1 mcg/mL. The activity of ceftolozane-tazobactam was also tested against species of *Klebsiella* and *Enterobacter*. As with other cephalosporins, the ceftolozane-tazobactam MIC90 values are somewhat higher against these two genera than with other members of the Enterobacteriaceae. The ceftolozane-tazobactam MIC50/90 for wild-type *K. pneumoniae* is 0.25/16 mcg/mL and for isolates with an ESBL phenotype, the MIC90 is >32 mcg/mL. The ceftazidime MIC90 for ESBL phenotype *K. pneumoniae* is also >32 mcg/mL and the meropenen MIC90 is >8 suggesting that KPC positive *K. pneumoniae* were included in this group of isolates. For *Enterobacter aerogenes*, the ceftolozane-tazobactam MIC50/90 is 0.25/4 mcg/mL while for *E. cloacae,* it is 0.25/8 mcg/mL. One exception to this trend was *Klebsiella oxytoca*, an organism that has a naturally occurring ESBL encoded in its chromosome. The ceftolozane-tazobactam MIC50/90 for this organism is 0.25/1 mcg/mL.

Table 5: Summary of the Activity of Ceftolozane-Tazobactam and Comparators Against *Klebsiella pneumoniae* and *K. pneumoniae* with ESBL Phenotype from 2012 US Surveillance

Genus/species (N)	Antibiotic	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)
***Klebsiella pneumoniae* (630)**	ceftolozane/ tazobactam	0.25	32	0.06->32
	piperacillin/ tazobactam	4	>64	≤0.5->64
	ceftazidime	0.12	>32	≤0.03->32
	meropenem	≤0.06	0.25	≤0.06->8
***Klebsiella pneumoniae* ESBL phenotype (127)[a]**	ceftolozane/ tazobactam	32	>32	0.25->32
	piperacillin/ tazobactam	>64	>64	1->64
	ceftazidime	>32	>32	1->32
	meropenem	0.25	>8	≤0.06->8

[a] ESBL defined using CLSI M100-S23 (2013) [17] criteria
Source: CXA.048.MC
Abbreviations: ESBL = extended spectrum beta-lactamase; N = number; MIC_{50} = minimum inhibitory concentration of 50%; MIC_{90} = minimum inhibitory concentration of 90%.

The activity of ceftolozane-tazobactam has been evaluated against genotypically characterized resistant isolates. These studies utilized genetically engineered or molecularly characterized isolates with diverse β-lactamases.
The addition of tazobactam potentiates the in vitro activity of ceftolozane against the majority of Enterobacteriaceae including isolates with AmpC overexpression or common ESBLs such as TEM, CTX-M and SHV. Genotypically defined resistance due to CTX-M-14 and CTX-M-15 β-lactamases in *E. coli* and *K. pneumoniae* are summarized in the table below.

The MIC50/90 for *E. coli* strains harboring CTX-M-14 and CTX-M-15 is <0.25/1 mcg/mL and 0.5/2 mcg/mL respectively while for *K. pneumoniae* harboring CTX-M-15, the MIC50/90 is 1/64 mcg/mL. Ceftolozane-tazobactam is not active against KPC-2 harboring *K. pneumoniae* for which the MIC50/90 is >16mcg/mL.

Table 6: Summary of Ceftolozane-Tazobactam against Enterobacteriaceae with Molecularly Characterized Markers of Resistance

Strain	Enzyme	Number of strains tested	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	Data pooled from referenced studies
E. coli	CTX-M-15	120	0.5	2	<0.25->64	[1],[2]
K. pneumoniae	CTX-M-15	12	1	64	0.5->64	[1]
E. coli	CTX-M-14	60	<0.25	1	<0.25-4	[1],[2], [4]
K. pneumoniae	KPC-2	53	>16	>16	16->16	[3]

[1]Source: Titleman et al., 2011 [20]
[2]Source: M5.3.5.4\CX-BD-001
[3]Source: Sader et al, 2011 [21]
[4]Source: M5.3.5.4\CXA.057.MC
Abbreviations: KPC = *Klebsiella pneumoniae* Carbapenemase; MIC_{50} = minimum inhibitory concentration of 50%; MIC_{90} = minimum inhibitory concentration of 90%.

Ceftolozane-tazobactam has activity against wild type and β-lactamase positive *Haemophilus influenzae*. The MIC50/90 for both is 0.12/0.25 mcg/mL.

Summary of Activity Against Wild-type and Resistant Non-fermentative Gram-negative Bacilli

The activity of ceftolozane-tazobactam was determined for recent clinical isolates of non-fermentative gram-negative bacilli. Ceftolozane-tazobactam has activity against *P. aeruginosa,* the sixth most frequently occurring pathogen according to the US Healthcare National Safety Network. Ceftolozane-tazobactam was the most active β-lactam agent tested against *P. aeruginosa* (MIC50/90 0.5/4 mcg/mL) and was 2 to 8-fold more active than ceftazidime or cefepime. In combined US and EU 2011 and 2012 surveillance of MDR *P. aeruginosa* isolates, the ceftolozane-tazobactam MIC50 is 4 mcg/mL, the lowest of all agents tested.

Table 7: Summary of Activity of Ceftolozane-Tazobactam and Comparators against Multi-Drug Resistant (MDR) *Pseudomonas aeruginosa*

Strain (number of strains tested)	Antibiotic	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)
Pseudomonas aeruginosa (940)[ab]	ceftolozane/ tazobactam	4	>32	0.25->32
	piperacillin/ tazobactam	>64	>64	1->64
	ceftazidime	32	>32	1->32
	meropenem	8	>8	≤0.06->8

[a] Combined 2011 and 2012 US and EU surveillance (M5.3.5.4\CXA.017.MC, M5.3.5.4\CXA.022.MC, M5.3.5.4\CXA.048.MC, M5.3.5.4\CXA.054.MC)
[b] Multi-drug resistance is defined as resistance to greater than or equal to 3 different classes of antibiotics
Abbreviations: MIC_{50} = minimum inhibitory concentration of 50%; MIC_{90} = minimum inhibitory concentration of 90%.

In addition, the ceftolozane-tazobactam MIC50/90 is 1/4 mcg/mL against carbapenem-resistant (non-MDR) *P. aeruginosa* isolates (MIC >8 mcg/mL for both imipenem and meropenem), while the ceftazidime MIC50/90 is 8/128 mcg/mL and the piperacillin/tazobactam MIC50/90 is 32/>64 mcg/mL. Ceftolozane-tazobactam also shows activity against clinical isolates with partial and fully derepressed AmpC. The MIC50/90 for ceftolozane-tazobactam against these strains is 2/8 mcg/mL while for ceftazidime, it is >32/>32 mcg/mL and for piperacillin/tazobactam it is >64/>64 mcg/mL. For clinical strains with decreased *oprD* expression, the MIC50/90 for ceftolozane-tazobactam is 1 /2 mcg/mL while for ceftazidime it is 8/>32 mcg/mL and for piperacillin-tazobactam it is 16/>64 mcg/mL. Of note, most of these clinical strains had additional resistance mechanisms such as AmpC derepression and over production of RND family efflux pumps.

In 2012 US surveillance, the MIC50/90 for wild type *P. aeruginosa* is 0.5 /2 mcg/mL while for 2012 EU surveillance it is 0.5/16 mcg/mL. This difference in MIC90 is due to regional differences within Europe in resistance patterns. This difference was noted for all agents tested except colistin with ceftolozane-tazobactam still being the second most active agent (next to colistin). Isolates from some countries, especially Poland, Russia and Ukraine, had high resistance rates to antipseudomonal cephalosporins and carbapenems. In contrast, isolates from five other EU countries (France, Germany, Italy, Spain and the UK) averaged greater than 96% susceptible at a ceftolozane-tazobactam MIC value ≤8 mcg/mL and had a MIC50/90 of 0.5/2 mcg/mL which is the same as the US MIC50/90 for *P. aeruginosa*.
From the 2011 European surveillance, 175 out of 991 (17.6%) *P. aeruginosa* isolates had ceftolozane-tazobactam MIC values ≥16 mcg/mL. These isolates were further characterized to understand what resistance mechanisms were involved. Analysis of the subset of strains with ceftolozane-tazobactam MIC ≥16 mcg/mL (n=139) revealed that

approximately 70.5% of these *P. aeruginosa* carried metallo-β-lactamase-encoding genes. Twenty-six per cent (25.9%) of these isolates carried genes encoding ESBLs and/or oxacillinases.

It is known that ceftolozane-tazobactam has no activity (MIC range 16->32 mcg/mL) against strains expressing metallo-β-lactamases including IMP, VIM, SPM and β-lactamases such as VEB, PER and GES. Comparator antibacterials such as piperacillin/tazobactam, ceftazidime, cefepime and imipenem also lack activity against isolates expressing these enzymes (MIC range 8 to >32 mcg/mL). Ceftolozane alone was tested against 100 *P. aeruginosa* isolates from 50 cystic fibrosis (CF) patients, as the first and last isolated strain from each patient, with a mean time between the two isolates of 67.6 ± 39.2 months. The overall MIC50/90 was 0.5/2 mcg/mL. Notably, ceftolozane was the only antibiotic tested in which the percent susceptibility based upon MIC value did not decrease in the set of early isolates as compared to late set of isolates. The percent susceptibility for ceftolozane-tazobactam was 95% for the first set of isolates and 96% for the last set of isolates. This contrasts with ceftazidime where the percentage of susceptible isolates dropped from 75% to 70% and piperacillin/tazobactam where the percentage of susceptible isolates dropped from 87% to 84%. Ceftolozane-tazobactam exhibited modest activity against *Acinetobacter* spp*, Acinetobacter calcoaceticus*/*baumannii* complex, *Achromobacter xylosoxidans* and *Stenotrophomonas maltophili*a. The MIC50/90 for these organisms was 1/ 32 mcg/mL, 16 / >32 mcg/mL, 32/>32 mcg/mL and 16/>32 mcg/mL respectively. Better activity was demonstrated against *Acinetobacter lwoffi* (MIC50/90 0.12 /32 mcg/mL) and *Burkholderia cepacia*, (MIC50/90 1 /4 mcg/mL), a pathogen associated with CF patients.

Summary of Activity Against Gram-positive Aerobic Bacteria
The in vitro activity of ceftolozane-tazobactam against *Streptococcus pneumoniae* varied according to susceptibility to penicillin. When tested against penicillin-susceptible strains, the MIC50/90 is ≤0.12/0.12 mcg/mL, for penicillin-intermediate strains, the MIC50/90 is 1/4 mcg/mL and for penicillin-resistant strains, MIC50/90 is 8/16 mcg/mL. Ceftolozane –tazobactam demonstrated activity against *S. pyogenes* and *S. agalactiae*. The MIC90 for both groups is ≤ 0.5 mcg/mL. Activity was also demonstrated against viridans streptococci including the *S. anginosus* group (*S. anginosus*, *S. constellatus* and *S. intermedius*) and *S. salavarius*/*vestibularis* group. The MIC50/90 for the *S.anginosus* group is 1/4 mcg/mL and the *S. salavarius*/*vestibularis* group 0.5/1 mcg/mL. Ceftolozane-tazobactam has limited activity against *Staphylococcus aureus* [MSSA (MIC50/90 16/32 mcg/mL) and MRSA (MIC50/90 64/>64 mcg/mL)]. It is also inactive against *Enterococcus faecalis* (VSE and VRE) and *Enterococcus faecium* (VSE and VRE) with a MIC50 of >64 mcg/mL. The MIC50/90 for *Staphylococcus epidermidis* MSSE is 8/8 mcg/mL and for *S. epidermidis* MRSE the MIC50/90 is 16/32 mcg/mL.

Summary of Activity Against Gram-positive and Gram-negative Anaerobic Bacteria

Ceftolozane-tazobactam has variable activity against anaerobic species. Studies have been conducted evaluating the activity of ceftolozane and ceftolozane-tazobactam against common anaerobe species. Ceftolozane-tazobactam has activity against the following species based on the MIC50/90 values: *Bacteroides fragilis* (1/4 mcg/mL), *Clostridium perfringens* (0.25/32 mcg/mL), *Fusobacterium* species (≤ 0.125 /0.25 mcg/mL) and *Prevotella* species (≤ 0.125 /1 mcg/mL). Lesser activity is seen against other species in the *Bacteroides fragilis* group (MIC90 values range from 8-32 mcg/mL) and activity was limited against both *C. difficile* and other *Clostridium* spp (MIC90 > 256 mcg/mL).

Table 8: In Vitro Activity of Ceftolozane-Tazobactam Against Clinical Isolates from US, EU, British and Canadian Surveillance Programs

		MIC (µg/mL)		
Genus/ Species	**Number of Isolates Tested (n)**	**Range**	**MIC_{50}**	**MIC_{90}**
Achromobacter xylosoxidans	22	0.5->32	32	>32
Acinetobacter calcoaceticus/baumannii complex	1260	≤0.015->64	16	>32
Acinetobacter lwoffii	44	≤0.015->32	0.12	32
Acinetobacter spp.	53	≤0.015->32	1	32
Burkholderia cepacia	21	0.25-8	1	4
Citrobacter braakii	46	0.06-16	0.25	8
Citrobacter freundii	544	≤0.06->32	0.25	8
Citrobacter freundii (CAZ susceptible)	403	≤0.06->32	0.25	0.5
Citrobacter freundii (CAZ non-susceptible)	141	0.25->32	8	32
Citrobacter koseri	325	0.06->32	0.25	0.5
Citrobacter spp.	61	0.06->32	0.25	8
Enterobacter aerogenes	607	≤0.12->32	0.25	4
Enterobacter aerogenes (CAZ susceptible)	445	≤0.12-2	0.25	0.5
Enterobacter aerogenes (CAZ non-susceptible)	162	0.25->32	2	8
Enterobacter asburiae	48	0.03-16	0.25	2
Enterobacter cloacae	2166	0.03->64	0.25	8
Enterobacter cloacae (CAZ susceptible)	1591	0.03->32	0.25	0.5
Enterobacter cloacae (CAZ non-susceptible)	575	0.12->64	4	32
Enterobacter spp.	29	0.12-16	0.25	4
Escherichia coli	9429	0.03->64	0.25	0.5
Escherichia coli (ESBL)	1146	0.06->64	0.5	4

		MIC (µg/mL)		
Genus/ Species	**Number of Isolates Tested (n)**	**Range**	**MIC_{50}**	**MIC_{90}**
Escherichia coli (CAZ susceptible)	8555	0.03->32	0.25	0.25
Escherichia coli (CAZ non-susceptible)	874	0.06->64	0.5	4
Haemophilus influenzae	1423	≤0.015-32	0.12	0.25
Haemophilus influenzae (β-lactamase positive)	243	≤0.015-0.25	0.12	0.25
Hafnia alvei	20	0.12-8	1	4
Klebsiella oxytoca	899	0.03->32	0.25	1
Klebsiella oxytoca (ESBL)	109	0.12->32	1	32
Klebsiella oxytoca (CAZ susceptible)	872	0.03-8	0.25	0.5
Klebsiella oxytoca (CAZ non-susceptible)	27	0.12->32	16	>32
Klebsiella pneumoniae	4410	0.03->64	0.25	16
Klebsiella pneumoniae (ESBL)	994	≤0.06->64	4	>32
Klebsiella pneumoniae (CAZ susceptible)	3458	0.06-16	0.25	0.5
Klebsiella pneumoniae (CAZ non-susceptible)	952	0.12->64	8	>32
Klebsiella spp.	46	0.12-8	0.25	1
Moraxella catarrhalis	472	≤0.015-1	≤0.015	0.12
Morganella morganii	608	0.06->32	0.25	0.5
Morganella morganii (CAZ susceptible)	518	0.06-4	0.25	0.5
Morganella morganii (CAZ non-susceptible)	90	≤0.12->32	0.5	32
Pantoea agglomerans	16	0.12-0.5	0.25	0.25
Proteus mirabilis	1600	0.03-32	0.5	0.5
Proteus mirabilis (ESBL)	83	0.25-32	1	8
Proteus vulgaris	132	0.25-4	0.5	1
Providencia rettgeri	54	0.03-4	0.12	0.5
Providencia stuartii	68	0.06->32	0.5	2
Pseudomonas aeruginosa	6316	0.03->64	0.5	4
Pseudomonas aeruginosa (CAZ susceptible)	4982	0.03->32	0.5	1

Genus/ Species	Number of Isolates Tested (n)	MIC (µg/mL)		
		Range	MIC_{50}	MIC_{90}
Pseudomonas aeruginosa (CAZ non-susceptible)	1334	0.25->64	4	>32
Pseudomonas aeruginosa (CAR non-susceptible)	1782	0.12->64	1	>32
Pseudomonas aeruginosa (TZP non-susceptible)	1738	0.12->64	2	>32
Pseudomonas putida	20	0.5->32	1	4
Pseudomonas spp.	59	0.25->32	1	4
Salmonella spp.	16	0.25-0.5	0.5	0.5
Serratia liquefaciens	56	0.12-4	0.5	1
Serratia marcescens	1614	0.12->64	0.5	1
Serratia marcescens (CAZ susceptible)	1571	0.12->32	0.5	1
Serratia marcescens (CAZ non-susceptible)	43	0.25->64	4	>32
Stenotrophomonas maltophilia	600	0.5->64	16	>32
Streptococcus pneumoniae	1600	≤0.015->32	≤0.12	2
Streptococcus pneumoniae (PEN-S)	1309	≤0.015-32	0.06	0.12
Streptococcus pneumoniae (PEN-I)	207	0.06-16	2	8
Streptococcus pneumoniae (PEN-R)	84	≤0.06->32	8	16
Streptococcus pyogenes (Group A)	388	≤0.06-2	0.12	0.25
Streptococcus agalactiae (Group B)	234	≤0.12-1	0.5	0.5
Enterococcus faecalis	223	4->64	64	>64
Enterococcus faecium	95	32->64	>64	>64
Staphylococcus aureus (MRSA)	382	32->64	64	>64
Staphylococcus aureus (MSSA)	1540	0.25-64	16	32
Staphylococcus epidermidis	144	0.5->64	16	64

Source: M5.3.5.4\CXA.022.MC 2011 US Surveillance
Source: M5.3.5.4\CXA.017.MC 2011 EU Surveillance
Source: M5.3.5.4\CXA201-M-003 2008 US Surveillance
Source: 2011 BSAC Bacteremia Resistance Surveillance and 2010/2011 Respiratory Resistance Surveillance
Source: M5.3.5.4\CXA.018.MC 2011 Canadian Surveillance (CANWARD)
Source: M5.3.5.4\CXA.048.MC 2012 US Surveillance
Source: M5.3.5.4\CXA.054.MC 2012 EU Surveillance
Source: 2012 BSAC Respiratory Resistance Surveillance

Various parameters that can affect the determination of the in vitro activity of antibacterials were investigated. Ceftolozane and tazobactam were shown to be stable under test conditions. Additionally, MIC values were not affected by variations such as agar versus broth microdilution, addition of serum or surfactants, pH, inoculum density, calcium ion (Ca2+) concentration, or CO2 tension.

Bactericidal Activity

Ceftolozane-tazobactam demonstrated time-dependent bactericidal activity against target gram-negative pathogens, including MDR strains. The bactericidal activity of ceftolozane and of ceftolozane-tazobactam was assessed with the minimum bactericidal concentration (MBC) method, with time kill experiments, and by visualizing live and dead cells microscopically using differential fluorescent staining techniques. Morphological changes associated with bactericidal effects were also visualized in bacteria treated with ceftolozane-tazobactam and ceftolozane alone using scanning electron microscopy (data not shown). Additionally, ceftolozane-tazobactam and ceftolozane alone were shown to be bactericidal in vivo in the neutropenic mouse thigh infection model.

Minimum Bactericidal Concentration

MBC values were determined according to CLSI document M26-A. For ceftolozane alone, the MBC values for 10 *E. coli* and 10 *K. pneumoniae* strains, all of which were ceftazidime-susceptible, ranged from 0.25 to 1 mcg/mL and were 1-2 times the MIC. Against 20 strains of *P. aeruginosa*, including 5 ceftazidime-resistant strains, the ceftolozane MBC values ranged from 0.5 to > 8 mcg/mL, and the MBC/MIC ratios ranged from 2 to > 8. Additionally, MBC/MIC ratios ranged from 1 to > 8 for *B. cepacia* and 1 to 8 for *S. pneumoniae* (including 1 penicillin-resistant and 6 penicillin-intermediate isolates). Finally, the MBC/MIC ratio for all 5 β-lactamase positive *M. catarrhalis* and 5 *S. pyogenes* was 1. The MBC values of ceftolozane-tazobactam (with fixed 4 mcg/mL tazobactam) was determined for clinical isolates of *E. coli* (N = 20; all ESBL-positive) and the same number of ESBL-positive clinical isolates of *K. pneumoniae*, as well as 10 clinical isolates of *P. aeruginosa* (5 ceftazidime-susceptible and 5 –resistant). The broadest range and the highest MBC values were observed for the *K. pneumoniae* isolates; these parameters were also reflected in the MIC distribution (data not shown). The MBC/MIC ratios were ≤ 4 for all but 2 of the isolates tested.

Table 9: Minimum Bactericidal Concentrations of Ceftolozane-Tazobactam Against Gram-negative Organisms

Organism	N	MBC (ug/mL)		
		Range	50%	90%
E. coli	20[a]	0.25 - 4	0.5	2
K. pneumoniae	20[a]	0.12 - > 32	4	> 32
P. aeruginosa	10[b]	0.5 - 32	2	4

[a] All isolates produced extended spectrum β-lactamases.
[b] 5 isolates were ceftazidime-susceptible and 5 were ceftazidime-resistant.
Source: M5.3.5.4\CXA201-M-003
Abbreviations: MBC = minimum bactericidal concentrations; N = number.

Time Kill Studies

Time kill studies are a more dynamic measure than MBC determination of the bactericidal potency of antibacterials because the bactericidal activity of antibacterials can be followed over time, in parallel with the growth of the unexposed control. Additionally, the use of different antibiotic concentrations and exposure times permits determination of whether the bactericidal activity is concentration-dependent or time-dependent, a factor that may affect the in vivo pharmacodynamics of an antibiotic and help determine the optimum regimen for a drug.

Ceftolozane

As expected for a β-lactam agent, the time kill kinetics of ceftolozane were shown to be time-dependent. Ceftolozane time kill kinetics were followed over 24 hours of exposure to concentrations equal to 1X, 4X and 8X the MIC values previously determined by broth microdilution. Experiments were performed according to CLSI Document M26-A. The overall results, summarized in demonstrated that exposure to concentrations of ceftolozane equal to 8X the MIC or less resulted in reductions of at least 3 log10 CFU/mL after ≤ 24 h in all strains tested. In addition, ceftolozane was bactericidal at 4X the MIC for the majority of strains evaluated.

Table 10: Summary of Bactericidal Activity of Ceftolozane in Time Kill Studies

Organism	Phenotype	Isolate Number	MIC (µg/mL)	Bactericidal activity at 24 h[a]		
				1X MIC	4X MIC	8X MIC
P. aeruginosa	CTZ-R	1731934	2	-	+	+
	CTZ-R	1731888	2	-	+	+
	CTZ-S	1731923	1	-	+	+
	CTZ-S	1731884	1	-	+	+
E. coli	CTZ-S	1732283	0.25	-	+	+
	CTZ-S	1732281	0.25	-	+	+
K. pneumoniae	CTZ-S	1732269	0.5	-	-	+
	CTZ-S	1732356	0.5	-	-	+
B. cepacia	NA	1732173	2	-	+	+
	NA	1732174	2	-	+	+
M. catarrhalis	NA	1732257	1	+	+	+
S. pneumoniae	Pen-S	1731673	2	-	+	+

[a] +, ≥ 3 $\log_{10}$ decrease in viable cells (CFU/mL) as compared with the initial inoculum; -, < 3 $\log_{10}$ decrease in CFU/mL.
Abbreviations: CTZ = ceftazidime; h = hours; MIC = minimum inhibitory concentration; NA = not applicable; Pen = penicillin; R = resistant; S = susceptible;
Source: M5.3.5.4\CXA101-M-001

In another study, the viable count of *P. aeruginosa* strain PAO1 (ceftolozane MIC value 0.5 mcg/mL) was reduced by > 3 log10 CFU/mL after 24-h exposure to 1X or 4X the MIC of ceftolozane. Ceftazidime and imipenem had < 3 log10 reductions CFU/mL at the same MIC multiples (data not shown). Similar results with *P. aeruginosa* strain PAO1 were demonstrated in another study, using a higher starting inoculum. In this study, the bactericidal activity of ceftolozane was also followed over time by fluorescence microscopy using live-dead staining (data not shown).

Ceftolozane-tazobactam
A time kill kinetics study of ceftolozane-tazobactam was conducted with 2 strains each of *P. aeruginosa* and *E. coli*, including one ATCC strain and one MDR strain of each species. The MDR strains were resistant to most β-lactam antibacterials, and to gentamicin and fluoroquinolones; the MDR *E. coli* strain (but not the *P. aeruginosa* strain) was susceptible to carbapenems. The ceftolozane-tazobactam MICs against the ATCC and MDR *P. aeruginosa* were 0.5 mcg/mL and 2 mcg/mL, respectively and against the ATCC and MDR *E. coli*, the MICs were 0.25 mcg/mL and 2 mcg/mL, respectively. Against both of the *E. coli* strains and the susceptible strain of *P. aeruginosa*, all tested concentrations of ceftolozane-tazobactam (i.e., as low as 1 mcg/mL ceftolozane + 4 mcg/mL tazobactam) produced at least a 3 log10 reduction in CFU/mL within ≤ 24 h. Against the MDR strain of *P. aeruginosa*, a sustained drop in CFU/mL of approximately 3log10 was seen within 4 h of exposure to concentrations of 16 mcg/mL or higher of ceftolozane in combination with 4 mcg/mL tazobactam.

In another time kill study, strains of *E. coli* and *K. pneumoniae* (two of each species were ESBL-negative and two were ESBL-positive), and *P. aeruginosa* (two each ceftazidime-susceptible, ceftazidime-resistant and imipenem-resistant) were exposed to 2X, 4X and 8X the MIC of ceftolozane-tazobactam. Greater than a 3 log10 drop in viable count was observed after 24-h exposure to 8X the MIC with all 4 *E. coli* strains and 3 of 4 of the *K. pneumoniae* strains. In several cases, extensive killing was seen with lower concentrations and/or after shorter exposure times. In the case of *P. aeruginosa*, the extent of killing varied from 2.5 to 3.3 log10 CFU/mL for 4 of the 6 strains, including one ceftazidime-resistant (MIC >128 mcg/mL) strain and both imipenem-resistant strains (data not shown).

Mechanism of Action
Ceftolozane
Ceftolozane shares the basic chemical and biological attributes and mechanism of action with other β-lactam antibacterials. The primary mechanism of action is inhibition of the transpeptidation step of bacterial peptidoglycan biosynthesis by inactivation of PBPs. The binding of β-lactams to PBPs leads to acylation of specific serine residues within the active site of these enzymes, resulting in inactivation of their transpeptidase activity. Affinity for PBPs is the principal variable determining the antibacterial spectrum and potency (growth inhibition and bactericidal activity) of β-lactam antibacterials. The

affinity for PBPs was analyzed by a competition assay using the fluorescent penicillin, BOCILLIN FL (Molecular Probes, Eugene, OR, USA). The binding affinities of drugs for PBPs were determined by an assay in which the membrane was incubated with a series of drug dilutions before being treated with BOCILLIN FL. PBPs were separated by SDS-polyacrylamide gel electrophoresis and detected by fluorography. The affinity of the antibiotic for PBPs was expressed in terms of the concentration required to inhibit fluorescent penicillin binding by 50% (IC50) as compared with a control in the absence of the tested antibacterials using the Quantity One software. Low IC50 values correspond to high affinity for PBPs.

Ceftolozane is a PBP3 inhibitor and has higher affinity than ceftazidime for the *P. aeruginosa* PBPs (1b, 1c and 3) that are essential for cell wall synthesis, cell replication and viability. Compared to imipenem, ceftolozane demonstrated higher affinity for PBP3 and PBP1b but a lower affinity for PBP2 and PBP1c (See table below). A second study to examine PBP binding by ceftolozane in *P. aeruginosa* and *E. coli* was conducted. PBP binding values for *P. aeruginosa* corroborated the earlier study, with the exception of PBP2 (PBP2 IC50 >50 mg/L). This discrepancy may be due to differences across labs, different membrane extracts used, or may due to a more limited range of values used to generate the IC50 curve in the initial study. For *E. coli*, ceftolozane showed comparable values to ceftazidime except for PBP1c.

Table 11: Inhibitory Concentration (IC50) Values of Ceftolozane, Ceftazidime, and Imipenem for *Pseudomonas aeruginosa* PAO1 Penicillin Binding Proteins (PBPs)

PBP	Ceftolozane (mg/L)	Ceftazidime (mg/L)	Imipenem (mg/L)
1b	0.07±0.01	0.12±0.03	0.13±0.01
1c	0.64±0.17	>2	0.08±0.005
2	1.36±0.56	>2	0.08±0.01
3	0.02±0.007	0.04±0.03	0.12±0.2
4	0.29±0.05	1.23±0.49	0.02±0.03
5/6	>2	>2	0.2±0.09
MIC (µg/mL)	0.5	1	1

Source: M5.3.5.4\CXA101-M-013

Reviewer's Comment
PBP2 binding affinities did not appear to be reproducible for PBP2 for *Pseudomonas aeruginosa*.

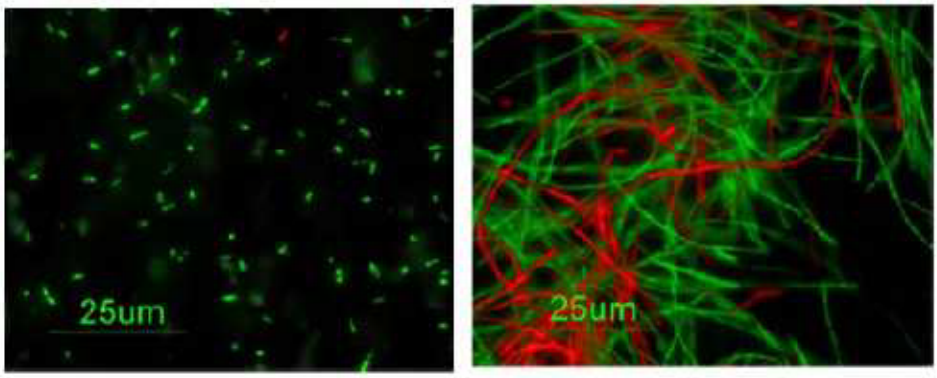
Table 12: Inhibitory Concentrations (IC50) Values of Ceftazidime, and imipenem for *Escherichia coli* Penicillin Binding Proteins (PBPs)

PBP	Ceftolozane (mg/L)	Ceftazidime (mg/L)	Imipenem (mg/L)
1b	2.22	0.300	0.12
1c	>50	1.72	0.025
2	>50	23.8	<0.005
3	0.0259	0.0057	4
4	>50	>50	<0.0015
5/6	>50	>50	0.07

Source: M5.3.5.4\CXA.080.MC

When *P. aeruginosa* PAO1 cells are incubated with sub-inhibitory concentrations of ceftolozane (0.5X MIC, 0.25 mcg/mL), live/dead staining shows the cells producing long filaments which indicates inhibition of cell septation and is correlated with inhibition of PBP3 activity (See figure below).

Figure 1: Morphological Changes in *Pseudomonas aeruginosa* after 2 hour Exposure to Ceftolozane



Left panel: T0.
Right panel: 2 hours of Exposure to Ceftolozane at 0.25 µg/mL (0.5X MIC).

Tazobactam

Beta-lactamase inhibitors such as tazobactam enhance and extend the activity of β-lactam antibacterials such as ceftolozane and piperacillin against organisms producing susceptibleβ-lactamases. Tazobactam has no intrinsic antibacterial activity (MIC >16 mcg/mL). Tazobactam is an irreversible inhibitor of β-lactamases and can bind covalently to chromosomal and plasmid-mediated bacterial β-lactamases. In a kinetic study, tazobactam demonstrated reversible binding kinetics prior to irreversible inactivation.

Enzyme Kinetics for Tazobactam

In a 1994 study, Payne et al. examined the β-lactamase inhibitory activities of clavulanic acid, sulbactam, and tazobactam against 35 isolated plasmid-mediated β-lactamases (20 ESBLs and the 15 conventional spectrum plasmid-mediated β-lactamases). Overall, clavulanic acid and tazobactam had equivalent activity against the sets of enzymes evaluated. However, each inhibitor had a distinct inhibition profile. Tazobactam was more active than clavulanic acid against TEM-2, OXA-2 and OXA-5 while clavulanic acid was more active for SHV-1, SHV-5 and TEM-5. In another study, the inhibition kinetics for tazobactam against three Class A beta-lactamase enzymes, TEM-1, CTX-M-15, and CTX-M-14, were examined. This study demonstrated that tazobactam is a superior inhibitor to clavulanic acid and sulbactam against these enzymes. Tazobactam has an IC50 that is up to 110-fold more potent, less tazobactam is hydrolyzed per enzyme inactivation event, and tazobactam remains bound to the enzyme to a comparable or better extent based upon the degree of deacylation measured.

Stability to Common Penicillinases

One common feature of cephalosporin antibacterials is their excellent stability to common penicillinases. Although no extensive enzymology analysis has been conducted to examine the stability of ceftolozane to these β-lactamases. Ceftolozane appears to be stable to most of the common penicillinases based on its activity against organisms carrying characterized β-lactamases. The activity of ceftolozane and other β-lactams against selected gram-negative strains expressing different β-lactamases was examined in two studies [CRE060042, CX-BD-001].

Table 13: Effect of Selected Beta-lactamases on the Susceptibility of Gram-negative Aerobic Organisms to Ceftolozane (CXA-101) and Comparators

Class of β-lactamase	Enzyme Type/Strains		MIC (µg/mL)		
			CXA-101	CAZ	IMP
Class A	TEM	*P. aeruginosa* FP2056	0.5	4	1
	OXA	*P. aeruginosa* FP1190	0.5	1	1
	PSE	*P. aeruginosa* FP2055	0.5	2	2
	TEM3	*E. coli* FP1714	2	32	0.25
	TEM7	*E. coli* FP1718	32	64	0.25
	CAZ2	*E. coli* FP1720	16	> 128	0.25
	SHV4	*E. coli* FP1723	32	> 128	0.25
Class B	IPM1	*P. aeruginosa* FP2058	> 128	> 128	64
Class C	AmpC (Id)	*P. aeruginosa* FP1380	4	> 128	0.5
	AmpC (Ia)	*S. marcescens* FP1184	1	1	0.5
	AmpC (Ib)	*E. coli* FP1186	0.125	0.5	0.125
	AmpC Inducible	*P. aeruginosa* FP1448	0.5	2	1
	AmpC Constitutive	*P. aeruginosa* FP1799	1	32	1
Class D	OXA2	*P. aeruginosa* FP1800	64	128	2
	OXA5	*P. aeruginosa* FP1801	2	2	1
	OXA6	*P. aeruginosa* FP1799	8	2	2
	PSE2	*P. aeruginosa* FP1802	1	2	1

Abbreviations: CAZ = Ceftazidime; IMP = Imipenem.
Source: CRE060042.

The tables below show the activity of ceftolozane against selected laboratory-engineered beta-lactamase-producing *E. coli* strains. Many strains producing class A enzymes remain susceptible to ceftolozane. Compared with ceftazidime, ceftolozane is slightly more active against most of these β-lactamase-producing strains. The MIC elevation, or shift, with ceftolozane in *P. aeruginosa* strains expressing Class C enzymes was much less pronounced than that with ceftazidime, indicating relatively greater stability of ceftolozane to hydrolysis by overexpressed AmpC.

Table 14: Activity of Ceftolozane (CXA-101) Against Selected Laboratory-Engineered Beta-lactamase-Producing *E. coli* Strains

E. coli Phenotype[1]	MIC (µg/mL)	
	CXA-101	Ceftazidime
J53 OXA-1	0.25	0.25
J53 OXA-2	0.25	0.25
J53 OXA-3	0.5	1
J53 OXA-4	0.25	0.25
J53 OXA-7	2	1
J53 SHV-1	0.25	0.5
J53 SHV-2	4	16
J62 TEM-1	0.125	0.25
J53 TEM-2	0.125	0.125
J62 TEM-3	0.5	8
DH5α CTX-M-15 (insert seq)	2	0.5
DH5α CTX-M-3	4	0.5
J53 HMS-1	0.25	0.25
J62 LXA-1	0.25	0.25
J53 PSE-4	0.5	0.5

1. All *E. coli* strains were molecularly engineered to include different and highly expressed β-lactamases.
Source: CX-BD-001.

Tazobactam

Tazobactam, a triazolymethyl penicillanic acid sulfone derivative, is structurally similar to sulbactam with the exception of a triazole ring, which facilitates binding of tazobactam to β-lactamases. Tazobactam, an irreversible inhibitor of β-lactamases, can bind covalently to chromosomal and plasmid-mediated bacterial β-lactamases. Half maximal inhibition values of tazobactam were 4- to 210-fold lower after 5 minutes of preincubation compared with no preincubation, indicating that tazobactam is either an irreversible inhibitor or a tight-binding competitive inhibitor [Bryson, 1994]. In a kinetic study with all major classes of β-lactamases, tazobactam demonstrated reversible binding kinetics prior to irreversible inactivation [Bush, 1983]. Similar to clavulanic acid, tazobactam is active against a broad range of gram-negative, plasma-mediated β-lactamase enzymes, including TEM, OXA, SHV, CTX, HMS and PSE enzymes [Cullmann, 1990; Payne, 1994]. Tazobactam also maintains some activity against derepressed AmpC. Tazobactam potentiates the activity of β-lactams, such as piperacillin, against Enterobacteriaceae, *Bacteroides* spp., and other gram-negative

bacteria that produce ESBLs or have hyper-production of AmpC. Beta-lactamase induction by a beta-lactamase inhibitor (BLI) could reduce the activity of a co-administered β-lactam. Tazobactam has not been shown to induce chromosomally mediated enzymes in Enterobacteriaceae to the same extent as a dose of clavulanate [Bryson, 1994]. An increase in concentration of clavulanic acid was associated with increased production of cephalosporinase, whereas increased enzyme production was only observed with very high concentrations of tazobactam. These findings show that tazobactam is only a weak inducer of β-lactamases. Detailed enzymatic kinetic studies are essential in elucidating the mode of action of mechanism based BLIs. Tazobactam was characterized in various enzymatic and microbiological studies [Cullmann, 1990; Payne, 1994; Fornara, 1997; Perilli, 1999]. In this document, some of the data for tazobactam are reviewed. The interactions of tazobactam and other BLIs with various plasmid and chromosomally mediated β-lactamases from clinical isolates were investigated [Cullmann, 1990]. As shown in the table below, the affinity of tazobactam for tested β-lactamases was 0- to 100-fold higher than that of sulbactam; its KI values ranged from 1.1×10^{-9} to 5.0×10^{-8} mol/L for class III and V enzymes and from 4.1×10^{-6} to 3.5×10^{-5} mol/L for Class I enzymes. Clavulanic acid had high affinity for all penicillinases, but the affinity was generally weaker than that of tazobactam for enzymes from gram-negative organisms.

Table 15: Affinity of Tazobactam and other Beta-lactamase Inhibitors for Various Beta-lactamases

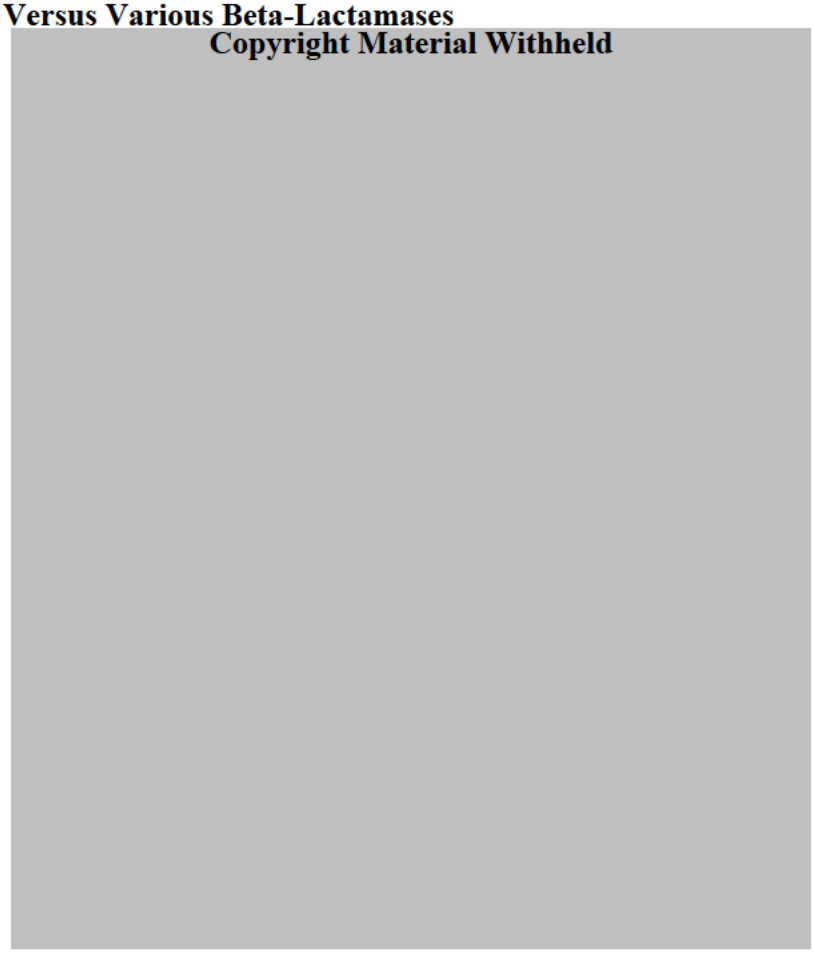
Source: Cullmann 1990.

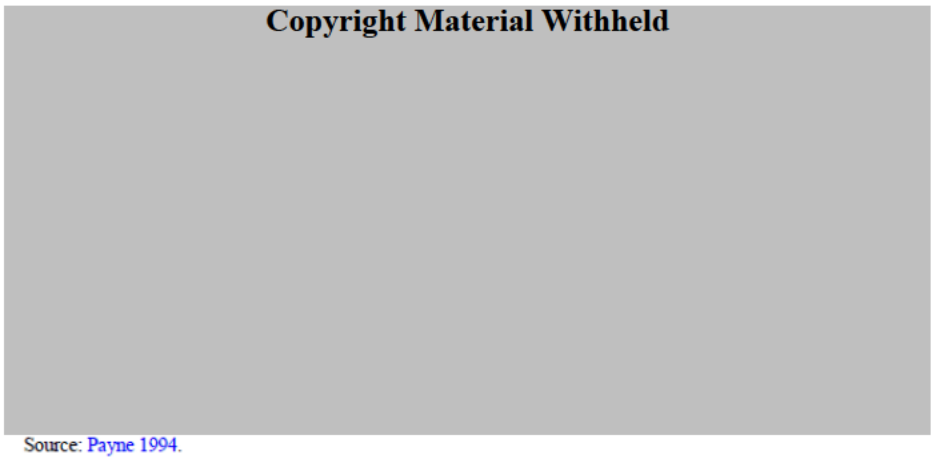
In a comprehensive study, Payne (1994) examined the β-lactamase inhibitory activities of clavulanic acid, sulbactam, and tazobactam against 35 isolated plasmid-mediated β-lactamases (20 ESBLs, and the 15 conventional spectrum plasmid-mediated, β-lactamases) [Payne, 1994]. The crude enzymes were prepared from cell lysates of *E. coli* transconjugants and other gram negative species with defined enzymes. The activities of inhibitors were measured as the IC50 values for the hydrolysis of nitrocefin by a

particular β-lactamase. Tazobactam and clavulanic acid had similar activity against the 35 different β-lactamases (see table below). Both tazobactam and clavulanic acid were more potent than sulbactam.

Reviewer's Comment
Clavulanic acid and tazobactam were both potent against the sets of enzymes evaluated. However, each inhibitor had a distinct inhibition profile. The IC50 value of clavulanic acid for *S. aureus* Russell, SHV-1, SHV-5, MJ-1, and TEM-5 was 8.1-, 4.7-, 5.9-, 4.8-, and 9.3-fold lower, respectively, than that of tazobactam. In contrast, tazobactam was more active than clavulanic acid against TEM-2, Enzyme C, Enzyme D, OXA-2 and OXA-5 (3.6-, 3.4-, 4.0-, 137- and 12.3-fold, respectively).

Table 16: Inhibitory Activity of Tazobactam and Other Beta-lactamase Inhibitors Versus Various Beta-Lactamases

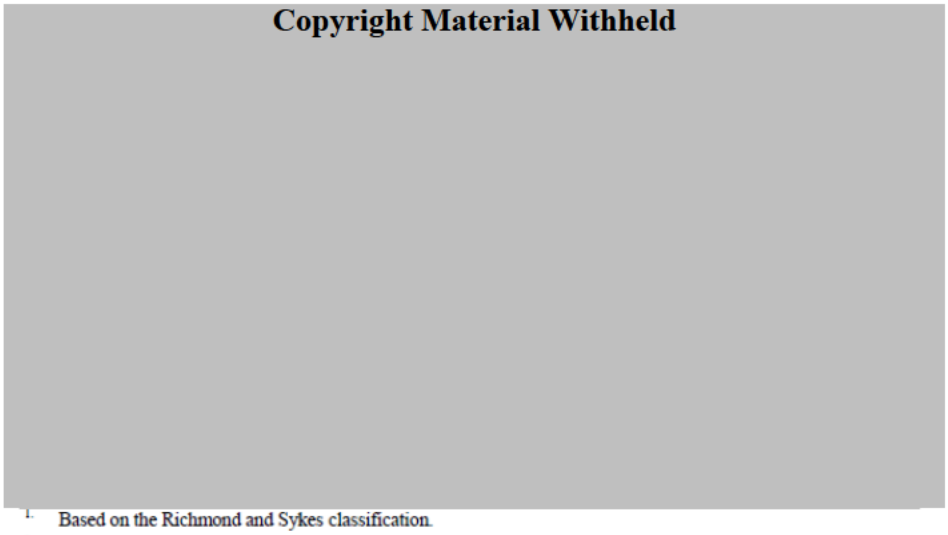




Source: Payne 1994.

The comparative spectra and activities of tazobactam and other inhibitors are summarized in the table below. In general, both tazobactam and clavulanic acid are more active than sulbactam against the majority of common β-lactamases. Tazobactam appears to be more active than clavulanic acid against some of the Class I, chromosomally mediated β-lactamases. Enzyme inactivation, as determined by enzyme activity half-life, was 8 to 1000 times faster with tazobactam than with clavulanic acid against all Class I, AmpC-type enzymes [Akova, 1990]. In addition, tazobactam was more potent than clavulanic acid against β-lactamases produced by *Bacteroides* spp.

Table 17: Summary of Inhibitory Activity of Tazobactam and Other Beta-Lactamase Inhibitors



1. Based on the Richmond and Sykes classification.
2. Graded based on IC_{50} as follows: +++ = IC_{50} < 0.05 µg/mL; ++ = IC_{50} >0.05 to < 0.5 µg/mL; + = IC_{50} >0.5 to < 5 µg/mL; 0 = IC_{50} > 5 µg/mL.

Source: Bryson 1994.

Reviewer's Comment
Tazobactam has inhibitory activity against staphylococcal penicillinases and many of the β-lactamases isolated from gram-negative bacteria, including Richmond and Sykes types II, III, IV, and V, as well as chromosomal I β-lactamases. Theses enzymes include TEM and SHV β-lactamases, and ESBLs.

Reviewer's Comment
The nomenclature and classification system of beta-lactamases may be expressed in terms of functional or structural similarities between the beta-lactamases, or as a combination of the two. These classification systems are also evolving as new beta-lactamases are being discovered, and as the relatedness between them is being evaluated.

Tazobactam also demonstrated moderate activity against the remaining Type I enzyme subtypes. However, like other inhibitors, it does not have meaningful activity against metallo-β-lactamases or Class D β-lactamases. The enzymatic inhibitory activity of tazobactam results in excellent synergistic activity of tazobactam and β-lactams against β-lactamase-producing organisms. In addition to piperacillin, tazobactam has been shown to have synergistic activity with many other β-lactam antibacterials including amoxicillin, ampicillin, carbenicillin, cefotaxime, cefoperazone, ceftriaxone and cefpirome [Bryson, 1994]. Tazobactam is expected to have good synergistic activity with ceftolozane against common ESBL- or AmpC-producing gram-negative organisms.

Ceftolozane -Tazobactam
The synergistic antibacterial mode of action of ceftolozane-tazobactam was evaluated in a comprehensive susceptibility study by examining the activity of ceftolozane-tazobactam against a special collection of Enterobacteriaceae isolates with characterized β-lactamase resistance mechanisms [CX-BD-001]. The study was performed using a set of genetically engineered or molecularly characterized clinical isolates with various β-lactamase producing mechanisms. As demonstrated in the tables below, the addition of tazobactam significantly enhanced the *in vitro* activity of ceftolozane or ceftazidime against the majority of Enterobacteriaceae, including isolates with AmpC or common ESBLs (including SHV, TEM, CTX-M and OXA); the MIC was reduced from ≥ 32 mcg/mL to ≤ 8 mcg/mL for most isolates tested. However, as expected, based on the spectrum of activity of tazobactam, ceftolozane-tazobactam was poorly active against isolates with class B enzymes, like PER-1 and IMP-1. The results were consistent with previous findings for tazobactam in combination with other β-lactams [Bryson, 1994; Schoonover, 1995; Gin, 2007].

Table 18: Activity of Ceftolozane (CXA-101) Against AmpC-Inducible Mutants

Bacterial Strain	Genus and Species	MIC (µg/mL)			
		CXA-101	CXA/TAZ	CAZ	CAZ/TAZ
C2-CON	*Citrobacter freundii*	16	4	64	32
C4-CON	*C. freundii*	32	4	128	32
C10-CON	*C. freundii*	16	2	64	32
84-CON	*E. cloacae*	32	8	128	64
100-CON	*E. cloacae*	1	0.25	16	1
684-CON	*E. cloacae*	16	4	128	64
M1-CON	*Morganella morganii*	2	0.125	4	≤0.030
M3	*M. morganii*	8	0.125	16	≤0.030
M6-CON	*M. morganii*	1	0.06	2	≤0.030
V3-CON	*P. vulgaris*	4	1	0.25	0.06

Abbreviations: CXA/TAZ = CXA-101/ tazobactam; CAZ = Ceftazidime; TAZ = Tazobactam; CON = Constitutively expressed.

Note: A fixed concentration of tazobactam (4 µg/mL) was used.

Source: CX-BD-001.

Table 19: Activity of Ceftolozane (CXA-101)-Tazobactam Against Transconjugate *E. coli* Strains

Strain of *E. coli*[1]	MIC (µg/mL)			
	CXA-101	CXA/TAZ	CAZ	CAZ/TAZ
J53 OXA-5	32	0.5	128	0.5
J53 SHV-2	4	2	16	8
J53 SHV-4	64	16	>128	128
J53 SHV-5	64	1	>128	4
J53 TEM-6	64	0.5	>128	2
J53 TEM-9	>128	8	>128	16
J53 TEM-10	64	1	>128	2
DH5α CTX-M-15	2	0.125	0.5	0.125
DH5α CTX-M-3	4	0.25	0.5	0.125
DH5α CTX-M-15	32	0.25	16	0.125
DH5α IMP-1	32	32	16	16
J53 PER-1	>128	16	>128	16

Abbreviations: CXA/TAZ = CXA-101/ tazobactam; CAZ = Ceftazidime; TAZ = Tazobactam.

1. Genetically engineered isolates.

Notes: A fixed concentration of tazobactam (4 µg/mL) was used.

Source: CX-BD-001.

Table 20: Activity of Ceftolozane (CXA-101)-Tazobactam Against ESBL-Producing Enterobacteriaceae Isolates

Strain	Enzyme/Phenotypes	MIC (µg/mL)			
		CXA-101	CXA/TAZ	CAZ	CAZ/TAZ
E. coli					
H041280204	CTX-M-3	16	0.5	2	0.5
H044020180	CTX-M-14	32	1	4	1
H045000482	CTX-M-gp8	16	0.5	4	1
H052600198	CMY + CTX-M-15	4	1	32	8
JAB FRENCH	CTX-M-2	>128	2	>128	2
EO499	CTX-M-15	32	2	8	1
EO553	CTX-M-15	32	0.25	2	0.25
EO550	CTX-M-15	64	0.5	32	0.5
Birmingham 1	CTX-M-26	>128	0.5	128	0.5
Birmingham 2	CTX-M-25c	2	0.5	1	0.25
LN07037	ESBL	64	0.5	32	0.25
LN06024	ESBL	32	0.5	>128	2
SE01031	ESBL	16	0.5	>128	1
SE01061	ESBL	8	0.25	64	0.5
SE02017	ESBL	2	0.25	16	0.25
H041280204	CTX-M-3	16	0.5	2	0.5
H044020180	CTX-M-14	32	1	4	1
H045000482	CTX-M-gp8	16	0.5	4	1
H050480301	CTX-M-gp2	16	0.5	4	0.5
H052600198	CMY + CTX-M-15	4	1	32	8
JAB FRENCH	CTX-M-2	>128	2	>128	2
EO499	CTX-M-15	32	2	8	1
EO553	CTX-M-15	32	0.25	2	0.25
EO550	CTX-M-15	64	0.5	32	0.5

Table 20: Activity of Ceftolozane (CXA-101)-Tazobactam Against ESBL-Producing Enterobacteriaceae Isolates (Continued)

Strain	Enzyme/Phenotypes	MIC (µg/mL)			
		CXA-101	CXA/TAZ	CAZ	CAZ/TAZ
K. pneumoniae					
LN01001	CTX-M 1 gp	>128	2	32	1
SE08055	CTX-M 1 gp	>128	4	64	0.5
SE08097	CTX-M 1 gp	128	1	32	0.5
SE08100	CTX-M1-gp	128	1	32	0.5
1	ESBL	4	0.125	32	0.25
9	ESBL	4	0.25	32	0.5
32	ESBL	32	8	128	2
18	ESBL	8	0.5	64	0.5
SE08073	CTX-M 1 gp g	32	0.25	8	0.25

Abbreviations: CXA/TAZ = CXA-101/ tazobactam; CAZ = Ceftazidime; TAZ = Tazobactam.
Note: A fixed concentration of tazobactam (4 µg/mL) was used.
Source: CX-BD-001.

Table 21: Activity of Ceftolozane (CXA-101)-Tazobactam Against Other Enterobacteriaceae

Strain	Species	Phenotype	MIC (µg/mL)			
			CXA-101	CAX/TAZ	CAZ	CAZ/TAZ
E395	*E. cloacae*	CTX-M gp-9	1	0.25	1	0.25
SE06069	*E. cloacae*	CTX-M 1 group	8	0.5	32	0.5
SE06009	*E. cloacae*	CTX-M 1 group	32	16	>128	8
SE06002	*E. sakazakii*	CTX-M 1 group	8	0.5	64	0.5
SE01077	*E. sakazakii*	CTX-M 1 group	32	0.5	16	0.5
SE03021	*E. cloacae*	ESBL	8	0.25	64	0.5
LN10096	*E. cloacae*	ESBL	0.25	0.25	0.25	0.25
LN09067	*E. cloacae*	ESBL	32	16	>128	8
LN09057	*E. cloacae*	ESBL	8	0.5	64	2
LN08011	*E. sakazakii*	ESBL	8	0.25	64	2
SE08075	*E. sakazakii*	ESBL	16	4	64	2
LN10022	*E. sakazakii*	ESBL	8	0.5	64	1
LN09004	*E. aerogenes*	AmpC	8	16	>128	128
LN08097	*C. freundii*	AmpC	4	1	32	8
LN08096	*C. freundii*	AmpC	8	0.5	32	1
LN08063	*C. freundii*	AmpC	8	4	64	32
LN08051	*C. freundii*	AmpC	8	4	64	32
LN08041	*C. freundii*	AmpC	8	2	64	32
LN03054	*C. freundii*	AmpC	4	1	16	4
LN04034	*C. freundii*	CTX-M 1 group	>128	4	128	2
LN07093	*C. freundii*	ESBL	32	0.5	128	0.5
LN03002	*C. freundii*	AmpC	8	4	32	2
LN10025	*M. morganii*	AmpC	2	0.125	2	≤0.030
LN08024	*M. morganii*	AmpC	16	0.25	32	0.06
SE06031	*M. morganii*	CTX-M 1gp	8	0.125	1	≤0.030
P959	*P. mirabilis*	ESBL	>128	4	>128	0.25
LN08012	*Serratia odorifera*	ESBL	8	0.25	64	0.25

Abbreviations: CXA/TAZ = CXA-101/ tazobactam; CAZ = Ceftazidime; TAZ = Tazobactam.
Note: A fixed concentration of tazobactam (4 µg/mL) was used.
Source: CX-BD-001.

Mechanisms of Resistance

Ceftolozane

The resistance mechanisms for ceftolozane are classified in the several main categories: drug inactivation, alteration of target sites or low affinity to PBPs, and decreased outer membrane permeability and active efflux. The mechanisms of resistance are detailed below.

Drug Inactivation

For cephalosporins, including ceftolozane, hydrolysis by β-lactamase is the most common resistance mechanism in gram-negative bacteria. The β-lactamases are a group of diversified enzymes that can be classified into four major classes (A, B, C, and D), according to their genomic background and enzymatic activities. Although β-lactamases have different affinities for different β-lactams, they inactivate these antibacterials by splitting the amide bond of the β- lactam ring. Ceftolozane is stable against most penicillinases, but it remains sensitive to enzymatic degradation by Class B metalloenzymes, Class D enzymes, and ESBLs produced by some Enterobacteriaceae and *Acinetobacter* isolates. It may also be degraded by some AmpC derepressed mutants of Enterobacteriaceae. In study CX-BD-001, ceftolozane was shown to be sensitive to degradation by various ESBL and AmpC enzymes, as well as by Class B β-lactamases. The addition of a β-lactamase inhibitor, such as tazobactam, was able to fully restore the activity of ceftolozane against the majority of ESBL-positive strains and some AmpC producing isolates.

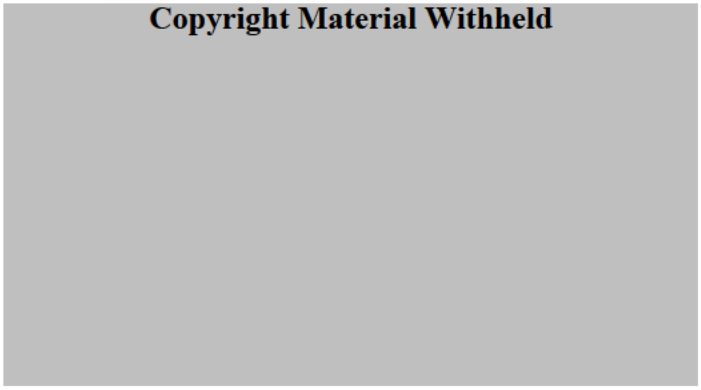
AmpC β-lactamase

Many gram-negative bacilli carry chromosomal *ampC* genes which can produce AmpC β-lactamases under certain inductive conditions. The overproduction of AmpC may result in different level of resistance to β-lactams. In one study, the induction of AmpC production was evaluated in *P. aeruginosa* [Takeda 2007b]. The induction level of AmpC β-lactamase in *P. aeruginosa* PAO1 is indicated in the figure below. Ceftolozane concentrations below 1 mcg/mL did not induce the expression of AmpC β-lactamase. Although ceftolozane induced expression of AmpC β-lactamase to a slightly greater extent than ceftazidime at concentrations of 10 mcg/mL and 100 mcg/mL, ceftolozane was more active than ceftazidime against AmpC-producing organisms because of its greater stability to AmpC β-lactamase.

Reviewer's Comment

The moderate *in vitro* induction of AmpC production in *P. aeruginosa* appears to have no impact on the activity of ceftolozane against this organism. However, *ampC* derepressed mutations in Enterobacteriaceae did result in high MICs of ceftolozane.

Figure 2: Induction of AmpC Enzyme Production in *Pseudomonas aeruginosa*



Source: Takeda 2007b

The effect of the level of AmpC β-lactamase expression on the antipseudomonal activity of ceftolozane and its stability to purified pseudomonal AmpC β-lactamases were examined (CRE060042, CXA101-M-014, CXA201-M-013, M5.3.5.4\CXA.021.MC). The activity of ceftolozane was tested against *P. aeruginosa* PAO1 and genetically modified PAO1strains that produce high levels of AmpC. Ceftolozane activity was conserved against all single and combined mutations leading to AmpC hyper-production with only the *ampD-dacB* (AmpC over-expression-PBP4) knockout producing a 4-fold increase in ceftolozane MIC value. Of note, this particular mutation shows the highest fold increase in AmpC expression. In contrast, ceftazidime, cefepime, piperacillin/tazobactam and aztreonam showed several-fold increases in MIC values, particularly in strains with multiple mutations (see table below).

Table 22: Activity of Ceftolozane and Comparator Antibacterials Against genetically Modified Strains of *Pseudomonas aeruginosa* PAO1 Producing Different Amounts of AmpC Beta-lactamase

P. aeruginosa strain designation	MIC (µg/mL)							Fold increase in *ampC* Expression[a]	
	CXA	CAZ	FEP	PTZ	ATM	IMP	MER	Baseline	Induced
PA01 (parent)	0.5	2	2	2	4	2	0.5	1	50±14
PAΔD (PA01 knockout mutant)	0.5	8	4	16	8	2	2	48±4	134±11
PAΔDDh3 (PA01 *ampD-AmpDh3* knockout mutant)	1	32	8	128	32	2	2	191±52	1014±297
PAADDh3Dh2 (PA01*ampD-ampDh2-ampDDh3*)	1	32	8	128	32	1	1	1020±87	1105±88
PAΔdB (PA01 *dacB* (PBP4) knockout mutant)	1	32	16	64	32	2	0.5	21±11	232±67
PAΔDdB (PA01 *ampD-dacB* knockout mutant	2	64	32	256	128	2	2	1770±414	1950±480

[a] Data represent *ampC* expression levels under basal and cefoxitin (50 µg/mL)-induced conditions
Abbreviations: ATM=aztreonam; CAZ=ceftazidime; CXA=ceftolozane; FEP=cefepime, IMP=imipenem, MER=meropenem MIC = minimum inhibitory concentration; PTZ=piperacillin/tazobactam.
Source: [9], M5.3.5.4\CXA101-M-014

Similar data were generated for ceftolozane using twelve clinical *P. aeruginosa* isolates that exhibited either partial or full derepression of the *ampC* gene. In general, ceftolozane was 8 to ≥64-fold more than ceftazidime and 16 to ≥128-fold more than piperacillin/tazobactam (CXA201-M-013). The kinetic parameters of *P.aeruginosa* AmpC with ceftolozane as a substrate were also analyzed using purified AmpC enzyme. The results suggest greater stability of ceftolozane to hydrolysis by AmpC due to its low affinity (high Km) for the AmpC enzyme compared to ceftazidime.

Table 23: Kinetic Parameters of *Pseudomonas aeruginosa* Purified AmpC Beta-lactamase with Ceftolozane and Ceftazidime as Substrates

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[a] Values are mean ± standard deviation of five independent experiments.
[b] Km value determined as the Ki value in competition experiments.
Source: [2]
Abbreviations: K_m = Michaelis-Menten rate constant; s = seconds; *k*cat=catalytic constant
Source: [2]= [Takeda, 2007b]

Other β-lactamases

Like other third generation cephalosporins, such as ceftazidime, ceftolozane remains vulnerable to most Class A ESBLs and Class B and Class D enzymes (see tables below). Addition of a beta-lactamase inhibitor, such as tazobactam, was able to inhibit the majority of ESBLs and increase the potency of ceftolozane against enzyme-producing Enterobacteriaceae. In *P. aeruginosa*, resistance resulting from ESBLs, Class D and Class A enzymes remains rare in most areas. Two mechanistic studies have been performed to examine ceftolozane -resistant *P. aeruginosa* [Giske 2009; CXA101-M-010]. The strains tested represented a low percentage of ceftolozane -resistant isolates identified during studies to examine carbapenem-resistant *P. aeruginosa.* The resistant *P. aeruginosa* isolates were carefully phenotyped and genotyped to define the precise resistance mechanisms. As demonstrated in the tables below, the β-lactamases that are responsible for resistance to ceftolozane included an OXA-10 variant, OXA- 144, OXA-17, OXA-14, OXA-32, PER-1, GES-2, GES-9, VEB-2,VIM-2 and some unknown β-lactamases. Moreover, most of the ceftolozane -resistant isolates were MDR, with broad resistance to β-lactams and other classes of antipseudomonal drugs. Only one isolate (OXA-32) had a reduced MIC when tazobactam was added. See also mechanism of action section of this review for data tables related to beta-lactamases.

Reviewer's Comment
The β-lactamases that are responsible for resistance to ceftolozane included an OXA-10 variant, OXA- 144, OXA-17, OXA-14, OXA-32, PER-1, GES-2, GES-9, VEB-2,VIM-2. Only one isolate (OXA-32) had a reduced MIC when tazobactam was added.

Table 24: Characterization of 11 Ceftolozane (CXA-101) Nonsusceptible Isolates of *P. aeruginosa*

P. aeruginosa		Acquired β-lactamase	AMK	MER	FEP	TOB	CAZ	CIP	IMP	PTZ	CXA	CXA/T4	CXA/T8
Isolate	Clone (PFGE)												
1C2	PTOL1	OXA-17	32	64	>64	32	>64	4	>64	>64	64	64	64
1C3	PTOL1	OXA-17	32	>64	>64	64	>64	4	>64	>64	64	64	64
1E1	PpA1	OXA-144[1]	16	16	16	64	>64	8	>64	>64	32	32	16
1E2	PpA1	OXA-144	32	32	32	64	>64	16	>64	>64	64	64	32
1E3	PpA2	OXA-10 variant	32	>64	>64	>64	64	1	16	>64	32	32	32
1B2	RCA2	PER-1	1	16	32	<0.12	>64	0.25	64	16	64	32	16
1D5	SA1	VIM-2	16	16	16	64	16	8	>64	16	64	64	64
3C1	VAL6	ND	2	4	64	<0.12	>64	0.5	>64	>64	64	8	8
3C8	GM1	OXA-144	4	4	4	16	32	2	>64	4	32	16	8
3C9	GM1	OXA-144	4	4	4	16	32	2	>64	4	32	16	8
3D2	GM2	ESBL pI 7.5[2]	8	4	32	8	>64	2	64	64	32	16	16

Abbreviations: AMK = Amikacin; CAZ = Ceftazidime; CIP = Ciprofloxacin; CXA = CXA-101; CXA/T4 = CXA-101/ tazobactam (4 µg/mL fixed concentration; CXA/T8 = CXA-101/ tazobactam (8 µg/mL fixed concentration); FEP = Cefepime; IMP = Imipenem; MER = Meropenem; ND = None detected; PTZ = Piperacillin/ tazobactam; TOB = Tobramycin.

1. New OXA ESBL (OXA-2 derivative), described for the first time in this study.
2. The ESBL gene was not identified with the set of PCR used for detection.

Source: CXA101-M-010.

Table 25: Activity of Ceftolozane (CXA-101)-Tazobactam and Comparators Against Drug Resistant Isolates of *P. aeruginosa*

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Abbreviations: CAZ = Ceftazidime; CAZ/T4 = Ceftazidime / tazobactam (4 µg/mL fixed concentration); CAZ/L2 = Ceftazidime/clavulanate (2 µg/mL fixed concentration); CIP = Ciprofloxacin; CXA = CXA-101; CXA/T4 = CXA-101/ tazobactam (4 µg/mL fixed concentration; CXA/T8 = CXA-101/ tazobactam (8 µg/mL fixed concentration); FEP = Cefepime; IMP=Imipenem; PTZ = Piperacillin/ tazobactam.

Source: Giske 2009.

Alteration of Target Sites or Low Affinity to Penicillin-binding Proteins
Alternation of target PBPs is the most common resistance mechanism in gram-positive bacteria. The production of PBP2A in staphylococci results in methicillin resistance. Other PBPs in pneumococci are associated with penicillin resistance, while the low affinity of β-lactams for PBPs in *E. faecium* causes intrinsic resistance to β-lactam antibiotics. *Enterococcus faecium* has six PBPs, and PBP5 seems to be the main target for β-lactam antibacterials. The β-lactam resistance observed in these strains is associated with increased production of PBP5, which has a relatively decreased affinity for imipenem and other β-lactams [Arbeloa, 2004]. In one study, the activity of ceftolozane against *P. aeruginosa* with mutated PBP4 was analyzed. As shown in the table below, the mutant with the *dacB*-encoded nonessential PBP4 deletion has less impact on ceftolozane

than other β-lactamase antibacterials tested [CXA101-M-014]. A recent study has indicated that PBP4 behaves as a trap target for β-lactams. The inactivation of this PBP is shown to determine an efficient and complex β-lactam resistance response, triggering overproduction of the chromosomal β-lactamase AmpC and the specific activation of the CreBC (BlrAB) two-component regulator, which in turn plays a role in resistance [Moya, 2009].

Decreased Outer Membrane Permeability and Active Efflux
Non-β-lactamase-mediated resistance of gram-negative bacteria, such as *P. aeruginosa*, can be caused by reduced drug concentrations at the target sites (e.g. PBPs) through two common mechanisms: reduced membrane permeability and increased efflux. Multidrug efflux pumps in the inner and outer membrane of *P. aeruginosa* may act in concert with periplasmic β-lactamases and membrane permeability components to protect these bacteria from β-lactam agents. A deficiency of OprD porin protein (a transporter channel involved in β-lactam uptake) and/or overexpression of certain efflux proteins in *P. aeruginosa* can decrease susceptibility to carbapenems [Masuda, 2000]. However, in order to achieve a high level of resistance to β-lactams, efflux systems have to collaborate with other mechanisms, such as OprD deficiency and increased β-lactamase production.

Outer Membrane Permeability
The passage of hydrophilic antibacterials through the outer membrane is facilitated by the presence of porins (water-filled diffusion channels). Mutations resulting in the loss of specific porins can occur in clinical isolates, which could cause increased resistance to β-lactam antibacterials. The effect of OprD, which is mostly involved in the permeation of carbapenems, was analyzed by measuring the antibacterial activity of ceftolozane against an OprD-deficient strain [CRE060042]. The ratio of the MIC for the OprD-deficient strain to that for the wild type strain for ceftolozane was equal to one, while that for imipenem was 16, suggesting that ceftolozane does not exhibit cross-resistance to carbapenems in terms of OprD deficiency mechanisms.

In a second study, multiple *oprD* mutants were used to evaluate the effect of the deficiency of porin OprD on the activity of ceftolozane. Sequencing of the three putative OprD mutants revealed in all cases mutations in *oprD* and each of the mutants had a different type of mutation: PAOD1 had a nonsense mutation .[(G194A (W65X)], PAOD2 had a frameshift mutation [1 bp (A) insertion in neucleotide 335] and PAOD3 a missense mutation [G3A (M1I)] affecting the initiation codon. As expected, *oprD* inactivation resulted in resistance to imipenem and 4-fold increased meropenem MICs. No major differences in terms of MICs were observed in the results for the three OprD mutants tested. Meropenem MICs were notably increased further in the *oprD-dacB* (mutants PAOD1dB to PAOD3dB) and *oprD-ampD* (mutants PAOD1AD to PAOD3AD) double mutants, surpassing the breakpoints for nonsusceptibility for this compound.

Reviewer's Comment
Ceftolozane was the only β-lactam tested in this study showing conserved activity against *oprD* mutants.

Activity of Ceftolozane-tazobactam against *Pseudomonas aeruginosa* Isolates with Loss of the Outer Membrane Protein OprD
The most common mechanism of resistance to the carbapenem class of antibacterials in *P. aeruginosa* is the loss or alteration of the outer membrane porin protein OprD. Ceftolozane was the only β-lactam tested that remained fully active against OprD, OprD-AmpC (*ampC*), and OprD-PBP4 (*dacB*) double mutants (CXA101-M-014).

Table 26: Activity of Ceftolozane and Comparator Antibacterials *Against Pseudomonas aeruginosa* Mutants with Mutations in Outer Membrane Protein OprD

P. aeruginosa strain designation	MIC (μg/mL)						
	CXA	CAZ	FEP	PTZ	ATM	IMP	MER
PA01 (parent)	0.5	2	2	2	4	2	0.5
PAOD1 (*oprD* spontaneous mutant)	0.5	2	2	2	4	16	1
PAOD2 (*oprD* spontaneous mutant)	0.5	2	2	4	4	8	2
PAOD3 (*oprD* spontaneous mutant)	0.5	2	2	4	4	16	2
PAOD1ΔD (*ampD* knockout mutants of the corresponding *oprD* spontaneous mutants)	0.5	16	4	32	8	16	8
PAOD2ΔD (*ampD* knockout mutants of the corresponding *oprD* spontaneous mutants)	0.5	16	8	32	8	16	8
PAOD3ΔD (*ampD* knockout mutants of the corresponding *oprD* spontaneous mutants)	0.5	16	8	32	8	16	8
PAOD1ΔDdB (dacB knockout mutants of the corresponding oprD spontaneous mutants	0.5	32	32	64	32	16	4
PAOD2ΔDdB (dacB knockout mutants of the corresponding oprD spontaneous mutants	0.5	32	32	64	32	16	4
PAOD3ΔDdB (dacB knockout mutants of the corresponding oprD spontaneous mutants	0.5	32	32	64	32	16	4

Abbreviations: ATM=aztreonam, CAZ=ceftazidime ; CXA=ceftolozane, , FEP=cefepime, IMP= imipenem, MER=meropenem, MIC = minimum inhibitory concentration; PTZ=piperacillin/tazobactam
Source: [4], M5.3.5.4\CXA101-M-014

Efflux

Active efflux is an important non-enzymatic mechanism of β-lactam resistance in *P. aeruginosa* and is mediated by four genetically different three component efflux systems that belong to the resistance-nodulation-division (RND) family: MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM. Ceftolozane is not a substrate of these efflux pumps as shown by the lack of increase in MIC values in strains over-expressing these efflux pumps as compared to parent strains (CXA201-M-013).

Active efflux of antibacterials is increasingly recognized as a common mechanism of resistance in many clinically relevant pathogens. Efflux is one of the most common resistance mechanisms of *P. aeruginosa*. Several efflux pumps play important roles in intrinsic and acquired resistance in *P. aeruginosa*. All carbapenems with the exception of imipenem are substrates for the MexAB-oprM, MexCD-OprJ and MexXY-OprM efflux pumps of *P. aeruginosa*, and overexpression of any of these pumps results in various levels of resistance to carbapenems [Masuda, 2000]. For ceftolozane, *in vitro* antibacterial activity was evaluated using several strains with overexpressed efflux pump activity [CRE060042]. The MIC of both ceftolozane and ceftazidime for strains with efflux pumps did not change, while the MIC of ciprofloxacin in some strains and imipenem in one strain increased compared to the parent strains. These data suggest that ceftolozane is not a substrate of common efflux pumps in *P. aeruginosa.*

Table 27: Effect of Active Efflux on MICs of Ceftolozane (CXA-101) and Comparators Using Strains with Over-expressed Efflux Pumps

Efflux System	Strain	MIC (µg/mL)			
		CXA-101	CAZ	IMP	CIP
MexAB-OprM	*P. aeruginosa* KG2212	0.5	2	1	0.25
MexCD-OprJ	*P. aeruginosa* KG3056	0.25	0.5	0.5	2
MexXY	*P. aeruginosa* KG4545	0.5	2	1	0.25
Parent of all above	*P. aeruginosa* PAO1	0.5	2	1	0.125
MexEF-OprN	*P. aeruginosa* KG4001	0.5	2	4	2
Parent of above	*P. aeruginosa* KG4222	0.5	2	1	0.125

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP = Imipenem.
Source: CRE060042.

Table 28: Impact of MexAB-OprM, MexEF-OprN and MexCD-OprJ efflux Pumps on Ceftolozane-Tazobactam and Comparator minimum Inhibitory Concentrations

	MIC (µg/mL)						
Strain Designation	Phenotype	CXA	CAZ	FEP	IPM	ATM	PTZ
PAO1	Parent	0.5	2	1	1	4	4
PA01-1455	*mexAB-oprM* overexpression	0.5	8	8	1	>16	32
PA01-Takai 1	*mexEF-oprN* overexpresson and decreased oprD	0.25	1	0.5	4	2	2
PS244	Parent	0.5	4	2	16	4	4
PS244-911C	*mexCD-oprJ* overexpression	0.5	2	8	1	2	8

Abbreviations: CXA=ceftolozane; CAZ=ceftazidime; FEP=cefepime; IPM=imipenem; ATM=aztreonam; PTZ=piperacillin/tazobactam

In a separate study, ceftolozane was tested with imipenem and ceftazidime using a special collection of clinical isolates (N=51) with different levels of intrinsic resistance driven by efflux pumps [CX-BD-001; Livermore 2009]. As shown in the figure below, unlike ceftazidime and imipenem, ceftolozane appears not to be affected by efflux mechanisms. Its MIC distribution was similar to that observed with wild type clinical *P. aeruginosa* isolates.

Figure 3: MIC Distributions of *P. aeruginosa* with Over-expressed Efflux Pumps

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Source: CA-BD-001 and Livermore 2009.

The results above indicate that ceftolozane enters bacterial cells independently of the OprD channel and that it is also not a substrate for the common efflux pumps found in *P. aeruginosa*. As a consequence, ceftolozane remains highly active against drug-resistant *P. aeruginosa* due to these mutations.

Tazobactam

Like other antibacterials, after introducing beta-lactamase inhibitors (BLIs) into clinical practice, the resistance to BLIs (including tazobactam) due to various mechanisms has been documented [Chaïbi, 1999; Yang, 1999]. Reports have established that the susceptibility of some Enterobacteriaceae isolates to BLIs can be affected by hyper-

production of unmodified TEM-type β-lactamase, or by the modification of the outer membrane proteins, or by both. Resistance may also be attributed to production of OXA-type enzymes, or to hyper-production of cephalosporinases. In the face of selective pressure arising from use of either newer cephalosporins or β-lactam plus BLI combinations, mutations arose among Class A β-lactamase genes, leading to resistance to the effects of BLIs. The recent emergence of bacterial strains producing inhibitor-resistant TEM (IRT) enzymes could be related to the frequent use of BLIs such as clavulanic acid, sulbactam, and tazobactam in hospitals and in general practice. The effect of BLIs has also been compromised by the emergence of mutant TEM-type β-lactamases, collectively designated inhibitor-resistant TEM or IRT β-lactamases [Yang, 1999].

The first IRT β-lactamases were described mainly in Europe, but they are spreading worldwide. The production of IRT has been detected in strains of Enterobacteriaceae, particularly in *E. coli, K. pneumoniae*, *P. mirabilis,* and *C. freundii*. The IRT β-lactamases differ from the parental enzymes TEM-1 or TEM-2 by one, two, or three amino acid substitutions at different locations. The key amino acid positions important for inhibitor resistance include Met69, Ser130, Arg244, Arg275, and Asn276. Ser130 is vital to the chemical mechanism of inhibition. Arg244 is involved in positioning β-lactams, especially penicillins and BLIs via their carboxyl groups. Site-directed mutagenesis studies confirm the role of Arg244 and its coordinating partners in β-lactam turnover and in the reactions leading to enzyme inactivation. This mechanism is dependent on the donation of a proton via a water molecule coordinated to Arg244 and Val216 to clavulanic acid to allow formation of a favorable leaving group. This proton donation is probably not required for formation of a favorable leaving group for the sulfone inhibitors sulbactam and tazobactam. Therefore, some amino acid substitutions have different effects on inhibition by clavulanic acid than the penicillanic acid sulfones. Met69 may play a more structural role in β-lactam positioning within the oxyanion hole.

Ceftolozane -Tazobactam
Resistance mechanisms specific to ceftolozane -tazobactam are likely to be similar to those for the individual components. Additional information pertaining to resistance is below.

Emergence of Resistance in Vitro

Single- and multiple-step selection of drug-resistant mutants was employed to explore the potential for emergence of resistance in *P. aeruginosa* after exposure to ceftolozane and comparator antibacterials (CRE060042, CXA101-M-012, CXA.012.MC, CXA.013.MC, CXA.031.MC,CXA.084.MC). Additionally, a stringent 10-day hollow-fiber model was used to evaluate the ceftolozane-tazobactam resistance incidence for both *P. aeruginosa* and ESBL positive *E. coli*. The data demonstrate that there is a low potential for ceftolozane-tazobactam drug resistance development in both *P. aeruginosa* and *E. coli* (CXA.044.MC, CXA.047.MC).

In the single-step resistance selection studies with *P. aeruginosa* PAO1, ceftolozane and three comparators were tested at 4X, 8X and 16X MIC (CRE060042,CXA.031.MC). Resistance incidence frequencies were calculated as the ratio of colonies on selection plates to the total amount of CFU plated. Ceftolozane has a lower resistance incidence frequency than imipenem at 4X MIC and ceftazidime at all concentrations tested and is comparable to imipenem at higher concentrations in *P. aeruginosa* PAO1 (see table below).

Table 29: Resistance Incidence Frequency of Ceftolozane in *Pseudomonas aeruginosa*

Multiple of ceftolozane MIC	ceftolozane	ceftazidime	cefepime	imipenem
4X MIC Exp 1	$< 6.1 \times 10^{-9}$	4.3×10^{-7}	No data	$> 6.1 \times 10^{-6}$
4X MIC Exp 2	1.10×10^{-8}	7.9×10^{-7}	$< 4.5 \times 10^{-8}$	No data
8X MIC Exp 1	$< 6.1 \times 10^{-9}$	3.7×10^{-7}	No data	$< 6.1 \times 10^{-9}$
8X MIC Exp 2	$< 4.5 \times 10^{-9}$	6.2×10^{-7}	$< 4.5 \times 10^{-8}$	No data
16X MIC Exp 1	$< 6.1 \times 10^{-9}$	1.2×10^{-8}	No data	$< 6.1 \times 10^{-9}$
16X MIC Exp 2	$< 4.5 \times 10^{-9}$	1.6×10^{-7}	No data	No data

Source: M5.3.5.4\CRE060042
Source: M5.3.5.4\CXA.031.MC
Abbreviations: Exp = experiment; MIC = minimum inhibitory concentration.

Resistant *P. aeruginosa* (PAO1) isolates from one resistance incidence frequency study (CXA.031.MC) were characterized by MIC antibiogram, chromosomal Amp C β-lactamase expression and whole genome sequencing. Whole genome sequencing shows a mutation in a gene encoding a probable two component sensor system for all three strains and an additional mutation in a gene encoding an aspartate decarboxylase precursor in one of the strains. There is no increase in chromosomal AmpC β-lactamase activity compared to the parent strain for these ceftolozane resistant isolates. However, ceftazidime resistant and cefepime strains demonstrate approximately 40 to 50-fold and 100 to150-fold increases in AmpC β-lactamase activity, respectively, compared to the parent strain.

Reviewer's Comment
These studies show that ceftolozane is stable to AmpC β-lactamase in *P. aeruginosa* (including strains with induced enzyme production). This property distinguishes ceftolozane from ceftazidime and other cephalosporins.

Table 30: Amp C Beta-lactamase Induction for *P. aeruginosa* Ceftolozane, Cefipime, and Ceftazidime Resistant Isolates

Isolate Designation	Antibiotic Selection	V_{max} mOD/min	Fold increase in activity compared to parent strain	CXA MIC (µg/mL)	CEP MIC (µg/mL)	CAZ MIC (µg/mL)
PAO1	NA	24	1	0.5	2	2
Aa2	ceftolozane	8	0.33	4	8	8
Ab1	ceftolozane	11	0.46	4	8	4
Ab2	ceftolozane	6	0.25	4	4	8
Ab3	ceftolozane	8.5	0.35	8	4	4
Ab4	ceftolozane	7.5	0.31	4	4	4
A3	cefepime	3619	151	4	32	128
B1	cefepime	4028	168	2	32	64
B3	cefepime	2932	122	2	32	64
B4	cefepime	2591	108	2	32	64
Fa2	ceftazidime	1233	51.38	2	32	64
Fb1	ceftazidime	1111.5	46.31	2	32	64
Fa1	ceftazidime	972	40.50	2	32	64
Fb2	ceftazidime	907.5	37.81	2	32	64
Fc1	ceftazidime	1039	43.29	16	32	64

Source: M5.3.5.4\CXA.031.MC
Abbreviations: CEP=cefepime, CAZ=ceftazidime; CXA=ceftolozane; MIC = minimum inhibitory concentration; NA = not applicable; V_{max} = maximum rate.

Additionally, five day serial passage of *P. aeruginosa* PAO1 was performed with ceftolozane and comparator compounds (CRE060042). After five serial passages, the ceftolozane MIC increased 4-fold to a final MIC value of 2 mcg/mL. In contrast, the MIC values for ceftazidime, imipenem and ciprofloxacin were increased 32-fold, 16-fold, and 16-fold, respectively. A second serial passage study using ceftolozane and comparators (ceftazidime and meropenem) was conducted by subjecting three *P. aeruginosa* strains with low (Pa 44, 0.5 mcg/mL), mid (Pa 2638, 2 mcg/mL) and high (Pa 2629, 16 mcg/mL) ceftolozane MIC values to as many as 16 passages (CXA.012.MC). In this study, ceftazidime shows highest absolute MIC values at the end of the study against all three strains (see table below). For all three test agents, resistance is stable after three passages in drug-free medium.

Table 31: Minimum Inhibitory Concentration MIC value (mcg/mL) of *P. aeruginosa* Strains at End of Serial Passage Study (Day 16)

Strain Designation	Ceftolozane (µg/mL)	Ceftazidime (µg/mL)	Meropenem (µg/mL)
Pa 44 (low MIC; 0.5µg/mL)	256	>2048	256
Pa 44 (low MIC; 0.5µg/mL)	64	>2048	256
Pa 44 (low MIC; 0.5µg/mL)	32	>2048	256
Pa 2638 (mid MIC; 2 µg/mL)	8	512	32
Pa 2638 (mid MIC; 2 µg/mL)	8	1024	32
Pa 2638 (mid MIC; 2 µg/mL)	8	1024	32
Pa 2629 (high MIC; 16 µg/mL)	512	2048	256
Pa 2629 (high MIC; 16 µg/mL)	512	>2048	256
Pa 2629 (high MIC; 16 µg/mL)	512	2048	256

Source: CXA.012.MC
Abbreviations: MIC = minimum inhibitory concentration.

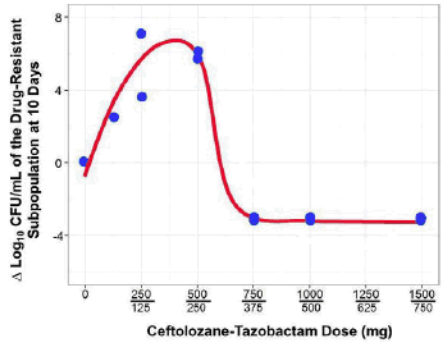
A study which examined the dynamics of resistance development to ceftolozane-tazobactam, ceftazidime, meropenem, and ciprofloxacin was undertaken in the *P.aeruginosa* wild type reference strain PAO1 and its mismatch repair deficient mutant strain (PAOMS, a *mutS* knockout mutant) by incubating the strains for 24h in 0.5X-64X the MIC concentration for up to 7 days (CXA.084.MC). PAO1 ceftolozane-tazobactam mutants reached only moderate resistance (MICs 4-8 mcg/mL) after the 7-day exposure experiments and 64X MIC concentrations were not reached in any of the cultures, even after extended 14-day exposure experiments. In contrast, ceftazidime reached 64 mcg/mL MIC by day 4 and ciprofloxacin and meropenem by day 6. Analysis of PAO1 ceftolozane-tazobactam mutants showed 2-4 mutations, whereas the mutator strain, PAOMS, showed 48-54 mutations. Major changes in global gene expression profiles were detected in all mutants but only the PAOMS mutants showed multiple mutations in conserved residues of AmpC, mostly in *ampC* regulators such *dacB* or *ampR*. The data in this study demonstrated that development of high-level resistance to ceftolozane-tazobactam occurred efficiently only in a *P. aeruginosa* mutant background. A study to evaluate the propensity of *E. coli*, *K. pneumonia*, and *E. cloacae* to develop mutational resistance was undertaken in both single step and serial passage studies using super-inhibitory and sub-inhibitory concentrations of ceftolozane-tazobactam, ceftazidime, cefepime, piperacillin/tazobactam or doripenem (CXA.072.MC). These strains included wild type and producers of TEM-1, CTX-M-15, AmpC (chromosomal inducible and de-repressed and plasmid-mediated inducible such as DHA-1), affected by the development of mutational resistance. However, in AmpC-producing isolates, ceftolozane-tazobactam selected for higher resistance rates compared to cefepime; in

contrast, ceftolozane-tazobactam selected for less resistance than cefepime in ESBL-producing isolates (data not shown).

Reviewer's Comment
Resistance to cetolozane-tazobactam was seen in the mismatch repair mutant PAOMS, a *P. aeruginosa* mutant background. High-level resistance that occurred in the mutator strain was associated with increased susceptibility to other antipseudomonals, particularly imipenem and ciprofloxacin.The fitness cost of high-level ceftolozane-tazobactam resistant PAOMS mutants was highly variable, possibly indicating the absence or presence of cost compensatory mutations in these mutants. For all the antibiotics tested, the impact on fitness of high-level antibiotic resistance was much lower for PAOMS mutants than for PAO1 mutants, likely reflecting the increased capacity of the mutator strain for acquiring cost-compensatory mutations. These mutants were described in the Applicant's final study report CXA.084.MC.

A rigorous 10-day hollow fiber model was used to evaluate the drug exposure needed to optimize cell kill and prevent the emergence of resistance in *P. aeruginosa* PA01 and PA 2638 (a clinical strain) and an ESBL (CTX-M-15, moderate producer) positive *E. coli*. Ceftolozane-tazobactam dosing regimens ranging from 125/62.5 mg to 1500/750 mg q8h were simulated as well as a control simulation with piperacillin/tazobactam 4500 mg q6h daily. For the ESBL positive *E. coli* strain, an inverted-U shaped function best described the relationship between selection of resistant bacterial populations and drug exposure. The lowest (125/62.5 mg) and the highest
(750/375, 1000/500 and 1500/750 mg) ceftolozane-tazobactam regimens did result in selection of resistant populations while resistance was observed with intermediate regimens (250/125, 500/250 mg). In contrast to lower doses, the higher ceftolozane-tazobactam regimens (750/375, 1000/500, 1500/750 mg), not only prevented resistance emergence but also sterilized the model system (see figure below). For the *P. aeruginosa* isolates studied, ceftolozane-tazobactam regimens evaluated ranged from 62.5/31.25 to 2000/1000 mg. For *P. aeruginosa* PAO1 (ceftolozane-tazobactam MIC 0.5 mcg/mL), no resistance was selected at any dose over the 10 day model. Thus, a second *P. aeruginosa* isolate (PA 2638) with a higher ceftolozane-tazobactam MIC (4 mcg/mL) was also tested in this model. Again, an inverted U shaped function best described the relationship between resistance selection and drug exposure. The lowest (62.5/31.25) and two highest (1000/500mg, 2000/100 mg) ceftolozane-tazobactam regimens did not select resistance; resistance was most pronounced with intermediate regimens (125/62.5, 250/125, 500/250 mg) and minimal with the 750/375 regimen. Additionally, the duration of time until the selection of drug resistance increased with dose regimen intensity (CXA.044.MC).

Figure 4: Change in log10 CFU/mL of Ceftolozane-Tazobactam Drug-Resistant Subpopulations at 10 Days



Source: 5.3.5.4\CXA.044.MC

Effect of Testing Dynamics on Ceftozolane-Tazobactam Activity

Ceftolozane

In order to evaluate the effect of various testing conditions on the *in vitro* activity of ceftolozane, 10 selected clinical isolates of gram-positive and gram-negative pathogens were examined [CXA101-M-001]. All isolates were tested for susceptibility by broth microdilution according to CLSI methods [CLSI 2006; CLSI, 2007] or modified testing procedures due to changes in testing conditions. All staphylococci and gram-negative isolates were grown in MHB, and all streptococci were grown in MHB with lysed horse blood as standard (control) medium. The control medium contained no plasma, was inoculated with 5×10^5 CFU/mL, contained 20 to 25 mg/L Ca2+, and had a neutral pH of 7.2 to 7.4. All isolates were tested in duplicate in the standard medium with the following modifications:

- Human serum was added to assess the appropriate test medium with either 20% serum or 50% serum.
- The effect of inoculum size was tested by adding an initial concentration of 5×10^4 CFU/mL and 5×10^6 CFU/mL.
- The effect of the medium was assessed by testing in cation-adjusted MHB supplemented with lysed horse blood (LHB), and *Haemophilus* testing medium (HTM), instead of the standard medium for staphylococci and gram-negative organisms, and in MHB and HTM for all streptococci.
- 50 mg/L Ca2+ was added to the appropriate media to assess the effects of increased divalent cation concentration.
- The pH of the appropriate media was adjusted to a pH of 5.0, 6.0, and 8.0.

Ceftolozane yielded consistent MIC values under various conditions, with five different gram-positive and gram-negative species tested, with one exception: the addition of human serum actually enhanced the activity of ceftolozane against selected gram-negative organisms, suggesting a potential synergy between ceftolozane and bioactive components in human serum.

Reviewer's Comment
In vitro testing conditions, reported by the Applicant, including pH, temperature, media, calcium concentration and inoculum size, had little effect on the activity of ceftolozane *in vitro*. However, the addition of human serum showed an enhanced activity of ceftozolane.

Protein binding affects the *in vivo* efficacy of antimicrobials because only free drug is available for effecting antibacterial activity. This effect can be predicted to a certain extent in an *in vitro* system; a significant MIC shift has been observed for some antibacterials (such as rifampin and fusidic acid) that exhibit high binding to human serum protein. Against isolates of *S. aureus*, *S. pneumoniae* and *K. pneumoniae*, the MICs of ceftolozane and ceftazidime showed little to no change (within one doubling dilution) when evaluated in the presence of 20% and 50% serum relative to standard conditions (no serum), with the exception of one *P. aeruginosa* strain. The MICs in this specific isolate were 4-fold lower when 50% human serum was present for both ceftolozane and ceftazidime. Against *E. coli* 1732281, a 1- to 4-fold doubling dilution decrease in ceftolozane MICs was observed in the presence of 20% serum, while a 2-fold doubling dilution drop in ceftolozane MIC was consistently observed in the presence of 50% serum. This effect was not apparent for ceftolozane against the other tested *E. coli* (1732283), and little effect from serum was observed for ceftazidime with either *E. coli* tested.
These findings reflected the low human plasma protein binding of ceftolozane (approximately < 10%), indicating that the major proportion of ceftolozane should be available *in vivo* as active free drug. Ceftolozane activity may be altered by interaction with human serum against some *P. aeruginosa* and *E. coli* isolates.

Reviewer's Comment
There were *E. coli* and *P. aeruginosa* strains that had lower MICs in the presence of 20% or 50% human serum.

Table 32: Effect of Human Serum on In Vitro Activity of Ceftolozane (CXA-101)

Organism	Isolate No.	Antibiotic	MIC (µg/mL) in the Presence of Human Serum[1]					
			No Serum		20% Serum		50% Serum	
			1	2	1	2	1	2
S. aureus	1731646	CXA-101	32	32	16	16	16	16
		Ceftazidime	8	8	4	4	8	4
	1731626	CXA-101	32	32	32	32	16	16
		Ceftazidime	4	8	8	8	4	8
S. pneumoniae	1731713	CXA-101	0.12	0.12	0.12	0.12	0.12	0.12
		Ceftazidime	2	2	1	1	1	1
	1731673	CXA-101	0.25	0.25	0.25	0.25	0.25	0.25
		Ceftazidime	2	2	2	2	2	2
P. aeruginosa	1731884	CXA-101	1	1	0.5	0.5	0.5	0.5
		Ceftazidime	4	4	2	2	1	1
	1731923	CXA-101	1	1	0.25	0.25	0.12	0.12
		Ceftazidime	2	2	0.5	0.5	0.25	0.25
E. coli	1732281	CXA-101	0.5	0.5	0.25	0.06	0.12	0.12
		Ceftazidime	0.25	0.25	0.12	0.25	0.12	0.12
	1732283	CXA-101	0.12	0.25	0.12	0.12	0.12	0.12
		Ceftazidime	0.12	0.25	0.12	0.12	0.06	0.12
K. pneumoniae	1732369	CXA-101	0.25	0.5	0.25	0.25	0.25	0.25
		Ceftazidime	0.25	0.5	0.12	0.12	0.12	0.12
	1732356	CXA-101	0.25	0.25	0.25	0.25	0.25	0.25
		Ceftazidime	0.25	0.25	0.25	0.25	0.12	0.25

1. Two replicates, "1" and "2", were tested at each serum concentration.

Source: CXA101-M-001.

Effect of Inoculum Size

The influence of the inoculum size on the MICs of ceftolozane is shown in the table below [CXA101-M- 001]. Overall, inoculum concentrations of 5×10^4 CFU/mL and 5×10^6 CFU/mL did not affect ceftolozane MICs, with either no change in MICs or only a doubling dilution decrease in MIC compared to the standard (5×10^5 CFU/mL).

Table 33: Influence of Inoculum Size on the Activity of Ceftolozane (CXA-101) and Ceftazidime

Organisms	Isolate ID	Antibiotic	MIC (µg/mL) at 3 Inoculum Concentrations[1]					
			5×10^5 CFU/mL		5×10^4 CFU/mL		5×10^6 CFU/mL	
			1	2	1	2	1	2
S. aureus	1731646	CXA-101	32	32	32	16	32	32
		Ceftazidime	8	8	4	4	8	8
	1731626	CXA-101	32	32	16	32	32	32
		Ceftazidime	4	8	4	4	8	8
S. pneumoniae	1731713	CXA-101	0.12	0.12	0.12	0.12	0.12	0.25
		Ceftazidime	2	2	1	1	2	2
	1731673	CXA-101	0.25	0.25	0.25	0.25	0.25	0.25
		Ceftazidime	2	2	2	2	2	2
P. aeruginosa	1731884	CXA-101	1	1	1	1	1	1
		Ceftazidime	4	4	1	1	4	4
	1731923	CXA-101	1	1	1	1	1	1
		Ceftazidime	2	2	2	2	4	4
E. coli	1732281	CXA-101	0.5	0.5	0.25	0.25	0.5	1
		Ceftazidime	0.25	0.25	0.25	0.25	0.5	1
	1732283	CXA-101	0.12	0.25	0.25	0.12	0.25	0.25
		Ceftazidime	0.12	0.25	0.12	0.12	0.25	0.25
K. pneumoniae	1732369	CXA-101	0.25	0.5	0.25	0.25	2	2
		Ceftazidime	0.25	0.5	0.25	0.25	0.5	0.5
	1732356	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5
		Ceftazidime	0.25	0.25	0.25	0.25	0.5	0.5

1. Two replicates, "1" and "2", were tested at each serum concentration.

Source: CXA101-M-001.

Effect of Culture Medium

The influence of the medium on the MICs of ceftolozane is shown in the table below [CXA101-M-001]. For *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, ceftolozane MICs did not change or were within one doubling dilution of the MIC observed under standard conditions Mueller-Hinton Broth (MHB) when tested in MHB supplemented with lysed horse blood (LHB) or with *Haemophilus* Testing Medium (HTM) (similar to ceftazidime). For *S. pneumoniae*, MICs were decreased 4- to -16-fold for ceftazidime when tested in HTM relative to standard medium (MHB with LHB), while ceftolozane MICs were unaffected by testing in HTM relative to standard conditions. One isolate of *S. pneumoniae* (1731673) did not grow in MHB alone, and a 2-fold and 32-fold decrease in the MICs of ceftolozane and ceftazidime, respectively, were observed when tested with MHB alone relative to standard conditions against the other isolate.

Table 34: Influence of Culture Medium on the Activity of Ceftolozane (CXA-101) and Ceftazidime

Organism	Isolate ID	Antibiotic	MIC (µg/mL) in 3 Media Formulations[1]					
			MHB		MHB+LHB		HTM	
			1	2	1	2	1	2
S. aureus	1731646	CXA-101	32	32	32	32	16	16
		Ceftazidime	8	8	8	8	4	4
	1731626	CXA-101	32	32	32	32	16	16
		Ceftazidime	4	8	4	4	4	4
S. pneumoniae	1731713	CXA-101	0.06	0.06	0.12	0.12	0.12	0.12
		Ceftazidime	0.06	0.06	2	2	0.12	0.12
	1731673	CXA-101	NG	NG	0.25	0.25	0.25	0.25
		Ceftazidime	NG	NG	2	2	0.5	0.5
P. aeruginosa	1731884	CXA-101	1	1	1	1	1	1
		Ceftazidime	4	4	2	2	4	4
	1731923	CXA-101	1	1	0.5	0.5	0.5	0.5
		Ceftazidime	2	2	2	4	2	2
E. coli	1732281	CXA-101	0.5	0.5	0.25	0.25	0.5	1
		Ceftazidime	0.25	0.25	0.5	0.5	0.5	1
	1732283	CXA-101	0.12	0.25	0.25	0.12	0.25	0.25
		Ceftazidime	0.12	0.25	0.25	0.25	0.25	0.25

Table 35: Influence of Culture Medium on the Activity of Ceftolozane (CXA-101) and Ceftazidime (Continued)

Organism	Isolate ID	Antibiotic	MIC (µg/mL) in 3 Media Formulations[1]					
			MHB		MHB+LHB		HTM	
			1	2	1	2	1	2
K. pneumoniae	1732369	CXA-101	0.25	0.5	0.5	0.5	0.5	0.5
		Ceftazidime	0.25	0.5	0.25	0.25	0.5	0.25
	1732356	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5
		Ceftazidime	0.25	0.25	0.25	0.25	0.5	0.5

Abbreviations: HTM = *Haemophilus* test medium; LHB = Lysed horse blood; MHB = Mueller-Hinton broth; NG = No growth.

1. Two replicates, "1" and "2", were tested at each serum concentration.

Source: CXA101-M-001.

Effect of pH

The influence of pH on the MICs of ceftolozane was evaluated with 10 organisms [CXA101-M-001]. At a pH of 5.0, *S. pneumoniae* showed no growth. Overall, at pH 5.0, 6.0, and 8.0, the activity of ceftolozane and ceftazidime showed no change or only a one doubling dilution change in MIC relative to the MIC observed under standard conditions (pH 7.2 to 7.4). Notable exceptions included ceftazidime against *S. aureus* isolate 1731646, where a 4-fold drop in MIC was noted at pH 5.0; all *E. coli* and *K. pneumoniae* had an MIC 2- to 4-fold higher at pH 5.0 relative to standard conditions for both ceftazidime and ceftolozane.

Reviewer's Comment

Changes in pH ranging from pH 5 to 8 had no significant effect on the MIC of ceftolozane for all isolates tested.

Table 36: Influence of pH on the Activity of Ceftolozane (CXA-101) and Ceftazidime

Organism	Isolate ID	Antibiotic	MIC (µg/mL) at 4 pH Ranges[1]							
			pH 7.2-7.4		pH 5.0		pH 6.0		pH 8.0	
			1	2	1	2	1	2	1	2
S. aureus	1731646	CXA-101	32	32	16	16	32	32	16	32
		Ceftazidime	8	8	2	2	4	4	8	8
	1731626	CXA-101	32	32	32	32	32	32	16	16
		Ceftazidime	4	8	8	4	8	8	8	8
S. pneumoniae	1731713	CXA-101	0.12	0.12	NG	NG	0.25	0.25	0.12	0.25
		Ceftazidime	2	2	NG	NG	2	2	1	1
	1731673	CXA-101	0.25	0.25	NG	NG	0.25	0.25	0.25	0.25
		Ceftazidime	2	2	NG	NG	2	2	2	2
P. aeruginosa	1731884	CXA-101	1	1	2	2	1	1	1	1
		Ceftazidime	4	4	8	4	4	4	2	2
	1731923	CXA-101	1	1	1	1	1	1	1	0.5
		Ceftazidime	2	2	4	4	2	4	2	2
E. coli	1732281	CXA-101	0.5	0.5	2	2	1	1	0.25	0.25
		Ceftazidime	0.25	0.25	2	2	1	1	0.5	0.25
	1732283	CXA-101	0.12	0.25	0.5	1	0.25	0.5	0.12	0.12
		Ceftazidime	0.12	0.25	1	1	0.5	0.5	0.12	0.12
K. pneumoniae	1732369	CXA-101	0.25	0.5	1	1	0.5	1	0.5	0.5
		Ceftazidime	0.25	0.5	1	0.5	0.5	0.5	0.25	0.5
	1732356	CXA-101	0.25	0.25	1	1	0.5	0.5	0.25	0.25
		Ceftazidime	0.25	0.25	1	1	0.5	0.5	0.25	0.25

Abbreviation: NG = No growth.

1. Two replicates, "1" and "2", were tested at each serum concentration.

Source: CXA101-M-001.

Effect of Divalent Cation Concentration

The influence of calcium concentration on the MICs of ceftolozane was evaluated with 10 organisms [CXA101-M-001]. The addition of 50 mg/L of calcium to the testing medium did not affect the activity of either ceftolozane or ceftazidime, as all MICs observed with calcium-supplemented media were the same or within one doubling dilution of the MIC under standard conditions (20-25 mg/L calcium). A change in divalent cation concentration has no effect on the *in vitro* activity of ceftolozane.

Table 37: Influence of Divalent Cation Concentration on the Activity of Ceftozolane (CXA-101) and Ceftazidime

Organism	Isolate ID	Antibiotic	MIC (µg/mL) at 2 Calcium Ion Concentrations[1]			
			20-25 µg/mL Ca^{2+}		50 µg/mL Ca^{2+}	
			1	2	1	2
S. aureus	1731646	CXA-101	32	32	32	32
		Ceftazidime	8	8	8	8
	1731626	CXA-101	32	32	32	32
		Ceftazidime	4	8	4	4
S. pneumoniae	1731713	CXA-101	0.12	0.12	0.12	0.12
		Ceftazidime	2	2	1	1
	1731673	CXA-101	0.25	0.25	0.25	0.25
		Ceftazidime	2	2	2	2
P. aeruginosa	1731884	CXA-101	1	1	1	1
		Ceftazidime	4	4	8	4
	1731923	CXA-101	1	1	1	1
		Ceftazidime	2	2	2	2
E. coli	1732281	CXA-101	0.5	0.5	0.25	0.25
		Ceftazidime	0.25	0.25	0.25	0.5
	1732283	CXA-101	0.12	0.25	0.12	0.25
		Ceftazidime	0.12	0.25	0.12	0.25
K. pneumoniae	1732369	CXA-101	0.25	0.5	0.5	0.5
		Ceftazidime	0.25	0.5	0.25	0.5
	1732356	CXA-101	0.25	0.25	0.25	0.5
		Ceftazidime	0.25	0.25	0.25	0.25

1. Two replicates, "1" and "2", were tested at each calcium ion concentration.

Source: CXA101-M-001.

Tazobactam

A limited number of publications address the effect of growth conditions on the activity of tazobactam. One clinically relevant observation is the effect of pH on certain β-lactamases [Fornara, 1997]. The pH affects the activity of some BLIs by changing their ionization and so altering their accumulation by bacteria. It was observed that penicillanic acid sulphones, particularly tazobactam, gave poorer potentiation of penicillins against *E. coli* strains with TEM-1 and TEM-2 -lactamases at pH 6.5–7.0 than at pH 7.5–8.0. MICs

of piperacillin with tazobactam or sulbactam (4 mg/L) were higher at pH 6.5 than at pH 8.0 for *E. coli* transconjugants with TEM-1 or TEM-2 enzymes, but not for those with TEM-3 or TEM-10 enzymes. Investigation showed that 1) all four enzymes were less sensitive to sulphones at pH 6.5 than at pH 8.0; 2) pH effects on MICs arose also for the TEM-3 and TEM-10 producers at lower sulphone concentrations; 3) the TEM-3 and TEM-10 producers formed less enzyme than those with TEM-1 and TEM-2 and 4) pH effects on the MICs for TEM-1 producers depended on enzyme quantity. It was concluded that all four TEM enzymes have pH-dependent susceptibility to sulphones, but whether this affects MICs depends on the ratio of inhibitor to enzyme achieved in the bacteria.

Ceftolozane-Tazobactam
Against *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922, there was no notable impact on the activity profile of ceftolozane-tazobactam when testing with nonstandard inocula (low or high), altered pH (low or high), incubation in 5% CO_2, and in the presence of serum/polysorbate, or supplemental Ca^{2+} (50 mcg/mL) (MICs were equal or within one doubling dilution under these nonstandard conditions relative to standard conditions).
• For *H. influenzae* ATCC 49247, ceftolozane-tazobactam MICs were 16-fold higher when elevated inocula (8.2×10^6 CFU/mL) were used to inoculate the panel. The activity of ceftazidime was also impacted to the same degree with elevated inocula. Apart from elevated inocula, no other evaluated nonstandard test condition had an impact on the activity profile of ceftolozane-tazobactam. The organism failed to grow on the test panels containing medium at pH 8.0.
• There was a trend towards 2-fold higher ceftolozane-tazobactam MICs when testing in the presence of polysorbate at either evaluated concentration (0.2 and 0.02%).
• With the exception of *H. influenzae* ATCC 49247, where increasing the initial inoculum resulted in higher MICs, ceftazidime activity was not notably altered for any non-standard condition against any of the evaluated organisms.

Effect of Miscellaneous Factors on Ceftozolane-Tazobactam Activity
Post-Antibiotic Effect In Vitro
Ceftolozane
No study has been performed to evaluate the postantibiotic effect (PAE) of ceftolozane alone.

Tazobactam
This study is not applicable to tazobactam, because tazobactam alone has little clinically relevant in vitro activity against bacteria due to its reduced affinity to penicillin-binding proteins.

Ceftolozane-Tazobactam

Postantibiotic effect is the suppression of bacterial growth after short exposure of organisms to antimicrobials, and is defined as PAE = T – C, where T is the time required for the CFU count in the test culture to increase 1 log10 above the count observed immediately after drug removal; and C is the time required for the CFU count in an untreated control culture to increase 1 log10 above the count observed immediately after completion of the same procedure used on the test culture for drug removal.

The PAEs for ceftolozane- tazobactam against *P. aeruginosa*, *E. coli*, and *K. pneumoniae* were evaluated [CXA201-M-006]. In this study, bacteria were exposed to 4 × MIC for 2 hours before antibacterials were removed. Postantibiotic effects were negative for ceftolozane-tazobactam against all strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* tested. In this test condition, no growth suppression was observed against any strain after the drug was removed from the culture. The results were consistent with similar findings for other β-lactam antibacterials; in gram-negative bacteria, no significant growth suppression, or even a negative PAE was found after exposure to β-lactams. Similar results have been observed with clinical isolates of various Enterobacteriaceae and *P. aeruginosa*.

SUSCEPTIBILITY TEST METHODS AND INTERPRETIVE CRITERIA

Development of Interpretive Criteria and Quality Control Parameters for MIC Testing

Interpretive criteria were based on proposed human therapeutic doses, PK and PD information and MIC distributions, as well as other attributes, All *in vitro* susceptibility tests for ceftolozane -tazobactam were performed in microbroth or agar dilution assays following CLSI protocols M100-S17 and M7-A7 [CLSI, 2006; CLSI, 2007] and using standard CLSI-recommended media and reagents. The results observed in these tests were generally consistent among different laboratories and addition of tazobactam appeared to have no effect on microbiological test methods. See the section of this review titled, "Susceptibility Interpretive Criteria (Breakpoints)" for proposed interpretive criteria.

To establish the QC limits for reference strains, an eight-laboratory collaborative study was undertaken to define QC parameters for broth microdilution susceptibility tests according to the CLSI guideline [Document M23-A2, 2001; CXA101-M-009]. Recommended quality control is found in the section of this review titled, "Quality Control Parameters".

Susceptibility Interpretive Criteria (Breakpoints)

Ceftolozane

The following tentative *in vitro* susceptibility interpretive criteria for ceftolozane alone (MIC microbroth assay) were proposed.

Table 38: Tentative In Vitro Susceptibility Interpretive Criteria for Ceftolozane (CXA-101)

Bacterial Group	MIC Breakpoints(µg/mL)		
	S	I	R
(b) (4)			

Abbreviations: I = Intermediate; R = Resistant; S = Susceptible.

Tazobactam

This study is not applicable to tazobactam because it does not have clinically meaningful activity against bacterial strains on its own.

Ceftolozane-Tazobactam
As for ceftolozane, all *in vitro* susceptibility tests for ceftolozane-tazobactam were performed in microbroth or agar dilution assays following CLSI protocols M100-S17 and M7-A7 [CLSI, 2006; CLSI, 2007] and using standard CLSI-recommended media and reagents. The results observed in these tests were generally consistent among different laboratories and addition of tazobactam appears to have no effect on microbiological test methods. Susceptibility results obtained suggest that the CLSI broth microdilution and agar dilution assays as well as disk diffusion test are applicable for ceftolozane-tazobactam.

Quality Control Parameters
Broth Microdilution
To establish the QC limits for reference strains, an eight-laboratory collaborative study was undertaken to define QC parameters for broth microdilution susceptibility tests according to the CLSI guideline [Document M23-A2, 2001, CXA101-M-009]. The results are shown in the table below. Quality control limits for standard aerobic reference strains were proposed and used for ensuring the QC of susceptibility tests in the clinical microbiology laboratory.

Ceftolozane

Table 39: Quality Control Ranges for Ceftolozane (CXA-101) versus ATCC Reference Strains

Quality Control Strain	MIC (µg/mL)	MICs Within Proposed Limits (%)[1]
E. coli ATCC 25922	0.12 – 0.5	99.2
P. aeruginosa ATCC 27853	0.25 – 1	100
S. aureus ATCC 29213	16 – 64	100
S. pneumoniae ATCC 49619	0.25 – 1	99.2

[1] This column shows the percentage of MICs that are within the proposed QC limits.
Source: CXA101-M-009.

Ceftolozane-Tazobactam
Significant lot-to-lot variability in Mueller-Hinton or Brucella base media was not observed with any of the broth or agar media. The mode for each lot was essentially the same. The guidelines for selecting MIC QC ranges recommended by the Clinical and Laboratory Standards Institute (M23-A3, 2008) were followed. For *B. fragilis* broth microdilution, there were 25 observations which were outside of the range proposed for ceftolozane-tazobactam. All of these observations were from the same laboratory. These observations resulted in only 89.6% of the values being included within the proposed 4-dilution range. The RangeFinder method of Turnidge & Bordash identified this laboratory as a statistical outlier for both the mean and the mode. Excluding all of the values from analysis resulted in a 4-dilution range which included 99.5% of the remaining values. The proposed range for broth microdilution was the same as that

proposed for the agar dilution for this control strain. For *B. fragilis* agar dilution, there were 19 instances in which observed values exceeded the recommended quality control ranges for the control drug. The majority of the out-of-range observations were from laboratory #2 and laboratory #4. Excluding all of the ceftolozane-tazobactam values associated with the out-of-range control results, there was no impact upon the proposed MIC ranges for ceftolozane-tazobactam.
The Applicant proposed the following QC limits for susceptibility tests of ceftolozane-tazobactam MICs in CAMHB, HTM, and supplemented Brucella broth or agar.

	Proposed QC Limits (% Included)
Control Strain	MIC (µg/ml) (*%)
S. aureus ATCC 29213	16/4 to 64/4 (100%)
E. coli ATCC 25922	0.12/4 to 0.5/4 (99.6%)
E. coli ATCC 35218	0.06/4 to 0.25/4 (100%)
P. aeruginosa ATCC 27853	0.25/4 to 1/4 (100%)
K. pneumoniae ATCC 700603	0.5/4 to 2/4 (100%)
H. influenzae ATCC 49247	0.5/4 to 2/4 (100%)
B. fragilis ATCC 25285 Broth Microdilution	0.12/4 to 1/4 (99.5%)**
B. fragilis ATCC 25285 Agar Dilution	0.12/4 to 1/4 (100%)
B. thetaiotaomicron ATCC 29741 Broth Microdilution	16/4 to 64/4 (98.3%)
B. thetaiotaomicron ATCC 29741 Agar Dilution	16/4to 128/4 (100%)

*( ) denotes the % of MICs within the proposed control limits.

Source: This submission.

Disk Diffusion
On each of ten (10) separate days, each of the 8 participating laboratories were asked to inoculate each of the control strains onto a series of agar plates representing prepared media from three different commercial media manufacturers for each media type. Two different lots of CXA101/tazobactam 30/10 µg disks and one lot of piperacillin/tazobactam 100/10 µg control disks were then applied to the media, incubated, and then read as recommended by the Clinical and Laboratory Standards Institute (CLSI). The laboratories were requested to perform repeat testing whenever the control disk produced zone diameters outside of the ranges recommended by the CLSI. There were 3 values for *E. coli* ATCC 35218 tested on MHA, 4 values for *H. influenza* ATCC 49247 and 16 values for *H. influenzae* NCTC8468 which were outside of established quality control ranges. All 16 of the out-of-control replicates for *H. influenzae* were from the same site. Eliminating all of the data for the study drug which

were associated with these out-of-control values had no impact upon the final proposed ranges.

Significant lot-to-lot variability in Mueller-Hinton base media was not observed with the plain Mueller-Hinton agar, HTM agar or the MHA+ horse blood + NAD against any of the QC strains against either of the drugs tested. Quality control ranges calculated by the RangeFinder method were all within 1 mm of those calculated by the Gavan method with the exceptions of *P. aeruginosa* ATCC 27853 (2 mm difference) and *E. coli* ATCC 35218 tested on HTM (3 mm difference). The following CXA101/tazobactam control limits were recommended by the Applicant for the 30/10 µg disks:

Proposed Zone Diameters Ranges (mm) for CXA101/tazobactam (% of Values Included with Range)

Control Strain	30/10 µg Disk Zone Diameters (mm)
S. aureus ATCC 25923	8 - 18 (99.2%)
S. aureus ATCC 29213	7 - 17 (96.3%)
E. coli ATCC 25922	24 - 32 (99.2%)
E. coli ATCC 35218 on MHA	24 - 32 (98.5%)
K. pneumoniae ATCC 700603	17 - 25 (99.8%)
P. aeruginosa ATCC 27853	25 - 31 (99.8%)
E. coli ATCC 35218 on HTM	25 - 31 (99.2%)
H. influenzae ATCC 49247	23 – 29 (99.4%)
H. influenzae NCTC 8468	23 – 29 (99.1%)

Source: This submission.

Reviewer's Comment
Quality control ranges for *Staphylococcus aureus* for ceftolozane-tazobactam were provided in the NDA submission and also published in the 2014 version of CLSI M100 (S24), but were not proposed in the Applicant's labeling. It is recommended that the labeling include Quality Control strains *S. aureus* ATCC® 25923 and *S. aureus* ATCC® 29213. See Agency's proposed labeling for recommended Quality Control ranges.

Ceftozolane-Tazobactam Testing Procedures
Ceftolozane
In vitro susceptibility tests for ceftolozane have been performed in microbroth or agar dilution assays following CLSI protocols M100-S17 and M7-A7 [CLSI, 2006; CLSI, 2007] and using standard CLSI-recommended media and reagents. The results observed

in these tests were generally consistent among different laboratories, with no major technical issues. Susceptibility results obtained have confirmed the applicability of the CLSI microdilution and agar dilution assays as well as disk diffusion test for ceftolozane.

Selection of the Tazobactam Concentration for Use *in Vitro* Susceptibility Testing
Fixed Ratio versus Fixed Concentration of Tazobactam

Whether a BLI should be used in a fixed concentration, or titrated in a fixed ratio with the partner compound in susceptibility tests has been debated. Early work with clavulanic acid universally favored the use of a fixed amount of the compound, but more recently, there has been a tendency, especially in the US, for fixed ratios to be adopted. In the case of clavulanic acid, the fixed ratio method has been approved by the CLSI in the US for tests with amoxicillin. A fixed ratio of sulbactam in combination with ampicillin is also established for *in vitro* testing. However, for other combinations, like piperacillin/ tazobactam and ticarcillin- clavulanate, a fixed amount of inhibitor is used. The table below lists all approved susceptibility test methods for the marketed β-lactam plus BLI combinations.

Table 40: Susceptibility Test Methods for Beta-Lactamase Inhibitor and Beta-Lactam Combinations

Combination of β-lactam With BLI	Broth Assay Drug:BLI Ratio or Fixed BLI Concentration	Disk Mass Drug / BLI (µg/mL)	Clinical Dose Drug:BLI Ratio
Amoxicillin/ clavulanate	2:1 ratio	20 / 10	2 to 16:1
Ampicillin/ sulbactam	2:1 ratio	10 / 10	2:1
Ticarcillin/ clavulanate	Fixed 4 µg/mL	75 / 10	30:1
Piperacillin/ tazobactam	Fixed 4 µg/mL	100 / 10	8:1
CXA-101/ tazobactam[1]	Fixed 4 or 8 µg/mL	30 / 10	2:1

Abbreviation: BLI = β-lactamase inhibitor.
[1]. Proposed test conditions and clinical dose.

It has been argued that a fixed concentration is preferred to a fixed ratio of β-lactam to BLI [Greenwood, 1996]. The evidence and scientific rationale for supporting a fixed concentration are summarized as follows: 1) any given experiment should only alter a single variable—the concentration of the active antibacterial; 2) if a fixed ratio is used, concentrations may be reached where the antibacterial activity of the inhibitor itself becomes significant; 3) inhibitor concentrations used in testing should reflect attainable serum concentrations of inhibitors with clinical doses. In plasma and tissues, the concentration ratio between β-lactam and BLI changes constantly over time, depending on differences in their respective PK profiles. Furthermore, because tazobactam binds irreversibly to β-lactamases, a fixed concentration of the BLI may better represent the PK/PD effect of the combination. Differences in results obtained by the two methods were noted and a fixed concentration of the inhibitor was recommended, partly because this approach facilitates recognition of inhibitor-resistant β-lactamases

[Thomson, 1995]. Although the regimens of β-lactam plus BLI combination in humans are always given at a fixed ratio, the plasma or tissue concentrations of the BLI and its β-lactam partner at any given time point are not always in the same fixed ratio as that seen in an *in vitro* test system. Therefore, *in vivo* dosing using a fixed ratio of β-lactam to BLI may not be a good argument for *in vitro* susceptibility testing to be performed at a fixed ratio. No direct correlation between the two different approaches was observed. An important determinant of the PK/PD activity of a β-lactam plus BLI combination is a sustainable plasma concentration for each individual component but not an exact ratio between the two molecules. After reviewing published information, the CLSI methodology for marketed combination drugs (including Zosyn®), and data generated for ceftolozane and ceftolozane -tazobactam, using a fixed concentration of tazobactam was thought to be a more appropriate than using a fixed ratio for *in vitro* susceptibility testing of ceftolozane-tazobactam. In addition to the scientific rationale described above, the Applicant also followed this method, since it was the current FDA and CLSI approved method for susceptibility testing of piperacillin- tazobactam.

Reviewer's Comment
In plasma and tissues, the concentration ratio between β-lactam and BLI changes over time, depending on differences in PK profiles. An important determinant of the PK/PD activity of a β-lactam plus BLI combination is the sustainable plasma concentration for each component.

Selection of the Appropriate Concentration of Tazobactam for Use in *In vitro* Susceptibility Testing
After determining that a fixed concentrations of a BLI, rather than a fixed ratio to ceftolozane, would be used, the appropriate concentration of tazobactam for use in *in vitro* susceptibility testing was determined. Three factors were considered: 1) the amount of BLI needed to inhibit most enzymes. Tazobactam is a BLI that inhibits most target enzymes at a concentration of 0.5 mcg/mL (IC50); 2) the intrinsic activity of the inhibitor itself. Tazobactam has intrinsic activity against some species; 3) the concentration achievable clinically, based on human PK information. The proposed clinical dose regimen, 1000 mg ceftolozane plus 500 mg tazobactam every eight hours, would achieve sufficiently high plasma concentrations for both drugs for an appropriate proportion of the dosing interval. For the piperacillin- tazobactam combination, a 4 mcg/mL fixed tazobactam is recommended by CLSI for *in vitro* susceptibility testing. This methodology appears to distinguish susceptible from resistant organisms for this drug combination. This method was proposed regardless of dosing regimens for (piperacillin-tazobactam) Zosyn®, from the lowest dose at 2 g piperacillin/0.25 g tazobactam, every six hours (q6h) to the highest dose at 4 g piperacillin/ 0.5 g tazobactam, q6h.

Reviewer's Comment
Tazobactam has intrinsic activity against some species, which may complicate the interpretation of test results against such species if too high a concentration of inhibitor is

used. The Applicant's proposed dosing regimen was 1000 mg ceftolozane plus 500 mg tazobactam every eight hours.

In a titration study to determine the tazobactam concentration required for *in vitro* testing with ESBL- or AmpC-producing organisms, MIC checkerboards were prepared between ceftolozane and tazobactam (1-32 mcg/mL), with 57 Enterobacteriaceae with ESBLs and 20 Enterobacteriaceae with derepressed AmpC [CXA201-M-001]. In this *in vitro* test system, the activity of ceftolozane against ESBL-producing or AmpC-hyper-producing Enterobacteriaceae increased with the concentration of tazobactam used. A dose-response relationship of this type is expected for a β-lactam plus BLI combination. The impact tazobactam concentrations had on the susceptibility to ceftolozane of these enzyme-producing Enterobacteriaceae is shown in the table below. Based on the results generated from these strain collections, tazobactam markedly increased the activity of ceftolozane against the majority of ESBL- and AmpC-producing organisms (depending on the concentration of tazobactam). It was assumed that a susceptibility breakpoint of 8 + 4 mcg/mL or 8 + 8 mcg/mL (ceftolozane + tazobactam) would predict clinical efficacy, and therefore, the ceftolozane-tazobactam combination should be effective for the majority of ESBL producers and AmpC over-producers.

Table 41: Proportions of Beta-lactamase-producing Enterobacteriaceae Inhibited by Various Concentrations of Tazobactam and Ceftolozane (CXA-101) in Combination

Tazobactam (µg/mL)	ESBL Producers (N=59)		Derepressed AmpC Producers (N=20)	
	CXA-101 ≤ 4 µg/mL	CXA-101 ≤ 8 µg/mL	CXA-101 ≤4 µg/mL	CXA-101 ≤ 8 µg/mL
0	10 (16.9%)	12 (20.3%)	3 (15%)	7 (35%)
1	24 (40.7%)	27 (45.8%)	7 (35%)	10 (50%)
2	30 (50.8%)	35 (59.3%)	8 (40%)	12 (60%)
4	43 (72.9%)	45 (75.3%)	7 (35%)	14 (70%)
8	53 (89.8%)	55 (93.2%)	16 (80%)	19 (95%)
16	57 (96.6%)	59 (100%)	19 (85%)	19 (95%)
32	59 (100%)	59 (100%)	20 (100%)	20 (100%

Abbreviation: ESBL = Extended-spectrum β-lactamase.
Source: CXA201-M-001.

More extensive *in vitro* MIC data have been accumulated for ceftolozane in combination with various concentrations of tazobactam against important clinical isolates. The general observations from those studies can be summarized as follows:
The concentration of tazobactam has a direct effect on the activity to ceftolozane of strains producing ESBLs or over-producing AmpC.

- The maximum synergistic effect was observed for a fixed concentrations of tazobactam of > 16 mcg/mL; synergy was significantly less with tazobactam concentrations < 2 mcg/mL.
- The MICs for ceftolozane-tazobactam against general surveillance isolates were similar for a 2:1 ratio of ceftolozane to tazobactam and a 4 mcg/mL fixed concentration of tazobactam, but slightly favored the fixed concentration.

The PK of tazobactam has been characterized in humans [Zosyn® package insert]. The achievable plasma concentrations of tazobactam were an important factor in the determination of an appropriate concentration of tazobactam to be used in an *in vitro* susceptibility test method. The maximum plasma concentration was approximately 34 mcg/mL following a 500-mg tazobactam, 30-minute infusion, and plasma concentrations exceed 4 mcg/mL for 2 to 3 hours. This was believed to provide sufficient time at adequate concentrations for tazobactam, an irreversible inhibitor, to act; and it supports the use of 4 to 8 mcg/mL (fixed concentration) of tazobactam for *in vitro* susceptibility testing. More favorable PK/PD targets (longer T>MIC) will be achieved when tazobactam is infused over 60 minutes, which is the proposed infusion time for administration of ceftolozane-tazobactam, rather than the 30-minute infusion of Zosyn® described above.

Based on the known PK profiles of ceftolozane and tazobactam, proposed human therapeutic doses of ceftolozane-tazobactam, *in vitro* susceptibility data against ESBL or AmpC producers, and proposed susceptibility breakpoints for ceftolozane, a fixed concentration of 4 mcg/mL or even 8 mcg/mL of tazobactam can be selected for *in vitro* susceptibility testing. The 4 mcg/mL fixed concentration of tazobactam was used in further *in vitro* studies for ceftolozane-tazobactam.

See also section of this review titled, "Proposed Susceptibility Interpretive Criteria and Quality Control" for additional information.

Comparison of Agar and Broth Dilution Methods

A direct comparative study of agar dilution versus broth microdilution methodology was performed to establish whether there is any potential for variation between the two methods, as recommended by CLSI guidelines [CXA101-M-001]. Approximately 74 gram-positive and gram-negative species have been tested. No significant inter-method MIC variation between agar and microbroth assays was detected; differences in MICs between the two methods were less than or equal to a 2-fold dilution for 91% of isolates (see table below).

Reviewer's Comment

The results suggest that both CLSI methods for broth and agar dilution may be used in measuring MICs of ceftolozane.

Table 42: Differences in MIC Between Agar Dilution Assays

Organism	Number of Isolates	No. of Isolates at 2-fold Difference Between Agar and Broth MIC				
		-2	-1	0	1	2
S. aureus	10	—	—	10	—	—
S. pneumoniae	19	1	—	12	5	1
P. aeruginosa	20	2	10	8	—	—
B. cepacia	10	3	4	2	1	—
E. coli	5	1	—	3	1	—
K. pneumoniae	10	—	4	3	3	—
Total	74	7	18	38	10	1
% of Total	100%	8%	24%	51%	14%	1%

Source: CXA101-M-001.

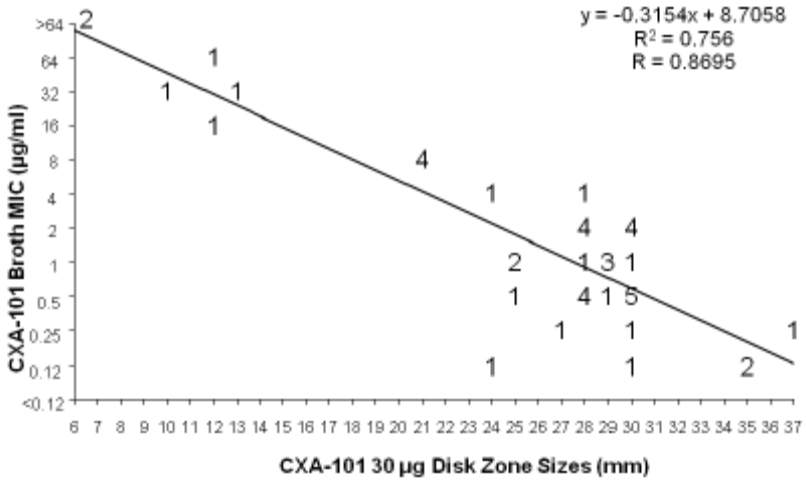
Development of Disk Diffusion Susceptibility Testing Methods

Agar disk diffusion is most commonly used in clinical microbiology laboratories for susceptibility testing of rapidly growing and certain fastidious bacterial pathogens. An exploratory study for an agar disk diffusion method for ceftolozane was performed to determine the appropriate disk mass and correlation of susceptibility data obtained by broth dilution methods and the disk inhibition zone sizes [CXA101-M-006].A total of 54 isolates selected for the study included *S. aureus* (4), streptococci (3), *H. influenza* (2), Enterobacteriaceae (6) and *P. aeruginosa* (41). All isolates were concurrently tested by broth microdilution and disk diffusion using CLSI methodology (M7-A7, M100-S18, and M2- A9). Ceftolozane was tested in duplicate using 10-mcg, 30-mcg, and 50-mcg disks, and ceftazidime (30-mcg) disks were used as a comparator. Disk zones correlated well with ceftolozane broth microdilution MICs for the evaluated isolates with an overall correlation coefficient of 0.88 for 10 mcg, 0.87 for 30 mcg, and 0.85 for 50 mcg. Accumulated data from 41 *P. aeruginosa* isolates confirmed that ceftolozane had lower MICs and larger zones of inhibition than ceftazidime. Although ceftolozane activity was slightly reduced against ceftazidime resistant isolates, ceftolozane maintained potency against these isolates by broth and disk diffusion. The activity of ceftolozane was not notably affected by other resistant phenotypes of *P. aeruginosa* and the varying disk loads did not show any notable difference in disk zone sizes.

Reviewer's Comment

Although zones of inhibition observed with the 10-mcg disk load and 50-mcg disk load were also suitable for disk testing of ceftolozane (data not shown), the 30-mcg disk load was selected as the ideal disk load for ceftolozane.

Figure 5: Ceftolozane (CXA-101) Broth Microdilution MICs (mcg/mL) versus 30 mcg Ceftolozane Disk Load Zone Sizes (mm)

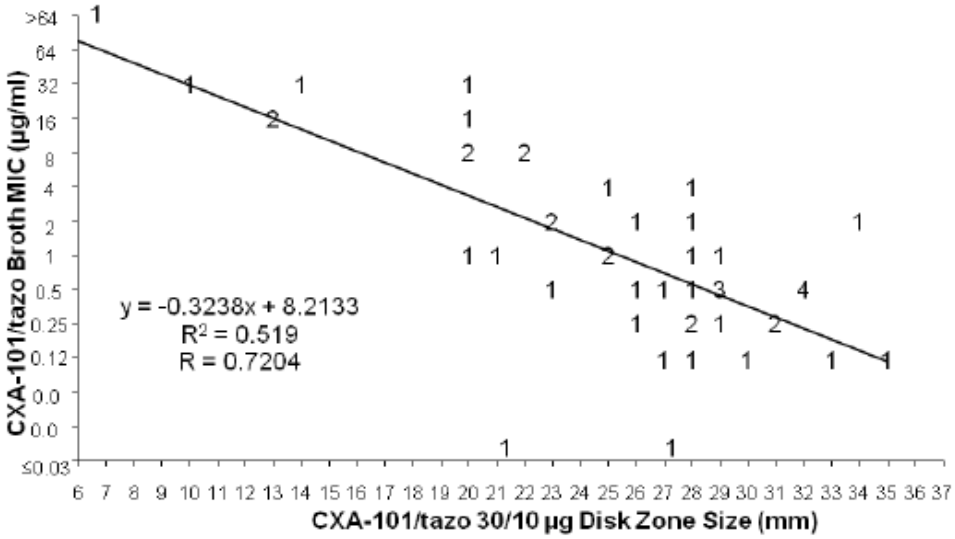


Source: CXA101-M-006.

A pilot study for an agar disk diffusion method was performed for ceftolozane-tazobactam to determine the appropriate disk mass and correlation of susceptibility data obtained by broth dilution methods and the disk inhibition zone sizes [CXA101-M-006]. After determining the appropriate disk mass for ceftolozane, the 30-mcg ceftolozane disk load was selected and tested in combination with varying concentrations of tazobactam (5, 10 and 20 mcg). A total of 54 isolates selected for the study included *S. aureus* (4), streptococci (3), *H. influenza* (2), Enterobacteriaceae (22) and *P. aeruginosa* (26). All evaluated concentrations of tazobactam in combination with ceftolozane yielded disk zones which correlated well with broth microdilution MICs of ceftolozane combined with a fixed concentration of tazobactam. The figure below shows the data for the 30/10-mcg disk (ceftolozane-tazobactam). For ESBL-producing *E. coli* and *K. pneumoniae*, disk zone size with ceftolozane alone increased when combined with tazobactam to zone sizes indicative of susceptibility (> 20 mm). These results correlated with broth microdilution results in which ceftolozane MICs decreased in combination with tazobactam against these isolates. Additional testing with more *P. aeruginosa* isolates with the indicated resistance phenotypes showed that the activity of ceftolozane -tazobactam was not different from that of ceftolozane alone by both broth and disk testing, regardless of resistance. Thus, ceftolozane at 30 mcg combined with either 10 mcg, 20 mcg, or 5 mcg tazobactam was suitable for disk testing ceftolozane-tazobactam.

Reviewer's Comment
Since 10 mcg is the approved disk mass for tazobactam in combination with piperacillin, this was one reason it was the chosen disk mass of tazobactam to test in combination with ceftolozane. For ESBL-producing *E.coli* and *K. pneumoniae*, a disk zone size with ceftolozane increased when combined with tazobactam

Figure 6: Ceftolozane (CXA-101)-Tazobactam 30/10 mcg Disk Load Zone Sizes (mm) Versus Ceftolozane-Tazobactam Broth Microdilution MICs (mcg/mL)



Antimicrobial Interactions and Fixed Combination Studies

Interactions with Other Antibacterials

Ceftolozane

To date, no study has been performed to examine the interaction of ceftolozane alone with other antibacterials.

Tazobactam

As a BLI, tazobactam has been shown to potentiate the activity of β-lactams (penicillins, cephalosporins and monobactams) against β-lactamase- gram-positive and gram-negative organisms [CX-BD-001,CXA201-M-003]. This is mainly due to its inhibition of β-lactamases so that the synergy is seen only with β-lactamase-producing organisms.

Ceftolozane-tazobactam

In an exploratory study, the *in vitro* synergistic effect of ceftolozane-tazobactam in combination with amikacin, meropenem, levofloxacin, tigecycline, and aztreonam against Enterobacteriaceae and *P. aeruginosa* was evaluated. A total of 12 isolates, including four *E. coli* (2 ESBL-positive), four *K. pneumoniae* (2 ESBL-positive), and six *P. aeruginosa* strains (exhibiting different resistance phenotypes to ceftazidime and imipenem), were studied [CXA201-M-006]. Ceftolozane and partner drugs were studied from 1/8 × to 8 × MIC and dispensed in a checkerboard fashion. As discussed before, tazobactam was used at a fixed concentration of 4 mcg/mL. Readings were performed, and the fractional inhibitory concentration (FIC) index was determined for each combination and each strain. The results are summarized in the table below. No antagonism was detected between ceftolozane-tazobactam and the antibacterials tested. An additive effect was the main interaction observed between ceftolozane-tazobactam

and studied partner drugs. Synergy was observed with the addition of aztreonam to ceftolozane -tazobactam against ESBL-producing Enterobacteriaceae, probably due to the inhibition of ESBL by the β-lactamase inhibitor. In addition, meropenem combined with ceftolozane exhibited a synergistic interaction against ESBL-positive *K. pneumoniae* strains. The combination of ceftolozane-tazobactam has some synergistic interactions with tigecycline against three *P. aeruginosa* strains, including ceftazidime-resistant isolates.

Table 43: Synergistic Activity of Ceftolozane (CXA-101) with other Gram-negative Antibacterials

Bacterial strains			CXA/TAZ MIC (µg/mL)	FIC Index / Synergistic Effect[1]				
				ATM	MER	AMK	LVX	TGC
E. coli	ESBL -	1	0.25	1.0 / AD	0.75 / AD	1.25 / I	0.75 / AD	0.75 / AD
		2	0.25	0.75 / AD	0.75 / AD	0.75 / AD	1.0 / AD	0.75 / AD
	ESBL +	3	0.25	<0.5 / S	0.62 / AD	0.62 / AD	1.0 / AD	1.0 / AD
		4	1	<0.5 / S	0.75 / AD	0.5 / S	1.0 / AD	0.52 / AD
K. pneumoniae	ESBL -	5	0.25	0.62 / AD	1.0 / AD	0.75 / AD	0.75 / AD	0.62 / AD
		6	0.25	0.75 / AD	0.75 / AD	0.62 / AD	1.12 / I	0.5 / S
	ESBL +	7	0.5	<0.5 / S	0.32 / S	0.28 / S	0.75 / AD	0.53 / AD
		8	1	<0.5 / S	0.37 / S	0.75 / AD	0.56 / AD	0.75 / AD
P. aeruginosa	CAZ-S	9	0.25	0.53 / AD	1.0 / AD	0.32 / S	0.75 / AD	1.0 / AD
		10	0.5	0.75 / AD	0.62 / AD	0.5 / S	0.75 / AD	1.0 / AD
	CAZ-R	11	2	1.0 / AD	0.75 / AD	0.56 / AD	0.5 / S	0.37 / S
		12	2	0.75 / AD	1.0 / AD	1.0 / AD	0.62 / AD	0.5 / S
	IMP-R	13	0.5	1.0 / AD	0.56 / AD	1.0 / AD	1.0 / AD	1.0 / AD
		14	0.5	0.62 / AD	0.62 / AD	0.62 / AD	0.62 / AD	0.31 / S

Abbreviations: ATM = Aztreonam; AMK = Amikacin; CAZ-R = Ceftazidime-resistant; CAZ-S = Ceftazidime-susceptible; CXA/TAZ = CXA-101/ tazobactam; ESBL = Extended-spectrum β-lactamase; FIC = Fractional inhibitory concentration; IMP-R = Imipenem-resistant; LVX = Levofloxacin; MER = Meropenem; TGC = Tigecycline.

1. Synergistic effects are symbolized by S = synergism; AD = additive effect; I = indifference.

Source: CXA201-M-006.

HUMAN AND ANIMAL STUDIES

Animal therapeutic and pharmacologic studies

Ceftolozane

Animal infection models are well recognized for their utility in predicting the clinical efficacy of antibacterial agents. The efficacy of ceftolozane has been evaluated in various animal models of clinically relevant infections. The effectiveness and potency of ceftolozane against aerobic gram-positive and gram-negative organisms has been demonstrated in multiple animal models, including sepsis, pneumonia, UTI, and wound infection [CRE060041, CXA101-M-004]. The table below lists the *in vivo* efficacy studies for ceftolozane. Most of these *in vivo* studies were conducted by Astellas Pharma Inc. The *in vivo* efficacy of ceftolozane against *P. aeruginosa*, including MDR isolates, was better than, or comparable to that of ceftazidime, imipenem, and ciprofloxacin. Ceftolozane also demonstrated activity against other species in different infection models. Furthermore, ceftolozane was efficacious in the presence of neutropenia. These findings support the further clinical development of ceftolozane for the treatment of a variety of bacterial infections.

Table 44: Infection Models Used in the Evaluation of Ceftolozane

Infection	Organism	Endpoint	Dose Regimen	Compounds Evaluated	Study Numbers
Sepsis (mouse)	*P. aeruginosa* (CAZ-S)	Lethality and ED_{50}	A total of 3 SC doses: 1, 3 and 5 h after infection	Ceftolozane Ceftazidime Imipenem/cilastatin Ciprofloxacin	M5.3.5.4\CRE060041 M4.2.2.2\CXA201-P-002 M5.3.5.4\CXA201-M-010
	P. aeruginosa (CAZ-R)				
	E. coli				
	K. pneumoniae				
	P. mirabilis				
	S. marcescens				
	E. cloacae				
	S. pneumoniae				
Pneumonia (immuno-competent mouse)	*P. aeruginosa*	Survival bacterial burden	SC q8h for 48 h	Ceftolozane Ceftazidime Piperacillin/tazobactam	M5.3.5.4\CXA.055.MC
Pneumonia (neutropenic mouse)	*P. aeruginosa* (CAZ-S)	Bacterial burden (4 days)	A total of 6 SC doses: 3 and 6 h after infection + twice daily on days 2 and 3.	Ceftolozane Ceftazidime Imipenem/cilastatin Ciprofloxacin	M5.3.5.4\CRE060041, Takeda, 2007 [34]
	P. aeruginosa (CAZ-R)				
	H. influenzae				
	K. pneumoniae				
	S. pneumoniae				
Complicated (polymicrobial) pneumonia (neutropenic mouse)	*P. aeruginosa* (CAZ-R) + *K. pneumoniae*				M5.3.5.4\CRE060041 Takeda, 2007 [34]
	P. aeruginosa (CAZ-R) + *H. influenzae*				

Infection	Organism	Endpoint	Dose Regimen	Compounds Evaluated	Study Numbers
Pneumonia (rabbit)	*P. aeruginosa*	Drug Penetration	IV infusion simulating human dosages	Ceftolozane	M4.2.2.2\CXA201-P-002
Urinary tract infection (mouse)	*P. aeruginosa* (CAZ-S)	Bacterial burden (4 days)	A total of 5 SC doses: 3 h after infection + twice daily on days 2 and 3	Ceftolozane Ceftazidime Imipenem/cilastatin Ciprofloxacin	M5.3.5.4\CRE060041 Takeda, 2007 [34]
	P. aeruginosa (CAZ-R)				
	P. aeruginosa (CAZ-R)				
Burn wound infection (mouse)	*P. aeruginosa* (CAZ-S)	Bacterial burden (4 days)	A total of 6 SC doses: 3 and 6 h after infection + twice daily on days 2 and 3.	Ceftolozane Ceftazidime Imipenem/cilastatin Ciprofloxacin	M5.3.5.4\CRE060041 Takeda, 2007 [34]
Thigh infection (neutropenic mouse)	*P. aeruginosa* (CAZ-S)	Bacterial burden (12 h)	A total of 4 SC doses every 3 h	Ceftolozane Ceftazidime	M5.3.5.4\CXA101-M-004
Thigh infection (neutropenic mouse)	*P. aeruginosa* (n=4) *E. coli* (n=2) *K. pneumoniae* (n=2)	Bacterial burden (24 h)	Various SC doses and regimens	Ceftolozane	M5.3.5.4\CXA101-M-004
Thigh infection (immuno-competent mouse)	*P. aeruginosa* (n=8) *K.-pneumoniae* (n=4) *E. coli* (n=4)	Bacterial burden (24 h)	SC regimens simulating human exposures	Ceftolozane/tazobactam Piperacillin/tazobactam	M5.3.5.4\CXA.009.MC

Infection	Organism	Endpoint	Dose Regimen	Compounds Evaluated	Study Numbers
Thigh infection (neutropenic mouse)	*P. aeruginosa* (N=15) *S. pneumoniae* (N=5)	Bacterial burden (24 h)	Various SC doses and regimens	Ceftolozane	M5.3.5.4\CXA.073.MC
Thigh infection (neutropenic mouse)	*E. coli* (n=6) *K. pneumoniae* (n=4)	Bacterial burden (24 hour)	Various IP doses and regimens	Ceftolozane/tazobactam	M5.3.5.4\CXA.083.MC
Thigh infection (neutropenic mouse)	N/A	Drug pharma-cokinetics (AUC, $t_{1/2}$)	Various SC/IP doses and regimens	Ceftolozane Tazobactam Ceftolozane/tazobactam	M5.3.5.4\CXA.042.MC M5.3.5.4\CXA.025.MC

AUC=area under the plasma concentration time curve; CAZ=ceftazidime; ED_{50}=effective dose protecting 50% of animals from a lethal inoculum; IP=intraperitoneal; IV=intravenous; N/A=not applicable; S=susceptible; R=resistant; SC=subcutaneous; $t_{1/2}$= elimination half-life.

Efficacy in a Mouse Sepsis Model

Sepsis in the mouse is the most commonly used efficacy model for evaluating and comparing the *in vivo* efficacy of antibacterials in protecting infected animals from mortality. Ceftolozane was investigated in this model along with ceftazidime, imipenem-cilastatin, and ciprofloxacin using various gram-positive or gram-negative bacterial strains grown in brain heart infusion broth or TSA, suspended in 5% porcine gastric mucin, and then injected intraperitoneally (IP) into ICR mice. Challenge doses up to approximately 1.8×10^7 CFU/mouse were evaluated. Drugs were administered subcutaneously (SC) 1, 3, and 5 hours after the bacterial challenge. Groups of eight mice were used for each drug dose level; deaths were recorded for 7 days after infection. The ED50 was calculated from the survival rate of mice on Day 7 after infection using Probit regression. Ceftolozane was active against both gram-positive and ram-negative bacterial infections (8 strains of 7 species) [CRE060041]. For all strains tested, the ED50 of ceftolozane was low (0.2 to 2.58 mg/kg). The organisms evaluated included ceftazidime-resistant *P. aeruginosa* with an ED50 for ceftolozane of 2.58 mg/kg, while ceftazidime and ciprofloxacin were ineffective against this strain. The *in vivo* activity of ceftolozane against other gram-positive or gram-negative pathogens was equal to that of ceftazidime and slightly better than that of imipenem. The ED50 values of ceftolozane for all

Enterobacteriaceae and *S. pneumoniae* were well below the anticipated equivalent human therapeutic doses.

Table 45: Summary of Ceftolozane in Mouse Sepsis induced by *P. aeruginosa*, Enterobacteriaceae and *S. pneumoniae*

Organism	Challenge (CFU/mouse)	Ceftolozane		CAZ	IMP/CS[a]	CIP
		MIC (µg/mL)	ED_{50} (mg/kg)[b]	ED_{50} (mg/kg)[b]		
P. aeruginosa 93 CAZ-S	6.9×10^5	0.25	1.07	3.16	0.85	0.91
P. aeruginosa 18064 CAZ- and CIP-R	4.5×10^6	1	2.58	>96	3.2	>48
E. coli 29	1.8×10^6	0.125	0.2	0.14	0.67	0.042
K. pneumoniae 1	2.8×10^5	0.125	0.26	0.21	1.46	0.21
P. mirabilis 4	2.5×10^6	0.5	0.33	0.092	1.11	0.14
S. marcescens 4003	8.2×10^4	0.5	1.57	0.78	4.42	0.4
E. cloacae 3020	1.8×10^7	0.25	0.26	0.34	1.36	0.085
S. pneumoniae FP1284	2.6×10^3	0.125	2.47	6.00	0.60	>48

CAZ=ceftazidime; CFU=colony forming units; CIP=ciprofloxacin; ED_{50}=effective dose protecting 50% of animals from a lethal inoculum, IMP/CS=imipenem/cilastatin; MIC=minimum inhibitory concentration; S=susceptible; SC=subcutaneous; R=resistant

[a] Concentrations represent imipenem.

[b] Determined on Day 7 after infection. Treatments were SC on Days 1, 3, and 5 after infection.

Source: M5.3.5.4\CRE060041

Tazobactam

Tazobactam alone demonstrates no in vitro antibacterial activity. Tazobactam also has no known in vivo antibacterial activity; therefore, no animal efficacy studies were performed with tazobactam alone.

Ceftolozane-Tazobactam

The mouse sepsis model was also used to evaluate the benefit of ceftolozane in combination with tazobactam against ESBL-positive *E. coli* and *K. pneumoniae*. The ED50 of ceftolozane-tazobactam (2:1 ratio) and of ceftolozane alone were determined and compared with ceftazidime and piperacillin-tazobactam (8:1 ratio), against 2 ESBL-positive *E. coli* strains (both CTX-M type) and 1 ESBL-negative strain and 3 different strains of *K. pneumoniae*, 1 of them ESBL-negative and 2 of them ESBL-positive (*bla*SHV, *bla*TEM and *bla*CTX-M) (CXA201-M-010; CXA201-M-014). The mice were challenged IP with a 100% lethal inoculum of a given strain, suspended in 5% porcine mucin. Subcutaneous treatments covered a wide range of dosages (8 mice per group) and were administered at 2, 4, and 6 hours after infection. The mice were scored for survival for 5 days, after which the ED50 values were determined. The table below presents the mouse sepsis model efficacy data. Although all of the test agents showed efficacy against the ESBL-negative strains, the ED50 of piperacillin-tazobactam was higher than that of the other agents tested. Against the ESBL-positive *E. coli* strains, ceftolozane-tazobactam was approximately 5 to 8 times more active than ceftolozane alone. Ceftolozane had

activity similar to that of ceftolozane-tazobactam against ceftazidime-susceptible *E. coli* strain C14.

Although the 2 ESBL-positive strains were susceptible to piperacillin-tazobactam in vitro, this combination drug was not efficacious against these 2 strains in mice (ED50 > 300 mg/kg/dose). All drugs had similar ED50 values against the ESBL-negative *K. pneumoniae* strain except for piperacillin-tazobactam. The ESBL-positive strain, C11, was resistant to ceftolozane, which was consequently not efficacious in this model. Ceftazidime, with an MIC of 1 mcg/mL, had an ED50 of 20.8 mg/kg while the ED50 of ceftolozane-tazobactam was 47.5 mg/kg against this strain. Against the C2 ESBL-positive strain of *K. pneumoniae*, only ceftolozane-tazobactam had in vivo activity, with an ED50 value of 44.9 mg/kg, similar to its activity against the other 2 strains of *K. pneumoniae*.

Reviewer's Comment
The *E. coli* ESBL-positive strain, C11, was resistant to ceftolozane, and was not efficacious in the mouse model described above.

Table 46: Efficacy of Ceftolozane-Tazobactam and Comparators in a Mouse Sepsis Model with ESBL-negative and ESBL-Positive Clinical Isolates of *E. coli* and *K. pneumoniae*

	E. coli C14 ESBL-negative		*E. coli* C11 ESBL-positive		*E. coli* C12 ESBL-positive	
Antibiotic	MIC (µg/mL)	ED_{50} (mg/kg)[a]	MIC (µg/mL)	ED_{50} (mg/kg)	MIC (µg/mL)	ED_{50} (mg/kg)
Ceftolozane	0.25	0.9	2	192.3	64	123.3
Ceftolozane/tazobactam	0.25	0.3	0.25	25.9	1	25.5
Ceftazidime	0.125	0.4	0.5-1	25.6	32-64	263.3
Piperacillin/tazobactam	1.5	14.7	1	>300	2	>300
	K. pneumoniae C4 ESBL-negative		*K. pneumoniae* C1 ESBL-positive		*K. pneumoniae* C2 ESBL-positive	
Ceftolozane	0.25	32.8	16	> 300	128	183.3
Ceftolozane/tazobactam	0.25	30.0	0.5	47.5	1	44.9
Ceftazidime	0.25	17.7	1	20.8	32	>300
Piperacillin/tazobactam	2	195.7	2	>300	16	>300

ESBL=extended spectrum β-lactamase; ED_{50}=effective dose protective of 50% of animals from a lethal inoculum; MIC=minimum inhibitory concentration
[a] Determined 5 days after infection. Treatment administered subcutaneously 2, 4, and 6 hours after infection.
Source: M5.3.5.4\CXA201-M-010; M5.3.5.4\CXA201-M-014

Reviewer's Comment
The mouse sepsis studies demonstrated that ceftolozane-tazobactam is effective in vivo against certain ESBL-positive *E. coli* and *K. pneumoniae*, and that ceftolozane alone is effective in vivo against wild-type Enterobacteriaceae, *S. pneumoniae,* and MDR *P. aeruginosa*.

Efficacy in a Mouse Pneumonia Model
The therapeutic effect of ceftolozane in experimentally induced pneumonia in mice caused by different pathogens was compared with those of several other antibacterials (CRE060041). All studies were performed in neutropenic mice. Mice (ICR) were pretreated to induce neutropenia by IP administration of cyclophosphamide (200 mg/kg four days before infection, with the exception of the *H. influenzae* group, in which animals were dosed on four and one days before infection). Mice, anesthetized with 50 mg/kg pentobarbital IV, were challenged intranasally with one or two isolates of *P. aeruginosa*, or *H. influenzae*, or *K. pneumoniae*, or *S. pneumoniae* suspended in saline at approximately the minimum lethal dose for the uncomplicated pulmonary infection model. The minimal lethal doses were predetermined independently for each organism in this testing system. For the evaluation of ceftolozane in a complicated pulmonary infection model, neutropenic mice treated with cyclophosphamide four days before infection at a dose of 200 mg/kg were anesthetized with 50 mg/kg pentobarbital IV and were challenged intranasally with a combination of *P. aeruginosa* with either *K. pneumoniae* or *H. influenzae*. Test agents were administered SC three and six hours post-challenge and twice a day on Days 2 and 3, with six mice per treatment group. Mice were sacrificed on Day 4. Lungs were removed aseptically and homogenized in saline. Residual viable bacterial cells in the homogenate were counted after culture on appropriate agar. The logarithmic mean numbers of residual viable cells in the lung were recorded.

Efficacy in Uncomplicated Murine Pneumonia
The in vivo efficacy of ceftolozane was investigated in immunocompetent and neutropenic mouse pneumonia models. Ceftolozane was shown to be highly efficacious against *P. aeruginosa* lung infection in immunocompetent mice. Using dosing regimens designed to simulate the human plasma AUC levels, ceftolozane reduced the bacterial burden by 3.44 and 2.43 log10 CFU/g of tissue in lung and spleen, respectively. Ceftolozane was significantly more efficacious than the comparators ($p < 0.05$) in reducing the lung bacterial burden (CXA.055.MC).

Table 47: Bacterial Counts in Lung and Spleen After 48 Hours of Treatment with Ceftolozane, Ceftazidime, and Piperacillin-Tazobactam

Regimen	MIC Value (µg/mL)	Bacterial Counts Mean ± SD log_{10} CFU/g of Organ	
		Lung	Spleen
Controls	NA	7.05 ± 0.86	5.06 ± 0.63
Ceftolozane	1	3.61 ± 0.35[a,b]	2.63 ± 0.46[a]
Ceftazidime	4	4.74 ± 1.01[a]	2.74 ± 0.49[a]
Piperacillin/tazobactam	64	5.04 ± 0.90[a]	2.80 ± 0.84[a]

CFU=colony-forming units; NA=not applicable; SD=standard deviation
[a] $P<0.001$ versus controls
[b] $P<0.05$ versus ceftazidime and piperacillin/tazobactam groups
Source: M5.3.5.4\CXA.055.MC

Additionally, neutropenic mouse pneumonia models were also used to evaluate the activity of ceftolozane against *P. aeruginosa* and *K. pneumoniae* strains (CRE060041). The table below shows that ceftolozane achieved more than a 3-log10 reduction in CFU in all 3 strains in the lungs at all doses tested by 72 hours post-infection as compared with untreated controls. Ceftolozane and the comparators were efficacious in the MDR *P. aeruginosa* lung infection model. Ceftolozane, with a 4-log10 reduction, had efficacy similar to that of imipenem, but was significantly more efficacious ($p \leq 0.01$) than ceftazidime or ciprofloxacin. Additionally, efficacy against *K. pneumoniae* was tested in the same infection model, using treatment with 0.5 and 2 mg/kg. At the higher dosage, the bacterial load after treatment with ceftolozane was significantly lower than that observed in the controls (more than a 5-log10 CFU reduction in the lungs). At this dose, efficacy was generally comparable among the drugs tested, with the exception of imipenem-cilastatin. At the lower dose, only ceftolozane and ciprofloxacin had a significant effect on decreasing the bacterial load.

Table 48: Efficacy of Ceftolozane and Comparators Against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in a Neutropenic Mouse Pulmonary Infection Model

Organism (inoculum)	Antibiotic	Bacterial Burden[a] (Median $\log_{10}$ CFU/lungs ± SD)		MIC (µg/mL)
		2 mg/kg	10 mg/kg	
P. aeruginosa 93 ceftazidime-S (2×10^3 CFU/mouse)	Untreated controls	6.93 ± 0.22		NA
	Ceftolozane	3.40 ± 0.28[bc]	2.54 ± 0.05[bc]	0.25
	Ceftazidime	4.93 ± 0.09[b]	3.59 ± 0.10[b]	1
	Imipenem/cilastatin	3.09 ± 0.18[b]	2.41± 0.15[b]	1
	Ciprofloxacin	3.04 ± 0.14[b]	2.38± 0.21[b]	0.125
		10 mg/kg	**50 mg/kg**	
P. aeruginosa 18064 MDR (2.2×10^4 CFU/mouse)	Untreated controls	7.27 ± 0.07		NA
	Ceftolozane	3.06 ± 0.20[bcd]	1.82 ± 0.07[bcd]	1
	Ceftazidime	6.12 ± 0.24[b]	4.63 ± 0.25[b]	32
	Imipenem/cilastatin	3.74 ± 0.19[b]	2.21 ± 0.04[b]	16
	Ciprofloxacin	6.84 ± 0.08	5.65 ± 0.26[b]	64
		0.5 mg/kg	**2 mg/kg**	
K. pneumoniae 19014 (1.6×10^6 CFU/mouse)	Untreated controls	7.84 ± 0.12		NA
	Ceftolozane	2.72 ± 0.08[b]	1.72 ± 0.19[e]	0.125
	Ceftazidime	4.51 ± 0.49	1.81± 0.17[e]	0.125
	Imipenem/cilastatin	5.34 ± 0.52	2.73 ± 0.18	0.25
	Ciprofloxacin	2.22 ± 0.29[b]	< 1.18[b]	≤ 0.03

CFU=colony-forming units; MDR=multidrug-resistant; MIC=minimum inhibitory concentration; NA=not applicable; s=susceptible; SD=standard deviation.

[a] Determined on the day after the last treatment. Treatment by subcutaneous injection twice daily, for 3 days, starting 3 hours after infection

[b] $p < 0.01$ versus control.

[c] $p < 0.01$ versus ceftazidime

[d] $p < 0.01$ versus ciprofloxacin

[e] $p < 0.05$ versus control.

Source: M5.3.5.4\CRE060041; Takeda, 2007 [35]

Ceftolozane was also tested in a model of complicated pneumonia in which neutropenic mice were infected simultaneously with 2 different organisms. In a co-infection with both susceptible *K. pneumoniae* and MDR *P. aeruginosa*, ceftolozane treatment (2, 10, and 50 mg/kg) produced the greatest reduction in bacterial load of both strains, as compared with ceftazidime, imipenem/cilastatin, and ciprofloxacin. Although ciprofloxacin was highly efficacious against the susceptible *K. pneumoniae* strain, it had little effect on the fluoroquinolone-resistant *P. aeruginosa*. Imipenem/cilastatin and ceftazidime were generally less active than ceftolozane against both pathogens, especially at the lowest dose (CRE060041; [Takeda, 2007]).

Ceftolozane was also tested in a model of complicated pneumonia in which neutropenic mice were infected simultaneously with 2 different organisms. In a co-infection with both susceptible *K. pneumoniae* and MDR *P. aeruginosa*, ceftolozane treatment (2, 10, and 50

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Activity against *Haemophilus influenzae* infection
In pulmonary infection caused by *H. influenzae*, the bacterial load after treatment with ceftolozane was significantly lower than that for the untreated control, and the efficacy of ceftolozane was generally comparable to that of ceftazidime and imipenem/ cilastatin. Consistent with *in vitro* data, ciprofloxacin was the most potent compound in this model.

Table 49: Efficacy in a Mouse Pulmonary Infection Caused by *H. influenzae*

Organism(Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/lung)[1]		MIC(µg/mL)
		2 mg/kg	10 mg/kg	
H. influenzae 18033 (1.8×10^7 CFU/mouse)	CXA-101	$5.43 \pm 0.30^{(*)}$	$4.83 \pm 0.11^{*}$	0.5
	CAZ	5.79 ± 0.06	$4.81 \pm 0.08^{*}$	0.25
	IMP/CS	$5.53 \pm 0.25^{(*)}$	$3.65 \pm 0.19^{*,**}$	8
	CIP	$2.18 \pm 0.11^{*,**}$	$1.44 \pm 0.12^{*,**}$	0.0156
	Untreated control	6.71 ± 0.17		NA

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.
1. Results are expressed as the mean ±SD for each dosage group.
* Significantly different from control ($p<0.01$).
(*) Significantly different from control ($p < 0.05$).
** Significantly different from CXA-101 ($p<0.01$).
Source: CRE060041.

Activity against *Streptococcus pneumoniae* Infection
In pulmonary infection caused by *S. pneumoniae*, the bacterial load after treatment with ceftolozane was significantly reduced in comparison to that of the control (more than a 4-log reduction). The efficacy of ceftolozane was generally comparable to that of imipenem/ cilastatin (see table below). However, neither ciprofloxacin nor ceftazidime achieved a statistically significant reduction in CFU for either dosing group compared to the untreated group.

Table 50: Therapeutic Efficacy in Murine Pulmonary Infection Caused by Penicillin-Susceptible *S. pneumoniae*

Organism (Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/lung)[1]		MIC (µg/mL)
		10 mg/kg	50 mg/kg	
S. pneumoniae 18241 (7.3×10^5 CFU/mouse)	CXA-101	$3.47 \pm 0.10^{(*)}$	$2.90 \pm 0.15^{(*)}$	2
	CAZ	4.07 ± 0.49	3.54 ± 0.37	2
	IMP/CS	$2.26 \pm 0.12^{*}$	$< 1.33^{*}$	0.0078
	CIP	6.39 ± 0.54	4.87 ± 0.49	1
	Untreated control	7.88 ± 0.08		NA

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.

1. Results are expressed as the mean ± SD for each dosage group.

* Significantly different from control ($p < 0.01$).

(*) Significantly different from control ($p < 0.05$).

Source: CRE060041.

Efficacy in Complicated Murine Pneumonia

In order to assess the potency of ceftolozane against complicated infections caused by multiple gram-negative pathogens, a mouse pneumonia model with two inoculated pathogens was used.

Complicated Pulmonary Infection Caused by *P. aeruginosa* and *K. pneumoniae*

In complicated pulmonary infection caused by MDR *P. aeruginosa* and susceptible *K. pneumoniae*, ceftolozane was the most potent compound evaluated (see table below). ceftolozane significantly reduced the bacterial load of both pathogens, even at the lowest dose. Although ciprofloxacin was highly active against the susceptible *K. pneumonia*, it had little effect on fluoroquinolone-resistant *P. aeruginosa*. Both imipenem and ceftazidime were generally less active than ceftolozane against both pathogens, especially at the low dose level.

Table 51: Therapeutic Efficacy in Murine Complicated Pulmonary Infection Caused by *P. aeruginosa* and *K. pneumoniae*

Organism (Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/lung)[1]			MIC (µg/mL)
		2 mg/kg	10 mg/kg	50 mg/kg	
P. aeruginosa 18064 (2.2×10^4 CFU/mouse)	CXA-101	4.55 ± 0.42*	3.73 ± 0.31*	2.77 ± 0.31*	1
	CAZ	7.23 ± 0.19(**)	5.87 ± 0.44	5.02 ± 0.25	32
	IMP/CS	6.74 ± 0.32	4.66 ± 0.29(*)	3.74 ± 0.38*	16
	CIP	7.12 ± 0.09(**)	6.18 ± 0.59	6.40 ± 0.27**	64
	Untreated control	7.91 ± 0.29			NA
K. pneumoniae 19014 (1.6×10^5 CFU/mouse)	CXA-101	2.16 ± 0.34*	<1.33*	< 1.00*	0.125
	CAZ	4.50 ± 0.51	2.63 ± 0.29	< 1.00(*)	0.125
	IMP/CS	5.17 ± 0.20	2.32 ± 0.23	< 1.00(*)	0.25
	CIP	1.48 ± 0.12*	<1.00*	< 1.00*	≤ 0.0313
	Untreated control	7.70 ± 0.29			NA

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.

1. Results are expressed as the mean ± SD for each dosage group.

* Significantly different from control ($p < 0.01$).

(*) Significantly different from control ($p < 0.05$).

** Significantly different from CXA-101 ($p < 0.01$).

(**) Significantly different from CXA-101 ($p < 0.05$).

Source: CRE060041.

Complicated Pulmonary Infection caused by *P. aeruginosa* and *H. influenzae*

In complicated pulmonary infection caused by MDR *P. aeruginosa* and *H. influenzae*, the efficacy of ceftolozane was superior to that of the other three agents tested against both pathogens (see table below). The other three comparators had weak activity against MDR *P. aeruginosa*, especially when treated with the lower dose regimen.

Table 52: Therapeutic Efficacy in Murine Complicated Pulmonary Infection Model Caused by *P. aeruginosa* and *H. influenzae*

Organism (Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/lung)[1]			MIC (µg/mL)
		2 mg/kg	10 mg/kg	50 mg/kg	
P. aeruginosa 18064 (1.1×10^4 CFU/mouse)	CXA-101	6.04 ± 0.40*	4.37 ± 0.35*	1.23 ± 0.11*	1
	CAZ	7.01 ± 0.16	6.18 ± 0.43*,**	5.47 ± 0.42*,**	32
	IMP/CS	7.22 ± 0.27	4.76 ± 0.38*	3.24 ± 0.26*,**	16
	CIP	7.12 ± 0.32	6.58 ± 0.14(*),**	6.29 ± 0.42*,**	64
	Untreated control	8.04 ± 0.18			NA
H. influenzae 18033 (5.9×10^6 CFU/mouse)	CXA-101	4.69 ± 0.40*	3.43 ± 0.23*	< 2.00*	0.5
	CAZ	6.13 ± 0.25**	4.33 ± 0.41	2.54 ± 0.25	0.25
	IMP/CS	5.89 ± 0.16	3.68 ± 0.18	< 2.00*	8
	CIP	3.02 ± 0.19*,**	< 2.18*	< 2.00*	0.0156
	Untreated control	7.24 ± 0.22			NA

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.

1. Results are expressed as the mean ± SD for each dosage group.

* Significantly different from control ($p < 0.01$).

(*) Significantly different from control ($p < 0.05$).

** Significantly different from CXA-101 ($p < 0.01$).

Source: CRE060041.

Reviewer's Comment

Ceftolozane demonstrated activity against both gram-positive and gram-negative respiratory pathogens in the mouse pneumonia model under neutropenic conditions. Ceftolozane was active against drug-resistant *P. aeruginosa* in this model. The bactericidal activity of ceftolozane in this model also suggested that ceftolozane had good tissue penetration in infected lungs.

Efficacy in a Mouse Burn Wound Infection Model

The efficacy of ceftolozane in wound infection caused by ceftazidime-susceptible or MDR *P. aeruginosa* was evaluated in a mouse model [CRE060041]. In brief, mice anesthetized with 50 mg/kg IV pentobarbital were submitted to an ethanol flame burn injury (0.1 mL of ethanol flame was applied twice on the shaved backs of the mice), and then challenged with *P. aeruginosa* suspended in saline into the SC burn wound. Test agents were administered SC on the other side of the burn wound at three and six hours post-challenge, then twice a day on Days 2 and 3 with six mice per treatment group. Mice were sacrificed on Day 4. The burn wound infected region was removed aseptically and homogenized with saline. The number of residual viable bacterial cells in the homogenate was determined by culture on agar plate. The logarithmic mean numbers of residual viable bacterial cells in the burn wound region were recorded. The therapeutic effects of ceftolozane in this burn-wound infection model are shown in the tables below. In burn-wound infection caused by ceftazidime-susceptible *P. aeruginosa*, the residual bacterial

load after treatment with ceftolozane was significantly lower than that with the untreated control. ceftolozane was slightly less active than ciprofloxacin, but more active than ceftazidime and imipenem-cilastatin.

Table 53: Therapeutic Effect in Murine Burn-Wound Infection Caused by a Susceptible Strain of *P. aeruginosa*

Organism (Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/wound)[1]		MIC µg/mL)
		10 mg/kg	50 mg/kg	
P. aeruginosa 93 (4.0×10^4 CFU/mouse	CXA-101	4.27 ± 0.26*	2.21 ± 0.33*	0.25
	CAZ	7.59 ± 0.16	5.58 ± 0.42	1
	IMP/CS	6.80 ± 0.42	5.31 ± 0.23	1
	CIP	2.15 ± 0.07*	<1.00*	0.125
	Untreated control	8.12 ± 0.05		NA

Abbreviations: CAZ = Ceftazidime; CPFX = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.

1. Results are expressed as the mean ± SD for each dosage group.

* Significantly different from control ($p<0.01$).

Source: CRE060041.

In a burn wound infection caused by ceftazidime-resistant *P. aeruginosa*, the efficacy of ceftolozane was superior to that of ceftazidime, imipenem-cilastatin, and ciprofloxacin (see table below). More than a 4-log reduction of bacterial count was observed after treatment with 50 mg/kg of ceftolozane. Ceftolozane was effective in this model, including against ceftazidime-resistant *P. aeruginosa*.

Table 54: Therapeutic Effect in Murine Burn Would Infection Caused by a MDR Strain of *P. aeruginosa*

Organism (Inoculum Size)	Antibiotic	Bacterial Count (Log_{10} CFU/wound)[1]		MIC (µg/mL)
		10 mg/kg	50 mg/kg	
P. aeruginosa 18064 (3.2×10^5 cfu/mouse)	CXA-101	$5.35 \pm 0.35^{*}$	$3.46 \pm 0.14^{*}$	1
	CAZ	$7.58 \pm 0.13^{**}$	$6.49 \pm 0.15^{*,**}$	32
	IMP/CS	$6.90 \pm 0.13^{(*),**}$	$6.16 \pm 0.32^{*,**}$	16
	CIP	$7.96 \pm 0.08^{**}$	$7.44 \pm 0.13^{**}$	64
	Untreated control	7.80 ± 0.18		NA

Abbreviations: CAZ = Ceftazidime; CIP = Ciprofloxacin; IMP/CS = Imipenem/cilastatin; NA = Not applicable.
1. Results are expressed as the mean ± SD for each dosage group.
* Significantly different from control ($p < 0.01$).
(*) Significantly different from control ($p < 0.05$).
** Significantly different from CXA-101 ($p < 0.01$).
Source: CRE060041.

A mouse burn wound infection model was used to evaluate ceftolozane and ceftazidime against either a ceftazidime-susceptible or MDR *P. aeruginosa* strain. In mice infected with a ceftazidime-susceptible strain of *P. aeruginosa* the bacterial burden at the burn site after treatment with ceftolozane was significantly ($P < 0.01$) lower than that of the vehicle-treated control. Ceftolozane was more active than ceftazidime and imipenem/cilastatin against this strain. Against the MDR strain, the efficacy of ceftolozane, with a greater than 4 log10 reduction in CFU relative to the vehicle-treated control, was superior to that of ceftazidime, imipenem/cilastatin, and ciprofloxacin (data not shown) [CRE060041].

Efficacy in the Mouse Urinary Tract Infection Model

The efficacy of ceftolozane in UTI caused by ceftazidime-susceptible or MDR *P. aeruginosa* was evaluated in a mouse model [CRE060041]. Female mice were denied water a day before bacterial challenge. Following anesthesia with 50 mg/kg IV pentobarbital, mice were challenged via the urethral opening with one of several bacterial pathogens suspended in saline. Test agents were administered SC at five hours post-challenge and twice a day on Days 2 and 3, with six mice per treatment group. Mice were sacrificed on Day 4. Both kidneys were removed aseptically and homogenized in saline. The number of viable bacterial cells in the homogenate was determined by culture on appropriate agar. The logarithmic mean numbers of residual viable bacterial cells in both kidneys were recorded. The therapeutic effects of ceftolozane in the urinary tract infection model are shown in the tables below. In urinary tract infection caused by a susceptible strain of *P. aeruginosa*, the efficacy of ceftolozane was comparable to that of ciprofloxacin, and superior to that of ceftazidime and imipenem/ cilastatin. In UTI caused by a ceftazidime-resistant strain of *P. aeruginosa*, the efficacy of ceftolozane was

superior to that of all three comparators. Ceftolozane demonstrated bactericidal activity in this UTI model against both ceftazidime-susceptible and ceftazidime-resistant strains (> 3-log reduction of bacterial counts).

Table 55: Efficacy of Ceftolozane and Comparators in a Murine Urinary Tract Infection Caused by *P. aeruginosa*

Organism (inoculum)	Antibiotic	Bacterial Burden[a] (Mean log_{10} CFU/kidneys ± SD)		MIC (µg/mL)
		0.5 mg/kg	2 mg/kg	
P. aeruginosa 93 ceftazidime-S (2.1×10^4 CFU/mouse)	Control	8.01 ± 0.05		NA
	Ceftolozane	4.52 ± 0.41[bc]	3.29 ± 0.14[bde]	0.25
	Ceftazidime	5.57 ± 0.16[b]	4.57 ± 0.41[b]	1
	Imipenem/cilastatin	6.08 ± 0.41[b]	4.73 ± 0.24[b]	1
	Ciprofloxacin	5.02± 0.24[b]	2.95 ± 0.35[b]	0.12
		2 mg/kg	10 mg/kg	
P. aeruginosa 18064 MDR (2.2×10^4 CFU/mouse)	Control	7.54 ± 0.26		NA
	Ceftolozane	3.97 ± 0.63[bf]	2.33 ± 0.33[bf]	1
	Ceftazidime	6.61± 0.37	5.66 ± 0.34[g]	32
	Imipenem/cilastatin	6.69 ± 0.22	4.58 ± 0.39[b]	16
	Ciprofloxacin	7.33± 0.34	6.35 ± 0.27	64

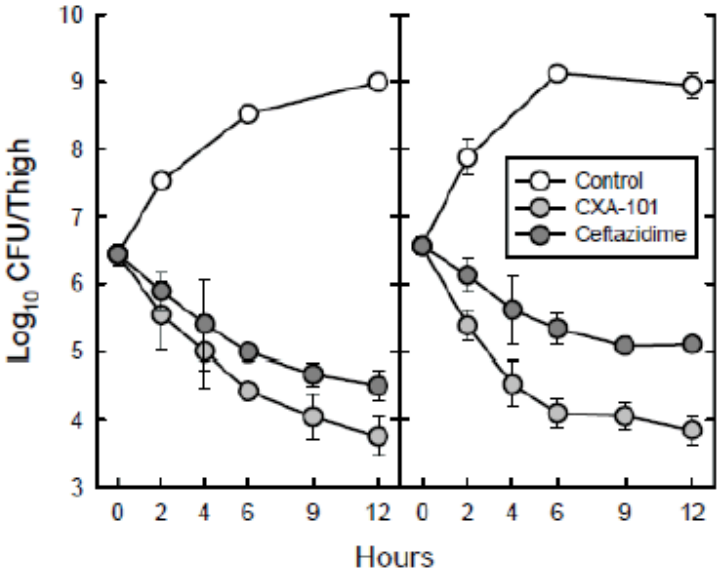
CFU=colony forming units; MDR=multidrug-resistant; MIC=minimum inhibitory concentration; NA=not applicable; s=susceptible; SD=standard deviation.
[a] Determined on Day 3 after infection. Test agents were administered SC at 5 hours post challenge and then twice daily on Days 1 and 2 after infection.
[b] $p < 0.01$ versus control.
[c] $p < 0.05$ versus imipenem.
[d] $p < 0.05$ versus ceftazidime.
[e] $p < 0.01$ versus imipenem.
[f] $p < 0.01$ versus ceftazidime, imipenem, and ciprofloxacin.
[g] $p < 0.05$ versus control.
Source: M5.3.5.4\CRE060041, Takeda, 2007 [35]

Efficacy in a Neutropenic Thigh Infection Model
Ceftolozane

The neutropenic mouse thigh infection study was used to evaluate the PK/PD driver of ceftolozane (CXA101-M-004; CXA.073.MC). As expected for a β-lactam agent, ceftolozane demonstrated bactericidal activity in vivo against susceptible strains of *E. coli*, *K pneumoniae* and *P. aeruginosa*. With a 6-hourly regimen, the mean maximal killing (as compared with the untreated control at the time treatment was initiated) for 2 strains each of *E. coli* and *K. pneumoniae* was 2.60 ± 0.24 and 4 strains of *P. aeruginosa* was 2.44 ± 0.46 log10 CFU/mL. Additionally, the total dose required for efficacy was lowest when dosing was more frequent, consistent with the short t½ of ceftolozane in animals and the %T>MIC PK/PD driver (data not shown). Additionally, when the bacterial load in the thighs was followed over time, it was demonstrated that treatment of 2 strains of *P. aeruginosa* with 200 mg/kg of ceftolozane every 3 hours produced a bactericidal effect in the neutropenic mouse thigh model. The extent and rate of killing of these organisms was greater with ceftolozane than with the same dosage of ceftazidime,

although their MIC values were similar (ceftolozane, 0.5 mcg/mL for both strains; ceftazidime, 2 mcg/mL for both strains) (see figure below).

Figure 7: Time Course of Bactericidal Activity of Ceftolozane and Ceftazidime Against *Pseudomonas aeruginosa*



ATCC=American Type Culture Collection; CFU=colony-forming units; CXA-101=ceftolozane; MIC=minimum inhibitory concentration
Note: Left panel, strain ATCC 27853; right panel, strain PO2.
Ceftolozane MIC values were 0.5 µg/mL for both strains; ceftazidime MIC values were 2 µg/mL for both strains.
Treatment was with 200 mg/kg every 3 hours.
Source: M5.3.5.4\CXA101-M-004.

Ceftolozane-Tazobactam

Using the same neutropenic mouse thigh infection model, the efficacy of ceftolozane-tazobactam against ESBL-producing Enterobacteriaceae was compared with that of ceftolozane alone and with a combination of ceftolozane with clavulanic acid, another BLI (CXA101-M-004). Different ratios of ceftolozane to the 2 BLIs were tested. The challenge organisms included 1 strain each of *E. coli* and *Enterobacter cloacae*, and 3 strains of *K. pneumoniae*, 1 of which carried both the CTX-M-3 ESBL and overproduced an AmpC enzyme. The effect of clavulanic acid and tazobactam was evaluated by MIC testing against the Enterobacteriaceae tested in the neutropenic mouse thigh model (see table below).

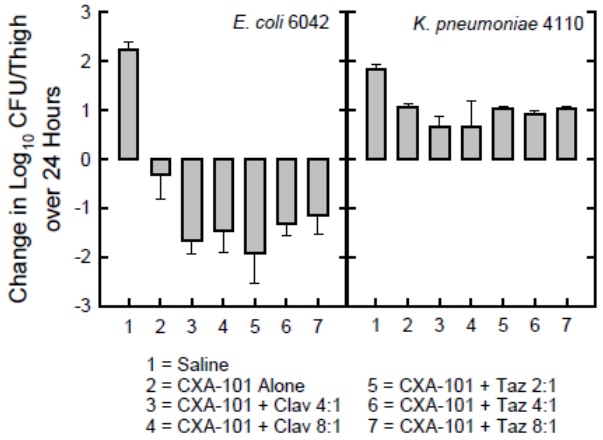
Table 56: MIC Values of Ceftolozane Alone and in Combination with a BLI Against the ESBL-Producing Strains Utilized in a Thigh Infection Model

Organism	MIC (µg/mL of ceftolozane)						Enzyme Genotype
	Ceftolozane	Ceftolozane/clavulanic Acid Ratio		Ceftolozane/tazobactam Ratio			
		4:1	8:1	2:1	4:1	8:1	
E. coli 6042	16 - 32	1	2	2	4	8	TEM-10
K. pneumoniae 4110	> 64	1 - 2	2 - 4	8 - 16	16	16 - 32	TEM-10
E. cloacae 81-1291A	8 - 16	2	4	2	1 - 2	4	SHV-5
K. pneumoniae 81-1260A	32 - 64	4	8	2	4	8	CTX-M-3 AmpC
K. pneumoniae 4105	> 64	2	4	4 - 8	8 - 16	32	TEM-29

BLI=β-lactamase inhibitor; ESBL=extended spectrum β-lactamase; MIC=minimum inhibitory concentration.
Source: M5.3.5.4\CXA101-M-004

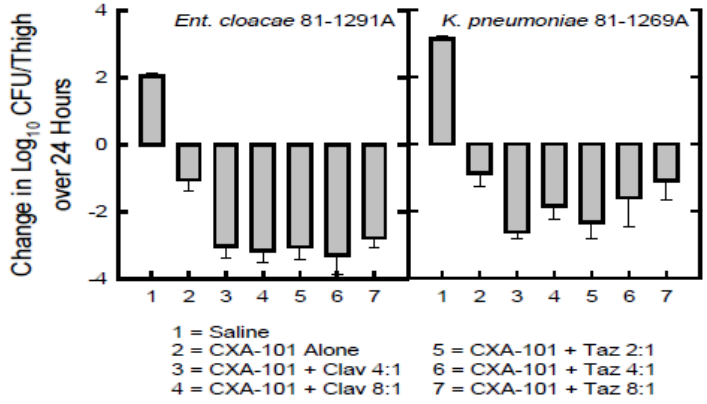
The effect of the various treatment regimens on the bacterial load in the mouse thigh is summarized in the figures below. Increasing the tazobactam dose, which results in lower ceftolozane/BLI ratios, correlated with increase in vivo bactericidal activity for *E. coli* 6042 and *K. pneumoniae* 81-1269A. In the thigh model, both ceftolozane-tazobactam and ceftolozane/clavulanic acid combinations had poor activity against the *K. pneumoniae* 4105 and 4110 strains. However, some activity was observed against *K. pneumoniae* 4110 with higher doses of the 2 combinations and the activity against strain 4105 was greater in the lung than in the thigh. As in other rodent models, relatively high doses of ceftolozane and BLIs were administered because of their poor PK profile (rapid elimination and short t½ in mice).

Figure 8: Efficacy of 400 mg/kg/6h Ceftolozane Alone and in Combination with Tazobactam or Clavulanic Acid in the Neutropenic Thigh Infection Model



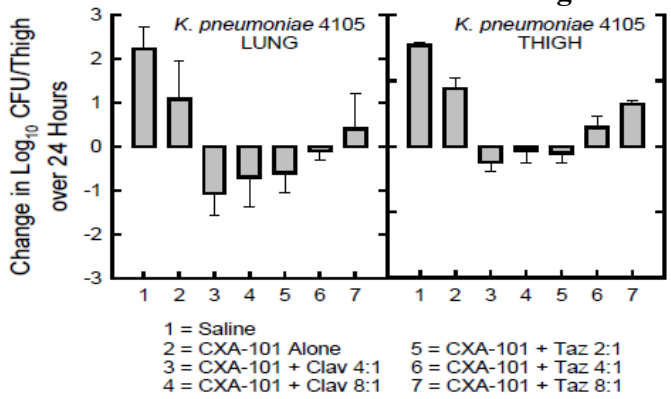
CFU=colony-forming units; Clav=clavulanic acid; CXA-101=ceftolozane; Taz=tazobactam.
Note: Treatment was subcutaneous.
Source: M5.3.5.4\CXA101-M-004

Figure 9: Efficacy of 800 mg/kg/6h Ceftolozane Alone and in Combination with Tazobactam or Clavulanic Acid in the Neutropenic Thigh Infection Model



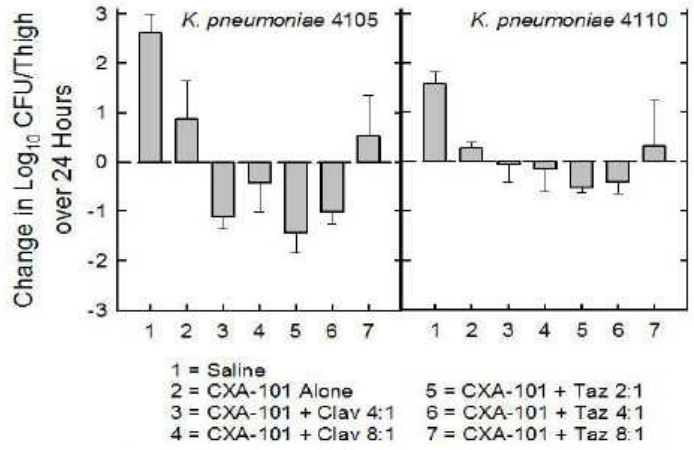
CFU=colony-forming units; Clav=clavulanic acid; CXA-101=ceftolozane; Taz=tazobactam.
Note: Treatment was subcutaneous.
Source: M5.3.5.4\CXA101-M-004.

Figure 10: Efficacy of 400 mg/kg/6h Ceftolozane Alone and in Combination with Tazobactam or Clavulanic Acid in the Lungs and Thigh of Neutropenic Mice



CFU=colony-forming units; Clav=clavulanic acid; CXA-101=ceftolozane; Taz=tazobactam.
Note: Treatment was subcutaneous.
Source: M5.3.5.4\CXA101-M-004

Figure 11: Efficacy for 800mg/kg/6h of Ceftolozane Alone and Combined with Clavulanic Acid and Tazobactam Against Two Strains of Enterobacteriaceae, Producing ESBLS, in the Thigh of Neutropenic Mice



CFU=colony-forming units; Clav=clavulanic acid; CXA-101=ceftolozane; Taz=tazobactam.
Note: Treatment was subcutaneous.
Source: M5.3.5.4\CXA101-M-004

Immunocompetent Mice

The efficacy of ceftolozane-tazobactam exposures approximating time as percentage of the dosing interval that the free drug concentration exceeds the MIC (%*f*T > MIC) of ceftolozane and ceftolozane-tazobactam and piperacillin/tazobactam against target gram-negative organisms was evaluated in an immunocompetent mouse thigh infection model (CXA.009.MC). The organisms tested included 6 strains of *P. aeruginosa*, 4 strains of *K. pneumoniae* (3 of them ESBL-positive), and 4 strains of *E. coli* (2 of them ESBL-positive). After infection, 3 mice per group were administered ceftolozane ± tazobactam at doses designed to approximate the *%f*T > MIC observed in humans given 1 g of ceftolozane ± 0.5 g tazobactam every 8 hours as a 1-hour infusion. As a comparison, groups of mice were administered piperacillin-tazobactam doses designed to approximate the %*f*T > MIC observed in humans given piperacillin/tazobactam 4.5 g every 6 hours as a 30-min infusion. The mice were euthanized after 24 hours. The thighs were homogenized and bacterial loads were determined by plating on agar medium.
This study demonstrated that predicted ceftolozane ± tazobactam *%f*T > MIC exposures of ≥ 37.5 resulted in 1- to 3-log decreases in CFU in *P. aeruginosa* and ESBL-negative strains of Enterobacteriaceae with MICs ≤ 16 mcg/mL while, in comparison, predicted piperacillin/tazobactam %*f*T > MIC exposures of >40 resulted in static to >1 log decreases in CFU in ESBL-negative strains with MICs ≤ 32 mcg/mL. With regard to the ESBL-positive strains, treatment with ceftolozane-tazobactam produced greater decreases in CFU than ceftolozane alone. Ceftolozane alone produced statistically significant reductions in CFU 4 of 8 *P. aeruginosa* isolates compared with piperacillin-tazobactam and in 8 of the isolates there was at least a 1-log reduction in colony counts (CXA.009.MC). The addition of tazobactam to ceftolozane produced significant reductions in CFU for 7 isolates as compared with piperacillin-tazobactam.

Overall, human simulated exposures of ceftolozane with or without tazobactam demonstrated greater efficacy versus piperacillin-tazobactam (data not shown).

Conclusions
Ceftolozane and ceftolozane-tazobactam were effective in systemic lethal infections in mice caused by *P. aeruginosa*, including MDR strains, and Enterobacteriaceae, including ESBLproducing strains. The efficacy of ceftolozane was demonstrated in severe infections, including sepsis, pneumonia, UTI, burn wound, and thigh infections. In general, ceftolozane and ceftolozane-tazobactam were comparable to or better than comparator antibacterials evaluated against all pathogens studied.

Reviewer's Comment
There was a difference in efficacy in animal models when ceftolozane alone was compared to the combination of ceftolozane with tazobactam. Examples of this include the following three models:

In the mouse sepsis model, the *E.coli* ESBL-positive strain (C11) was resistant to ceftolozane. Ceftazidime, with an MIC of 1 mcg/mL, had an ED50 of 20.8 mg/kg while the ED50 of ceftolozane-tazobactam was 47.5 mg/kg against this strain. Against the ESBL-positive *E. coli* strains, ceftolozane-tazobactam was approximately 5 to 8 times more active than ceftolozane alone. Against the (C2) ESBL-positive strain of *K. pneumoniae*, ceftolozane alone had an ED50 of 183.3 mg/kg, while ceftolozane-tazobactam had in vivo activity, with an ED50 value of 44.9 mg/kg.

In the neutropenic mouse thigh infection model, the efficacy of ceftolozane-tazobactam against ESBL-producing Enterobacteriaceae was compared with that of ceftolozane alone. The challenge organisms included *E. coli, Enterobacter cloacae*, and *K. pneumoniae*, 1 of which carried both the CTX-M-3 ESBL and overproduced an AmpC enzyme. Increasing the tazobactam dose, correlated with increase in vivo bactericidal activity for *E. coli* 6042 and *K. pneumoniae* 81-1269A.

In the immunocompetent mouse thigh infection model, the efficacy of ceftolozane-tazobactam exposures approximating MIC (%*f*T > MIC) of ceftolozane and ceftolozane-tazobactam target gram-negative organisms was evaluated. The organisms tested included *P. aeruginosa*, *K. pneumoniae* (3 of them ESBL-positive), and *E. coli* (2 of them ESBL-positive). With regard to the ESBL-positive strains, treatment with ceftolozane-tazobactam produced greater decreases in CFU than ceftolozane alone. Ceftolozane alone produced statistically significant reductions in CFU for 4 of 8 *P. aeruginosa* isolates. The addition of tazobactam to ceftolozane produced significant reductions in CFU for 7 isolates.

Pharmacokinetics and Pharmacodynamics

Rabbit Penetration Model

A rabbit model was used to evaluate the penetration of ceftolozane into plasma, bone, and lung when simulating human PK. The PK of ceftolozane was evaluated in the rabbit after a 60-min IV infusion. Plasma half-life of ceftolozane into the rabbit was 0.75 hour as compared to 2.38 hours in humans; therefore requiring simulation to accurately evaluate drug penetration (CXA201-P-002). Rabbits were dosed using a computer controlled pump simulating the human kinetic profiles of ceftolozane in rabbits. After simulation of a one hour infusion, concentrations of ceftolozane in plasma, lungs, marrow, and bone were determined at 0 (Cmax), 30, 60, and 120 min. The penetration of ceftolozane was assessed as the ratio between homogenized tissues concentrations of ceftolozane and plasma concentrations (see table below). The highest penetration was noted at 1.5h and ranged from 25% (in lung) to 9% (in bone).

Table 57: Ratio of Ceftolozane Concentrations Between Tissues and Plasma in the Rabbit After Simulation of the Human Pharmacokinetics of 1 g Administered Every 8 Hours

	Concentration of Ceftolozane (Tissue to Plasma Ratio) (%)			
	Lungs		Bone Tissue	
Time (hours)	Right Lung	Left Lung	Marrow	Bone
Study 1				
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	14.5 ± 2.3	14.8 ± 0.4	7.9 ± 2.1	5.2 ± 0.4
1.5	19.2 ± 3.5	15.8 ± 1.8	14.1 ± 1.4	6.1 ± 2.2
2	18.0 ± 1.9	13.6 ± 0.8	11.7 ± 3.4	5.6 ± 1.1
3	9.4 ± 3.6	7.7 ± 1.7	6.8 ± 4.4	0.0 ± 0.0
Study 2				
1.5	24.8%	25.0%	17.5%	9.0%

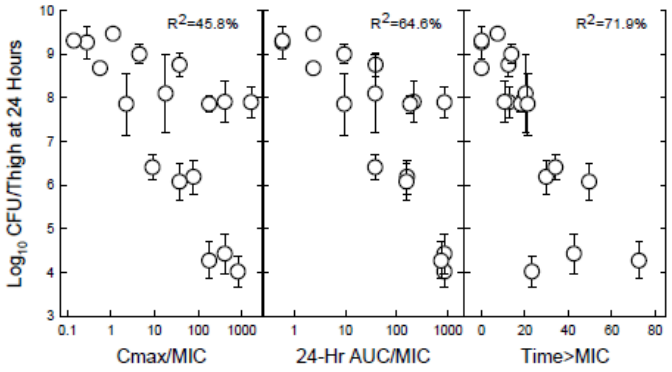
Note: 3 animals were used per time point. Data presented as mean percentage plus or minus standard deviation.
Source: M4.2.2.2\CXA201-P-002

Ceftolozane

The % T>MIC has been established as the PK/PD index that correlates best with the therapeutic efficacy of cephalosporins and other β-lactam antibacterials. The % T>MIC required to achieve a bacteriostatic effect in the neutropenic mouse thigh infection model is considered the target for predicting clinical efficacy of cephalosporin antibacterials [Ambrose, 2007; Craig, 1998]. To confirm that this relationship is also applicable to ceftolozane, a study using multiple isolates of *P. aeruginosa* and Enterobacteriaceae was conducted in a neutropenic mouse thigh infection model [CXA101-M-004]. The objectives for this study were to determine the PK/PD indices and their magnitudes for ceftolozane in the animal model, and to provide a guideline for estimation of the dosing regimen(s) suitable for human studies. The relationships between microbiological effect

and each of the PD indices (% T>MIC, 24-hour area under the plasma drug concentration-time curve (AUC)/MIC and peak/MIC) were investigated for several bacterial strains. As with other β-lactam antibacterials, the microbiological effect correlated most strongly with the % T>MIC, with coefficient of determination (R2) values of > 70% (see figure below).

Figure 12: Relationship of Different PK/PD Indices to the Activity of Ceftolozane (CXA-101) Against *K. pneumoniae* ATCC 4316



Abbreviations: AUC = Area under the curve of concentration vs. time; C_{max} = Maximum concentration in plasma; R^2 = Coefficient of determination.
Source: CXA101-M-004.

In general, the shape of the dose-response curves was similar for all strains. The % T>MIC values for bacteriostasis, 1-log kill, and maximum bactericidal effect are shown in the table below. A high extent of bacterial killing was achieved in neutropenic mice for most strains of Enterobacteriaceae and *P. aeruginosa*. The doses required to achieve stasis varied from 5.69 to 61.2 mg/kg/6 hours. The free-drug % T>MIC values corresponding to the bacteriostatic doses varied from 21% to 29% (1.3-fold variance). A 1-log reduction of bacterial count was achieved with T>MIC values ≤ 38%. The T>MIC values required for a static effect were similar for strains of Enterobacteriaceae and strains of *P. aeruginosa*.

Table 58: Maximum Killing and T>MIC Values Required for Static and Bactericidal effects with 6-Hourly Dosing of Ceftolozane (CXA-101) Against Eight Organisms

Organism	T>MIC Static Dose (%)	T>MIC 1-log kill (%)	Maximum Killing Log_{10} CFU/thigh
Enterobacteriaceae			
E. coli ATCC 25922	28.1	32.8	-2.95
E. coli NIH-J	28.0	32.3	-2.49
K. pneumoniae ATCC 43816	25.2	32.0	-2.52
K. pneumoniae 216	24.0	29.2	-2.42
Mean	26.3 ± 2.1	31.6 ± 1.6	-2.60 ± 0.24
P. aeruginosa			
ATCC 27853	24.3	33.9	-1.92
4034A	28.5	35.3	-2.61
PO2	21.7	30.1	-2.24
313	21.4	26.7	-2.99
Mean	24.0 ± 3.3	31.5 ± 3.9	-2.44 ± 0.46

Abbreviation: T>MIC = Time above minimum inhibitory concentration.
Source: CXA101-M-004.

In summary, the cephalosporin T>MIC target for free drug to produce a net bacteriostatic effect has been reported most commonly in the range of 30% to 40% [Craig 1998]. The T>MIC with ceftolozane (21% to 28%) required to produce a bacteriostatic effect appeared to be shorter than that reported with other cephalosporins but will need to be verified in comparative studies. Data from this study confirmed that 1) T>MIC was the best PK/PD predictor of efficacy of ceftolozane; 2) T>MIC was not affected by changes in MICs and 3) similar PK/PD indices for ceftolozane were observed against Enterobacteriaceae and *P. aeruginosa*. The PK/PD index of % T>MIC is regarded as the most predictive in selecting a dosing regimen for clinical studies

Proposed Susceptibility Interpretive Criteria and Quality Control Parameters
The Applicant's proposed susceptibility interpretive criteria and quality control parameters for ceftolozane-tazobactam are shown in the figures below:

Table 59: Proposed Interpretive Criteria for Ceftolozane-Tazobactam

(b) (4)

Source: M2.7.2.4.8\Table 127

Proposed Quality Control Parameters
See Applicant and Agency's proposed labeling at the end of this review and also the section of this review titled, "Development of Interpretive Criteria and Quality Control Parameters" for further information,

Human Pharmacokinetics
An overview of the studies that evaluated the clinical pharmacology of ceftolozane alone or ceftolozane-tazobactam in healthy volunteers, special populations, and subjects with infection, and the studies that evaluated microbiology was provided by the Applicant.

General Clinical Pharmacology
Pharmacokinetics
Ceftolozane exposure (maximum [peak] plasma drug concentration [Cmax] and area under the plasma concentration-time curve [AUC]) was approximately dose-proportional when administered IV over a 1-hour period to healthy volunteers with normal renal function following single doses ranging from 250 mg to 3 g and multiple (10-day) doses of 500 mg to 2 g every 8 hours and 1.5 g every 12 hours. The PK parameters for ceftolozane-tazobactam were similar following single and multiple doses, given alone or co-administered (see table below), demonstrating lack of accumulation or PK interaction. Ceftolozane elimination half-life (t½) was independent of dose and ranged from approximately 2 to 3 hours with no observed accumulation, thus supporting 3 times daily administration.

Table 60: Mean (%CV) Ceftolozane and Tazobactam Plasma Pharmacokinetics Parameters After Single and Multiple Ceftolozane-Tazobactam 1.5 g Intravenous 1-hour Infusions Every 8 Hours in Healthy Adults

	Ceftolozane/Tazobactam (1.5 g every 8 hours)			
	Ceftolozane (1 g)		Tazobactam (500 mg)	
PK parameters	Day 1 (n=9)[a]	Day 10 (n=10)	Day 1 (n=9)[a]	Day 10 (n=10)
C_{max} (µg/mL)	69.1 (11)	74.4 (14)	18.4 (16)	18.0 (8)
t_{max} (h)[b]	1.02 (1.01, 1.1)	1.07 (1.0, 1.1)	1.02 (0.99, 1.03)	1.01 (1.0, 1.1)
AUC (µg•h/mL)[c]	172 (14)	182 (15)	24.4 (18)	25.0 (15)
$t_{½}$ (h)	2.77 (30)	3.12 (22)	0.91 (26)[d]	1.03 (19)

AUC=area under the plasma concentration-time curve; AUC_{last}=area under the plasma concentration-time curve from time zero to the last measurable concentration (plasma samples were obtained through 24 hours); $AUC_{\tau,ss}$=area under the plasma concentration-time curve for a dosing interval at steady state; C_{max}=maximum (peak) plasma drug concentration; CV=coefficient of variation; PK=pharmacokinetic; $t_{½}$=elimination half-life; t_{max}=time to reach maximum (peak) plasma concentration following drug administration
[a] N=9, one outlier subject excluded from descriptive statistics
[b] Median (minimum, maximum) presented
[c] AUC for Day 1=AUC_{last} and AUC for Day 10=steady state AUC ($AUC_{\tau,ss}$)
[d] N=8, one subject excluded from descriptive statistics as the concentration-time profile did not exhibit a terminal log-linear phase and $t_{½}$ could not be calculated
Source: M2.7.2.2.1.2\Table 6 and Table 7

Distribution
The plasma protein binding of ceftolozane in humans ranges from approximately 16% to 21%; plasma protein binding for tazobactam is approximately 30%.
The apparent volume of distribution at steady state after IV administration (Vss) of ceftolozane and of tazobactam administered to healthy volunteers in multiple-dose studies (approximately 12 to 17 L and 14 to 19 L, respectively) was similar to extracellular fluid volume, suggesting distribution of both compounds in the extravascular space. In infected patients, Vss appeared to increase, consistent with a previously published report showing that the volume of distribution of β-lactam antibacterials can be increased significantly in the presence of intra-abdominal disease [Adan, 2012]. Both ceftolozane and tazobactam penetrate into ELF in concentrations likely to be clinically relevant.

Metabolism and Excretion
Ceftolozane undergoes minimal metabolism following IV administration in humans with most (mean of approximately 99%) of the administered dose excreted unchanged in the urine, indicating that it is metabolically stable. Ceftolozane is predominantly eliminated by glomerular filtration and that tubular secretion-related drug interactions observed with other antibacterials (Zosyn®, [2012]) are not expected with ceftolozane.
Less than 20% of a tazobactam dose is converted to a single metabolite (M1) that lacks pharmacological activity. Tazobactam and its M1 metabolite are eliminated primarily by renal excretion with <20% as the M1 metabolite and >80% as unchanged drug through glomerular filtration and tubular secretion.

Special Populations
The PK of ceftolozane-tazobactam were evaluated in subjects with mild, moderate, and severe renal impairment, as well as subjects with end-stage renal disease (ESRD) on hemodialysis (HD). Given that ceftolozane-tazobactam is primarily eliminated by renal excretion, creatinine clearance (CLCR) was shown to influence and predict the CLR and exposure to ceftolozane-tazobactam. Relative to ceftolozane-tazobactam exposures in subjects with normal renal function (CLCR ≥90 mL/min), the slightly increased exposures observed in subjects with mild renal impairment (CLCR >50 to 89 mL/min) were not clinically relevant, whereas exposures increased approximately 2- to 2.5-fold and 3- to 5-fold in subjects with moderate (CLCR 30 to 50 mL/min) and severe (CLCR 15 to 29 mL/min) renal impairment, respectively.

Reviewer's Comment
Dose adjustments were recommended by the Applicant for certain special populations.

Pharmacokinetics/Pharmacodynamics and Exposure/Response
Like other β-lactam antibacterials, the PK/PD parameter that most closely correlates with efficacy is the time, as a percentage of the dosing interval, that the plasma concentration of ceftolozane exceeds the minimum inhibitory concentration (MIC) of the infecting

organism (%T>MIC). In the mouse neutropenic thigh infection model, the mean (median) %T>MIC values based on total drug concentration for bacteriostasis and 1-log-kill were 25.2 ((b) (4) and 31.5 ((b) (4) respectively. These data support a bactericidal %T>MIC value of approximately 30%. Monte Carlo simulation analysis of clinical PK data revealed that, using a PD target of 30% T>MIC, a dose of ceftolozane-tazobactam 1.5 g every 8 hours administered as a 1-hour IV infusion provides sufficient drug concentrations to cover target pathogens, with a probability of target attainment (PTA) of ≥90% for pathogens with an MIC of up to 8 mcg/mL. These results led to the selection of the dose regimen of ceftolozane-tazobactam 1.5 g every 8 hours as a 1-hour IV infusion for Phase 2 and Phase 3 trials.

The relationship between exposure and efficacy or safety was assessed based on data from the Phase 2 cUTI and cIAI studies. The dose of 1.5 g ceftolozane-tazobactam was associated with a small number of clinical failures. No trend was observed between exposure and clinical response (success or failure). With respect to safety, a relationship between exposure and select adverse events (AEs) or changes in laboratory values from baseline was not apparent.

Reviewer's Comment
The dose of 1.5 g ceftolozane-tazobactam was associated with a small number of clinical failures. No trend was observed between exposure and clinical response (success or failure). This may suggest that the reasons for failure were not related to inadequate exposures.

Since ceftolozane is an antibacterial agent that targets both gram-negative and gram-positive bacterial pathogens by binding the essential bacterial PBPs and tazobactam is an inhibitor of many bacterial class A and C β-lactamases, all primary pharmacodynamic (PD) effects are directed towards bacteria as opposed to animal or human organs or tissues.

The Applicant's conclusions from PK-PD target attainment analysis are below:
• The results of the PK-PD target attainment analyses described herein, which are based on free-drug % T>MIC targets of (b) (4) and (b) (4) associated with net bacterial stasis and a 1-log10 CFU reduction from baseline, respectively, support *in vitro* susceptibility test interpretive criteria for ceftolozane-tazobactam against *P. aeruginosa* of 8 mg/L for the dosing regimens by renal function categories described below:

- For patients with high normal renal function administered ceftolozane-tazobactam 1000/500 mg q8h, a PK-PD MIC cut-off value of 4 to 8 mg/L was identified;
- For patients with normal renal function and mild renal impairment administered ceftolozane-tazobactam 1000/500 mg q8h, a PK-PD MIC cut-off value of 8 mg/L was identified;

- For patients with moderate renal impairment administered ceftolozane-tazobactam 500/250 mg q8h, a PK-PD MIC cut-off value of 8 mg/L was identified; and
- For patients with severe renal impairment administered ceftolozane-tazobactam 250/125 mg q8h, a PK-PD MIC cut-off value of 8 mg/L was identified.

• The results of the PK-PD target attainment analyses described for 2000 mg ceftolozane adjusted for renal function group, which are based on free-drug % T>MIC targets of (b) (4) and (b) (4) associated with net bacterial stasis and a 1-log10 CFU reduction from baseline, respectively, support *in vitro* susceptibility test interpretive criteria for ceftolozane-tazobactam against *P. aeruginosa* of 16 mg/L.

CLINICAL TRIALS

Overview

Primary data in support of the effectiveness of ceftolozane-tazobactam in cUTI and in cIAI are each derived from two Phase 3 studies in adult subjects, incorporated into 1 well-controlled, adequately-powered, pooled analysis in each indication, cUTI (cUTI\CXA-cUTI-10-04 and -05) and cIAI (cIAI\CXA-cIAI-10-08 and -09).

Study Designs

Studies CXA-cUTI-10-04 and -05 and CXA-cIAI-10-08 and -09 were multicenter, multinational, randomized, double-blind, active-controlled noninferiority studies. Subject participation comprised 3 phases: Screening, Treatment, and Post-treatment (comprising the end-of-therapy [EOT], TOC, and LFU visits).

Subjects in both indications received IV therapy for the entire duration of treatment. All subjects were initially treated as inpatients; outpatient IV therapy could have been considered after a minimum duration of treatment (9 doses, 3 days).

Cubist obtained agreement from the FDA and the Committee for Medicinal Products for Human Use (CHMP) to proceed with a single-study strategy for the cUTI and cIAI indications achieved by pooling data from the 2 identical Phase 3 cUTI protocols and the 2 identical Phase 3 cIAI protocols, providing one database per indication with appropriate total sample size and adequate power. The data from the individual protocols for each indication were pooled after database lock, analyzed as 1 dataset, and are reported in 1 clinical study report per indication (cUTI\CXA-cUTI-10-04 and -05, cIAI\CXA-cIAI-10-08 and -09).

Phase 1 Studies

Nine phase 1 studies were conducted with FDA guidance that evaluated clinical pharmacology. Single and multiple ascending doses of ceftolozane alone and in combination with tazobactam were evaluated. Safety, tissue distribution and investigations into special populations (impaired renal function) were examined.

Phase 2 Studies

Phase 2 studies were intended to assess the safety profile and provide a preliminary evaluation of efficacy. The studies also provided important PK data in the cUTI and cIAI populations. The designs of the Phase 2 studies were similar to those of the Phase 3 studies; both were multicenter, prospective, double-blind, active-controlled studies assessing only 1 dose of ceftolozane or ceftolozane-tazobactam. One Phase 2 study (Ceftolozane-03) investigated ceftolozane alone compared with ceftazidime for the treatment of cUTI and a second (cIAI\CXA-cIAI-10-01) explored ceftolozane-tazobactam compared with meropenem for the treatment of cIAI.

The Phase 2 study in cUTI (Ceftolozane-03) was the first and only safety and efficacy study conducted with ceftolozane alone in a patient population. Subsequent development

of ceftolozane as a single agent was discontinued in favor of ceftolozane-tazobactam in the Phase 3 studies. The results from this Phase 2 study supported the therapeutic effectiveness and safety of the 1 g every 8 hours regimen of ceftolozane administered as an IV infusion for 7 to 10 days in adults with cUTI. The Phase 2 cUTI study was similar to the Phase 3 study in terms of the population and diagnostic criteria.

The primary efficacy endpoint in the Phase 2 study was microbiological eradication in the microbiologically evaluable (ME) at TOC population consistent with the prevailing regulatory guidance at the time the study was conducted. Ceftazidime was used as the comparator in Phase 2 whereas levofloxacin was used in Phase 3. This change was made to ensure the Phase 3 program was more widely accepted globally because ceftazidime is not commonly used as empiric therapy for cUTI and more ESBL producing organisms were anticipated as sites were expanded in Phase 3.

The Phase 2 study in cIAI (CXA-cIAI-10-01) was similar to the Phase 3 cIAI study in the range of infections eligible for participation, inclusion and exclusion criteria, doses of ceftolozane-tazobactam plus metronidazole, comparator agent, and definitions of clinical response. However, the 2 studies differed slightly in the treatment duration allowed, the timing of the assessment for the primary and secondary endpoints, and stratification. In the Phase 2 study, the treatment duration was 4 to 7 days (up to 14 days in Phase 3), the primary endpoint was assessed at the TOC visit 7 to 14 days after the end of study drug therapy (compared with 24 to 32 days after the initiation of study drug in Phase 3), and stratification was based on localized complicated appendicitis versus other sites of infection (compared with bowel versus other sites of infection in Phase 3).

Complicated Urinary Tract Infections (cUTI) Studies (Studies CXA-cUTI-10-04 and CXA-cUTI-10-05), and Complicated Intra-abdominal Infections (cIAI) Studies (StudiesCXA-cIAI-10-08 and CXA-cIAI-10-09)

Phase 3 Studies

Treatments Administered and Duration of Therapy

Only 1 dosing regimen of ceftolozane-tazobactam (1.5 g of ceftolozane-tazobactam every 8 hours) was evaluated throughout the Phase 3 clinical development program. To ensure an adequate but not unnecessarily lengthy duration of drug exposure, 7 days of therapy was selected in the cUTI indication and 4 to 10 days of therapy was selected in the cIAI indication (which could be extended to 14 days in more serious infections with slower rate of clinical response). In both the cUTI and cIAI indications, the full course of treatment was administered as an IV infusion, with no oral switch permitted. The studies were designed to test non-inferiority against concurrent active control arms: levofloxacin 750 mg every 8 hours administered as an IV infusion in the cUTI indication and meropenem 1 g every 8 hours administered as an IV infusion in the cIAI indication.

Levofloxacin: Comparator Agent in cUTI

Levofloxacin was chosen as the comparator in cUTI because it is approved for, and widely used in, the treatment of cUTI worldwide. The 750 mg once daily dose of levofloxacin is the highest approved dose for the treatment of cUTI. This high-dose regimen was chosen to ensure the maximum possible activity of the comparator in the setting of increasing resistance, although at the time of initial planning of the cUTI study, levofloxacin resistance was not known to be as prevalent globally as observed in the study.

Levofloxacin 750 mg once daily has proven efficacy in cUTI, a favorable safety profile in various infection types, and is cited in various clinical practice guidelines as the first choice therapy for the treatment of cUTI [Hooton, 2009; Grabe; 2012, Nicolle 2005]. Although levofloxacin 750 mg once daily is approved as a 5-day course in the United States for cUTI, there is no evidence-based consensus on the appropriate duration of therapy for cUTI. Most infectious disease experts and various clinical practice guidelines recommend a minimum of 7 days of treatment for patients with serious cUTIs [Hooton, 2009; Grabe, 2012; Nicolle, 2005]. For this reason and considering the increasing rates of fluoroquinolone resistance worldwide [Bouchillon, 2012], Cubist elected to extend the duration of levofloxacin therapy to 7 days for this study. Selecting the highest approved levofloxacin dose, and extending the approved duration, were intended to ensure global acceptance of the comparator regimen, and provide investigators with an ethically appropriate, clinically relevant, effective comparator against which the activity of ceftolozane-tazobactam could be assessed. Not allowing oral switch therapy reduced the potential confounding of additional antimicrobial therapy and ensured accrual of more seriously ill patients.

Meropenem: Comparator Agent in cIAI

Meropenem is a broad-spectrum antimicrobial agent with excellent activity against pathogens associated with cIAI. Meropenem was chosen as the comparator for cIAI based on proven safety and efficacy in this indication. It is approved as monotherapy for the treatment of cIAI in the United States and other regions and is recommended as monotherapy in treatment guidelines for cIAI [Solomkin, 2010]. The 1g IV every 8 hour dose of meropenem chosen for this study is the approved dose for the treatment of IAI (Merrem®, [2013]). The optimal duration of therapy for patients with IAI has not been determined in randomized controlled studies; however, antibacterials approved for use in cIAI were generally studied for 5 to 14 days of therapy and 4 to 7 days of therapy is recommended in the evidence-based guidelines for the treatment of cIAI [Solomkin, 2010].

Metronidazole: Adjunct Therapy with Ceftolozane-tazobactam in cIAI

Metronidazole, a limited-spectrum, anaerobe-specific antibiotic commonly used in the treatment of cIAI in combination with a cephalosporin, was used as an adjunct in the ceftolozane-tazobactam arm of the cIAI studies. Its use in this manner is recommended in

evidence-based guidelines for the treatment of cIAI [Solomkin, 2010]. In vitro studies have shown no antagonism with ceftolozane-tazobactam.

Reviewer's Comment
In vitro studies have shown no antagonism between metronidazole and ceftolozane-tazobactam, which is important because metronidazole was used in combination with study drug in the cIAI studies.

For both indications, the primary objective of the Phase 3 studies was demonstration of noninferiority of ceftolozane-tazobactam versus the respective comparator based on a 10% noninferiority margin and 95% confidence interval. In the cUTI indication, the primary efficacy endpoint was the composite microbiological eradication and clinical cure rate in the microbiological modified intent-to-treat (mMITT) population at the test-of-cure (TOC) visit 7 (± 2) days after the last treatment. The mMITT population was defined as all randomized subjects who received any amount of study drug and had at least 1 qualifying causative uropathogen from a pretreatment baseline urine specimen. Subjects who were cured at the TOC visit were reassessed at the late follow-up (LFU) visit (28 to 35 days after end of therapy).

In the cIAI indication, the primary efficacy endpoint was the clinical cure rate in the microbiological intent-to-treat (MITT) population at the TOC visit 24 to 32 days after the initiation of study drug. The MITT population was defined as all randomized subjects with cIAI with at least 1 baseline intra-abdominal pathogen, regardless of susceptibility to study drug. Subjects who were clinically cured at the TOC visit were reassessed at the LFU visit (38 to 45 days after the initiation of study drug). For each indication, non-inferiority was concluded when the lower bound of the 95% confidence interval was greater than -10%.

The table below summarizes the primary and key secondary efficacy analysis populations in the Phase 3 studies; the primary and key secondary efficacy objectives for the 2 indications are provided in the tables below. In the analysis of both the cUTI and cIAI studies, a primary analysis population and several secondary populations were defined based on satisfying clinical and microbiological criteria.

Table 61: Primary and Key Secondary Efficacy Populations

Efficacy population	cUTI Indication	cIAI Indication
Primary	mMITT: All randomized subjects who received any amount of study drug and who had at least 1 acceptable causative uropathogen from a study-qualifying pretreatment baseline urine specimen.	MITT: All randomized subjects who had IAI as evidenced by identification of at least 1 baseline intra-abdominal pathogen, regardless of susceptibility to study drug.
Key Secondary	ME: Subset of mMITT population who had interpretable urine culture result at the TOC visit, adhered to study procedures, and had a TOC visit within the specified visit window.	ME: Subset of MITT population who received adequate amount of study drug, met the protocol-specific disease definition of cIAI, had at least 1 baseline infecting pathogen identified that was susceptible to study drug, adhered to study procedures and had a TOC visit within the specified visit window.

ITT=intent-to-treat; ME=microbiologically evaluable; MITT=microbiological intent-to-treat; mMITT=microbiological modified intent-to-treat; TOC=test-of-cure

Table 62: Primary and Key Secondary Efficacy Objectives

Efficacy objective	cUTI Indication	cIAI Indication
Primary	To demonstrate noninferiority of ceftolozane/tazobactam versus levofloxacin in composite microbiological eradication and clinical cure rate in the mMITT population at the TOC visit. Noninferiority to levofloxacin was concluded if the lower bound of the 2-sided 95% stratified Newcombe CI around the difference in composite microbiological eradication and clinical cure rates (ceftolozane/tazobactam minus levofloxacin) was greater than -10.0%.	To demonstrate noninferiority of ceftolozane/tazobactam plus metronidazole versus meropenem in the clinical cure rate in the MITT population at the TOC visit. Noninferiority to meropenem was concluded if the lower bound of the 2-sided 95% stratified Newcombe CI of the difference in the clinical cure rates ([ceftolozane/tazobactam and metronidazole] minus meropenem) was greater than -10.0%.
Key Secondary	To demonstrate noninferiority of ceftolozane/tazobactam versus levofloxacin based on the difference in composite microbiological eradication and clinical cure rate in the ME population at the TOC visit (ceftolozane/tazobactam minus levofloxacin), using the 10% noninferiority margin.	To demonstrate noninferiority of ceftolozane/tazobactam plus metronidazole versus meropenem based on the difference in clinical cure rates in the ME population at the TOC visit ([ceftolozane/tazobactam and metronidazole] minus meropenem), using the 10% noninferiority margin.

CI=confidence interval; ME=microbiologically evaluable; MITT=microbiological intent-to-treat; mMITT=microbiological modified intent-to-treat; TOC=test-of-cure

In addition to the primary and secondary analyses, several prespecified sensitivity analyses were performed for the primary efficacy endpoints to confirm the overall robustness of the study results, including adjustments for protocol number, region, and baseline diagnosis. As well, analyses were conducted at different time points, including EOT and LFU. Subgroup analyses based on various demographic and baseline disease characteristics were also conducted for the primary and key secondary efficacy endpoints in the cUTI and cIAI indications. A discussion of the rationale for pooling the data across protocols within each indication was provided (not shown).

The table below presents a summary of the number of subjects included in the key populations for analysis in the Phase 3 studies by indication and treatment arm. Across the Phase 3 studies, 2076 subjects were randomized and 2047 received study drug (Safety population), including 1015 who received at least one dose of ceftolozane-tazobactam. A total of 1083 subjects were randomized in the cUTI study and 993 subjects were randomized in the cIAI study. The percentage of subjects included in the primary and key secondary efficacy populations were balanced between the treatment arms in the Phase 3 studies. In the cUTI studies, 800 subjects were included in the primary mMITT population and in the cIAI study, 806 subjects were included in the primary MITT population. The most common reason for exclusion from the primary efficacy population in the cUTI and cIAI indications was lack of a qualifying baseline infecting pathogen . Exclusion from the ME population was primarily related to lack of a TOC visit (cUTI) or indeterminate response at TOC (cIAI).

Table 63: Analysis Populations in the Phase 3 cUTI and cIAI Studies by Indication and Treatment Arm

	Phase 3 cUTI		Phase 3 cIAI	
Populations for Analysis	Ceftolozane/ Tazobactam n (%)	Levofloxacin n (%)	Ceftolozane/ Tazobactam + Metronidazole n (%)	Meropenem n (%)
Number of Subjects Randomized	543 (100)	540 (100)	487 (100)	506 (100)
Subjects in Safety Population	533 (98.2)[a]	535 (99.1)[a]	482 (99.0)[a]	497 (98.2)[a]
Subjects in mMITT/MITT Population[b]	398 (73.3)	402 (74.4)	389 (79.9)	417 (82.4)
Subjects in ME Population	341 (62.8)	353 (65.4)	275 (56.5)	321 (63.4)

cIAI=complicated intraabdominal infection; cUTI=complicated urinary tract infection; ME=microbiologically evaluable; MITT=microbiological intent-to-treat; mMITT=microbiological modified intent-to-treat

[a] One subject in each indication was randomized to ceftolozane/tazobactam but received comparator drug. These subjects are included in the ceftolozane/tazobactam arm for efficacy analyses and in the comparator arm for safety analyses.

[b] Primary analysis populations for efficacy analyses in the cUTI and cIAI studies, respectively; see Table 4.

Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.1.1.1 and M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.1.1.2.3

The demographic and baseline characteristics were comparable across study populations, including the intent-to-treat population, and between treatment arms in both the cUTI and cIAI indications. Mean age was 48 to 50 years old across treatment arms and indications; a broad age range was evaluated across the studies (18 to 92 years old). In both indications, about 25% of the subjects were 65 years of age or older. The majority of subjects (75%) enrolled in the cUTI study were female and most subjects (58%) in the cIAI study were male.

The majority of subjects (>70%) in both the cUTI and cIAI indications were enrolled in Eastern Europe. Subjects from North America comprised 3% of the mMITT population in the cUTI study and 6% of the MITT population in the cIAI study. Enrollment was low in the United States likely due to the IV only nature of the study and the requirement that subjects remain hospitalized for the entire duration of IV study therapy if a site did not have outpatient parenteral antibiotic therapy capabilities. Despite the relative high

enrollment of subjects outside the United States, the spectrum of diagnoses, bacteriology, and the surgical procedures performed (in cIAI) in these studies were representative of the epidemiology and standard-of-care in the United States [Bouchillon, 2012; Mazuski, 2009; Hoban, 2007; Foxman, 2013]. Baseline disease characteristics were also comparable between the ceftolozane-tazobactam and comparator arms in both the cUTI and cIAI indications.

Reviewer's Comment
Despite the relative high enrollment of subjects outside the United States, the spectrum of diagnoses, bacteriology, and the surgical procedures performed (in cIAI) in these studies were representative of the epidemiology and standard-of-care in the United States. The criteria for qualifying culture for the cUTI study is described below:

Complicated Urinary Tract Infection Studies (cUTI) Studies (CXA-cUTI-10-04 and CXA-cUTI-10-05)
In Study CXA-cUTI-10-04 and -05, adult subjects with clinical signs and/or symptoms of cUTI with pyuria were randomly assigned (1:1 ratio) to receive ceftolozane-tazobactam or levofloxacin administered as IV infusions for 7 days, stratified by investigational site. Baseline urine cultures were mandatory to establish the diagnosis. Blood samples for culture were drawn in subjects with pyelonephritis or suspected bacteremia and in subjects with an indwelling catheter. Specific eligibility requirements ensured enrollment was limited to seriously ill subjects requiring inpatient IV antibiotic therapy for the entire duration of treatment.

The primary efficacy endpoint in cUTI was the composite microbiological eradication and clinical cure rate in the mMITT population at the TOC visit 7 (± 2) days after the last treatment.For the primary efficacy endpoints in each indication, missing data were handled using a treatment failure approach for the MITT/mMITT populations and with a data-as-observed approach for the ME population. In the treatment failure approach, subjects with a missing (including indeterminate) efficacy endpoint were categorized as treatment failures.

The following information was described by the Applicant in Protocol CXA-cUTI-10-04: A study-qualifying pretreatment baseline urine culture must grow at least 1 and not more than 2 bacterial isolates at $\geq 10^5$ CFU/mL each. If more than 2 bacterial isolates are identified, the culture will be considered contaminated regardless of colony count unless 1 of the isolates that grows in the urine at $\geq 10^5$ CFU/mL is also isolated from a blood culture obtained at the same visit. Coagulase-negative *Staphylococci* and non-Group D *Streptococci* will not be considered causative pathogens of cUTI in this study.

Catheterized Subjects Only
Eligible subjects who are catheterized at study entry are required to have pretreatment baseline blood culture specimens (1 aerobic bottle from 2 separate sites for a total of 2 aerobic bottles) obtained at the same time as the baseline urine culture specimen. A catheter is defined as any tube, stent, or foreign body conduit that extends from inside anywhere within the urogenital system to the outside of the body.
If a catheterized subject's urine culture grows more than 1 organism at any colony count, the urine culture will be considered contaminated regardless of the colony count unless 1 of the isolates that grows in the urine at $\geq 10^5$ CFU/mL is also isolated from a blood culture obtained at the same visit.

Requalification for Study Entry
Subjects who have been screened but have not been previously randomized to this study may be rescreened for participation if their eligibility characteristics have changed and (a) they have not received any antibacterial therapy for the current cUTI or (b) their previous cUTI has been successfully treated and they present with signs and symptoms of a new cUTI. Subjects may not be randomized to this study more than once. Subjects who have participated in any previous study of ceftolozane or ceftolozane-tazobactam may not be randomized to this study.

Microbiological outcome categories as described by the Applicant are as follows:

Outcome	Definition
Eradication	A urine culture at the TOC visit shows all uropathogens found at baseline at $\geq 10^5$ CFU/mL are reduced to $< 10^4$ CFU/mL
Persistence	A urine culture, taken any time after the completion of therapy, grows $\geq 10^4$ CFU/mL of the uropathogen found at baseline
Indeterminate	No urine culture available

Microbiological outcome categories at Late Follow-up Visit were described by the Applicant as follows:

Outcome	Definition
Sustained Eradication	A urine culture obtained within the 28- to 35-day post-therapy window shows all uropathogens found at baseline at $\geq 10^5$ CFU/mL remain $< 10^3$ CFU/mL
Persistence	A urine culture, taken any time after the completion of therapy, grows $\geq 10^3$ CFU/mL of the uropathogen found at baseline. The outcomes for pathogens that persisted at the TOC visit are carried forward to the LFU visit.
Recurrence	A urine culture taken any time after documented eradication at the TOC visit, up to and including the LFU visit, grows $> 10^3$ CFU/mL of the uropathogen found at baseline
Indeterminate	No urine culture available at the LFU visit

Reviewer's Comment
The definition of persistence different in the two tables above. This is likely because the definition of eradication is also different.

Complicated Intra-abdominal Infections (cIAI) Studies (CXA-cIAI-10-08 and CXA-cIAI-10-09)
In Study CXA-cIAI-10-08 and -09, adult subjects with cIAI with evidence of intraperitoneal infection who required surgical intervention (e.g., laparotomy, laparoscopic surgery, or percutaneous drainage of an abscess) within 24 hours of the first dose of study drug were randomly assigned (1:1 ratio) to receive ceftolozane-tazobactam plus metronidazole or meropenem plus placebo administered as IV infusions for 4 to 10 days (or up to 14 days for subjects with a slow rate of clinical response), stratified by investigational site and primary site of infection. Intra-abdominal specimens were collected for culture of both aerobes and anaerobes at the time of the initial interventional procedure. Blood samples for culture were drawn in subjects with hospital-acquired infections, those who had failed prior antibacterial therapy, or those who had signs of severe sepsis.

In the cIAI indication, the primary efficacy endpoint was the clinical cure rate in the MITT population at the TOC visit 24 to 32 days after the initiation of study drug. Noninferiority was concluded if the lower bound of the 95% CI was greater than -10%.

Baseline Infecting Pathogens and Susceptibility
Table 64: Baseline Disease Characteristics in the Pooled Phase 3 cIAI study by Treatment Arm (MITT Population)

Disease Characteristic	Ceftolozane/Tazobactam + Metronidazole (N=389) n (%)	Meropenem (N=417) n (%)
Primary Site of Infection		
Bowel (small or large)	77 (19.8)	80 (19.2)
Other Site of cIAI	312 (80.2)	337 (80.8)
Diagnosis		
Appendiceal perforation or periappendiceal abscess	175 (45.0)	203 (48.7)
Cholecystitis[a]	72 (18.5)	69 (16.5)
Diverticular disease with perforation or abscess	29 (7.5)	36 (8.6)
Acute gastric or duodenal perforation	38 (9.8)	33 (7.9)
Traumatic perforation of the intestine	5 (1.3)	7 (1.7)
Other Peritonitis[b]	41 (10.5)	33 (7.9)
Other Intra-abdominal abscess (including liver/spleen)	29 (7.5)	36 (8.6)
Other Disease Characteristics		
APACHE II $\geq$10	78 (20.1)	70 (16.8)
Presence of Diffuse Peritonitis	139 (41.2)	137 (40.3)
Localized Complicated Appendicitis	115 (29.6)	142 (34.1)
Etiology: Spontaneous Rupture	277 (71.2)	302 (72.4)

cIAI=complicated intra-abdominal infection; MITT=microbiological intent-to-treat
[a] Comprising gangrenous cholecystitis, with rupture, perforation, or progression of the infection beyond the gallbladder wall
[b] Due to other perforated viscus or following a prior operative procedure.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.1.2.2.1 and Table 14.1.2.6.1

The incidence and distribution of urinary tract and intra-abdominal baseline infecting pathogens, were similar between treatment arms in the cUTI and cIAI indications, respectively (see tables below) and consistent with results in prior pivotal trials in the indications, with the exception that a higher percentage of subjects had infections involving ESBL-producing or levofloxacin-resistant pathogens [Mazuski, 2009; Naber, 2009; Babinchak, 2005].

As expected, Enterobacteriaceae were the most common pathogens isolated in both indications, with *E. coli* being the most common pathogen overall, followed by *K. pneumoniae* and *P. aeruginosa* (CXA-cUTI-10-04 and -05, CXA-cIAI-10-08 and -09). In cIAI, the most common gram-negative anaerobes were *B. fragilis*, *B. ovatus*, and *B. thetaiotaomicron*. In addition, *Streptococcus* spp. were isolated in approximately 28% of subjects with cIAI, with *S. anginosus* and *S. constellatus* being the most common species (CXA-cIAI-10-08 and -09). As expected, most cIAIs (68%) were polymicrobial.

In the cUTI study, 97% of all gram-negative pathogens isolated at baseline (with MIC information available) were susceptible to ceftolozane-tazobactam (defined as MIC ≤8 mcg/mL); this is in contrast to levofloxacin where only 72% of gram-negative pathogens isolated at baseline were susceptible (CXA-cUTI-10-04 and -05). Of note, >99% of all *E. coli* isolates were susceptible to ceftolozane-tazobactam (MIC50 and MIC90 of 0.25 and 0.5 mcg/mL, respectively). Almost 15% of subjects in the mMITT population had ESBL-producing Enterobacteriaceae by phenotypic criteria (CXA-cUTI-10-04 and -05). Approximately 80% of these isolates exhibited levofloxacin resistance. Among ESBL-producing *E. coli* and *K. pneumoniae*, 100% and 80%, respectively, were susceptible to ceftolozane-tazobactam (CXA-cUTI-10-04 and -05).

In the cIAI study, 96% and 98% of all gram-negative aerobes isolated at baseline were susceptible to ceftolozane-tazobactam and meropenem, respectively (CXA-cIAI- 10-08 and -09), including 97% and 75% of ESBL-producing *E. coli* and *K. pneumoniae*, respectively (CXA-cIAI-10-08 and -09). For both ceftolozane-tazobactam and meropenem, the rates of susceptibility in streptococci were >90%. The majority of *B. fragilis* (96%) were susceptible to ceftolozane-tazobactam at baseline.

Reviewer's Comment
Ceftolozane-tazobactam had limited activity against gram-negative anaerobes, however, according to surveillance data, metronidazole was expected to have activity against most of these pathogens [Citron, 2012; Löfmark, 2010], and susceptibility was not specifically evaluated in the cIAI study. Ninety-eight percent of gram-negative anaerobes were susceptible to meropenem. Among subjects with bacteremia at baseline, the most common pathogen isolated from the blood in both indications was *E. coli*.

Primary and Key Secondary Efficacy Endpoints

Ceftolozane-tazobactam was effective in the treatment of subjects with cUTI, and, in combination with metronidazole, was effective in the treatment of subjects with cIAI. The Phase 3 studies in both indications met their primary efficacy endpoints: the ceftolozane-tazobactam arm demonstrated noninferiority to the comparator arm in treatment response. In the cUTI indication, ceftolozane-tazobactam achieved high composite microbiological and clinical cure rates at the TOC visit in the primary (mMITT) and key secondary (ME) efficacy populations (see table below). For both populations, the lower bound of the 95% CI around the treatment difference, ceftolozane-tazobactam minus levofloxacin, was greater than -10%, indicating noninferiority. Notably, the 2-sided 95% CI around the treatment differences excluded zero in both primary and key secondary analysis populations indicating superiority over levofloxacin. This conclusion was retained even with a 99% CI, a requirement for demonstration of superiority in a single study, confirming the robustness of the results in the cUTI study.

Table 65: cUTI Indication: Primary and Key Secondary Efficacy Endpoints: Summary and Analysis of Composite By-Subject Microbiological and Clinical Response at TOC by Population

Analysis Population Composite Response	Ceftolozane/ Tazobactam n (%)	Levofloxacin n (%)	% Difference in Success Rate (95% CI)	(99% CI)[a]
Primary Analysis:[b,c] *mMITT Population*	*N=398*	*N=402*		
Success	306 (76.9)	275 (68.4)	8.5 (2.31, 14.57)	(0.36, 16.46)
Failure	66 (16.6)	103 (25.6)		
Indeterminate[d]	26 (6.5)	24 (6.0)		
Secondary Analysis:[b,e] *ME Population*	*N=341*	*N=353*		
Success	284 (83.3)	266 (75.4)	8.0 (1.95, 13.97)	(0.01, 15.84)
Failure	57 (16.7)	87 (24.6)		

CI=confidence interval (based on stratified Newcombe); ME=microbiologically evaluable; mMITT= microbiological modified Intent-to-Treat; TOC=test-of-cure.
[a] 99% CI for determination of superiority from a single study.
[b] The analysis is stratified by region.
[c] Treatment Failure approach, indeterminate is classified as failure.
[d] Indeterminate responses included missing outcome assessments and those that could not be classified as cure or failure.
[e] Data-as-Observed approach, indeterminate is excluded from analysis.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.2, Table 14.2.2a, and Table 14.2.5

In the cIAI indication, both ceftolozane-tazobactam plus metronidazole and meropenem achieved high clinical cure rates at the TOC visit in both the primary (MITT) and key secondary (ME) populations (see table below). For both populations, the lower bound of the 95% CI around the treatment difference, ceftolozane-tazobactam plus metronidazole minus meropenem, was greater than -10%, indicating noninferiority of ceftolozane-tazobactam plus metronidazole to meropenem. Of note, failure rates were identical in the 2 treatment arms in the MITT population (8.2%) and were similar in the ME population (5.8% and 5.3%).

Table 66: cIAI Indication: Primary and Key Secondary Efficacy Endpoints: Summary and Analysis of By-Subject Clinical Response at TOC by Population

Analysis Population Clinical Response	Ceftolozane/Tazobactam + Metronidazole n (%)	Meropenem n (%)	Percentage Difference in Success Rate (95% CI)
Primary Analysis: MITT Population[a,b]	*N=389*	*N=417*	
Cure	323 (83.0)	364 (87.3)	-4.2 (-8.91, 0.54)
Failure	32 (8.2)	34 (8.2)	
Indeterminate[c]	34 (8.7)	19 (4.6)	
Secondary Analysis: ME Population[a,d]	*N=275*	*N=321*	
Cure	259 (94.2)	304 (94.7)	-1.0 (-4.52, 2.59)
Failure	16 (5.8)	17 (5.3)	

CI=confidence interval (based on stratified Newcombe with Minimum Risk weights); ME=microbiologically evaluable; MITT= microbiological intent-to-treat.
[a] The analysis is stratified by region and primary site of infection as recorded on the electronic case report form.
[b] Treatment Failure Approach, indeterminate is classified as failure.
[c] Indeterminate responses included missing outcome assessments and those that could not be classified as cure or failure.
[d] Data-as-Observed, indeterminate is excluded from analysis.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.2.1.1

Other Secondary Endpoints and Additional Analyses
In both indications, the analyses of additional efficacy endpoints were consistent with the primary analysis indicating the robustness of the results. In cUTI, rates of clinical success and microbiological success were high at the EOT and TOC visits in both treatment arms. Notably, ceftolozane-tazobactam was superior to levofloxacin in microbiological success at both EOT (95% versus 85%) and TOC (80% versus 72%).

Reviewers' Comments
Microbiological eradication rates were lower than clinical success rates at TOC. This difference represented cases of asymptomatic bacteriuria

Outcome assessments at the LFU visit in the cUTI indication showed high sustained clinical cure rates (>95%) in both treatment arms, illustrating the durability of the treatment effect. In cIAI, clinical cure rates at EOT and microbiological success at TOC were consistent with the primary analyses. Clinical cure at the EOT visit was noted in 89% and 92% of subjects in the ceftolozane-tazobactam plus metronidazole and meropenem treatment arms, respectively. At the TOC visit, microbiological success was observed in 85% and 89% of subjects in the ceftolozane-tazobactam plus metronidazole and meropenem treatment arms, respectively. All subjects in the ceftolozane-tazobactam plus metronidazole treatment arm who were clinical cures at the TOC visit and had an LFU assessment were sustained clinical cures at the LFU visit, illustrating the durability of the treatment effect. Similarly, a high percentage of subjects in the meropenem arm were sustained clinical cures at LFU.

Reviewer's Comment
Microbiological responses in cIAI closely matched clinical responses because most microbiological eradications are presumed from the clinical response, given the absence of specimens to culture.

Response by Baseline Infecting Pathogen
In both indications, efficacy was demonstrated against common pathogens in both treatment arms. The tables below provide summaries of the microbiological and clinical response at the TOC visit by baseline infecting pathogen in the ME population for the cUTI and cIAI indications, respectively. In the cUTI indication, ceftolozane-tazobactam was superior to levofloxacin in terms of microbiological eradication rates against the most common uropathogen, *E. coli*.
Ceftolozane-tazobactam also showed activity (per pathogen eradication rates) against *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*, comparing favorably with levofloxacin. Ceftolozane-tazobactam had higher microbiological eradication rates against characterized ESBL-positive uropathogens compared with levofloxacin, including *E. coli* and *K. pneumoniae* producing CTX-M-14 or CTX-M-15, the most common ESBLs identified in this study. Ceftolozane-tazobactam achieved superior microbiological eradication rates in the ME population compared with levofloxacin against levofloxacin-resistant *E. coli* (eradication rates of 73% for ceftolozane-tazobactam and 44% for levofloxacin) and also compared favorably against levofloxacin-resistant *K. pneumoniae* (82% versus 30%) and *P. aeruginosa* (100% versus 38%).

Table 67: cUTI Indication: Per Pathogen Microbiologic Eradication Rates in the ME Population at the TOC Visit

Organism Group Pathogen	Ceftolozane/Tazobactam (N=341) n/N1[a] (%)	Levofloxacin (N=353) n/N1[a] (%)
Aerobic Gram-Negative	287/323 (88.9)	263/340 (77.4)
Escherichia coli	237/262 (90.5)	226/284 (79.6)
Escherichia coli (ESBL producers)	27/36 (75.0)	18/36 (50.0)
Escherichia coli (CTX-M-14/15 producers)[b]	20/27 (74.1)	13/25 (52.0)
Klebsiella pneumoniae	21/25 (84.0)	14/23 (60.9)
Klebsiella pneumoniae (ESBL producers)	7/10 (70.0)	2/7 (28.6)
Klebsiella pneumoniae (CTX-M-15 producers)[b]	5/8 (62.5)	1/4 (25.0)
Proteus mirabilis	10/10 (100)	8/11 (72.7)
Pseudomonas aeruginosa	6/7 (85.7)	7/12 (58.3)

ESBL=extended spectrum β-lactamase
[a] n=Number of subjects with pathogens eradicated or presumed eradicated; N1=Number of subjects with the specified baseline pathogen or group.
[b] CTX-M-14/15 includes CTX-M-14, CTX-M-15, and CTX-M-15-like enzymes. CTX-M-15 includes CTX-M-15 and CTX-M-15-like enzymes.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.12.2 and Table 14.2.28.2

The Analysis populations described in the submission by the Applicant, for the cIAI study, were as follows:
Intent-to-Treat (ITT): The ITT population consisted of all randomized subjects regardless of whether or not the subjects went on to receive study drug. Subjects in the ITT population were categorized based on the treatment that the subjects were randomized to, irrespective of what they actually received.
Microbiological Intent-to-treat (MITT): The MITT population consisted of all randomized subjects who had IAI as evidenced by identification of at least 1 baseline intra-abdominal pathogen identified, regardless of susceptibility to study drug.
Clinically Evaluable (CE): The CE population was a subset of the ITT population of subjects who received an adequate amount of study drug, met the protocol-specific disease definition of cIAI, adhered to study procedures, and had a TOC visit within the specified visit window.
Microbiologically Evaluable (ME): The ME population was the subset of the CE subjects who had at least 1 baseline infecting intra-abdominal pathogen identified that was susceptible to study drug.
Expanded Microbiologically Evaluable (ME): The expanded ME consisted of all subjects in the MITT population who met all CE population criteria.
Safety: The safety population included all subjects who received any amount of study drug. Subjects in the Safety population were categorized based on the actual treatment that the subjects received, irrespective of the treatment to which they were randomized.

The analysis populations described by the Applicant, for the cUTI study, were as follows:
Intent-to-Treat (ITT): All randomized subjects regardless of whether or not the subjects went on to receive study drug.
Modified Intent-to-Treat (MITT): All randomized subjects who received any amount of study drug.
Microbiological Modified Intent-to-Treat (mMITT): A subset of the MITT that included subjects who had at least 1 qualified uropathogen from a study-qualifying pretreatment baseline urine specimen.
Clinically Evaluable at Test-of-Cure (CE at TOC): A subset of the mMITT population who adhered to study procedures and had a TOC visit within the specified visit window. All subjects in the CE at TOC population had to have an evaluable clinical outcome.
Microbiologically Evaluable at Test-of-Cure (ME at TOC): A subset of the CE at TOC population who adhered to study procedures and had an appropriately collected urine culture specimen and interpretable urine culture result at the TOC visit.
Clinically Evaluable at Late Follow-Up (CE at LFU): A subset of the CE at TOC population and included all subjects who were clinical cures at the TOC visit, and had an LFU assessment, 28 to 35 days (expanded to 21 to 42 days) after the last dose of study medication, (or were classified as a clinical failure after the TOC visit but prior to the LFU visit).
Microbiologically Evaluable at Late Follow-Up (ME at LFU): A subset of the ME at TOC population and included all subjects who were microbiological successes at the

TOC visit, and had an LFU assessment 28 to 35 days (expanded to 21 to 42 days) after the last dose of study medication, (or were classified as a microbiological failure after the TOC visit but prior to the LFU visit).
Safety: All subjects who received any amount of the study drug. Subjects in the Safety population were categorized based on the actual treatment that the subjects received, irrespective of the treatment to which they were randomized.

Table 68: Per-Pathogen Microbiologic Eradication Rates (Outcomes) at the TOC Visit (ME at TOC Population)

Baseline Pathogen Category Organism Group Pathogen Response	Ceftolozane/ Tazobactam (N=341) n (%)	Levofloxacin (N=353) n (%)	% Difference (95% CI)
Gram-Negative Aerobes			
Gram-Negative Aerobes	N1=323	N1=340	
Eradication	287 (88.9)	263 (77.4)	11.5 (5.82, 17.10)
Persistence	36 (11.1)	77 (22.6)	
Enterobacteriaceae	N1=316	N1=327	
Eradication	281 (88.9)	255 (78.0)	10.9 (5.22, 16.61)[a]
Persistence	35 (11.1)	72 (22.0)	
Escherichia coli	N1=262	N1=284	
Eradication	237 (90.5)	226 (79.6)	10.9 (4.91, 16.77)[a]
Persistence	25 (9.5)	58 (20.4)	
Escherichia coli (ESBL Producers)	N1=36	N1=36	
Eradication	27 (75.0)	18 (50.0)	Not available
Persistence	9 (25.0)	18 (50.0)	
Escherichia coli (CTX-M-14/15 Producers)[b]	N1=27	N1=25	
Eradication	20 (74.1)	13 (52.0)	Not available
Persistence	7 (25.9)	12 (48.0)	
Klebsiella pneumoniae	N1=25	N1=23	
Eradication	21 (84.0)	14 (60.9)	23.1 (-2.09, 45.39)
Persistence	4 (16.0)	9 (39.1)	
Klebsiella pneumoniae (ESBL Producers)	N1=10	N1=7	
Eradication	7 (70.0)	2 (28.6)	Not available
Persistence	3 (30.0)	5 (71.4)	
Klebsiella pneumoniae (CTX-M-14/15 Producers)[b]	N1=8	N1=4	
Eradication	5 (62.5)	1 (25.0)	Not available
Persistence	3 (37.5)	3 (75.0)	
Proteus mirabilis	N1=10	N1=11	
Eradication	10 (100)	8 (72.7)	27.3 (-5.55, 56.56)
Persistence	0	3 (27.3)	

Baseline Pathogen Category Organism Group Pathogen Response	Ceftolozane/ Tazobactam (N=341) n (%)	Levofloxacin (N=353) n (%)	% Difference (95% CI)
Pseudomonas aeruginosa	N1=7	N1=12	
Eradication	6 (85.7)	7 (58.3)	27.4 (-15.86, 56.25)
Persistence	1 (14.3)	5 (41.7)	
Enterobacter cloacae	N1=6	N1=7	
Eradication	2 (33.3)	6 (85.7)	-52.4 (-78.78, -0.27)
Persistence	4 (66.7)	1 (14.3)	
Gram-Positive Aerobes			
Gram-Positive Aerobes	N1=21	N1=20	
Eradication	8 (38.1)	16 (80.0)	-41.9 (-62.96, -11.76)
Persistence	13 (61.9)	4 (20.0)	
Enterococcus faecalis	N1=16	N1=16	
Eradication	5 (31.3)	12 (75.0)	-43.8 (-66.37, -9.21)
Persistence	11 (68.8)	4 (25.0)	
Enterococcus faecium	N1=2	N1=3	
Eradication	1 (50.0)	3 (100)	-50.0 (-90.55, 19.26)
Persistence	1 (50.0)	0	

CI = Confidence interval (based on Wilson score); ESBL = Extended spectrum β-lactamase; ME = Microbiologically evaluable; TOC = Test-of-cure.
[a] Ceftolozane/tazobactam was superior to levofloxacin based on a 99% CI (M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.12.2a).
[b] CTX is a subset among all ESBL producers (M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.28.2).
Notes: n=Number of subjects in specific category; N=Number of subjects in population; N1=Number of subjects with baseline pathogen category/pathogen or N1=Number of subjects with the specified ESBL status for the specified pathogen. Percentages are calculated as 100 x (n/N1).
Subjects are counted in the worst response category within baseline pathogen category and organism group.
Eradication and presumed eradication count as eradication.
All ESBL producer includes any enzyme. All CTXM14-15 includes CTX-M-14, CTX-M-15, CTX-M-15-like.
All CTXM14-15 is a subgroup of all ESBL producers. No ESBL = All negative ESBL.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.12.2 and Table 14.2.28.2.

In the cIAI indication, ceftolozane-tazobactam plus metronidazole demonstrated high clinical cure rates against common intra-abdominal pathogens, including *E. coli, K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *K. oxytoca*, *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus, S. anginosus*, *S. constellatus,* and *S. salivarius* (see table below). Among gram-negative aerobes, clinical cure rates for *E. coli* were 95% and 94% in the ceftolozane-tazobactam plus metronidazole and meropenem arms, respectively, for *K. pneumoniae*, 93% and 88%, respectively, and for *P. aeruginosa*, 100% and 93%, respectively. Ceftolozane-tazobactam plus metronidazole had similar clinical cure rates to meropenem against characterized ESBL-positive intra-abdominal pathogens, including *E. coli* and *K. pneumoniae* producing CTX-M-14 or CTX-M-15.

Table 69: cIAI Indication: Per Pathogen Clinical Cure Rates in the Expanded ME Population at the TOC Visit

Organism Group Pathogen	Ceftolozane/Tazobactam + Metronidazole (N=307) n/N1[a] (%)	Meropenem (N=345) n/N1[a] (%)
Gram-negative Aerobes	238/252 (94.4)	273/291 (93.8)
Escherichia coli	197/208 (94.7)	216/231 (93.5)
Escherichia coli (ESBL-producing)	14/14 (100)	18/20 (90.0)
Escherichia coli (CTX-M-14/15)[b]	9/9 (100)	7/9 (77.8)
Klebsiella pneumoniae	28/30 (93.3)	22/25 (88.0)
Klebsiella pneumoniae (ESBL-producing)	7/8 (87.5)	3/4 (75.0)
Klebsiella pneumoniae (CTX-M-15)[b]	5/5 (100)	0/1 (0)
Pseudomonas aeruginosa	26/26 (100)	27/29 (93.1)
Enterobacter cloacae	19/22 (86.4)	22/22 (100)
Klebsiella oxytoca	12/12 (100)	21/22 (95.5)
Gram-positive Aerobes	153/168 (91.1)	170/185 (91.9)
Streptococcus anginosus	25/30 (83.3)	23/23 (100)
Streptococcus constellatus	17/18 (94.4)	20/23 (87.0)
Streptococcus salivarius	9/10 (90.0)	8/8 (100)
Gram-negative Anaerobes	104/109 (95.4)	132/137 (96.4)
Bacteroides fragilis	39/41 (95.1)	56/57 (98.2)
Bacteroides ovatus	36/37 (97.3)	42/42 (100)
Bacteroides thetaiotaomicron	20/20 (100)	40/43 (93.0)
Bacteroides vulgatus	12/13 (92.3)	21/22 (95.5)

cIAI=complicated intra-abdominal infection; ESBL=extended spectrum β-lactamase; ME=microbiologically evaluable
Note: Expanded ME population includes all subjects in the MITT population who met all clinically evaluable criteria, irrespective of baseline pathogen susceptibility to study drugs.
[a] n=Number of subjects with pathogens eradicated or presumed eradicated; N=number of subjects in the expanded ME population; N1=Number of subjects with specified baseline pathogen or group.
[b] CTX-M-14/15 includes CTX-M-14, CTX-M-15, and CTX-M-15-like enzymes. CTX-M-15 includes CTX-M-15 and CTX-M-15-like enzymes.
Source: M5.3.5.3\ISM\Table 9.3.1

An integrated analysis of clinical cure rates by pathogen across the Phase 3 studies for *E. coli* and *K. pneumoniae*, as well as the ESBL-producing isolates is provided in the table below. Across the Phase 3 studies, clinical cure rates for ESBL-producing *E. coli* and *K. pneumoniae* were 98% and 94%, respectively, for ceftolozane-tazobactam and 88% and 73%, respectively, for the combined comparators. Clinical cure rates for *P. aeruginosa* in the integrated analysis were 100% (33 of 33 isolates) for ceftolozane-tazobactam and 93% (38 of 41) for the combined comparators.

Table: 70: Clinical Cure Rates for ESBL-Producing Pathogens in the Phase 3 Clinical Trials (ME Population)

Pathogen	Ceftolozane/Tazobactam (N=648) n/N1[a] (%)	All Comparators (N=698) n/N1[a] (%)
Escherichia coli	452/470 (96.2)	483/515 (93.8)
Escherichia coli (ESBL-producing)	49/50 (98.0)	48/56 (87.5)
Escherichia coli (CTX-M-14/15)[b]	35/36 (97.2)	28/34 (82.4)
Klebsiella pneumoniae	51/55 (92.7)	41/48 (85.4)
Klebsiella pneumoniae (ESBL-producing)	17/18 (94.4)	8/11 (72.7)
Klebsiella pneumoniae (CTX-M-15)[b]	13/13 (100)	2/5 (40.0)

ESBL=extended spectrum β-lactamase; ME=microbiologically evaluable
[a] n=Number of subjects with pathogens eradicated or presumed eradicated; N=number of subjects in the ME population; N1=Number of subjects with specific baseline pathogen or group.
[b] CTX-M-14/15 includes CTX-M-14, CTX-M-15, and CTX-M-15-like enzymes. CTX-M-15 includes CTX-M-15 and CTX-M-15-like enzymes.
Source: M5.3.5.3\ISM\Table 9.3.1

Emergent Infections

The occurrence of emergent infections was uncommon in both indications. The incidence of emergent infections in subjects with cUTI was comparable in the 2 treatment arms. Overall, superinfections were recorded in only 3.5% in the ceftolozane-tazobactam arm and 5.2% in the levofloxacin arm. In the context of cUTI, where subjects frequently have risk factors predisposing to recurrent infections, the incidence of new infections was low; 9.0% and 6.7% of subjects in the ceftolozane-tazobactam and levofloxacin arms, respectively.

Similarly, in the cIAI indication, superinfections and new infections were uncommon in each treatment arm and largely comprised organisms intrinsically resistant to ceftolozane-tazobactam (*Enterococcus* spp.). In the MITT population, superinfections were detected in 2.6% and 3.1% of subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem arms, respectively. Likewise, new infections developed in only 3.1% and 2.2% of subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively.

In the cUTI study, emergence of decreased susceptibility and frank resistance to therapy was rare in subjects who received ceftolozane-tazobactam, but was more common among subjects who received levofloxacin.

Reviewer's Comment

Two (0.5%) of the persisting pathogens included *E. coli* and *P. aeruginosa*. One isolate of each pathogen developed resistance to ceftolozane-tazobactam among the 51 microbiological failures in the ceftolozane-tazobactam treatment arm. There was 1 *E. coli* isolate (Subject ID: 1005-5309-008) that had an MIC value shift of 0.5 mcg/mL at baseline to a MIC of 64 mcg/mL at TOC, and 1 *P. aeruginosa* isolate (Subject ID: 1005-5104-015) that had an MIC value shift from 16 mcg/mL to >64 mcg/mL. Levofloxacin-

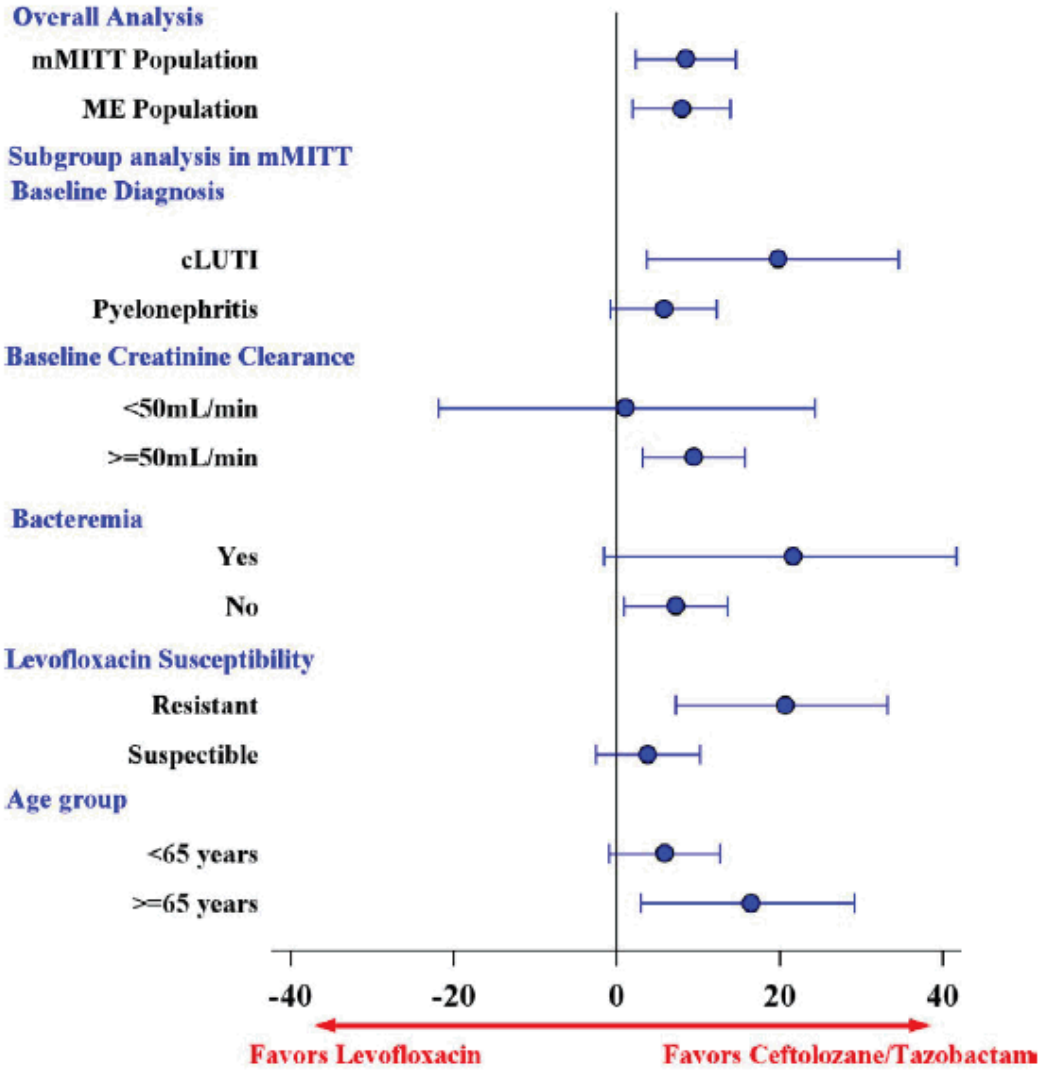
resistance on therapy developed in 16 (4.0%) of the levofloxacin-treated subjects, and included 15 *E. coli* isolates and one *Enterococcus faecalis*.

In the cIAI indication, emergence of decreased susceptibility or resistance to the study drugs was not detected in either treatment arm.

Sensitivity Analyses
In the cUTI study, several pre-planned sensitivity analyses for the primary endpoint were performed in the mMITT and ME populations controlling for: protocol and region, baseline diagnosis, and region alone. Sensitivity analyses between ceftolozane-tazobactam and levofloxacin were consistent with the primary and key secondary efficacy outcomes, indicating the robustness of the key efficacy findings. Similarly in the cIAI study, several pre-planned sensitivity analyses were performed on the MITT and ME populations; these included an analysis using a data-as-observed approach and an unistratified analysis. In all analyses, the results were consistent with the primary and key secondary analyses, indicating the robustness of the key efficacy findings.

Efficacy Results in Subgroups
The composite response rate in the ceftolozane-tazobactam arm compared favorably with levofloxacin in all high-risk subgroups, including subjects with complicated lower urinary tract infection (cLUTI), renal impairment, bacteremia at baseline, levofloxacin-resistant uropathogens, and the elderly, and in some cases showed superiority. Further, ceftolozane-tazobactam was efficacious in the treatment of cUTI across geographic regions with results generally consistent with the primary outcome and comparing favorably with levofloxacin. Composite outcomes favored levofloxacin slightly in North American subjects; however, results were interpreted with caution given the wide 95% CI around the treatment difference.

Figure 13: cUTI Indication: Differences (95% CI) Between Treatment Arms in Composite By-Subject Microbiological and Clinical Response Rates at TOC in the mMITT and ME Populations Overall and in Key Subgroup in the mMITT Population



CI=confidence interval; cLUTI=complicated lower urinary tract infection; cUTI=complicated urinary tract infection; ME=microbiologically evaluable; mMITT=microbiological modified intent-to-treat; TOC=test of cure
Notes: for the overall analysis 95% stratified Newcombe CI is presented; for the subgroup analysis, 95% Wilson score is presented. For mMITT population, a treatment failure approach was used where indeterminate responses were imputed as failure; for ME population a data-as-observed approach was used.
Source: M2.7.3 cUTI\In-text Figure 3

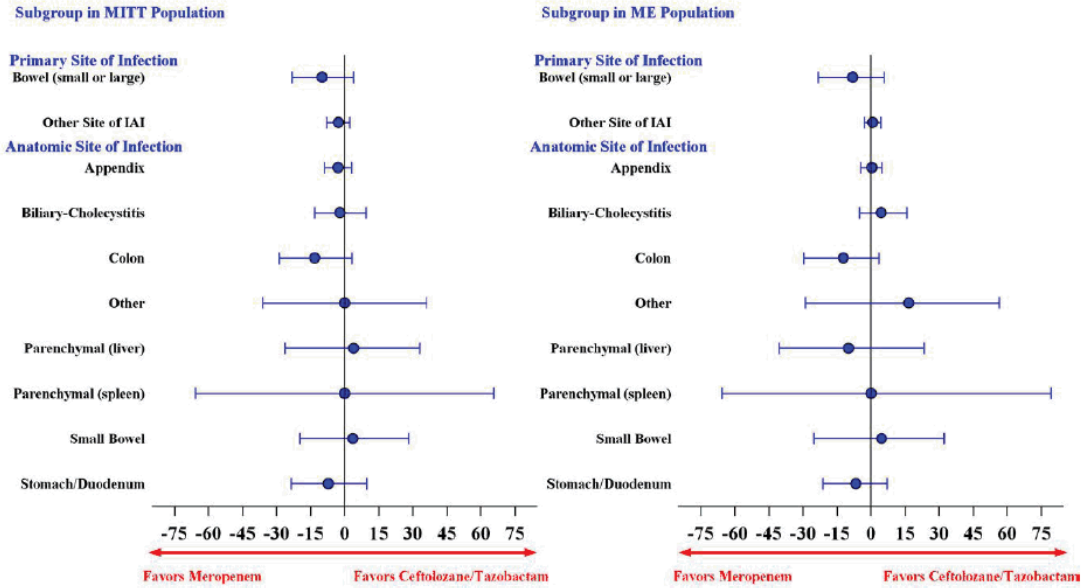
Plots which showed differences in clinical cure rates in the cIAI indication were presented. In the subgroups analyzed, results were generally consistent with the primary and key secondary efficacy analyses, demonstrating the consistency and robustness of the results. As expected, in both treatment arms, clinical cure rates were lower in high-risk

subjects (e.g., the elderly, those with elevated baseline APACHE II scores, and those with baseline renal impairment) and those with disease characteristics historically linked to poorer outcomes (e.g., infections originating in the small or large bowel), while clinical cure rates were marginally higher in subjects with infections originating in the appendix compared with overall. In most subgroups, clinical responses were similar between the 2 treatment arms in the MITT and ME populations. However, in the MITT population, differences (≥10%) were observed in favor of meropenem in the elderly (age ≥65 years), subjects with moderate renal impairment, subjects with an APACHE II score of ≥10, and subjects with an infection originating in the colon.

The treatment difference by age was not consistent, as clinical cure rates in subjects >75 years of age were similar between the 2 treatment arms (70% versus 73% for ceftolozane-tazobactam plus metronidazole versus meropenem, respectively) demonstrating lack of trend by age. In addition, outcomes in the elderly favored ceftolozane-tazobactam in the cUTI study. These findings suggest the poorer outcome in subjects specifically between 65 and 75 years in the cIAI indication may be a chance occurrence.

In cIAI, the numbers of subjects with a missing or indeterminate clinical response assessment were disproportionately greater in subgroup analyses. The impact of this imbalance can be appreciated by reviewing the same analyses in the ME population, where the point estimates for the difference in clinical cure rates were closer to zero in all of the subgroups. In the ME population, clinical cure rates were comparable across treatment arms with efficacy rates of >80% in most subgroups. Thus, evidence regarding decreased treatment effect in specific subgroups in the cIAI indication was not conclusive or consistent with findings in cUTI. Overall clinical cure rates in the cIAI indication by treatment arm were similar to those in the 2 highest enrolling regions, Eastern Europe and South America. Clinical cure rates in other regions (North America, Rest of World, and Western Europe) were lower compared with Eastern Europe and South America with wider CIs due to the relatively smaller sample sizes.

Figure 14: cIAI Indication: Summary of the Difference (95% CI) Between Treatment Arms in Clinical Response Rates at TOC by Site of infection in the MITT and ME Populations



CI=confidence interval; cIAI=complicated intra-abdominal infection; IAI=intra-abdominal infection; ME=microbiologically evaluable; MITT=microbiological intent-to-treat; TOC=test of cure
Notes: the 95% CI of the difference (ceftolozane/tazobactam + metronidazole – meropenem) was calculated using Wilson score. A subject can have more than 1 anatomic site of infection. For MITT population, a treatment failure approach was used where indeterminate responses were imputed as failure; for ME population a data-as-observed approach was used.
Source: M2.7.3 cIAI\In-text Figure 6 and Figure 7

Table 71: Summary and Analysis of Clinical Response at the Test-of-Cure Visit by Study (MITT and ME Populations; Studies CXA-cIAI-10-08 and CXA-cIAI-10-09)

Protocol	Clinical Response	MITT			ME		
		Ceftolozane/ Tazobactam + Metronidazole n (%)	Meropenem n (%)	% Difference[a] (95% CI)	Ceftolozane/ Tazobactam + Metronidazole n (%)	Meropenem n (%))	% Difference[a] (95% CI)
CXA-cIAI-10-08		N=198	N=213		N=150	N=167	
	Cure	170 (85.9)	188 (88.3)	-2.4 (-9.04, 4.12)	142 (94.7)	158 (94.6)	0.1 (-5.40, 5.29)
	Failure	15 (7.6)	16 (7.5)		8 (5.3)	9 (5.4)	
	Indeterminate	13 (6.6)	9 (4.2)		NA	NA	
CXA-cIAI-10-09		N=191	N=204		N=125	N=154	
	Cure	153 (80.1)	176 (86.3)	-6.2 (-13.60, 1.22)	117 (93.6)	146 (94.8)	-1.2 (-7.46, 4.45)
	Failure	17 (8.9)	18 (8.8)		8 (6.4)	8 (5.2)	
	Indeterminate	21 (11.0)	10 (4.9)		NA	NA	

CI=confidence interval, ME=microbiologically evaluable, MITT=microbiological intent-to-treat; N=number of subjects in population; n=number of subjects in specific category; NA=not applicable.
[a] The 95% CI of the difference of (ceftolozane/tazobactam plus metronidazole) - meropenem is calculated as a Wilson Score CI.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.2.2.4 and Table 14.2.2.2.19

Comparison Between Phase 2 and Phase 3 Efficacy Results

Although not powered for efficacy, the results of the Phase 2 cUTI and cIAI studies support the findings of the Phase 3 studies. Efficacy in the Phase 2 and Phase 3 cUTI studies at the TOC visit in the mMITT population was similar, with clinical and

microbiological cure rates of 90.8% and 83.1%, respectively, for ceftolozane, and 92.1% and 76.3%, respectively, for the comparator, ceftazidime. Similarly, efficacy results for ceftolozane-tazobactam plus metronidazole were similar in the Phase 2 and Phase 3 cIAI studies with clinical cure rates at the TOC visit of 83.0% in Phase 2 and 83.6% in Phase 3. The observed response to meropenem at the TOC visit of the Phase 2 study was higher than in the Phase 3 study (96.0% versus 87.0%, respectively).

Quality Control Analysis for Clinical Studies

Clinical Trial Quality Control

During the Phase 3 CXA-cUTI-10-04 and -05 and CXA-cIAI-10-08 and -09 studies, all aerobic pathogens and the *B. fragilis* group anaerobic clinical isolates were tested at a central laboratory, (b) (4) All other anaerobes were tested at (b) (4) At (b) (4), each isolate was identified and susceptibility testing was done by broth microdilution according to CLSI document M7-A9 [36].

Quality control testing was performed using the appropriate ATCC organism(s) on each day that clinical isolates were tested. For MIC testing of all aerobic organisms, dried-form reference panels prepared by (b) (4) were used. For the *B. fragilis* group (*B. caccae, B. cellulosilyticus, B. clarus, B. coprocola, B. coprophilus, B. dorei, B. eggerthii, B. faecis, B. finegoldii, B. fluxus, B. fragilis, B. intestinalis, B. massiliensis, B. nordii, B. oleiciplenus, B. ovatus, B. plebeius, B. salyersiae, B. stercoris, B. thetaiotaomicron, B. uniformis, B. vulgatus, B. xylanisolvens*) frozen panels prepared by (b) (4) were utilized. For all other anaerobes, agar dilution was performed at (b) (4) according to CLSI document M11-A7. Disk diffusion was used for all aerobic pathogens using a ceftolozane-tazobactam 30/10 mcg disk produced by Mast Group Ltd (Bootle UK). Disk QC ranges for comparator antibacterials were based on CLSI document M100-S22. All QC isolates were within the CLSI approved QC ranges for ceftolozane-tazobactam.

Reviewer's Comment

All aerobic pathogens, the *B. fragilis* group, and anaerobic clinical isolates were tested at a central laboratory, (b) (4). All other anaerobes were tested at (b) (4) (b) (4). The Applicant stated that methods by the Clinical and laboratory Standards Institute (CLSI) were used at the (b) (4), and that each isolate was identified and susceptibility testing was done.

Quality Control for Disk Diffusion Susceptibility Testing

A series of studies were performed to select the amounts of ceftolozane and tazobactam for Kirby-Bauer disks, to correlate inhibition zones with MIC values, and to establish QC ranges for the disk diffusion susceptibility test.

Disk Diffusion Quality Control results
A study to establish the inhibition zone diameter QC ranges of ceftolozane-tazobactam 30 mcg/10 mcg Kirby-Bauer disks was conducted in 8 laboratories. Three different commercial sources of agar media and 2 different manufacturers of susceptibility disks were utilized, as prescribed by CLSI document M23-A3 [CLSI, 2008]. Inhibition zone ranges were determined for CLSI reference strains using methodology according to CLSI M02-A10 [CLSI, 2009]. The approved QC limits included at least 98.5% of valid inhibition zones for each reference strain and growth medium tested.

(b) (4)), the central microbiology laboratory for CXAcUTI-10-04 and -05 and CXA-cIAI-10-08 and -09 studies, performed QC testing concurrent with subject isolate testing. All QC testing data were within the approved QC ranges for ceftolozane-tazobactam and levofloxacin. Out of 869 results, there was 1 meropenem result that was 1 mm below the CLSI approved QC ranges for meropenem; all other data points were within QC.

***E. coli* ATCC 25922**
The *E. coli* ATCC 25922 testing for ceftolozane-tazobactam has a proposed QC range of 24 to 32 mm. The QC zone diameters for ceftolozane-tazobactam at the central laboratory (513 results) were 100% within this range. The modal ceftolozane-tazobactam zone size is 27 mm. All results for QC testing, with all study antibacterials by disk diffusion, were 100% within the QC ranges for the respective antibacterials.

***E. coli* ATCC 35218**
The *E. coli* ATCC 35218 testing for ceftolozane-tazobactam has a proposed QC range of 25 to 31 mm. The QC zone diameters for ceftolozane-tazobactam at the central laboratory (513 results) were 100% within this range. The modal ceftolozane-tazobactam zone size is 27 mm. All results for QC testing, with all study antibacterials by disk diffusion, were 100% within the QC ranges for the respective antibacterials.

***P. aeruginosa* ATCC 27853**
The *P. aeruginosa* ATCC 27853 testing for ceftolozane-tazobactam has a proposed QC range of 25 to 31 mm. The QC zone diameters for ceftolozane-tazobactam at the central laboratory (513 results) were 100% within this range. The modal ceftolozane-tazobactam zone size is 26 mm. All results for QC testing, with ceftolozane-tazobactam by disk diffusion, were 100% within the QC ranges for the respective antibacterials. Out of 231 results, there was 1 meropenem result that was 1 mm below the CLSI approved QC ranges for meropenem, all other data points were within QC.

***S. pneumoniae* ATCC 49619**
The *S. pneumoniae* ATCC 49619 testing for ceftolozane-tazobactam has a proposed QC range of 21 to 29 mm. The QC zone diameters for ceftolozane-tazobactam at the central laboratory (181 results) were 100% within this range. The modal ceftolozane-tazobactam

zone size is 22 mm. All results for QC testing, with all study antibacterials by disk diffusion, were 100% within the QC ranges for the respective antibiotic.

***S. aureus* ATCC 25923**
The *S. aureus* ATCC 25923 testing for ceftolozane-tazobactam has a proposed QC range of 10 to 18 mm. The QC zone diameters for ceftolozane-tazobactam at the central laboratory (512 results) were 100% within this range. The modal ceftolozane-tazobactam zone size is 11 mm. All results for QC testing, with all study antibacterials by disk diffusion, were 100% within the QC ranges for the respective antibacterials.

Overall, the modal QC results were generally left of the middle of the CLSI approved QC range for each appropriate QC strains. These data will continue to be monitored to determine if future changes in QC ranges are warranted.

Reviewer's Comment
One hundred percent of the zone diameter QC results for ceftolozane-tazobactam and levofloxacin were in range for the CXA-cUTI-10-04 and -05, and CXA-cIAI-10-08 and -09 studies. Out of 869 results, there was 1 meropenem result that was 1 mm below the CLSI approved QC ranges for meropenem; all other data points were within QC.

Determination of Appropriate Kirby-Bauer Disk Mass
A study was performed and the Kirby-Bauer disk masses selected in accordance with the CLSI M23-A3 guidelines. Kirby-Bauer disk inhibition zones were first determined, in duplicate, for ceftolozane alone against a panel of susceptible and resistant bacterial isolates, including 5 ATCC strains used for QC in CLSI methodology, 15 clinical isolates of different species, and 25 isolates of *P. aeruginosa* with different antibiotic-resistance profiles. Kirby-Bauer disks containing 10, 30, and 50 mcg of ceftolozane were prepared. The inhibition zones obtained, which were similar for the 3 disk potencies, were evaluated against the broth microdilution MIC values obtained with the same isolates. Standard, commercially prepared 30-mcg Kirby-Bauer disks of ceftazidime were tested in parallel. The 30-mcg disk load, a common cephalosporin disk load, was chosen for ceftolozane because it produced excellent correspondence between the zone sizes and the MIC values for the isolates tested (correlation coefficient 0.87). The correlation coefficients were similar for the other 2-disk masses tested. Representative supporting data, including ceftolozane susceptible and nonsusceptible strains, are summarized in the table below.

Table 72: Evaluation of Different Kirby-Bauer Disk Loads of Ceftolozane

Species	Isolate	Inhibition Zone (mm)			MIC (µg/mL)
		10-µg disk	30-µg disk	50-µg disk	
S. aureus	ATCC 25923	8/8[a]	12/12	13/13	16
E. coli	ATCC 25922	25/25	27/27	29/29	0.25
P. aeruginosa	ATCC 27853	24/25	28/28	29/30	0.5
H. influenzae	ATCC 49247	21/22	25/25	28/28	1
S. pneumoniae	ATCC 49619	20/20	25/25	27/27	0.5
S. aureus	2014183	6/6	10/10	13/13	32
S. aureus	2014184	6/6	6/6	6/6	>64
S. pneumoniae	2014179	29/29	32/32	34/35	0.12
S. pneumoniae	2014180	18/18	23/24	25/26	4
S. pyogenes	2014181	21/21	23/24	25/25	0.12
H. influenzae	2014182	28/27	30/30	32/31	0.12
E. coli	2014193	6/6	6/6	6/6	>64
E. coli	2015983	8/8	13/13	14/14	32
K. pneumoniae	2014185	6/7	12/12	14/14	64
K. pneumoniae	2014186	15/14	20/20	23/23	8

Table 73: Evaluation of Different Kirby-Bauer Disk Loads of Ceftozolane (Continued)

Species	Isolate	Inhibition Zone (mm)			MIC (µg/mL)
		10-µg disk	30-µg disk	50-µg disk	
P. aeruginosa	2014187	24/24	28/28	31/31	0.5
P. aeruginosa	2014188	15/14	21/21	25/25	8
P. aeruginosa	2014189	25/25	27/27	31/31	0.5
P. aeruginosa	2014190	27/27	30/30	32/32	0.5
P. aeruginosa	2014191	16/16	20/21	24/24	8

ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration
Note: Testing was conducted according to CLSI methodology [27,28,29].
[a] Inhibition zones for duplicate disks.
Source: M5.3.5.4\CXA101-M-006

After selection of the 30-mcg Kirby-Bauer disk mass for ceftolozane, this load was tested in combination with 5, 10, and 20 mcg tazobactam per disk. ESBL-positive and ESBL-negative strains of *E. coli* and *K. pneumoniae* were included in the test panel. Good correlation between zone diameter and MIC value was observed for all categories of isolates tested with correlation coefficients of 0.84 for ceftolozane-tazobactam 30/5 mcg, 0.72 for ceftolozane-tazobactam 30/10 mcg, and 0.79 for ceftolozane-tazobactam 30/20 mcg Kirby-Bauer disks. For tazobactam, 10-mcg/disk was chosen, the same content as in the standard piperacillin/tazobactam disk used in the United States.

Therefore, the final disk mass selected was 30 mcg ceftolozane and 10 mcg tazobactam per disk. An independent study, conducted by the European Union Committee on Antimicrobial Susceptibility Testing [EUCAST, 2013] Laboratory for Antimicrobial Susceptibility Testing, supported the use of the 30 mcg/10 mcg (ceftolozane-tazobactam) disk mass for susceptibility testing in countries that follow EUCAST methodology. In addition to the QC strains, a number of clinical isolates were tested. The mean inhibition zones for each species obtained with the ceftolozane-tazobactam disk and an appropriate cephalosporin comparator were determined. The mean zone diameter for the ceftolozane-tazobactam 30/10 mcg Kirby-Bauer disk ranged from 22-28 mm, making the disk easy to read and in line with zone diameters of other cephalosporin disks which ranged from 17-34 mm.

Table 74: CLSI Approved Quality Control Limits for Susceptibility testing of Ceftolozane-Tazobactam with Kirby-Bauer Disk Diffusion Methodology

Bacterial Strain	Inhibition Zone Diameter (mm)	Percent Within Range
Escherichia coli ATCC 25922	24-32	99.2
Escherichia coli ATCC 35218	24-32	98.5
Pseudomonas aeruginosa ATCC 27853	25-31	99.8
Klebsiella pneumoniae ATCC 700603[a]	17-25	99.8
Haemophilus influenzae ATCC 49247	23-29	99.4
Staphylococcus aureus ATCC 25923	8-18	99.2
Streptococcus pneumoniae ATCC 49619	21-29	98.8

ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute
The disks contained 30 µg of ceftolozane and 10 µg of tazobactam.
[a] This strain is not required for routine testing.
Source: M5.3.5.4\CXA.004.MC; M5.3.5.4\CXA.016.MC CLSI, 2012 [26].

Additionally, two strains utilized for QC by EUCAST were tested: *S. aureus* ATCC 29213 and *Haemophilus influenzae* National Collection of Type Cultures (NCTC) 8468. The proposed inhibition zone ranges are in the table below.

Table 75: CLSI Approved Quality Control Limits for Kirby-Bauer Disk Diffusion Susceptibility Testing of Ceftolozane-Tazobactam with the EUCAST Quality Control Pathogens

Bacterial Strain	Inhibition Zone Diameter (mm)	Percent Within Range
Haemophilus influenzae NCTC 8468[a]	23-29	99.1
Staphylococcus aureus ATCC 29213	7-17	96.3

ATCC=American Type Culture Collection; EUCAST=European Union Committee on Antimicrobial Susceptibility Testing; NCTC=National Collection of Type Cultures
The disks contained 30 µg of ceftolozane and 10 µg of tazobactam.
[a] *H. influenzae* was tested on Mueller-Hinton agar supplemented with 5% horse blood and 20 µg/mL nicotinamide adenine dinucleotide (NAD).
Source: M5.3.5.4\CXA.004.MC

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Broth Microdilution Susceptibility Tests

(b) (4) the central microbiology laboratory for CXA-cUTI- 10-04 and -05 and CXA-cIAI-10-08 and -09 studies, performed QC testing concurrent with subject isolate testing. All QC testing data were within the approved QC ranges for each antimicrobial. Data for all the aerobic pathogens was collected using dried form microdilution panels prepared by (b) (4) which were validated according to standard validation methods (CXA.019.MC). *B. fragilis* group anaerobes were tested using frozen broth microdilution panels prepared by (b) (4)

***E. coli* ATCC 25922**
The modal MIC of ceftolozane-tazobactam was 0.25 mcg/mL, which is in the middle of the current QC range of 0.12 to 0.5 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

***E. coli* ATCC 35218**
The modal MIC of ceftolozane-tazobactam was 0.12 mcg/mL, which is in the middle of the current QC range of 0.06 to 0.25 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

***P. aeruginosa* ATCC 27853**
The modal MIC of ceftolozane-tazobactam was 1 mcg/mL, which is at the higher end of the current QC range of 0.25 to1 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

***S. pneumoniae* ATCC 49619**
The modal MIC of ceftolozane-tazobactam was 0.5 mcg/mL, which is in the middle of the current QC range of 0.25 to 1 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

***B. fragilis* ATCC 25285**
The modal MIC of ceftolozane-tazobactam was 0.12 mcg/mL, which is at the lower end of the current QC range of 0.12 to 1 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

Table 76: Phase 3 Ceftolozane-Tazobactam Broth Microdilution QC Results

Quality Control Organism	N	Minimum Inhibitory Concentration (µg/mL)							
		0.03	0.06	0.12	0.25	0.5	1	2	4
***E. coli* ATCC 25922**	430			3	369	58			
CXA-cUTI-10-04 CXA-cUTI-10-05	249			1	205	43			
CXA-cIAI-10-08 CXA-cIAI-10-09	181			2	164	15			
***E. coli* ATCC 35218**	430		1	359	70				
CXA-cUTI-10-04 CXA-cUTI-10-05	249		1	203	45				
CXA-cIAI-10-08 CXA-cIAI-10-09	181		--	156	25				
***P. aeruginosa* ATCC 27853**	430				7	177	246		
CXA-cUTI-10-04 CXA-cUTI-10-05	249				--	101	148		
CXA-cIAI-10-08 CXA-cIAI-10-09	181				7	76	98		
***S. pneumoniae* ATCC 49619**									
CXA-cIAI-10-08 CXA-cIAI-10-09	178				7	136	35		
***B. fragilis* ATCC 25285**									
CXA-cIAI-10-08 CXA-cIAI-10-09	26			18	6	2			

ATCC=American Type Culture Collection; QC=quality control
Note: Shaded cells represent CLSI-approved QC range. All organisms were within QC range. Dash (--)=no isolates at this value.
Source: M5.3.5.4\CXA.062.MC and M5.3.5.4\CXA.063.MC

***S. aureus* ATCC 29213**

The modal MIC of ceftolozane-tazobactam was 32 mcg/mL, which is in the middle of the current QC range of 16 to 64 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; 100% of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

***B. thetaiotaomicron* ATCC 29741 (broth)**

The MICs of ceftolozane-tazobactam were evenly distributed between 16 and 32 mcg/mL, which is the lower to middle portion of the current QC range of 16 to 64 mcg/mL. The current range encompassed 100% of the ceftolozane-tazobactam values reported; One hundred percent of the levofloxacin and meropenem MIC values also fell within the established QC ranges for these agents.

Table 77: Phase 3 Ceftolozane-Tazobactam Broth Microdilution QC Results for *S. aureus* and *B. thetaiotaomicron*

Quality Control Organism	N	Minimum Inhibitory Concentrations (µg/mL)						
		4	8	16	32	64	128	>128
S. aureus ATCC 29213	275			13	246	16		
CXA-cUTI-10-04 CXA-cUTI-10-05	128			1	124	3		
CXA-cIAI-10-08 CXA-cIAI-10-09	147			12	122	13		
B. thetaiotaomicron ATCC 29741 (broth)								
CXA-cIAI-10-08 CXA-cIAI-10-09	26			13	13	-		

ATCC=American Type Culture Collection; QC=quality control
Note: Shaded cells represent CLSI-approved QC range. All organisms were within QC range.
Source: M5.3.5.4\CXA.062.MC and M5.3.5.4\CXA.063.MC

Summary
Overall, the MIC QC results were generally in the middle of the CLSI approved QC range for the appropriate QC strains. The only exception was the *P. aeruginosa* ATCC 27853 strain which ran at the higher end of the QC range.

Reviewer's Comment
One hundred percent of the MIC QC results for all QC strains tested were in range for the CXA-cUTI-10-04 and -05 and CXA-cIAI-10-08 and -09 studies.

Quality Control for Broth Dilution (MIC) Methods
A study to establish the MIC quality control (QC) ranges for ceftolozane-tazobactam was conducted in 8 laboratories. Broth microdilution ranges were determined for CLSI reference strains of Enterobacteriaceae, *P. aeruginosa,* and other species of aerobic bacteria commonly used in susceptibility testing methods. Both broth and agar dilution QC ranges were determined for reference strains of anaerobic bacteria. The study was conducted according to CLSI M23-A3 methodology [CLSI, 2008]. As shown in the table below, the approved QC limits included at least 98% of valid MIC values for each reference strain and growth medium tested. Another study, conducted using the same CLSI methodology, determined the QC limits of ceftolozane-tazobactam for broth microdilution testing of *S. pneumoniae* American Type Culture Collection (ATCC) 49619. A laboratory (Lab #5) was identified as a statistical outlier for the mean, median and mode and was thus excluded from further analysis. With Lab #5 excluded, 100% of MIC determinations were within this range.

Table 78: CLSI-Approved Control Limits for Susceptibility Testing of Ceftolozane-Tazobactam with Broth Microdilution and Agar Dilution Methodology

Bacterial Strain		Number of strains tested by all laboratories	MIC Range (µg/mL ceftolozane with 4 µg/mL tazobactam)	Percent (%) of MICs Within Range
			Broth Microdilution	
Escherichia coli	ATCC 25922	240	0.12-0.5	99.6
Escherichia coli	ATCC 35218	240	0.06-0.25	100
Pseudomonas aeruginosa	ATCC 27853	240	0.25-1	100
Klebsiella pneumoniae [a]	ATCC 700603	240	0.5-2	100
Haemophilus influenzae	ATCC 49247	240	0.5-2	100
Staphylococcus aureus	ATCC 29213	240	16-64	100
Streptococcus pneumoniae	ATCC 49619	210	0.25-1	100
Bacteroides fragilis	ATCC 25285	210	0.12-1	99.5
Bacteroides thetaiotaomicron	ATCC 29741	240	16-64	98.3
			Agar Dilution	
Bacteroides fragilis	ATCC 25285	480	0.12-1	100
Bacteroides thetaiotaomicron	ATCC 29741	470	16-128	100

ATCC=American Type Culture Collection; CLSI=Clinical and Laboratory Standards Institute; MIC=minimum inhibitory concentration
Note: Broth microdilution MIC determinations were performed utilizing *Haemophilus* test medium (HTM) broth for *H. influenzae*, Brucella broth supplemented with hemin, vitamin K1, and 5% lysed horse blood (LHB) for anaerobes, and cation-adjusted Mueller-Hinton broth (CAMHB) for the other organisms. Agar dilution testing of the *Bacteroides spp.* strains was performed using Brucella agar supplemented with hemin, vitamin K1, and 5% laked sheep blood under anaerobic conditions.
[a] This strain is not required for routine testing.
Sources: M5.3.5.4\CXA.003.MC; M5.3.5.4\CXA.016.MC; CLSI, 2012 [26].

Correlation of Provisional Interpretive Criteria with Clinical Outcome

Primary Endpoint Outcomes versus MIC and Zone Diameter

In the sections that follow, the primary endpoint outcome of favorable microbiological response and clinical response are discussed by pathogen, MIC value, and zone diameters.

Pathogen Eradication, Outcome by MIC and Outcome by Zone Diameter Analysis for cUTI

The primary efficacy endpoint for the cUTI study was the composite microbiological and clinical cure rates at the TOC visit. The primary analysis was based on the mMITT population and the key secondary analysis was based on the ME at TOC population. Ceftolozane-tazobactam achieved noninferiority compared to levofloxacin for the primary efficacy variable in the treatment of adult subjects with cUTI (including pyelonephritis) (see table below). In addition, ceftolozane-tazobactam demonstrated statistical superiority over levofloxacin in terms of the composite endpoint, as evidenced

by the lower bound of the 2-sided 95% CI around the treatment differences excluding zero in both the primary and secondary analysis populations.

Table 79: Primary and Key Secondary Analysis: Composite Microbiological and Clinical Response Rate at TOC Visit by Population in cUTI

Analysis	Response	Ceftolozane/ Tazobactam n (%)	Levofloxacin n (%)	% Difference (95% CI)	% Difference (99% CI)[a]
Primary Analysis[b]		N=398	N=402		
mMITT	Success	306 (76.9)	275 (68.4)	8.5 (2.31, 14.57)[c]	8.5 (0.36, 16.46)[c]
	Failure	66 (16.6)	103 (25.6)		
	Indeterminate	26 (6.5)	24 (6.0)		
Secondary Analysis[b]		N=341	N=353		
ME at TOC	Success	284 (83.3)	266 (75.4)	8.0 (1.95, 13.97)[d]	8.0 (0.01, 15.84)[d]
	Failure	57 (16.7)	87 (24.6)		

CI=confidence interval (based on stratified NewCombe); cUTI=complicated urinary tract infection; ME at TOC=microbiologically evaluable at test of cure; mMITT=microbiological modified intent-to-treat.
[a] 99% CI per Food and Drug Administration's request for determination of superiority from a single study.
[b] Stratified by region.
[c] Treatment Failure Approach, indeterminate is classified as failure (refer to M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Section 9.7.1.1.3 for details).
[d] Data-as-Observed, indeterminate is excluded from analysis (refer to M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Section 9.7.1.1.3 for details).
Notes: n=Number of subjects in specific category. N=Number of subjects in specified population. Percentages are calculated as 100 x (n/N).
Success: Per-subject microbiological response is microbiological success and the clinical response is clinical cure.
Failure: Per-subject microbiological response is microbiological failure or the clinical response is clinical failure.
Indeterminate: Per-subject microbiological response is non-evaluable and/or the clinical response is indeterminate.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.2a, Table 14.2.2 and Table 14.2.5.

Pathogen Eradication Rates (cUTI)

The table below presents the microbiologic eradication rates by baseline uropathogen at the TOC visit for the ME at TOC population for the most common cUTI pathogens. Overall, ceftolozane-tazobactam was efficacious at eradicating gram-negative pathogens causing cUTI. The highest microbiological eradication rates were noted for ceftolozane-tazobactam against *E. coli*, and these were superior to the levofloxacin eradication rates. The 95% confidence interval (CI) did not include zero for *E. coli*, indicating ceftolozane-tazobactam had superior microbiological eradication rates compared with levofloxacin against this pathogen. Of the Enterobacteriaceae, the lowest microbiological eradication rates were noted for *E. cloacae*. Ceftolozane-tazobactam also compared favorably to levofloxacin against *P. aeruginosa* with eradications rates of 85.7% and 58.3%, respectively.

As previously reported, enterococcal isolates are inherently resistant to cephalosporins; as a consequence low per-pathogen eradication rates against *E. faecalis* and *E. faecium* were observed for ceftolozane-tazobactam. The results in the mMITT population were similar to the ME at TOC population. The microbiological eradication rates were higher for both treatment arms in both the mMITT and ME at TOC populations at EOT compared to the TOC visit.

Table 80: Per-Pathogen Microbiologic Rates (Outcomes) at the TOC Visit (ME at TOC Population) in cUTI

Baseline Pathogen Category	Ceftolozane/ Tazobactam (N=341)		Levofloxacin (N=353)		% Difference (95% CI)
	n/N1	(%)	n/N1	(%)	
Gram-Negative Aerobes	287/323	88.9	263/340	77.4	11.5 (5.82, 17.10)
Enterobacteriaceae	281/316	88.9	255/327	78.0	10.9 (5.22, 16.61)
Enterobacter cloacae	2/6	33.3	6/7	85.7	-52.4 (-78.78, -0.27)
Escherichia coli	237/262	90.5	226/284	79.6	10.9 (4.91, 16.77)
Escherichia coli (CTX-M-14/15)	20/27	74.1	13/25	52.0	Not available
Klebsiella pneumoniae	21/25	84.0	14/23	60.9	23.1 (-2.09, 45.39)
Klebsiella pneumoniae (CTX-M-14/15)	5/8	62.5	1/4	25.0	Not available
Proteus mirabilis	10/10	100	8/11	72.7	27.3 (-5.55, 56.56)
Pseudomonas aeruginosa	6/7	85.7	7/12	58.3	27.4 (-15.86, 56.25)
Gram-Positive Aerobes	8/21	38.1	16/20	80.0	-41.9 (-62.96, -11.76)
Enterococcus faecalis	5/16	31.3	12/16	75.0	-43.8 (-66.37, -9.21)
Enterococcus faecium	1/2	50.0	3/3	100	-50.0 (-90.55, 19.26)

CFU=colony-forming unit; CI=confidence interval (based on Wilson score); cUTI=complicated urinary tract infection; ME=microbiologically evaluable; n=number of subjects in specific category; N=number of subjects in population; N1=number of subjects with baseline pathogen category/pathogen; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N1).
Subjects are counted in the worst response category within baseline pathogen category and organism group.
Eradication and presumed eradication count as eradication.
Indeterminate: No interpretable urine culture available, and no previous urine culture (after at least 3 days of study drug administration) that is negative (no growth) or $< 10^4$ CFU/mL colony count.
All CTX-M-14/15 includes CTX-M-14, CTX-M-15, CTX-M-15-like. All CTX-M14/15 is a subgroup of all ESBL producers.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.12.2 and Table 14.2.28.2.

Ceftolozane-tazobactam achieved significantly higher microbiological eradication rates than levofloxacin against levofloxacin-resistant Enterobacteriaceae, including levofloxacin-resistant *E. coli,* and levofloxacin-resistant *K. pneumoniae* (see table below). Against the small number of *E. cloacae*, the ceftolozane-tazobactam eradication rate was 33%. The results in the mMITT population were similar to the ME at TOC population. In the mMITT population, ceftolozane-tazobactam achieved eradication rates of 71.2%, 69.2%, and 75% for levofloxacin-resistant *E. coli*, *K. pneumoniae*, and *P. aeruginosa,* respectively. The equivalent microbiological eradication rates for levofloxacin were 41%, 36.4%, and 37.5%, respectively.

Table 81: Per Pathogen Microbiologic Eradication Rates (Outcomes) of Levofloxacin-resistant Pathogens at the TOC Visit (ME and TOC Population) in cUTI

Baseline Pathogen Category	Ceftolozane/ Tazobactam (N=341)		Levofloxacin (N=353)		% Difference (95% CI)
	n/N1	%	n/N1	%	
Gram-Negative Aerobes	58/80	72.5	41/92	44.6	27.9 (13.21, 40.91)
Enterobacteriaceae	55/77	71.4	38/84	45.2	26.2 (10.96, 39.72)
Enterobacter cloacae	1/5	20.0	2/2	100	-80.0 (-96.38, -1.73)
Escherichia coli	43/59	72.9	30/68	44.1	28.8 (11.59, 43.55)
Klebsiella pneumoniae	9/11	81.8	3/10	30.0	51.8 (9.50, 75.05)
Proteus mirabilis	0	-	2/2	100	Not Calculated
Pseudomonas aeruginosa	3/3	100	3/8	37.5	62.5 (-2.09, 86.32)
Gram-Positive Aerobes	2/11	18.2	6/10	60.0	-41.8 (-68.42, -0.63)
Enterococcus faecalis	1/9	11.1	4/8	50.0	-38.9 (-68.79, 4.24)
Enterococcus faecium	1/2	50.0	2/2	100	-50.0 (-90.55, 27.26)

CFU=colony-forming unit; CI=confidence interval (based on Wilson score); cUTI=complicated urinary tract infection; ME= microbiologically evaluable; n=number of subjects in specific category; N=number of subjects in population; N1=number of subjects with baseline pathogen category/pathogen; TOC=Test of cure.
Notes: Percentages are calculated as 100 x (n/N1).
Subjects are counted in the worst response category within baseline pathogen category and organism group.
Eradication and presumed eradication count as eradication.
Indeterminate: No interpretable urine culture available, and no previous urine culture (after at least 3 days of study drug administration) that is negative (no growth) or < 10^4 CFU/mL colony count.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.12.2.1.

Ceftolozane-tazobactam had higher clinical cure and microbiological eradication rates against characterized ESBL-positive isolates compared to levofloxacin in the mMITT and ME at TOC populations. Clinical cure rates for ceftolozane-tazobactam for *E. coli* and *K. pneumoniae* producing CTX-M-14 or CTX-M-15 were 87% and 90% respectively. The equivalent clinical cure rates for levofloxacin were 71% and 40%, respectively. In the levofloxacin treatment arm, more than 50% of isolates with a levofloxacin MIC ≥4 mcg/mL were not eradicated. Thus, despite the high dose of levofloxacin used in this study and high urinary concentrations, resistance to levofloxacin was a predictor of composite failure with levofloxacin therapy. Failure rates for levofloxacin-resistant pathogens compared with overall results were also lower with ceftolozane-tazobactam therapy, despite most isolates being susceptible to ceftolozane-tazobactam. This likely reflects that levofloxacin resistance is an indicator of subjects at particular risk of recurrent UTI.

Reviewer's Comment
In the levofloxacin treatment arm, more than 50% of isolates with a levofloxacin MIC ≥4 mcg/mL were not eradicated.

Table 82: Summary of Per-Pathogen Clinical Cure by ESBL Status of Baseline pathogen at TOC Visit (mMITT Population) in cUTI

Baseline Pathogen ESBL Status	Ceftolozane/ Tazobactam (N=398)		Levofloxacin (N=402)	
	n/N1	%	n/N1	%
Escherichia coli				
All ESBL Producers	36/41	87.8	32/43	74.4
All CTX-M14/15	27/31	87.1	22/31	71.0
No ESBL	4/4	100	2/2	100
Klebsiella pneumoniae				
All ESBL Producers	11/12	91.7	5/8	62.5
All CTX-M-14/15	9/10	90.0	2/5	40.0
No ESBL	0	-	0	-

cUTI=complicated urinary tract infection; ESBL=extended spectrum β-lactamase; mMITT=microbiologically modified Intent-Treat; n=number of subjects in specific category; N=number of subjects in mMITT Population; N1=number of subjects with the specified ESBL status for the specified pathogen; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N1).
All ESBL producer includes any enzyme. All CTX-M14-15 includes CTX-M-14, CTX-M-15, CTX-M-15-like. All CTX-M14-15 is a subgroup of all ESBL producers. No ESBL=All negative ESBL.
Only pathogens with molecular testing complete at database lock are included in the table.
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.26.1.

In total, 4 *P. aeruginosa* overexpressed AmpC and the 1 ceftolozane-tazobactam-treated subject with *P. aeruginosa* AmpC overexpression was a composite cure at the TOC visit.

Correlation of MIC Values With Clinical and Microbiological Response in the cUTI Study

Results in the mMITT population were similar to the ME at TOC population.
The ceftolozane-tazobactam MIC values ranged from 0.0625 mcg/mL to >64 mcg/mL in the ceftolozane-tazobactam treated subjects and in the comparator-treated subjects. The clinical success and microbiological cure rates were high in the ceftolozane-tazobactam arm for pathogens with ceftolozane-tazobactam MIC values ≤8 mcg/mL. In both treatment arms, clinical response rates were slightly higher than the microbiological response rates, which is expected in this indication and reflective of the common occurrence of asymptomatic bacteriuria at the TOC visit [Nicolle, 2005]. The fact that all 46 subjects with asymptomatic bacteriuria in the ceftolozane-tazobactam treatment arm did not require nonstudy antibiotic treatment through the LFU visit indicates that these microbiological failures were not clinically meaningful, but rather that positive urine cultures at the TOC visit represent colonization.

Reviewer's Comment

Summaries for pathogen eradication and clinical cure for the key cUTI pathogens are listed below. The Applicant described information pertaining to clinical success and

microbiological outcome at two MIC cut-off values of ≤8 mcg/mL and ≥16 mcg/mL for all of the pathogens discussed.

Enterobacteriaceae (N=353)
• Ceftolozane-tazobactam MIC values ranged from 0.0625 mcg/mL to >64 mcg/mL in the ceftolozane-tazobactam-treated subjects with most of the isolates between an MIC of 0.125 to 2 mcg/mL (see table below).
• There was no apparent association between clinical cure or pathogen eradication rates and MIC values in the ceftolozane-tazobactam treatment arm. Success rates overall were high. The number of pathogens was small at higher MIC values.
• Clinical cure rates were high regardless of MIC values.
• The distribution of MIC values in the combined surveillance studies for the Enterobacteriaceae were similar to the MIC values obtained in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 94% for Enterobacteriaceae with MIC values ≤8 mcg/mL.
− Clinical success rates were 90% for Enterobacteriaceae with MIC values ≥16 mcg/mL.
− Microbiological eradication rates were 84.6% for Enterobacteriaceae with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 50% for Enterobacteriaceae with MIC values ≥16 mcg/mL, mostly accounted for by isolates with MICs >64 mcg/mL.

Table 83: Clinical Cure and Pathogen Eradication Rates at TOC by Ceftolozane-Tazobactam MIC value for Gram-negative Aerobes and Enterobacteriaceae from the Phase 3 cUTI Study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Gram-Negative Aerobes	0.0625->64	338/360	93.9	301/360	83.6
	0.0625	2/2	100	2/2	100
	0.125	83/85	97.6	73/85	85.9
	0.25	161/176	91.5	154/176	87.5
	0.5	53/56	94.6	45/56	80.4
	1	14/14	100	11/14	78.6
	2	8/8	100	5/8	62.5
	4	4/5	80.0	2/5	40.0
	8	3/ 3	100	2/3	66.7
	16	4/4	100	3/4	75.0
	32	0/1	0	0/1	0
	64	1/1	100	1/1	100
	>64	7/7	100	4/7	57.1
Enterobacteriaceae	0.0625->64	332/353	94.1	296/353	83.9
	0.0625	2/2	100	2/2	100
	0.125	83/85	97.6	73/85	85.9
	0.25	161/176	91.5	154/176	87.5
	0.5	51/54	94.4	44/54	81.5
	1	13/13	100	10/13	76.9
	2	8/8	100	5/8	62.5
	4	4/4	100	2/4	50.0
	8	3/3	100	2/3	66.7
	16	3/3	100	2/3	66.7
	32	0/1	0	0/1	0
	64	1/1	100	1/1	100
	>64	5/5	100	2/5	40.0

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations)

E. coli (N=292)

• Ceftolozane-tazobactam MIC values ranged from 0.0625 mcg/mL to >64 mcg/mL in the ceftolozane-tazobactam-treated subjects with the majority of isolates between an MIC of 0.125 to 1 mcg/mL (see table below).
• There was no apparent association between clinical cure or pathogen eradication rates and MIC values in the ceftolozane-tazobactam treatment arm. Success rates were high overall. The number of pathogens was small at higher MIC values.
• Clinical cure rates were high (>90%) for isolates at each MIC value.
• The distribution of MIC values in the combined surveillance studies for *E. coli* was similar to the MIC values obtained for *E. coli* in the clinical studies.
• Applying the provisional breakpoint of ≤ (b) (4)
− Clinical success rates were 93.8% for *E. coli* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% for *E. coli* with MIC values ≥16 mcg/mL.
− Microbiological eradication rates were 84.6% for *E. coli* with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 50% for *E. coli* with MIC values ≥16 mcg/mL.

E. coli CTX-M-14/15 (N=31)
• For the CTX-M-14/15 subpopulation of *E. coli,* the ceftolozane-tazobactam MIC values ranged from 0.25 mcg/mL to >64 mcg/mL in the ceftolozane-tazobactam-treated subjects with the majority of isolates between 0.25 mcg/mL and 1 mcg/mL (N=28) (see table below).
• High microbiological eradication rates and high clinical success rates were apparent at MIC values ≤ 1 mcg/mL, though there were only single isolates at MIC values ≥2 mcg/mL.

Table 84: Clinical Cure and Pathogen eradication Rates by TOC by Ceftolozane-Tazobactam MIC Value for *Escherichia coli* from the Phase 3 cUTI study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Escherichia coli	0.0625->64	274/292	93.8	248/292	84.9
	0.0625	2/2	100	2/2	100
	0.125	81/83	97.6	72/83	86.7
	0.25	146/160	91.3	139/160	86.9
	0.5	31/33	93.9	27/33	81.8
	1	8/8	100	6/8	75.0
	2	2/2	100	1/2	50.0
	4	1/1	100	0/1	0
	8	1/1	100	0/1	0
	>64	2/2	100	1/2	50.0
Escherichia coli (CTX-M)	0.25->64	27/31	87.1	21/31	67.7
	0.25	4/7	57.1	4/7	57.1
	0.5	15/16	93.8	13/16	81.3
	1	5/5	100	4/5	80
	2	1/1	100	0/1	0
	8	1/1	100	0/1	0
	>64	1/1	100	0/1	0

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations), M5.3.5.1\cUTI\CXA-cUTI-10-04 and-05\ Listings 16.2.6.1.2 and M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Listing 16.2.6.3

K. pneumoniae (N=30)
• Ceftolozane-tazobactam MIC values ranged from 0.25 mcg/mL to 64 mcg/mL in the Ceftolozane-tazobactam-treated subjects with the majority of isolates between an MIC of 0.25 to 8 mcg/mL (see table below).
• There was no apparent association between clinical cure or pathogen eradication rates and MIC values in the ceftolozane-tazobactam treatment arm. Success rates were generally high. The number of pathogens was small at each MIC value.
• The distribution of MIC values in the combined surveillance studies for the *K. pneumoniae* were similar to the MIC values obtained for *K. pneumoniae* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 96.3% for *K. pneumoniae* with MIC values ≤8 mcg/mL.
− Clinical success rates were 66.7% for *K. pneumoniae* with MIC values ≥16 mcg/mL.

− Microbiological eradication rates were 77.8% for *K. pneumoniae* with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 66.7% for *K. pneumoniae* with MIC values ≥16 mcg/mL.

K. pneumoniae CTX-M-14/15 (N=10)
• For the CTX-M-14/15 subpopulation of *K. pneumoniae*, the ceftolozane-tazobactam MIC values ranged from 0.25 mcg/mL to 32 mcg/mL in the ceftolozane-tazobactam treated subjects (see table below).
• Microbiological eradication and clinical cures were comparable at all of the MIC values, though there were generally only single isolates at each MIC value.
• Outcome by MIC was not predictive for CTX-M-14/15 *K. pneumoniae*. Presence or absence of CTX-M-14/15 does not appear to affect microbiological eradication or clinical cure rates.

Table 85: Clinical Cure and Pathogen Eradication Rates at TOC by Ceftolozane-Tazobactam MIC Value for *Klebsiella pneumoniae* from the Phase 3 UTI study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Klebsiella pneumoniae	0.25-64	28/30	93.3	23/30	76.7
	0.25	7/8	87.5	7/8	87.5
	0.5	9/9	100	6/9	66.7
	1	3/3	100	2/3	66.7
	2	4/4	100	3/4	75.0
	4	1/1	100	1/1	100
	8	2/2	100	2/2	100
	16	1/1	100	1/1	100
	32	0/1	0	0/1	0
	64	1/1	100	1/1	100
Klebsiella pneumoniae (CTX-M)	0.25-32	9/10	90	5/10	50
	0.25	2/2	100	1/2	50
	0.5	1/1	100	0/1	0
	1	1/1	100	0/1	0
	2	3/3	100	2/3	66.7
	8	1/1	100	1/1	100
	16	1/1	100	1/1	100
	32	0/1	0	0/1	0

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations), M5.3.5.1\cUTI\CXA-cUTI-10-04 and-05\ Listings 16.2.6.1.2 and M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Listing 16.2.6.3

P. mirabilis (N=12)
• Ceftolozane-tazobactam MIC values ranged from 0.25 mcg/mL to 0.5 mcg/mL in the Ceftolozane-tazobactam-treated subjects (see table below).
• There was no apparent association between clinical cure or pathogen eradication rates and MIC values in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at each MIC value.
• Clinical cure rates were high for isolates at each MIC value.
• Pathogen eradication rates in the ceftolozane-tazobactam treatment arm were 100% at

each MIC value.
• The distribution of MIC values in the combined surveillance studies for the *P. mirabilis* were similar to the MIC values obtained for *P. mirabilis* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 91.7% for *P. mirabilis* with MIC values ≤8 mcg/mL.
− Clinical success rates were not evaluated for *P. mirabilis* with MIC values ≥16 mcg/mL, since no *P. mirabilis* isolates had MIC values ≥16 mcg/mL.
− Microbiological eradication rates were 100% for *P. mirabilis* with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were not evaluated for *P. mirabilis* with MIC values ≥16 mcg/mL, since no *P. mirabilis* isolates had MIC values ≥16 mcg/mL.

Table 86: Clinical Cure and Pathogen Eradication Rates at TOC by Ceftolozane-Tazobactam MIC Value for *Proteus mirabilis* from the Phase 3 cUTI Study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Proteus mirabilis	0.25-0.5	11/12	91.7	12/12	100
	0.25	3/3	100	3/3	100
	0.5	8/9	88.9	9/9	100

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations)

P. aeruginosa (N=7)
• Ceftolozane-tazobactam MIC values ranged from 0.5 mcg/mL to ≥ 64 mcg/mL in the Ceftolozane-tazobactam-treated subjects (see table below).
• There was no apparent association between clinical cure or pathogen eradication rates and MIC values in the ceftolozane-tazobactam treatment arm. Overall 6/7 subjects with *P .aeruginosa* achieved clinical cure and 5/7 achieved microbiological eradication. The number of pathogens was small at each MIC value.
• The distribution of MIC values in the combined surveillance studies for the *P. aeruginosa* were more susceptible than the MIC values obtained for *P. aeruginosa* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 75% for *P. aeruginosa* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% *P. aeruginosa* with MIC values ≥16 mcg/mL.
− Microbiological eradication rates were 100% for *P. aeruginosa* with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 100% for *P. aeruginosa* with MIC values ≥16 mcg/mL.

Table 87: Clinical Cure and Pathogen Eradication Rates at TOC by Ceftolozane-tazobactam MIC Value for *Pseudomonas aeruginosa* from the Phase 3 cUTI Study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Pseudomonas aeruginosa	0.5->64	6/7	85.7	5/7	71.4
	0.5	2/2	100	1/2	50.0
	1	1/1	100	1/1	100
	4	0/1	0	0/1	0
	16	1/1	100	1/1	100
	>64	2/2	100	2/2	100

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations)

E. faecalis (N=22)
• Ceftolozane-tazobactam MIC values were ≥32 mcg/mL for the gram-positive cocci including *E. faecalis* (see table below).
• *E. faecalis* with MIC values ≥32 mcg/mL had poor microbiological eradication rates (33.3% eradication).
• Clinical cure rates were higher than the microbiological eradication rates for this population (83.3%).
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were not evaluated for *E. faecalis* with MIC values ≤8 mcg/mL, because no *E. faecalis* isolates had MIC values ≤8 mcg/mL.
− Clinical success rates were 83.3% for all *E. faecalis* with MIC values ≥16 mcg/mL.
− Microbiological eradication rates were not evaluated for *E. faecalis* with MIC values ≤8 mcg/mL because no *E. faecalis* isolates had MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 33.3% for *E. faecalis* with MIC values ≥16 mcg/mL.

Reviewer's Comment
E. faecalis had poor microbiological eradication rates, however, this this not one of the pathogens that the Applicant has listed in their indications.

Table 88: Clinical Cure and Pathogen Eradication Rates at TOC by Ceftolozane-Tazobactam MIC Value for Gram-Positive Aerobes and *Enterococcus faecalis* from the Phase 3 cUTI Study

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	Clinical Success		Microbiological Eradication	
		n/N1	%	n/N1	%
Gram-Positive Aerobes	32->64	17/22	77.3	9/22	40.9
	32	4/5	80.0	4/5	80.0
	64	6/6	100	4/6	66.7
	>64	8/12	66.7	2/12	16.7
Enterococcus faecalis	32->64	15/18	83.3	6/18	33.3
	32	2/2	100	1/2	50.0
	64	6/6	100	4/6	66.7
	>64	7/10	70.0	1/10	10.0

cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=number of subjects in specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.18.1 and Table 14.2.20.1 (mMITT populations)

Summary

Ceftolozane-tazobactam susceptibility (≤8 mcg/mL) was associated with high rates of microbiological eradication and clinical cure. Intrinsic resistance (MIC values ≥32 mcg/mL) to ceftolozane-tazobactam in the enterococci predicted microbiological persistence and, therefore, composite failure in subjects treated with ceftolozane-tazobactam. No label indication for *E. faecalis* is being requested. There was a better association of microbiological eradication and MIC values than clinical cure and MIC value.

Reviewer's Comment

The CTX-M-14/15-producing Enterobacteriaceae isolates fell within the wild-type MIC distribution. The number of isolates with higher MIC values was small, however, the provisional susceptibility breakpoint criteria of susceptible (b) (4) was generally predictive of higher clinical success rates and higher microbiological eradication rates than MIC values ≥16 mcg/mL.

Correlation of Kirby-Bauer Zone Diameter Values With Clinical and Microbiological Response in cUTI Study

Results in the mMITT population were similar to the ME at TOC population. The ceftolozane-tazobactam zone diameter sizes ranged from 6 to 35 mm in the ceftolozane-tazobactam-treated subjects and in the comparator-treated subjects. The clinical success and microbiological cure rates were high in the ceftolozane-tazobactam treatment arm for pathogens with ceftolozane-tazobactam zone diameters ≥ 19 mm. In both treatment arms, clinical response rates were higher than the microbiological response rates, which are expected in this indication and due to asymptomatic bacteriuria that was detected at the TOC visit [Nicolle, 2005]. No provisional susceptibility interpretive criteria were set for the disk diffusion assay; therefore, these data were evaluated only after regression

analysis was completed for the MIC to zone correlations from isolates from the Phase 3 program. Summaries for pathogen eradication and clinical cure for the key cUTI pathogens are listed below:

Reviewer's Comment
Disk Diffusion correlations are tentative since discussions are still ongoing within the Agency regarding MIC and disk diffusion breakpoints. A more complete discussion of disk diffusion correlation studies may be discussed in a subsequent review.

Enterobacteriaceae (N=353)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 35mm in the ceftolozane-tazobactam-treated subjects with the majority of isolates between 18 and 30 mm.
• The number of pathogens was small at both zone size extremes, making correlations with zone size and outcome difficult.
• Clinical cure rates and microbiological eradication rates were high for isolates at zone diameters ≥19 mm.
• Applying the zone diameter proposed breakpoints resulted in differentiation in microbiological eradication rates between susceptible (≥19 mm), indeterminate (16 to 18 mm) and resistant (≤15 mm) populations.

Reviewer's Comment
The number of Enterobacteriaceae pathogens was small at both zone size extremes, making correlations with zone size and outcome difficult.

E. coli (N=292)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 35 mm in the ceftolozane-tazobactam-treated subjects with the majority of isolates between 18 and 30 mm.
• The number of pathogens was small at both zone size extremes.
• Clinical cure rates and microbiological eradication rates were high for isolates at zone diameters ≥ 19 mm.
• Applying the zone diameter proposed breakpoints resulted in differentiation in microbiological eradication rates between susceptible (≥19 mm), indeterminate (16 to18 mm), and resistant (≤15 mm) populations.

E. coli CTX-M-14/15 (N=31)
• For the CTX-M-14/15 subpopulation of *E. coli,* the ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 28 mm in the ceftolozane-tazobactam-treated subjects.
• There was no apparent association between clinical cure or pathogen eradication rates and zone diameters in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at each zone size.

K. pneumoniae (N=30)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 8 to 29 mm in the ceftolozane-tazobactam-treated subjects with the majority of isolates between 18 and 30 mm.
• The number of pathogens was small at both zone size extremes.
• Clinical cure rates and microbiological eradication rates were high for isolates at each zone diameter value.
• Applying the zone diameter proposed breakpoints differentiated between susceptible and resistant populations.
K. pneumoniae CTX-M-14/15 (N=10)
• For the CTX-M-14/15 subpopulation of *K. pneumoniae*, the ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 12 to 22 mm in the Ceftolozane-tazobactam-treated subjects.
• There was no apparent association between clinical cure or pathogen eradication rates and zone diameters in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at each zone size.

P. mirabilis (N=12)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 10 to 29 mm in the ceftolozane-tazobactam-treated subjects.
• There was no apparent association between clinical cure and zone diameter in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at both zone size extremes.
• Clinical cure rates and microbiological eradication rates were high for isolates at each zone diameter value.
• Pathogen eradication rates in the ceftolozane-tazobactam treatment arm were 100%. Therefore, applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure or microbiological eradication rates between susceptible, intermediate and resistant populations.

P. aeruginosa (N=7)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 29 mm in the ceftolozane-tazobactam-treated subjects.
• There was no apparent association between clinical cure or pathogen eradication rates and zone diameters in the ceftolozane-tazobactam treatment arm.
• The number of pathogens was small at each zone diameter.
• Overall, 6/7 subjects with *P. aeruginosa* were a clinical cure and 5/7 were a microbiological eradication.
• Applying the zone diameter proposed breakpoints resulted in no differentiation in clinical cure or microbiological eradication rates between susceptible, intermediate, and resistant populations.

E. faecalis (N=18)
• Gram-positive cocci with ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 12 mm in the ceftolozane-tazobactam-treated subjects.
• *E. faecalis* with ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 12 mm in the ceftolozane-tazobactam-treated subjects.
• Zone diameters ≤12 mm had poor microbiological eradication rates (33.3% eradication).
• Clinical cure rates were higher (83.3%) and did not correlate well with zone diameters.
• Applying the zone diameter proposed breakpoints resulted in differentiation between microbiological eradication rates susceptible (≥16 mm), indeterminate (12 to 15 mm), and resistant (≤11 mm) populations.

Summary
Overall, among the pathogens tested, Ceftolozane-tazobactam zone sizes of ≥19 mm were associated with higher rates of microbiological eradication and clinical cure. Zone diameters ≤11 mm were associated with intrinsic resistance and lower microbiological success rates.

Pathogen Eradication, Outcome by MIC, and Outcome by Zone Diameter Analysis for Complicated Intra-abdominal Infections
The primary efficacy endpoint for the cIAI study was to demonstrate the noninferiority of ceftolozane-tazobactam plus metronidazole versus meropenem in adult subjects with cIAI based on the difference in clinical cure rates at the TOC visit (26 to 30 days after the initiation of study drug administration) in the Microbiological Intent-to-Treat (MITT) population. In the analysis of clinical response for the primary efficacy outcome measure, ceftolozane-tazobactam plus metronidazole met the statistical criteria for noninferiority to meropenem at the TOC visit in the MITT and ME populations. Ceftolozane-tazobactam plus metronidazole and meropenem achieved comparably high microbiological success rates across the different populations.

Reviewer's Comment
Based on the nature of the infection, few subjects had follow-up cultures taken and microbiological outcomes were mostly presumed. The results for pathogen eradication, outcome by MIC, and outcome by zone diameter were presented by clinical cure.

Table 89: Primary and Secondary Analysis for Noninferiority of Clinical Response at the TOC Visit (MITT and ME Populations) in the Phase 3 cIAI Study

Analysis	Clinical Response	Ceftolozane/ Tazobactam + Metronidazole n (%)	Meropenem n (%)	Percentage Difference[a] (95% CI)
Primary Analysis[b]		N=389	N=417	
MITT Population	Cure	323 (83.0)	364 (87.3)	-4.2 (-8.91, 0.54)
	Failure	32 (8.2)	34 (8.2)	
	Indeterminate	34 (8.7)	19 (4.6)	
Secondary Analysis[c]		N=275	N=321	
ME Population	Cure	259 (94.2)	304 (94.7)	-1.0 (-4.52, 2.59)
	Failure	16 (5.8)	17 (5.3)	

eCRF=electronic case report form; CI=confidence interval; cIAI=complicated intra-abdominal infection; N=number of subjects in the specified population; n=number of subjects in specific category; ME=microbiologically evaluable; MITT=microbiological intent-to-treat; TOC=test of cure.
[a] The 95% CI of the difference of (ceftolozane/tazobactam plus metronidazole) - meropenem is calculated as a 95% stratified Newcombe CI with Minimum Risk weights.
[b] Using a treatment failure approach, where indeterminate clinical responses are imputed as clinical failures.
The analysis is stratified by region and primary site of infection as recorded on the eCRF.
[c] Using a data-as-observed approach , the analysis is stratified by region and primary site of infection as recorded on the eCRF.
Note: Subjects from Site 1008-4024 and Site 1009-4227 are excluded from the analysis.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.2.1.1

Pathogen Eradication Rates (cIAI)

The table below presents the per-subject microbiological success rates at the TOC visit in the MITT, ME, and Expanded ME populations. Consistent with the clinical response findings at the TOC visit, due to limited number of subjects with a follow-up intra-abdominal culture, ceftolozane-tazobactam plus metronidazole and meropenem achieved comparably high microbiological success rates across the different populations.

Table 90: Per-Subject Microbiologic Success Rates at the TOC Visit in the Phase 3 cIAI Study (MITT, ME, and Expanded ME Populations)

Microbiological Response	Ceftolozane/Tazobactam + Metronidazole	Meropenem	Percent (%) Difference in Eradication Rate (95% CI)[a]
MITT Population			
	(N=389)	(N=417)	
Microbiological Success[b]	332 (85.3)	370 (88.7)	-3.4 (-8.09, 1.26)
Microbiological Failure[c]	25 (6.4)	28 (6.7)	
Indeterminate	32 (8.2)	19 (4.6)	
ME Population			
	(N=275)	(N=321)	
Microbiological Success[b]	264 (96.0)	307 (95.6)	0.4 (-3.13, 3.69)
Microbiological Failure[c]	11 (4.0)	14 (4.4)	
Expanded ME Population			
	(N=307)	(N=345)	
Microbiological Success[b]	293 (95.4)	326 (94.5)	0.9 (-2.59, 4.40)
Microbiological Failure[c]	14 (4.6)	19 (5.5)	

CI=confidence interval; cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MITT=modified intent-to-treat; n=number of subjects in specific category; N=number of subjects; Success=per-pathogen microbiological eradication for all baseline infecting pathogens; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
[a] The 95% CI of the difference of ceftolozane/tazobactam plus metronidazole - meropenem are calculated as Wilson Score CIs.
[b] Microbiological Success includes entries of "Eradication" and "Presumed Eradication".
[c] Microbiological Failure includes entries of "Persistence" and "Presumed Persistence".
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.4.1, Table 14.2.4.2, and Table 14.2.4.3

The table below presents the microbiologic eradication rates by baseline intra-abdominal pathogen at the TOC visit for the ME at TOC population for the most common cIAI pathogens. Overall, ceftolozane-tazobactam was efficacious at eradicating gram-negative pathogens causing cIAI. The highest microbiological eradication rates (100%) were noted for ceftolozane-tazobactam against *P. aeruginosa, K. pneumoniae* and *S. salivarius* and these were comparable to meropenem eradication rates. The 95% CI included zero for all pathogens except the *Staphylococcus* spp. group. Of the Enterobacteriaceae, the lowest microbiological eradication rates were noted for *E. cloacae* (85.7%). The CI intervals tended to be broad, reflecting the relatively small sample size of some of these subgroups of pathogens. The results in the MITT population were similar to the Expanded ME and ME populations.

Table 91: Per-Pathogen Microbiologic Eradication Rates (Outcomes) at the TOC Visit in the Phase 3 cIAI Study (ME Population)

Baseline Pathogen Category	Ceftolozane/ Tazobactam + Metronidazole (N=275)		Meropenem (N=321)		Percent (%) Difference (95% CI)
	n/N1	%	n/N1	%	
Organism Group	**Gram-Negative Aerobes**				
Gram-Negative Aerobes	234/243	96.3	269/282	95.4	0.9 (-2.80, 4.48)
Enterobacteriaceae	223/232	96.1	253/266	95.1	1.0 (-2.88, 4.77)
Enterobacter cloacae	18/21	85.7	22/22	100	-14.3 (-34.64, 3.25)
Escherichia coli	193/201	96.0	214/225	95.1	0.9 (-3.34, 5.05)
Klebsiella pneumoniae	28/28	100	22/25	88.0	12.0 (-2.38, 29.96)
Proteus mirabilis	10/11	90.9	9/10	90.0	0.9 (-28.89, 32.23)
Pseudomonas aeruginosa	25/25	100	28/28	100	0.0 (-13.32, 12.06)
	Gram-Positive Aerobes				
Gram-Positive Aerobes	131/141	92.9	158/167	94.6	-1.7 (-7.73, 3.84)
Enterococcus faecalis	28/32	87.5	33/35	94.3	-6.8 (-22.89, 8.14)
Enterococcus faecium	18/20	90.0	36/37	97.3	-7.3 (-27.52, 5.96)
Streptococcus anginosus	28/30	93.3	23/23	100	-6.7 (-21.32, 8.43)
Streptococcus constellatus	17/18	94.4	21/23	91.3	3.1 (-18.01, 21.81)
Streptococcus salivarius	10/10	100	8/8	100	0.0 (-27.75, 32.44)
	Gram-Negative Anaerobes				
Gram-Negative Anaerobes	107/109	98.2	134/137	97.8	0.4 (-4.48, 4.62)
Bacteroides fragilis	39/41	95.1	56/57	98.2	-3.1 (-14.48, 5.20)

CI=Confidence interval (based on Wilson score); cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; n=number of subjects in specific category; N=number of subjects in population; N1=number of subjects with baseline pathogen category/pathogen; TOC=test of cure
Notes: Percentages are calculated as 100 x (n/N1).
Subjects are counted in the worst response category within baseline pathogen category and organism group.
Eradication and presumed eradication count as eradication.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.3.2.

A total of 58 subjects in the MITT population had baseline intra-abdominal pathogens that were confirmed to be ESBL-positive, including 29 in each arm (7.5% versus 7.0% of subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively).

Of the 58 subjects with ESBL-positive pathogens, clinical cure rates were comparable across treatment arms with 25/29 (86.2%) versus 24/29 (82.8%) subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively. Clinical cure rates for subjects with *E. coli* with CTX-M-14 or CTX-M-15

ESBLs (with or without other ESBLs) were 9/11 (81.8%) and 8/11 (72.7%) for the ceftolozane-tazobactam plus metronidazole and meropenem treatment arms, respectively.

Table 92: Summary of By-Pathogen Clinical Cure by ESBL Status of Baseline Pathogen at TOC Visit in Phase 3 cIAI Study (MITT Population)

Baseline Pathogen ESBL Status	Ceftolozane/ Tazobactam + Metronidazole (N=389)		Meropenem (N=417)	
	n/N1	%	n/N1	%
Escherichia coli				
All ESBL Producer	14/16	87.5	19/23	82.6
All CTX-M-14/15	9/11	81.8	8/11	72.7
No ESBL	3/4	75.0	5/5	100
Klebsiella pneumoniae				
All ESBL Producer	8/9	88.9	3/4	75.0
All CTX-M-14/15	6/6	100	0/1	0
No ESBL	0	0	0	0

cIAI=complicated intra-abdominal infection; ESBL=Extended spectrum β-lactamase; MITT=modified intent-to-treat; n=number of subjects in specific category; N=number of subjects in MITT population; N1=number of subjects with the specified ESBL status for the specified pathogen; TOC=test of cure
Notes: Percentages are calculated as 100 x (n/N1).
All ESBL producer includes any enzyme. All CTX-M-14/15 includes CTX-M-14, CTX-M-15, CTX-M-15-like. All CTX-M-14/15 is a subgroup of all ESBL producers. No ESBL=All negative ESBL based on molecular characterization.
Only pathogens with molecular testing complete at database lock are included in the table.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.18.1

• A total of 8 subjects in the MITT population had baseline intra-abdominal *P. aeruginosa* overexpressing AmpC, including 5 and 3 subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively.
• Of the 8 subjects in the MITT population, clinical cure rates were comparable across treatment arms (80% versus 100% in ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively), but the low incidence of these pathogens limited the ability to draw any conclusions. The clinical cure rate was 100% in both treatment arms in the ME population.

Correlation of MIC Values With Clinical and Microbiological Response in cIAI
The results in the MITT population were similar to the expanded ME population. The ceftolozane-tazobactam MIC values ranged from 0.03 to 256 mcg/mL in the Ceftolozane-tazobactam-treated subjects and in the comparator-treated subjects. Overall, baseline resistance to ceftolozane-tazobactam or meropenem was rare among Gram-negative aerobic pathogens. In each analysis population, ceftolozane-tazobactam had MIC values of ≤2 mcg/mL against the vast majority of aerobic Gram-negative pathogens, with very few pathogens having an MIC of ≥16 mcg/mL. High cure rates were associated with ceftolozane-tazobactam MIC values ≤8 mcg/mL. Outcomes by MIC for anaerobes

were summarized, but the MIC values to ceftolozane-tazobactam may not have been relevant as the majority were susceptible to metronidazole. Summaries for pathogen eradication and clinical cure for the key baseline cIAI pathogens in the expanded ME population are listed below:

Enterobacteriaceae (N=233)

• Ceftolozane-tazobactam MIC values ranged from 0.06 to 128 mcg/mL in the ceftolozane-tazobactam plus metronidazole-treated subjects with the majority of values between 0.125 to 2 mcg/mL.
• The overall clinical cure rate was 94.0% for subjects in the ceftolozane-tazobactam plus metronidazole arm with Enterobacteriaceae at baseline.
• The number of pathogens was small at the higher MIC values.
• The distribution of MIC values in the combined surveillance studies for the Enterobacteriaceae were similar to the MIC values obtained in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 94.4% for Enterobacteriaceae with MIC values ≤8 mcg/mL.
− Clinical success rates were 85.7% Enterobacteriaceae with MIC values ≥16 mcg/mL.

Table 93: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for Enterobacteriaceae from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Enterobacteriaceae	0.06 - 128	219/233	94.0
	0.06	3/3	100
	0.125	79/81	97.5
	0.25	151/162	93.2
	0.5	48/51	94.1
	1	23/23	100
	2	9/11	81.8
	4	3/3	100
	8	3/4	75.0
	16	3/3	100
	64	2/3	66.7
	128	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

C. freundii (N=7)

• Ceftolozane-tazobactam MIC values ranged from 0.25 to 2 mcg/mL in the ceftolozane-tazobactam plus metronidazole-treated subjects.
• The overall clinical cure rate was 100% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *C. freundii* at baseline.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 100% for *C. freundii* with MIC values ≤8 mcg/mL.

− Clinical success rates were not evaluated for *C. freundii* with MIC values ≥16 mcg/mL, because no *C. freundii* isolates had MIC values ≥16 mcg/mL.

Table 94: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Citrobacter freudii* from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Citrobacter freundii	0.25 - 2	7/7	100
	0.25	5/5	100
	0.5	3/3	100
	1	1/1	100
	2	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

E. coli (N=200)
• Ceftolozane-tazobactam MIC values ranged from 0.06 to 128 mcg/mL in the ceftolozane-tazobactam plus metronidazole-treated subjects with the majority of isolates between 0.125 and 0.5 mcg/mL.
• The overall clinical cure rate was 94.5% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *E. coli* at baseline.
• Clinical cure rates were high at MIC values ≤128 mcg/mL; isolates at MIC values ≥0.5 mcg/mL were not common.
• The number of pathogens was small at higher MIC values making correlations between failure and higher MICs difficult.
• The distributions of MIC values in the combined surveillance studies for *E. coli* were similar to the MIC values obtained for *E. coli* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 95.2% for all *E. coli* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% for all *E. coli* with MIC values ≥16 mcg/mL.

E. coli CTX-M-14/15 (N=9)
• For the CTX-M-14/15 subpopulation of *E. coli,* the ceftolozane-tazobactam MIC values ranged from 0.25 to 4 mcg/mL in the ceftolozane-tazobactam- plus metronidazole treated subjects.
• All subjects with a CTX-M-14/15 positive *E. coli* were clinical cures.
• It is notable that the presence or absence of CTX-M-14/15 does not appear to correlate with any change in clinical cure rates compared with the general *E. coli* population.

Table 95: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Escherichia coli* From the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Escherichia coli	0.06 - 128	189/200	94.5
	0.06	2/2	100
	0.125	72/73	98.6
	0.25	125/135	92.6
	0.5	25/25	100
	1	8/8	100
	2	5/6	83.3
	4	1/1	100
	64	1/1	100
	128	1/1	100
Escherichia coli (CTX-M-14/15)	0.25 - 4	9/9	100
	0.25	2/2	100
	0.5	1/1	100
	1	3/3	100
	2	2/2	100
	4	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= mininum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2, M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Listing 16.2.6.2 and M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Listing 16.2.6.3

K. oxytoca (N=12)
• Ceftolozane-tazobactam MIC values ranged from 0.125 to 1 mcg/mL in the ceftolozane-tazobactam plus metronidazole- treated subjects with the majority of isolates at 0.25 mcg/mL.
• Overall clinical response was 100% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *K. oxytoca* at baseline.
• Clinical cure rates were high at MIC values ≤1 mcg/mL.
• The distribution of MIC values in the combined surveillance studies for *K. oxytoca* was similar to the MIC values obtained for *K. oxytoca* in the clinical studies.
• Applying the provisional breakpoint of [redacted] (b) (4):
− Clinical success rates were 100% for *K. oxytoca* with MIC values ≤8 mcg/mL.
− Clinical success rates were not evaluated for *K. oxytoca* with MIC values ≥16 mcg/mL, because no *K. oxytoca* isolates had MIC values ≥16 mcg/mL.

Table 96: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Klebsiella oxytoca* from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Klebsiella oxytoca	0.125 - 1	12/12	100
	0.125	4/4	100
	0.25	9/9	100
	0.5	1/1	100
	1	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

K. pneumoniae (N=28)
• Ceftolozane-tazobactam MIC values ranged from 0.06 to 64 mcg/mL in the ceftolozane-tazobactam plus metronidazole- treated subjects with the majority of isolates at an MIC of 0.25 mcg/mL.
• The number of pathogens was small at each MIC value.
• Overall clinical response was 92.9% for subjects in the ceftolozane-tazobactam plus metronidazole treatment arm with *K. pneumoniae* at baseline.
• Clinical cure rates were high at MIC values ≤8 mcg/mL; isolates at the higher MIC values were not common.
• The distribution of MIC values in the combined surveillance studies for the *K. pneumoniae* were similar to the MIC values obtained for *K. pneumoniae* in the clinical studies.
• Applying the provisional breakpoint of (b) (4)
− Clinical success rates were 96.4% for *K. pneumoniae* with MIC values ≤8 mcg/mL.
− Clinical success rates were 66.7% for *K. pneumoniae* with MIC values ≥16 mcg/mL.

K. pneumoniae CTX-M-14/15 (N=4)
• For the CTX-M-14/15 subpopulation of *K. pneumoniae,* the ceftolozane-tazobactam MIC values ranged from 1 to 16 mcg/mL in the ceftolozane-tazobactam-treated subjects.
• All subjects with a CTX-M-14/15 positive *K. pneumoniae* were clinical cure.
• High clinical cure rates were associated with MIC values ≤8 mcg/mL.
• The presence or absence of CTX-M-14/15 did not appear to correlate with any change in clinical cure rates.

Table 97: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Klebsiella pneumoniae* from the Phase 3 cIAI (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Klebsiella pneumoniae	0.06 - 64	26/28	92.9
	0.06	1/1	100
	0.25	15/16	93.8
	0.5	6/6	100
	1	2/2	100
	2	2/2	100
	8	1/1	100
	16	2/2	100
	64	0/1	0
Klebsiella pneumoniae (CTX-M-14/15)	1 - 16	4/4	100
	1	1/1	100
	2	1/1	100
	8	1/1	100
	16	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2 M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Listing 16.2.6.2 and M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Listing 16.2.6.3

P. mirabilis (N=11)
• Ceftolozane-tazobactam MIC values ranged from 0.25 to 16 mcg/mL in the ceftolozane-tazobactam plus metronidazole-treated subjects with the majority of isolates at 0.5 mcg/mL.
• Overall clinical response was 90.9% for subjects in the ceftolozane-tazobactam plus metronidazole treatment arm with *P. mirabilis* at baseline.
• The number of pathogens were small at most MIC values.
• Clinical cure rates were high at MIC values ≤8 mcg/mL; isolates at higher MIC values were not common.
• The distribution of MIC values in the combined surveillance studies for the *P. mirabilis* were similar to the MIC values obtained for *P. mirabilis* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 83.3% for *P. mirabilis* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% for *P. mirabilis* with MIC values ≥16 mcg/mL.

Table 98: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Proteus mirabilis* from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Proteus mirabilis	0.25 - 16	10/11	90.9
	0.25	0/1	0
	0.5	9/10	90.0
	1	1/1	100
	16	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

E. cloacae (N=22)
• Ceftolozane-tazobactam MIC values ranged from 0.125 to 64 mcg/mL in the ceftolozane-tazobactam plus metronidazole-treated subjects, with the majority of isolates between MIC values of 0.25 and 1 mcg/mL.
• Overall clinical cure rates were 86.4% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *E. cloacae* at baseline.
• Clinical cure rates were high at MIC values ≤8 mcg/mL; isolates at higher MIC values were not common.
• The number of pathogens was small at most MIC values.
• The distribution of MIC values in the combined surveillance studies for the *E. cloacae* were similar to the MIC values obtained for *E. cloacae* in the clinical studies.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 78.6% for all *E. cloacae* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% for all *E. cloacae* with MIC values ≥16 mcg/mL.

Table 99: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Enterobacter cloacae* From the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Enterobacter cloacae	0.125 - 64	19/22	86.4
	0.125	0/1	0
	0.25	9/10	90.0
	0.5	6/7	85.7
	1	4/4	100
	2	0/2	0
	4	2/ 2	100
	8	1/ 2	50.0
	16	1/ 1	100
	64	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

P. aeruginosa (N=24)
• Ceftolozane-tazobactam MIC values ranged from 0.03 to 4 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 0.5 and 1 mcg/mL.
• The overall clinical cure rate was 100% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *P. aeruginosa* at baseline.
• Clinical cure rates were high at MIC values ≤8 mcg/mL.
• Because of the high success rate, no association between clinical cure rates and MIC values could be made.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 100% for all *P. aeruginosa* with MIC values ≤8 mcg/mL.
− Clinical success rates were not evaluated for *P. aeruginosa* with MIC values ≥16 mcg/mL, because there were no *P. aeruginosa* isolates with MIC values ≥16 mcg/mL.

Table 100: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Pseudomonas aeruginosa* from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Pseudomonas aeruginosa	0.03 - 4	24/24	100
	0.03	1/1	100
	0.5	10/10	100
	1	13/13	100
	2	1/1	100
	4	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2 (expanded ME population)

Gram-negative Anaerobes (N=101)
• Ceftolozane-tazobactam MIC values ranged from 0.03 to 256 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects.

Reviewer's Comment
Outcomes by MIC for anaerobes are summarized, but the ceftolozane-tazobactam MIC may not be related to success as the majority of isolates were susceptible to metronidazole.

B. fragilis (N=40)
• Ceftolozane-tazobactam MIC values ranged from 0.06 to 32 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 0.125 to 1 mcg/mL.
• The overall clinical cure rate was 97.5% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *B. fragilis* at baseline.
• Clinical cure rates were high at MIC values ≤ 32 mcg/mL

Reviewer's Comment
Because of the high success rate for *B. fragilis*, no association between clinical cure rates and MIC values could be made.

Table 101: Clinical Cure Rates at TOC by Ceftolozane-Tazobactam MIC Values for *Bacteroides* spp. from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/ Tazobactam MIC (µg/mL)	n/N1	Percent (%)
Bacteroides fragilis	0.06 - 32	39/40	97.5
	0.06	2/2	100
	0.125	9/10	90.0
	0.25	13/13	100
	0.5	15/15	100
	1	7/7	100
	2	1/1	100
	4	1/1	100
	8	1/1	100
	32	1/1	100
Bacteroides ovatus	0.06 - 256	36/37	97.3
	0.06	1/1	100
	0.125	1/1	100
	0.5	3/3	100
	2	2/2	100
	4	6/6	100
	8	12/12	100
	16	7/7	100
	32	8/8	100
	64	7/7	100
	256	0/1	0
Bacteroides thetaiotaomicron	0.125 - 64	18/18	100
	0.125	1/1	100
	0.5	1/1	100
	16	2/2	100
	32	14/14	100
	64	2/2	100
Bacteroides vulgatus	1 - 16	12/13	92.3
	1	1/1	100
	4	2/2	100
	8	5/5	100
	16	5/6	83.3

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

B. ovatus (N=37)
• Ceftolozane-tazobactam MIC values ranged from 0.06 mcg/mL to 256 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 4 mcg/mL to 64 mcg/mL.
• The overall clinical cure rate was 97.3% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *B. ovatus* at baseline.
• Clinical cure rates were high at MIC values ≤ 64 mcg/mL.

B. thetaiotaomicron (N=18)
• Ceftolozane-tazobactam MIC values ranged from 0.125 mcg/mL to 64 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 16 mcg/mL to 64 mcg/mL.
• The overall clinical cure rate was 100% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *B. thetaiotaomicron* at baseline.
• Clinical cure rates were high at MIC values ≤64 mcg/mL.

B. vulgatus (N=13)
• Ceftolozane-tazobactam MIC values ranged from 1 mcg/mL to 16 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 4 mcg/mL to 16 mcg/mL.
• The overall clinical cure rate was 92.3% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *B. thetaiotaomicron* at baseline.
• Clinical cure rates were high at MIC values ≤16 mcg/mL.

E. avium, E. faecalis and E. faecium
• Ceftolozane-tazobactam MIC values were ≥32 mcg/mL for *E. avium, E. faecalis* and *E. faecium*.
• Despite the lack of activity of ceftolozane-tazobactam against *E. avium, E. faecalis* and *E. faecium* high microbiological eradication rates of 63/72 (87.5%) and 83/90 (92.2%) were demonstrated in the ceftolozane-tazobactam plus metronidazole and meropenem treatment arms, respectively.

S. anginosus (N=30)
• Ceftolozane-tazobactam MIC values ranged from 0.125 mcg/mL to 16 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 2 mcg/mL to 8 mcg/mL.
• The overall clinical cure rate was 83.3% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *S. anginosus* at baseline.
• Clinical cure rates were high at MIC values ≤16 mcg/mL.
• The distribution of MIC values in the combined surveillance studies for the *S. anginosus* were similar to the MIC values obtained for *S. anginosus* in the cIAI clinical study.
• Applying the provisional breakpoint of (b) (4):

− Clinical success rates were 86.8% for *S. anginosus* with MIC values ≤8 mcg/mL.
− Clinical success rates were 100% for *S. anginosus* with MIC values ≥16 mcg/mL.

S. constellatus (N=18)
• Ceftolozane-tazobactam MIC values ranged from 0.06 mcg/mL to 8 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects with the majority of isolates with MIC values of 2 mcg/mL to 8 mcg/mL.
• The overall clinical cure rate was 94.4% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *S. constellatus* at baseline.
• Clinical cure rates were high at MIC values ≤8 mcg/mL.
• The distribution of MIC values in the combined surveillance studies for the *S. constellatus* were similar to the MIC values obtained for *S. constellatus* in the cIAI clinical study.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 94.4% for *S. constellatus* with MIC values ≤8 mcg/mL.
− Clinical success rates were not evaluated for *S. constellatus* isolates with MIC values ≥16 mcg/mL, because there were no *S. constellatus* with MIC values ≥16 mcg/mL.

S. salivarius (N=10)
• Ceftolozane-tazobactam MIC values ranged from 0.25 mcg/mL to 2 mcg/mL in the ceftolozane-tazobactam plus metronidazole treated subjects.
• The overall clinical cure rate was 90.0% for subjects in the ceftolozane-tazobactam plus metronidazole arm with *S. salivarius* at baseline.
• Clinical cure rates were high at MIC values ≤2 mcg/mL.
• The distribution of MIC values in the combined surveillance studies for the *S. salivarius* were similar to the MIC values obtained for *S. salivarius* in the cIAI clinical study.
• Applying the provisional breakpoint of (b) (4):
− Clinical success rates were 90% for *S. salivarius* with MIC values ≤8 mcg/mL.
− Clinical success rates were not evaluated for *S. salivarius* with MIC values ≥16 mcg/mL, because there were no *S. salivarius* isolates with MIC values ≥16 mcg/mL.

Table 102: Clinical Cure rates at TOC by Ceftolozane-Tazobactam MIC Values for *Streptococcus* spp from the Phase 3 cIAI Study (Expanded ME)

Baseline Infecting Pathogen	Ceftolozane/Tazobactam MIC (µg/mL)	n/N1	Percent %
Streptococcus anginosus	0.125 - 16	25/30	83.3
	0.125	1/1	100
	0.25	1/1	100
	1	1/1	100
	2	8/10	80.0
	4	13/15	86.7
	8	9/10	90.0
	16	2/2	100
Streptococcus constellatus	0.06 - 8	17/18	94.4
	0.06	1/1	100
	0.125	1/1	100
	0.25	2/2	100
	0.5	1/1	100
	1	1/1	100
	2	3/3	100
	4	11/12	91.7
	8	4/4	100
Streptococcus salivarius	0.25 - 2	9/10	90.0
	0.25	2/2	100
	0.5	7/7	100
	1	1/2	50.0
	2	1/1	100

cIAI=complicated intra-abdominal infection; ME=microbiologically evaluable; MIC= minimum inhibitory concentration; n=number of subjects within a specific category; N1=number of subjects in specified population with baseline pathogen categories with the corresponding ceftolozane/tazobactam MIC value; TOC=test of cure.
Notes: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.12.2

Summary

Ceftolozane-tazobactam susceptibility (≤8 mcg/mL) was associated with high rates of clinical cure across species. The presence of CTX-M-14/15 did not adversely affect the cure rate and the CTX-M-14/15 *E. coli* and *K. pneumoniae* MIC distribution fell into the Enterobacteriaceae distribution. Though the number of isolates with higher MIC values was small, the provisional susceptibility interpretive breakpoints were predictive of generally higher clinical cure rates for isolates with MIC values (b) (4) compared to MIC values ≥16 mcg/mL.

Correlation of Kirby-Bauer Zone Diameter Values with Clinical and Microbiological Response in cIAI Study

A summary of the clinical cure rates by ceftolozane-tazobactam zone diameter values is shown in their respective sections below. Summaries of clinical response at the TOC visit by zone diameter of the baseline pathogens in the MITT and expanded ME populations were provided. Overall, the results in the MITT population were similar to the expanded ME population.

The ceftolozane-tazobactam Kirby-Bauer zone diameters ranged from 6 to 42 mm in the ceftolozane-tazobactam plus metronidazole-treated subjects and in the comparator-treated subjects. Overall, baseline resistance to ceftolozane-tazobactam or meropenem was rare among Gram-negative aerobic pathogens. The clinical cure rates were high in the ceftolozane-tazobactam arm for pathogens with ceftolozane-tazobactam zone diameters ≥19 mm. Summaries for the clinical cure rates by zone diameter for the key baseline cIAI pathogens in the expanded ME population are listed below:

Enterobacteriaceae (N=233)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 36 mm in the ceftolozane-tazobactam treated subjects with the majority of isolates with ceftolozane-tazobactam zone diameters ≥19 mm.
• There was no apparent association between clinical cure and zone diameter in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at both zone size extremes.
• Clinical cure rates were high for isolates at each zone diameter value.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible (≥19 mm), intermediate (16-18mm) and resistant (≤15 mm) populations.

C. freundii (N=7)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 19 to 28 mm in the ceftolozane-tazobactam treated subjects.
• Overall clinical response was 100% for subjects in the ceftolozane-tazobactam arm with *C. freundii* at baseline.
• Clinical Cure rates were high for isolates with zone diameters ≥19 mm.
E. coli (N=200)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 15 to 36 mm in the ceftolozane-tazobactam treated subjects with the majority of isolates between 20 and 30 mm.
• There was no apparent association between clinical cure and zone diameter in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at both zone size extremes.
• Clinical Cure rates were high for isolates with zone diameters ≥15 mm.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate, and resistant populations.

E. coli CTX-M-14/15 (N=9)
• In the CTX-M-14/15 sub-population of *E. coli* the ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 17 to 26 mm in the ceftolozane-tazobactam treated subjects.
• All subjects with a CTX-M positive *E. coli* were clinical cure.
• High clinical cure rates were associated with ceftolozane-tazobactam zone diameters

of ≥17 mcg/mL.
• The presence or absence of CTX-M-14/15 does not appear to correlate with any change in clinical cure rates.

K. oxytoca (N=12)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 18 to 29 mm in the ceftolozane-tazobactam treated subjects.
• Overall clinical response was 100% for subjects in the ceftolozane-tazobactam arm with *K. oxytoca* at baseline.
• Clinical Cure rates were high for isolates with zone diameters ≥18 mm.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate, and resistant populations.

K. pneumoniae (N=28)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 30 mm in the ceftolozane-tazobactam treated subjects with the majority of isolates with zone diameters ≥ 15 mm.
• There was no apparent association between clinical cure and zone diameter in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at both zone size extremes.
• Clinical cure rates were high for isolates at each zone diameter value.

K. pneumoniae CTX-M-14/15 (N=4)
• In the CTX-M sub-population of *K. pneumoniae* the ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 6 to 18 mm in the ceftolozane-tazobactam treated subjects.
• All subjects with a CTX-M positive *K. pneumoniae* were clinical cure.
• High clinical cure rates were associated with all zone diameters.
• The presence or absence of CTX-M-14/15 does not appear to correlate with any change in clinical cure rates.

P. mirabilis (N=11)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 12 to 27 mm in the ceftolozane-tazobactam treated subjects.
• There was no apparent association between clinical cure and zone diameter in the ceftolozane-tazobactam treatment arm. The number of pathogens was small at all zone diameters.
• Clinical Cure rates were high for isolates with zone diameters ≥16 mm.
• Applying the zone diameter proposed breakpoints resulted in clear differentiation in clinical cure rates between susceptible, intermediate, and resistant populations.

E. cloacae (N=22)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 9 to 29 mm in the ceftolozane-tazobactam treated subjects.
• The number of pathogens were small at each zone diameter.

• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate and resistant populations.

P. aeruginosa (N=24)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 20 to 33 mm in the ceftolozane-tazobactam treated subjects.
• The overall clinical cure rate was 100% for subjects in the ceftolozane-tazobactam arm with *P. aeruginosa* at baseline.
• Clinical cure rates were high for all zone diameters.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate, and resistant populations as all isolates were susceptible to ceftolozane-tazobactam.

E. avium, E. faecalis and *E. faecium*
• In the ceftolozane-tazobactam treatment arm, the ceftolozane-tazobactam zone diameters were ≤ 11 mm for *E. avium, E. faecalis* and *E. faecium*.
• Despite the lack of activity of ceftolozane-tazobactam against *E. avium, E. faecalis* and *E. faecium* (zone diameters < 11mm), high microbiological eradication rates were demonstrated in the ceftolozane-tazobactam plus metronidazole and meropenem treatment arms, respectively. Therefore no correlation between zone diameter and outcome was noted.

S. anginosus (N=30)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 13 to 27 mm in the ceftolozane-tazobactam treated subjects.
• The overall clinical cure rate was 83.3% for subjects in the ceftolozane-tazobactam arm with *S. anginosus* at baseline.
• Clinical cure rates were high at zone diameters ≥16 mm.
• Applying the zone diameter proposed breakpoints resulted in differentiation in clinical cure rates between susceptible and intermediate. No resistant population was present.

S. constellatus (N=18)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 13 to 28 mm in the ceftolozane-tazobactam treated subjects.
• The overall clinical cure rate was 94.4% for subjects in the ceftolozane-tazobactam arm with *S. constellatus* at baseline.
• Clinical cure rates were high at zone diameters ≥13 mm.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate, and resistant populations.

S. salivarius (N=10)
• Ceftolozane-tazobactam Kirby-Bauer disk zone diameter values ranged from 15 to 29 mm in the ceftolozane-tazobactam treated subjects.
• The overall clinical cure rate was 90.0% for subjects in the ceftolozane-tazobactam arm with *S. salivarius* at baseline.
• Clinical cure rates were high at zone diameters ≥15 mm.
• Applying the zone diameter proposed breakpoints did not result in differentiation in clinical cure rates between susceptible, intermediate and resistant populations.
Anaerobes were not tested by disk diffusion at the central laboratory.

Summary
Ceftolozane-tazobactam zone sizes of ≥19 mm for Gram-negative pathogens was associated with high rates of clinical cure and zone sizes of ≥16 mm for gram-positive pathogens was associated with high rates of clinical cure. Overall, clinical cure rates were high making correlations with clinical outcome and zone diameter difficult. Zone diameters ≤11 mm were associated with intrinsic resistance such as in *Enterococcus* spp. The majority of isolates had zone diameters that were ≥17 mm. In the cIAI study, all *E. coli* and *K. pneumoniae* with a CTX-M-14/15 were clinical cures; therefore, no association with zone diameter or MIC values and CTX-M-14/15 expression could be made.

Reviewer's Comment
Disk diffusion correlations are tentative since discussion is still ongoing within the Agency regarding MIC and disk breakpoints. Further discussion regarding disk diffusion correlations may be in a subsequent review.

Reviewer's Comment
For the cUTI and cIAI studies, tables showing pathogen eradication versus zone size (per pathogen), and clinical cure versus zone size (per pathogen) were not located in the submission, and the Applicant will be asked to provide the information.

Pathogen Eradication, Outcome by MIC and Outcome by Zone Diameter Analysis for Combined Phase 3 Studies
The pathogen eradication rates, outcome by MIC value, and outcome by zone diameter will be summarized for the combined Phase 3 studies in the sections below. Overall, the microbiological success rates in the ceftolozane-tazobactam treatment arm in the mMITT populations were high and were comparable to the combined comparators in both the mMITT and ME populations (see table below). The only regional difference was noted in North America in the mMITT population, where the microbiological outcomes were lower than in the comparator arm. This may be attributed to higher variability due to the smaller number of subjects from North America and the larger number of indeterminate responses in the ceftolozane-tazobactam treatment arm. In general, the anaerobe data and

the *S. anginosus, S. constellatus,* and *S. salivarius* data will not be discussed in this section as these pathogens were specific only to the cIAI study.

Table 103: Per-Subject Microbiological Response at the TOC Visit for combined Phase 3 Studies (mMITT and ME Population)

Population Response	Ceftolozane/Tazobactam n (%)	All Comparators n (%)
mMITT Population, N	787	819
Microbiological Success	652 (82.8)	660 (80.6)
Microbiological Failure	76 (9.7)	113 (13.8)
Microbiological Non-evaluable	59 (7.5)	46 (5.6)
ME Population, N	648	698
Microbiological Success	587 (90.6)	600 (86.0)
Microbiological Failure	61 (9.4)	98 (14.0)

N=number of subjects in the modified microbiological intent to treat (mMITT) or microbiologically evaluable (ME) population; n=number of subjects in the specific category; TOC=test of cure
Note: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.3\ISM\Table 8.1.

Table 104: Per-Subject Microbiological Response at the TOC Visit in the Combined Phase 3 Studies, by Geographic Region (mMITT and ME Population)

	North America		European Union		Rest of World	
Population Response	Ceftolozane/ tazobactam n (%)	All Comparators n (%)	Ceftolozane/ tazobactam n (%)	All Comparators n (%)	Ceftolozane/ tazobactam n (%)	All Comparators n (%)
mMITT Population, N	41	35	369	378	377	406
Microbiological Success	26 (63.4)	28 (80.0)	302 (81.8)	312 (82.5)	324 (85.9)	320 (78.8)
Microbiological Failure	9 (22.0)	4 (11.4)	36 (9.8)	45 (11.9)	31 (8.2)	64 (15.8)
Microbiological Non-evaluable	6 (14.6)	3 (8.6)	31 (8.4)	21 (5.6)	22 (5.8)	22 (5.4)
ME Population, N	23	20	299	317	326	361
Microbiological Success	17 (73.9)	17 (85.0)	271 (90.6)	279 (88.0)	299 (91.7)	304 (84.2)
Microbiological Failure	6 (26.1)	3 (15.0)	28 (9.4)	38 (12.0)	27 (8.3)	57 (15.8)

N=number of subjects in the modified microbiological intent to treat (mMITT) or microbiologically evaluable (ME) population in each geographic region; n=number of subjects in the specific category; TOC=test of cure
Note: Percentages are calculated as 100 x (n/N).
Source: M5.3.5.3\ISM\Table 8.1.1

Pathogen Eradication Rates Combined Phase 3 Studies

The table below presents the microbiologic response rates by baseline pathogen at the TOC visit for the mMITT and ME populations for the most common pathogens in the combined Phase 3 program. A list of microbiologic response rates in the mMITT population by pathogen, by geographic region, and by ESBL status was provided. Microbiologic response rates for the ME population are provided by geographic region and by ESBL status.

Overall, ceftolozane-tazobactam was efficacious at eradicating key Gram-negative pathogens, *Streptococcus* spp., and *Bacteroides* spp. in the combined Phase 3 trials. In general, the eradication rates were similar between ceftolozane-tazobactam treatment arm and the comparator treatment arms.

• Eradication rates for ceftolozane-tazobactam were higher for the ESBL-positive subgroup of *E. coli* and *K. pneumoniae* compared with the comparator in both the mMITT and the ME Populations.

− CTX-M-14/15 *E. coli* eradication rates for ceftolozane-tazobactam and comparators in the ME population were 80.6% and 58.8%, respectively.

− CTX-M-14/15 *K. pneumoniae* eradication rates for ceftolozane-tazobactam and comparators in the ME population were 76.9% and 20.0%, respectively.

• Ceftolozane-tazobactam eradication rates were high and similar for all *P. aeruginosa* and the over-expressing AmpC *P. aeruginosa* subset.

• In general, the overall *E. coli* eradication rates were lower in North America in the mMITT population compared with the other geographic regions but this was interpreted with caution because of the small numbers of pathogens from subjects enrolled in the North America; this difference was not notable in the ME population.

• Generally, the eradication rate for ceftolozane-tazobactam was lower against *E. cloacae* as compared with the combined comparator group.

• Eradication rates were lower for ceftolozane-tazobactam against the enterococci. Differences in eradication against the enterococci would be expected as enterococci are intrinsically resistant to cephalosporins.

• Eradication rates in the ME population were generally higher than in the mMITT population; though overall trends and conclusions were similar.

Table 105: Per-Pathogen Microbiologic Eradication Rates at the TOC Visit for Pathogens from the Site of Infection from the Combined Phase 3 Studies (mMITT and ME Populations)

Pathogen Group Pathogen	mMITT Population				ME Population			
	Ceftolozane/ Tazobactam (N=787) n (%)		All Comparators (N=819) n (%)		Ceftolozane/ Tazobactam (N=648) n (%)		All Comparators (N=698) n (%)	
	n/N1	%	n/N1	%	n/N1	%	n/N1	%
Gram-negative aerobes	**583/691**	**84.4**	**588/732**	**80.3**	**529/575**	**92.0**	**539/631**	**85.4**
Enterobacteriaceae	566/668	84.7	559/693	80.7	512/557	91.9	513/599	85.6
Citrobacter freundii	8/10	80.0	13/17	76.5	8/8	100.0	12/16	75.0
Enterobacter cloacae	23/32	71.9	31/33	93.9	21/28	75.0	28/29	96.6
Escherichia coli	480/560	85.7	481/594	81.0	437/470	93.0	445/515	86.4
ESBL Producers[a]	42/57	73.7	36/65	55.4	41/50	82.0	35/55	63.6
CTX-M-14/15	30/42	71.4	21/42	50.0	29/36	80.6	20/34	58.8
Klebsiella oxytoca	16/18	88.9	26/27	96.3	14/14	100.0	23/24	95.8
Klebsiella pneumoniae	56/74	75.7	42/60	70.0	50/55	90.9	36/48	75.0
ESBL Producers[a]	15/21	71.4	6/12	50.0	14/18	77.8	5/11	45.5
CTX-M-14/15	11/16	68.8	2/6	33.3	10/13	76.9	1/5	20.0
Proteus mirabilis	23/24	95.8	18/22	81.8	20/21	95.2	17/21	81.0
Pseudomonas aeruginosa	38/46	82.6	40/49	81.6	32/33	97.0	36/41	87.8
AmpC Overexpression[b]	5/6	83.3	5/6	83.3	5/5	100.0	5/6	83.3
Gram-negative anaerobes[c]	**117/137**	**85.4**	**145/154**	**94.2**	**107/109**	**98.2**	**134/137**	**97.8**
Bacteroides fragilis[c]	42/47	89.4	59/64	92.2	39/41	95.1	56/57	98.2
Bacteroides thetaiotaomicron[c]	22/25	88.0	42/46	91.3	20/20	100.0	41/43	95.3
Bacteroides uniformis[c]	7/7	100.0	8/8	100.0	7/7	100.0	7/7	100.0
Bacteroides vulgatus[c]	13/15	86.7	24/26	92.3	13/13	100.0	21/22	95.5
Gram-positive aerobes	**189/247**	**76.5**	**216/245**	**88.2**	**164/189**	**86.8**	**189/205**	**92.2**
Streptococcus anginosus[c]	29/36	80.6	25/27	92.6	28/30	93.3	23/23	100.0
Streptococcus constellatus[c]	18/24	75.0	21/25	84.0	17/18	94.4	21/23	91.3
Streptococcus salivarius[c]	10/11	90.9	9/11	81.8	10/10	100.0	8/8	100.0
Enterococcus faecalis	45/71	63.4	53/62	85.5	37/53	69.8	49/56	87.5
Enterococcus faecium	26/37	70.3	46/49	93.9	24/27	88.9	42/44	95.5

(Footnotes on next page)

cIAI=complicated intra-abdominal infection; ESBL=extended spectrum β-lactamase N=number of subjects in the microbiological modified intent-to-treat (mMITT) or microbiologically evaluable (ME) population; N1=number of subjects with the specified pathogen; n=number of subjects in the specific category; TOC=test of cure.
[a] Not all *E. coli* and *K. pneumoniae* were tested for ESBL production All ESBL producer includes any enzyme. All CTX-M-14/15 includes CTX-M-14, CTX-M-15, CTX-M-15-like.
[b] Not all *Pseudomonas aeruginosa* isolates were tested for AmpC overexpression
[c] Isolates were primarily from the cIAI study
Notes: Percentages are calculated as 100 x (n/N).
If a subject has more than one pathogen in any of the higher level summaries (eg, Gram-negative aerobes, Enterobacteriaceae), the subject will be counted once and at the worse per-pathogen microbiological response.
Source: M5.3.5.3\ISM\Table 9.1, Table 9.1.2, Table 9.2, and Table 9.2.2

Reviewer's Comment
The Applicant mentions CTX-M-15-like ESBLs in the footnote to the table above. These likely refer to beta-lactamases that have sequence homology to CTX-M-15 since structure/ function studies were not done in the clinical trials.

Per-Pathogen Clinical Cure Rates Combined Phase 3 Studies
The table below presents clinical response rates by baseline pathogen at the TOC visit for the ME population for the most common pathogens in the combined Phase 3 program. Overall, ceftolozane-tazobactam achieved high (>83%) clinical cure rates in subjects with key Gram-negative pathogens, *Streptococcus* spp., and *Bacteroides* spp. in the combined Phase 3 studies. In general, the eradication rates were similar between the ceftolozane-tazobactam and comparator treatment arms.
• Per-pathogen clinical cure rates for ceftolozane-tazobactam and the combined comparator group were generally higher than the per-pathogen eradication rates, but treatment differences remained generally similar across pathogens.
• Per-pathogen clinical cure rates for ceftolozane-tazobactam were higher for the ESBL-positive subgroup of *E. coli* and *K. pneumoniae* compared with the comparator in the ME Population.
− CTX-M-14/15 *E. coli* eradication rates for ceftolozane-tazobactam and comparators in the ME population were 97.2% and 82.4%, respectively.
− CTX-M-14/15 *K. pneumoniae* eradication rates for ceftolozane-tazobactam and comparators in the ME population were 100% and 40.0%, respectively.
• The clinical response for ceftolozane-tazobactam against the *E. cloacae* was improved (89.3%) as compared with the ceftolozane-tazobactam microbiological eradication rate (75%).

Table 106: Per-pathogen Clinical Success rates at the TOC Visit for Pathogens from the Infection Site from the Combined Phase 3 Studies (ME Population)

Pathogen Group Pathogen	Ceftolozane/ Tazobactam (N=648) n (%)		All Comparators (N=698) n (%)	
	n/N1	%	n/N1	%
Gram-negative aerobes	551/575	95.8	589/631	93.3
Enterobacteriaceae	533/557	95.7	559/599	93.3
Citrobacter freundii	8/8	100	13/16	81.3
Enterobacter cloacae	25/28	89.3	29/29	100
Escherichia coli	452/470	96.2	483/515	93.8
ESBL Producers[a]	49/50	98.0	48/56	85.7
CTX-M-14/15	35/36	97.2	28/34	82.4
Klebsiella oxytoca	14/14	100	23/24	95.8
Klebsiella pneumoniae	51/55	92.7	41/48	85.4
ESBL Producers[a]	17/18	94.4	8/11	72.7
CTX-M-14/15	13/13	100	2/5	40.0
Proteus mirabilis	20/21	95.2	17/21	81.0
Pseudomonas aeruginosa	33/33	100	38/41	92.7
Gram-negative anaerobes[b]	104/109	95.4	132/137	96.4
Bacteroides fragilis[b]	39/41	95.1	56/57	98.2
Bacteroides thetaiotaomicron[b]	20/20	100	40/43	93.0
Bacteroides uniformis[b]	7/7	100	7/7	100
Bacteroides vulgatus[b]	12/13	92.3	21/22	95.5
Bacteroides ovatus[b]	36/37	97.3	42/42	100
Gram-positive aerobes	169/189	89.4	190/205	92.7
Streptococcus anginosus[b]	25/30	83.3	23/23	100
Streptococcus constellatus[b]	17/18	94.4	20/23	87.0
Streptococcus salivarius[b]	9/10	90.0	8/8	100
Enterococcus faecalis	45/53	84.9	53/56	94.6
Enterococcus faecium	24/27	88.9	41/44	93.2

ESBL=extended spectrum β-lactamase N=number of subjects in the microbiologically evaluable (ME) population; N1=number of subjects with the specified pathogen; n=number of subjects in the specific category; TOC=test of cure.
[a] Not all *E. coli* and *K. pneumoniae* were tested for ESBL production. All ESBL producers includes any enzyme. All CTX-M-14-15 includes CTX-M-14, CTX-M-15, CTX-M-15-like.
[b] Isolates were from the complicated intra-abdominal infection studies
Notes: Percentages are calculated as 100 x (n/N1).
If a subject has more than one pathogen in any of the higher level summaries (eg, Gram-negative aerobes, Enterobacteriaceae), the subject will be counted once and at the worse per-pathogen clinical response.
Source: M5.3.5.3\ISM\Table 9.3.1

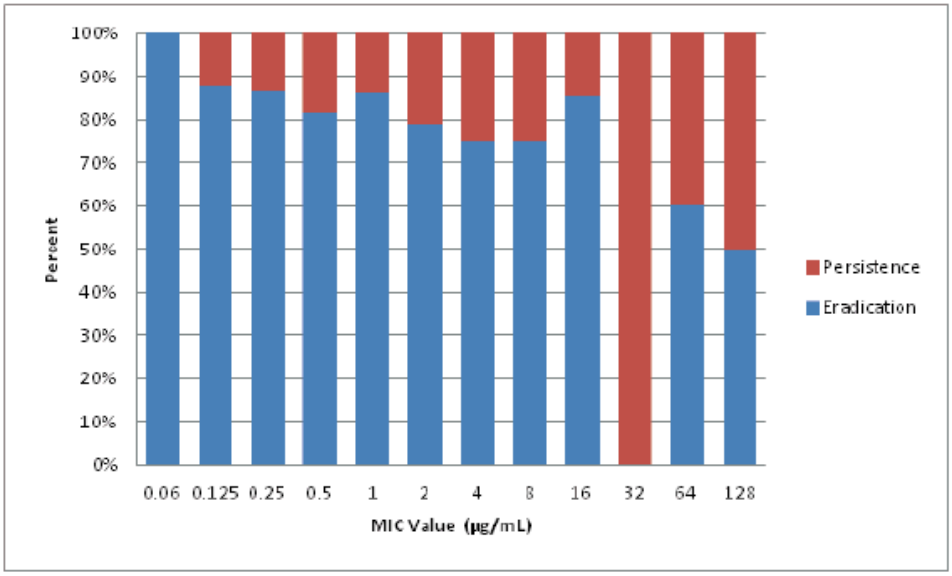
Correlation of MIC Values With Clinical and Microbiological Response in Combined Phase 3 Studies

A summary of the microbiological eradication rates at TOC by ceftolozane-tazobactam MIC values for key cIAI and cUTI pathogens is shown in the table below. Results for the mMITT population were similar to the ME population. The ceftolozane-tazobactam MIC values ranged from 0.03 to 256 mcg/mL in the ceftolozane-tazobactam-treated subjects.

Overall, baseline resistance to ceftolozane-tazobactam was rare among Gram-negative aerobic pathogens. The microbiological eradication rates were high in the ceftolozane-tazobactam treatment arm for pathogens with ceftolozane-tazobactam MIC values ≤16 mcg/mL. Summaries of the microbiological eradication rates for ceftolozane-tazobactam by MIC value for the Gram negative pathogen categories are listed below:

Enterobacteriaceae (N=731)
• Data for the Enterobacteriaceae are displayed in the figure below.
• At MIC values ≥32 mcg/mL, there appears to be a decrease in the percent eradicated.
• Values ≤ 16 mcg/mL appeared to have similar, high eradication rates.
• These combined data support an Enterobacteriaceae breakpoint of susceptible of ≤16 mcg/mL.
• Applying the provisional breakpoint of (b) (4):
− Microbiological eradication rates were 85.5% for Enterobacteriaceae with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 63.2% for Enterobacteriaceae with MIC values ≥16 mcg/mL.
For the ESBL-positive *E. coli* and *K. pneumoniae*
− Microbiological response rates remained high for the ESBL subgroup.
− No correlation with outcome and MIC was noted as there were few isolates at higher MIC values.

Figure 15: Summary of Microbiological Eradication and Persistence at TOC by MIC for Enterobacteriaceae (mMITT population)



MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; TOC=test of cure
Source: M5.3.5.3\ISM\Table 10.1

P. aeruginosa (N=43)
• Eradication rates in general were high.
• Eradication rates varied inconsistently with MIC values (100% microbiological eradication in isolates with MIC values of 0.03, 16, and 128 mcg/mL).
• Applying the provisional breakpoint of (b) (4)
− Microbiological eradication rates were 80% for all *P. aeruginosa* with MIC values ≤8 mcg/mL.
− Microbiological eradication rates were 100% for all *P. aeruginosa* with MIC values ≥16 mcg/mL.

Correlation of Kirby-Bauer Zone Diameter Values With Clinical and Microbiological Response in Combined Phase 3 Studies
A summary of the microbiological eradication rates at TOC by ceftolozane-tazobactam zone diameters for significant cIAI and cUTI pathogens is shown in the table below. Per-pathogen microbiological eradication at TOC by zone diameter for the mMITT population were presented for all pathogens, by geographic region and by ESBL status. Overall, the results in the mMITT population were similar to the ME population.

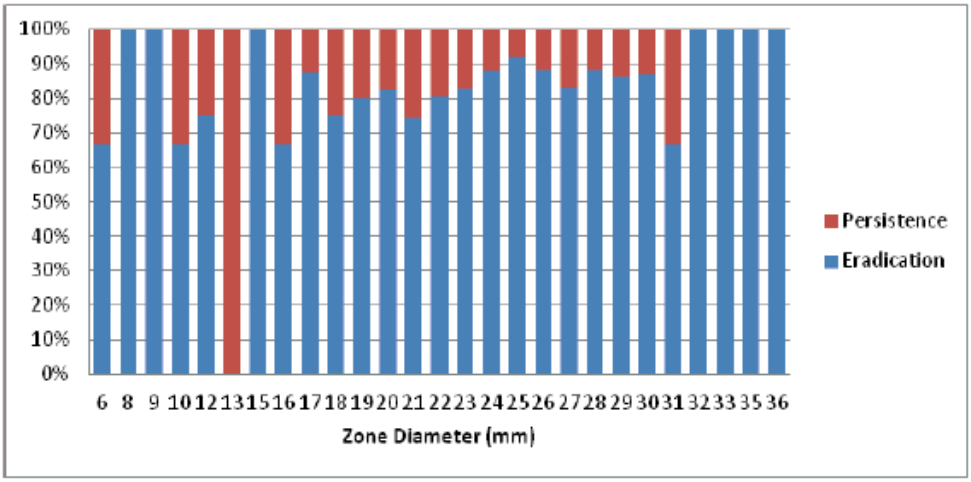
The ceftolozane-tazobactam zone diameter values ranged from 6 to 36 mm in the Ceftolozane-tazobactam-treated subjects. Overall, baseline resistance to ceftolozane-tazobactam was rare among Gram-negative aerobic pathogens. The clinical cure rates were high in the ceftolozane-tazobactam arm for pathogens with ceftolozane-tazobactam zone diameter values ≥17 mm. Summaries of the microbiological eradication rates for ceftolozane-tazobactam by zone diameter value are listed below:

Enterobacteriaceae (N=731)
• Data for the Enterobacteriaceae are displayed in the figure below.
• At zone diameters around 16 mm, there may be a difference in outcome; higher rates of microbiological persistence.
• Zone diameters ≥17 mm appear to be associated with higher eradication rates.
• These combined data support the Enterobacteriaceae interpretive criteria for susceptible of ≥17 mm.

For the ESBL-positive *E. coli* and *K. pneumoniae*
• Microbiological response rates remained high for the ESBL subgroup.
• No correlation with outcome and zone was noted as there were few isolates at the smaller zone diameters.

P. aeruginosa (N=43)
• Eradication rates in general were high.
• Eradication rates varied inconsistently with zone diameters.

Figure 16: Summary of Microbiological Eradication and Persistence at TOC by Zone Diameter (mm) for Enterobacteriaceae (mMITT Population)



mMITT=microbiological modified intent-to-treat; TOC=test of cure
Source: M5.3.5.3\ISM\Table 11.1

Emergence of Decreased Susceptibility to Study Drug
Emergence of Decreased Susceptibility to Study Drugs in cUTI

The table below summarizes the emergence of decreased susceptibility across the 2 treatment arms for the mMITT population in the cUTI study. Emergence of decreased susceptibility is defined as a ≥2 dilution increase in MIC from the baseline MIC value compared to EOT, TOC or LFU uropathogen MIC value (the post-baseline MIC value must be ≥2 mcg/mL). Both ceftolozane-tazobactam and levofloxacin had a low incidence of emergence of decreased susceptibility with only 4 (1.0%) instances in the ceftolozane-tazobactam arm compared to 14 (3.5%) in the levofloxacin arm.

Table 107: Summary of Emergence of Decreased Susceptibility (mMITT Population)

Baseline Pathogen	Ceftolozane/ Tazobactam (N=398) n	Levofloxacin (N=402) n
Number of Subjects with Emergence of Decreased Susceptibility[a]	4 (1.0%)	14 (3.5%)
Total number of Baseline Pathogens with Emergence of Decreased Susceptibility	N1=4	N1=14
Enterococcus faecalis	1	1
Escherichia coli	2	13
Pseudomonas aeruginosa	1	0

cUTI=complicated urinary tract infection; EOT=end of therapy; LFU=late follow-up; MIC=minimum inhibitory concentration; mMITT=microbiologically modified Intent-to-Treat; n=number of subjects in specific category; N=number of subjects in mMITT population; N1=number of baseline pathogens with emergence of decreased susceptibility; TOC=test of cure
Notes: Decreased susceptibility is defined as pathogen with ≥2 dilution difference (2 two-fold dilutions) in MIC for study drug between susceptibility of pathogen recovered at baseline compared to EOT, TOC or LFU pathogen MIC, ie, post-baseline MIC/baseline MIC ≥4 µg/mL and post-baseline MICs ≥2 µg/mL.
[a] Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.30.1.

The table below summarizes the emergence of resistance across the 2 treatment arms for the mMITT population. Resistance was defined by CLSI criteria for levofloxacin and per the provisional breakpoints for ceftolozane-tazobactam. Emergence of resistance was rare in the ceftolozane-tazobactam treatment arm.

Reviewer's Comment
Two (0.5%) of the persisting pathogens developed resistance among the 51 microbiological failures in the ceftolozane-tazobactam treatment arm with baseline and post-baseline isolates available for susceptibility testing. Levofloxacin-resistance on therapy developed in 16 (4.0%) of the levofloxacin-treated subjects.

Table 108: Summary of Emergence of Resistance (mMITT Population) cUTI

Baseline Pathogen	Ceftolozane/ Tazobactam (N=398) n	Levofloxacin (N=402) n
Number of Subjects with Emergence of Resistance[a]	2 (0.5%)	16 (4.0%)
Total Number of Baseline Pathogens with Emergence of Resistance	N1=2	N1=16
Enterococcus faecalis	0	1
Escherichia coli	1	15
Pseudomonas aeruginosa	1	0

CLSI=Clinical and Laboratory Standards Institute; cUTI=complicated urinary tract infection; MIC=minimum inhibitory concentration; mMITT=microbiological modified intent-to-treat; n=Number of subjects in specific category; N=number of subjects in mMITT population; N1=number of baseline pathogen with emergence of resistance
Notes: For ceftolozane/tazobactam, Susceptible/Indeterminate/Resistant (S/I/R) breakpoints are defined as MIC≤8 µg/mL; MIC=16 µg/mL; and MIC≥32 µg/mL. Levofloxacin-resistance MIC cut-offs are based on CLSI definitions and breakpoints [37]
[a] Percentages are calculated as 100 x (n/N).
Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.31.1.

Ceftolozane-tazobactam resistance developed in 1 *E. coli* isolate (Subject No. 1005-5309-008) that had a MIC value shift from 0.5 mcg/mL (susceptible) at baseline to a MIC of 64 mcg/mL (resistant) at TOC and 1 *P. aeruginosa* isolate (Subject ID: 1005-5104-015) that had a MIC value shift from 16 mcg/mL (intermediate) to 64 mcg/mL (resistant). These isolates were characterized to evaluate the genetic relatedness and to elucidate the resistance mechanism . The MIC results at the central laboratory for the *E. coli* isolate from Subject No.1005-5309-008, demonstrated an increase in MIC at the TOC visit from 0.5 to 64 mcg/mL, which then decreased to 4 mcg/mL at the LFU visit. This MIC increase followed by decrease was confirmed by triplicate MIC testing at (b) (4) (b) (4) s, suggesting that the susceptibility of the TOC isolate towards ceftolozane-tazobactam is markedly different from that of the baseline isolate. Molecular testing identified the same pair of resistance enzymes in all 4 isolates (CTX-M-15 like, OXA-1/30), no new enzyme could be found to explain the change in activity. Pulsed-field gel electrophoresis (PFGE) testing indicated that the isolates with altered MIC values were identical to the baseline isolate while the EOT isolate was unrelated (with a ceftolozane-

tazobactam MIC similar to that of the baseline isolate). These results do not provide an explanation of the observed ceftolozane-tazobactam MIC change during therapy for isolates from Subject No. 1005-5309-008. It is possible that transient changes in ESBL expression, permeability or efflux resulted in the MIC increase at TOC, as evidenced by the observation that the MIC decreased at LFU in an isolate found to be identical by PFGE.

The MIC results at the central laboratory for the *P. aeruginosa* isolate from Subject No. 1005- 5104-015, demonstrated an increase in MIC at the TOC visit from 16 to 64 mcg/mL. The observed MIC increase was confirmed by triplicate testing at (b) (4) laboratories. The baseline isolate was shown to express the ESBL enzyme OXA-2. In addition, increased expression of the efflux pumps MexCD and MexXY with concurrent loss of the expression of the OprD porin was observed. In contrast, the TOC isolate was found to have wild-type expression levels of MexXY but retained increased expression of MexCD. The OXA-2 expression and OprD loss remained unchanged. The 2 isolates were found to be identical based on PFGE testing. In vitro studies have shown that ceftolozane-tazobactam is not a substrate for the *P. aeruginosa* efflux pumps and the explanation for the increased MIC is unclear.

Reviewer's Comment
The Applicant made an attempt to characterize *E. coli* and *P. aeruginosa* isolates that had a change in MIC under therapy with ceftolozane-tazobactam. Molecular testing was used to determine resistance mechanisms such as enzymes and the relatedness of isolates was determined by PFGE. The exact mechanism of resistance for these isolates could not clearly be determined.

Table 109: Molecular Characterization of Ceftolozane-Tazobactam Resistance Emergence

Subject No.	Organism	Visit	Ceftolozane/ Tazobactam MIC (µg/mL) ICON	Ceftolozane/ Tazobactam MIC (µg/mL) JMI	ESBL Results	PFGE Results
1005-5104-015	*P. aeruginosa*	Screening	16	8/8/8	OXA-2, MexCD and MexXY up, OprD loss	baseline
		TOC	64	>32/>32/>32	OXA-2, MexCD up, OprD loss	identical
1005-5309-008	*E. coli*	Screening	0.5	2/2/4	CTX-M-15 like, OXA-1/30	baseline
		EOT	0.5	0.5/0.5/0.5	CTX-M-15 like, OXA-1/30	unrelated
		TOC	64	32/32/>32	CTX-M-15 like, OXA-1/30	identical
		LFU	4	2/2/2	CTX-M-15 like, OXA-1/30	identical

EOT=End of therapy; ESBL=extended-spectrum β-lactamase; LFU=late Follow-up; MIC=minimum inhibitory concentration; PFGE=pulsed-field gel electrophoresis; TOC=test of cure
Source: M5.3.5.4\CXA.068.MC

Supplemental Molecular Characterization for CXA-cUTI-10-04 and CXA-cUTI-10-05 Clinical studies

One hundred and forty six isolates of Enterobacteriaceae were found to express ESBLs, with CTX-M-15 as the most common enzyme. Of 24 *P. aeruginosa* isolates tested, 17 (70.8%) were found to possess changes in AmpC, efflux pumps or porin expression levels alone or in combination with ESBL enzymes. Isolate sets from 109 patients met the criteria for PFGE testing and the majority of the post-baseline isolates (66.1%) were found to be identical to the corresponding baseline isolate. The table below shows the summary of ESBL and carbapenemase positive Enterobacteriaceae identified in the cUTI studies.

Table 110: Summary of ESBL and Carbapenemase Positive Enterobacteriaceae Identified in the cUTI Studies

Organism	Genotype	N	Ceftolozane/Tazobactam MIC Range (µg/mL)
Escherichia coli	**Any CTX-M-15**	**59**	**0.25-8**
	CTX-M-15 only	8	0.25-1
	CTX-M-15, OXA-1/30	28	0.25-8
	CTX-M-15, TEM	10	0.25-1
	CTX-M-15, OXA-1/30, TEM	12	0.25-8
	CTX-M-15, CTX-M-27	1	0.5
	Any CTX-M-14	**12**	**0.25-1**
	CTX-M-14 only	4	0.25-1
	CTX-M-14, TEM-1	8	0.25-0.5
	Other CTX-M	**13**	**0.25-2**
	Alone	6	0.25-1
	With TEM	7	0.25-2
	SHV or TEM	**13**	**0.12-1**
	Carbapenemase	**3**	**1->64**
	NDM-5, CTX-M-15, OXA-1/30, TEM-1	1	>64
	KPC-2, TEM-1	2	1-8
Klebsiella pneumoniae	**Any CTX-M-15**	**19**	**0.25->64**
	CTX-M-15, OXA, SHV	2	0.5-8
	CTX-M-15, OXA, SHV, TEM	17	0.25->64
	Other ESBL	**7**	**1-64**
Proteus mirabilis	**Any CTX-M-15**	**1**	**1**
	CTX-M-15, OXA-1/30, TEM	1	1
	Other CTX-M	**1**	**1**
	TEM-1	**2**	**0.5**
	Carbapenemase	**1**	**4**

Organism	Genotype	N	Ceftolozane/Tazobactam MIC Range (µg/mL)
	KPC-2	1	4
Enterobacter species	Any CTX-M-15	8	1->64
	CTX-M-15 only	2	1-2
	CTX-M-15, SHV, TEM	2	4
	CTX-M-15, OXA-1/30, TEM	4	1->64
	CTX-M-3 +/-TEM	3	1-16
Other Enterobacteriaceae[a]	Any CTX-M-15	3	4 - >64
	CTX-M-15, OXA-1/30	1	4
	CTX-M-15, OXA-1/30, SHV, TEM	1	32
	CTX-M-15, TEM	1	>64
	CTX-M-3, TEM	1	16

[a] Includes two isolates of *Serratia marcescens* and one isolate each of *Citrobacter freundii* and *Klebsiella oxytoca*

Source: This submission.

Table 111: KPC-2-positive Isolates Identified in the CXA-cUTI-10-04 Clinical Study

Patient No.	Accession No.	Organism	Ceftolozane/ Tazobactam MIC (µg/mL)	Imipenem MIC (µg/mL)	ESBL	Treatment Arm	Microbiological Outcome	Clinical Outcome
4309-001	U691262-ISO-1	*E. coli*	8	2	KPC-2, TEM-1	Levofloxacin	failure	cure
4309-002	U691260-ISO-2	*E. coli*	1	2	KPC-2, TEM-1	Ceftolozane/ Tazobactam	N/A	N/A
4309-002	U691260-ISO-1	*P. mirabilis*	4	4	KPC-2	Ceftolozane/ Tazobactam	N/A	N/A

Source: This submission.

Table 112: Summary of Efflux pump/Porin Changes, ESBL, and Carbapenemase positive *P. aeruginosa* identified in cUTI studies

Organism	Genotype	N	Ceftolozane/Tazobactam MIC Range (µg/mL)
P. aeruginosa	OXA	4	1->64
	Alone	1	1
	With pump/porin changes	3	16->64
	AmpC upregulated	8	0.5-4
	Alone	6	0.5-4
	With pump/porin changes	2	0.5-2
	OprD loss	1	>64
	Metallo-β-lactamase	4	>64
	Alone	1	>64
	With pump/porin changes	2	>64
	With pump/porin changes and AmpC	1	>64

Source: This submission.

Supplemental Molecular Characterization for CXA-cIAI-10-08 and CXA-cIAI-10-09 Clinical studies

In total, 129 isolates of Enterobacteriaceae and 70 isolates of *P. aeruginosa* were further characterized. Of these, 44/129 (34.1%) Enterobacteriaceae were positive for at least a CTX-M-14 or CTX-M-15 enzyme and 11/70 (15.7%) *P. aeruginosa* were positive for upregulated AmpC expression. The table below shows a summary of ESBL positive Enterobacteriaceae identified in the cIAI studies.

Table 113: Summary of ESBL Positive Enterobacteriaceae Identified in the cIAI studies

Organism	Genotype	N	Ceftolozane-tazobactam MIC Range (µg/mL)
Escherichia coli	Any CTX-M-15	26	0.25-64
	CTX-M-15 only	2	0.25-2
	CTX-M-15, OXA-1/30	8	0.25-32
	CTX-M-15, TEM	7	0.25-64
	CTX-M-15, OXA-1/30, TEM	9	1-4
	Any CTX-M-14	5	0.5-2
	CTX-M-14, TEM-1	5	0.5-2
	Other CTX-M	5	0.25-0.5
	OXA-1/30	3	0.25-16
	TEM	17	0.12->64
	SHV and TEM	1	0.25
Klebsiella pneumoniae	Any CTX-M-15	8	1->64
	CTX-M-15, OXA-1/30, SHV	1	4
	CTX-M-15, OXA-1/30, SHV, TEM	7	1->64
	SHV +/- TEM	6	0.25-64
Proteus mirabilis	Any CTX-M-15	1	0.5
	CTX-M-15, TEM	1	0.5
	TEM-1	2	0.5-1
Enterobacter species	Any CTX-M-15	3	0.5-16
	CTX-M-15, OXA-1/30, TEM	3	0.5-16
	Other CTX-M	1	1
	SHV	2	0.25-0.5
	SHV and TEM	3	4-64
Other Enterobacteriaceae[a]	Any CTX-M-15	1	2
	CTX-M-15, OXA-1/30, TEM	1	2
	Other CTX-M	2	0.25-32
	TEM	2	1-2

[a] Two isolates of *Citrobacter freundii* and one isolate each of *Providencia stuartii*, *Serratia fonticola* and *Serratia marcescens*

Source: This submission.

Table 114: Summary of ESBLs, pump/porin and AmpC Changes in *P. aeruginosa* Identified in the cIAI Studies

Organism	Genotype	N	Ceftolozane-tazobactam MIC Range (µg/mL)
P. aeruginosa	**OXA**	**4**	**4-16**
	With AmpC upregulated	2	4
	With efflux pumps and AmpC upregulated	2	4-16
	AmpC upregulated	**7**	**0.5-8**
	Alone	6	0.5-8
	With pump/porin changes	1	1
	MexAB upregulated	**1**	**0.5**

Organism	Genotype	N	Ceftolozane-tazobactam MIC Range (µg/mL)
	OprD loss	**1**	**1**

Source: This submission.

Emergence of Decreased Susceptibility to Study Drugs in cIAI
There was no emergence of decreased susceptibility or resistance in either treatment arm.

Assessment of Superinfecting Pathogens and New Infecting Pathogens
The incidence rate of superinfections and new infections are summarized with the number and percentage by treatment group. The 95% Wilson score CI was provided for each group. The summary is based on the mMITT population.

Assessment of Superinfecting Pathogens and New Infecting Pathogens (cUTI)
The table below presents subjects with emergent infections and the uropathogens isolated. The incidence of emergent infections was comparable in the 2 treatment arms. In general, new infections were reported more frequently than superinfections in both treatment arms. Superinfections were low with an incidence of 3.5% in the ceftolozane-tazobactam treatment arm and 5.2% in the levofloxacin treatment arm. Likewise, the incidence of new infections was also low, 9.0% and 6.7% of subjects, in the ceftolozane-tazobactam and levofloxacin treatment arms, respectively. As expected, enterococci were the most common pathogens isolated from subjects with superinfections and new infections. In the ceftolozane-tazobactam arm, the majority of the emergent bacterial isolates were pathogens known to be intrinsically resistant to cephalosporins (*E. faecalis* and *E. faecium*), and did not represent acquired resistance during study therapy.

Table 115: Summary of Emergent Infections (mMITT Population)

New Pathogen	Ceftolozane/ Tazobactam (N=398) n	Levofloxacin (N=402) n	Percent (%) Difference (95% CI)
Number of Subjects with a Superinfection[a]	14 (3.5%)	21 (5.2%)	-1.7 (-4.69, 1.20)
Total New Pathogens Classified as Superinfections	N1=17	N1=25	
Enterococcus faecalis	7	8	
Enterococcus faecium	6	0	
Escherichia coli	2	7	
Enterobacter cloacae	1	1	
Klebsiella pneumoniae	0	3	
Number of Subjects with a New Infection[a]	36 (9.0%)	27 (6.7%)	2.3 (-1.45, 6.15)
Total New Uropathogens Classified as New Infections	N2=42	N2=30	
Enterococcus faecalis	12	11	
Escherichia coli	8	10	
Enterococcus faecium	5	1	
Klebsiella pneumoniae	4	4	
Klebsiella oxytoca	2	0	
Pseudomonas aeruginosa	1	1	
Enterobacter cloacae	1	1	

CFU=colony-forming unit; CI=Confidence interval (based on Wilson score); mMITT=microbiologically modified intent-to-treat; n=number of subjects within a specific category; N=number of subjects in mMITT population; N1=number of pathogens classified as superinfections; N2=number of pathogens classified as new infections.

[a] Percentages are calculated as 100 x (n/N).
Superinfection=A urine culture grows $\geq 10^5$ CFU/mL of a pathogen other than the baseline pathogen(s) during the course of study drug therapy. New Infection=A urine culture grows $\geq 10^5$ CFU/mL of a pathogen other than the baseline pathogen(s) after administration of the last dose of study drug therapy.

Source: M5.3.5.1\cUTI\CXA-cUTI-10-04 and -05\Table 14.2.17.

Assessment of Superinfecting Pathogens and New Infecting Pathogens (cIAI)

The table below presents the number of subjects with a superinfection and the number of subjects with a new infection for the MITT and ME populations, respectively. Superinfections and new infections were uncommon in each treatment arm and largely comprised organisms intrinsically resistant to ceftolozane-tazobactam (*Enterococcus* spp.). Superinfections were seen in 10/389 (2.6%) versus 13/417 (3.1%) of subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively. Likewise, only 12/389 (3.1%) versus 9/417 (2.2%) of subjects in the ceftolozane-tazobactam plus metronidazole versus meropenem treatment arms, respectively, had new infections.

Table 116: Summary of Emergent Infections in cIAI (MITT population) (Continued)

New Pathogen Category New Pathogen	Ceftolozane/Tazobactam + Metronidazole (N=389) n	Meropenem (N=417) n
Gram-positive Aerobes		
Enterococcus spp.	11	6
Staphylococcus spp.	6	5
Streptococcus spp.	0	2
Other	3	0
Gram-negative Anaerobes	2	4
Gram-positive Anaerobes	0	0

CI=confidence interval; cIAI=complicated intra-abdominal infection; MITT=microbiological intent-to-treat; n=number within a specific category; N=number of subjects in MITT population; N1=number of subjects with intra-abdominal pathogens classified as superinfections; N2=number of subjects with intra-abdominal pathogens classified as new infections.
Notes: Percent calculated as N1/N
Superinfection=Isolation of a pathogen, other than the original baseline pathogen(s), from an intra-abdominal specimen taken from a subject while on study drug.
New Infection=Isolation of a pathogen, other than the original baseline pathogen(s), from an intra-abdominal specimen in a subject after completion of study drug.
Subjects with more than 1 new intra-abdominal pathogen are counted only once in the overall population N, once in the superinfection/new infection rate and once within each intra-abdominal pathogen.
Source: M5.3.5.1\cIAI\CXA-cIAI-10-08 and -09\Table 14.2.11.1.

Table 117: Summary of Emergent Infections in cIAI (MITT population)

New Pathogen Category New Pathogen	Ceftolozane/Tazobactam + Metronidazole (N=389) n	Meropenem (N=417) n
Number of Subjects with a Superinfection, N1 (%)	10 (2.6%)	13 (3.1%)
Total New Pathogens Classified as Superinfections	14	18
Gram-negative Aerobes		
Enterobacteriaceae	3	4
Other	1	0
Gram-positive Aerobes		
Enterococcus spp.	6	5
Staphylococcus spp.	3	5
Streptococcus spp.	2	4
Other	0	1
Gram-negative Anaerobes	1	1
Gram-positive Anaerobes	0	2
Number of Subjects with a New Infection, N2	12 (3.1%)	9 (2.2%)
Total New Pathogens Classified as New Infections	18	16
Gram-negative Aerobes		
Enterobacteriaceae	6	4
Other	0	0

Correlation Between Phase 3 MIC Distributions and Recent Surveillance Study

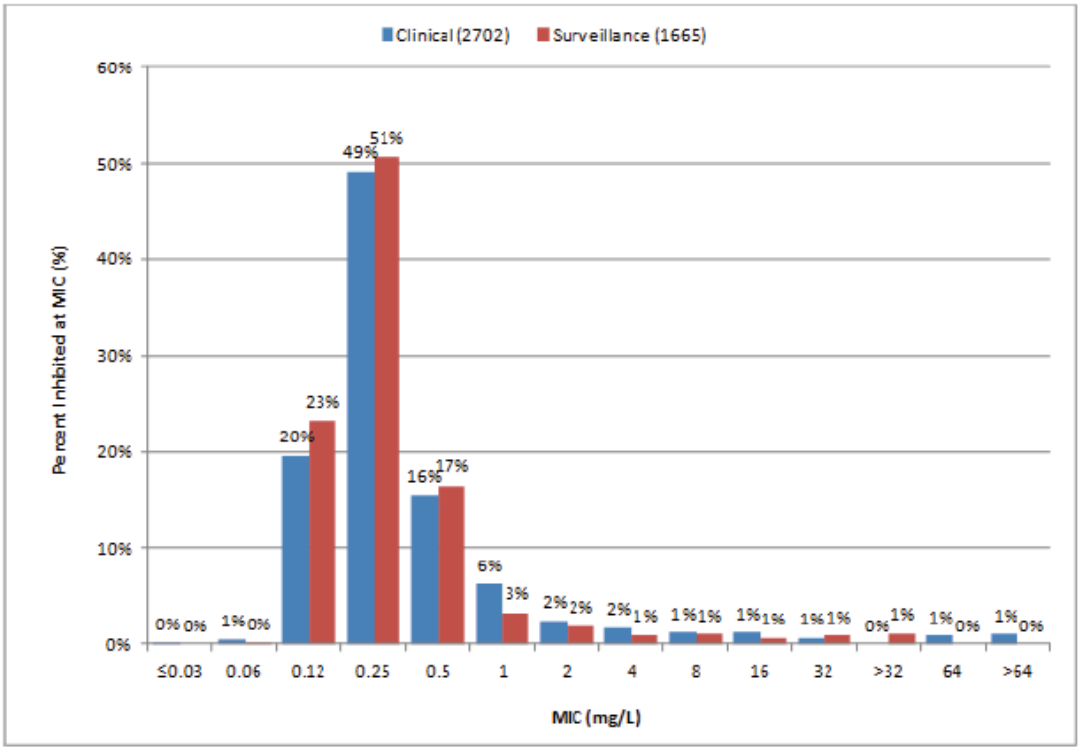
MIC Frequency Distributions

The susceptibility of clinical trial and surveillance isolates to ceftolozane-tazobactam for all target organisms is presented in this section as side-by-side MIC frequency distributions. The distributions, presented as histograms, are composed of ceftolozane-tazobactam MIC values for baseline isolates from the ITT population from the combined Phase 3 clinical efficacy studies that used a central microbiology reference laboratory (CXA-cUTI-10-04, -05 and CXA-cIAI-10- 08,-09 studies) and the combined 2011 and 2012 surveillance studies for ceftolozane-tazobactam.

Enterobacteriaceae

For the 4367 isolates of Enterobacteriaceae, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=2702) was similar to that of the surveillance study isolates (n=1665) (the figure below). The majority of the isolates fell in the range of 0.12 to 0.5 mcg/mL. It was notable that approximately 50% of both the clinical and surveillance Enterobacteriaceae isolates were inhibited by ceftolozane-tazobactam at 0.25 mcg/mL.

Figure 17: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against Enterobacteriaceae

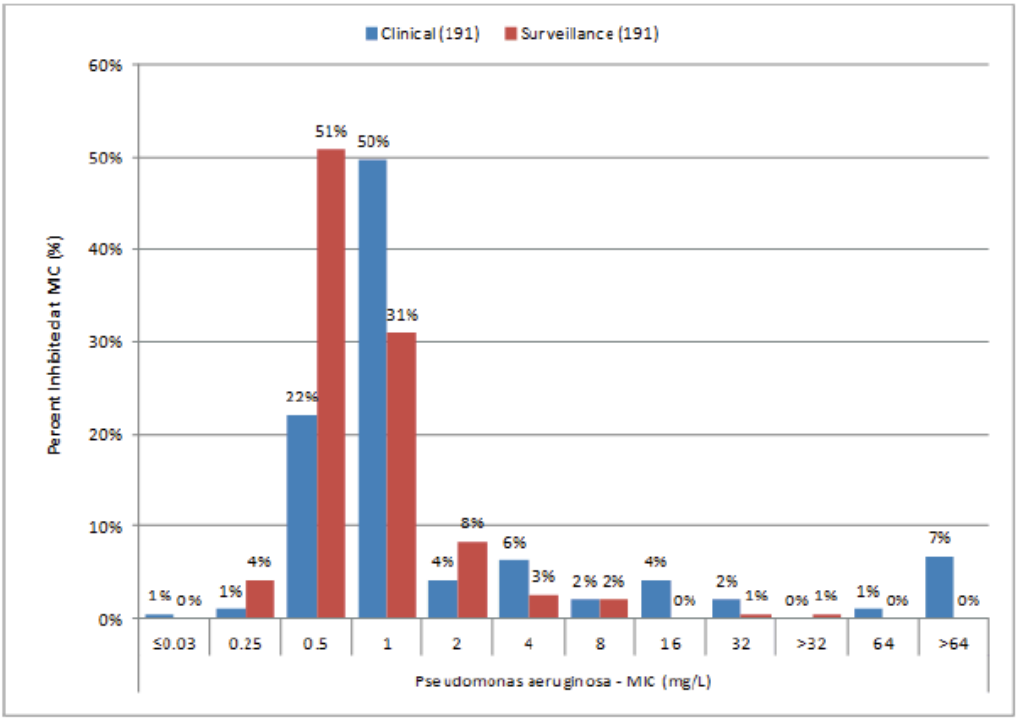


MIC=minimum inhibitory concentration
Note: Total Enterobacteriaceae N=4367
Source: M5.3.5.4\CXA.060.MC

Pseudomonas aeruginosa

For the 382 isolates of *P. aeruginosa*, the overall distribution of ceftolozane-tazobactam for clinical trial isolates (n=191) was similar to that of the surveillance study isolates (n=191) (see figure below). The majority of the isolates fell between MIC values of 0.5 and 1 mcg/mL. However, a somewhat higher percentage of the clinical isolates were at the higher end of the distribution, which was driven by the Eastern European *P. aeruginosa* with carbapenemases. This resulted in clinical isolate MIC50/MIC90 values of 1 and 16 mcg/mL while surveillance isolate MIC50/MIC90 values were 0.5 and 2 mcg/mL. In addition, it was noted that approximately 51% of the *P. aeruginosa* surveillance isolates were inhibited by ceftolozane-tazobactam at 0.5 mcg/mL while approximately 50% of the *P. aeruginosa* clinical isolates were inhibited by ceftolozane-tazobactam at 1 mcg/mL.

Figure 18: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Pseudomonas aeruginosa*



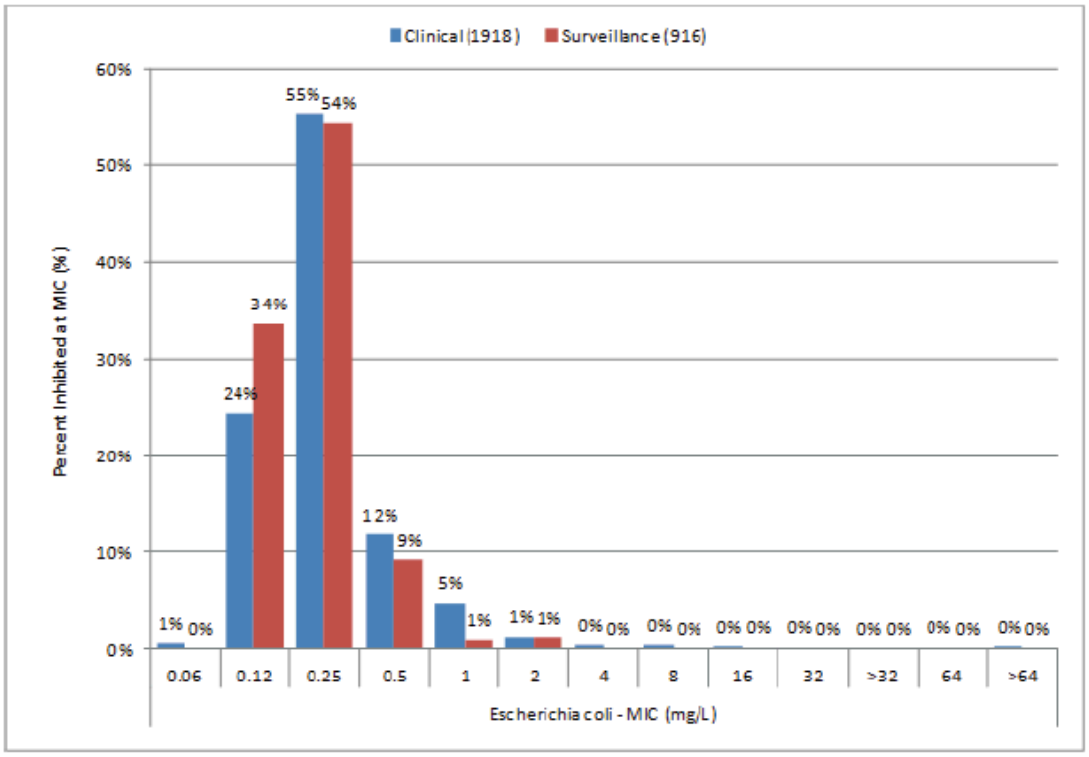
MIC=minimum inhibitory concentration
Note: Total *P. aeruginosa* N=382
Source: M5.3.5.4\CXA.060.MC

Escherichia coli

For the 2834 isolates of *E. coli*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=1918) was similar to that of the surveillance study isolates (n=916) (see figure below). The majority of the isolates fell within the range of 0.12 to 0.25 mcg/mL. The clinical and surveillance isolates had identical MIC50 and MIC90 values of 0.25 and

0.5 mcg/mL, respectively. It was notable that approximately 50% of both the clinical and surveillance *E. coli* isolates were inhibited by ceftolozane-tazobactam at 0.25 mcg/mL.

Figure 19: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Escherichia coli*



MIC=minimum inhibitory concentration
Note: Total *E. coli* N=2834
Source: M5.3.5.4\CXA.060.MC

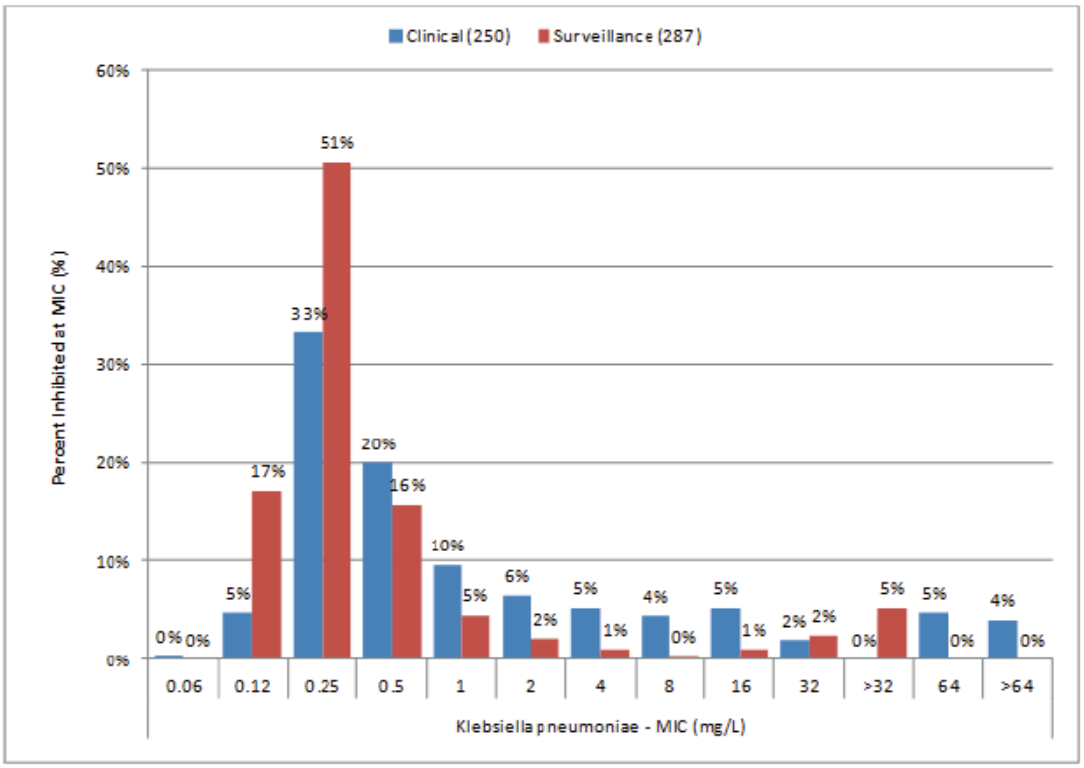
Klebsiella pneumoniae

For the 537 isolates of *K. pneumoniae*, the overall distribution of ceftolozane-tazobactam for clinical trial isolates (n=250) was similar to that of the surveillance study isolates (n=287) (see figure below). The majority of the isolates fell between the range of 0.12 to 0.5 mcg/mL. However, a somewhat higher percentage of the clinical isolates were at the higher end of the distribution. This resulted in clinical isolate MIC50/MIC90 values of 0.5 and 32 mcg/mL while surveillance isolate MIC50 /MIC90 values were 0.5 and 4 mcg/mL.

Reviewer's Comment

A somewhat higher percentage of the clinical isolates were at the higher end of the distribution. This resulted in clinical isolate MIC50/MIC90 values of 0.5 and 32 mcg/mL while surveillance isolate MIC50 /MIC90 values were 0.5 and 4 mcg/mL.

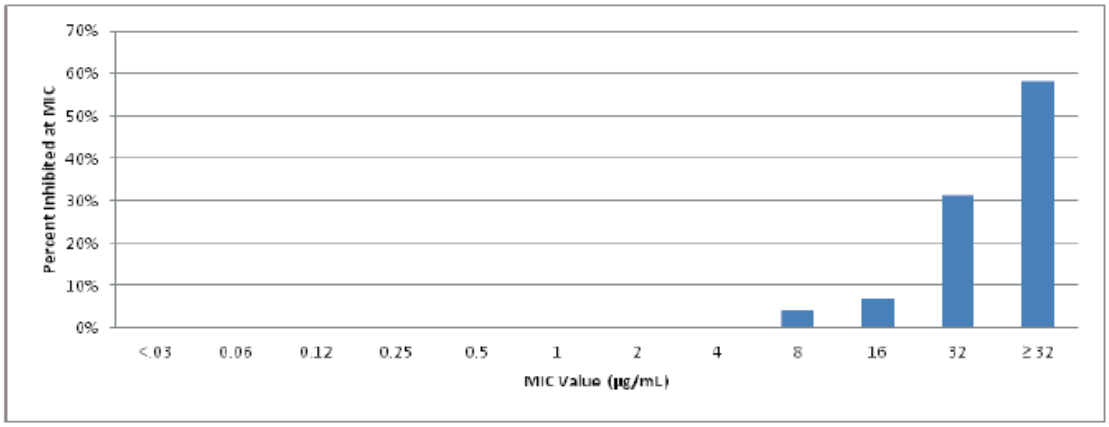
Figure 20: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Klebsiella pneumoniae*



MIC=minimum inhibitory concentration
Note: Total *K. pneumoniae* N=537
Source: M5.3.5.4\CXA.060.MC

To confirm that the current proposed susceptibility interpretive criteria can distinguish between ceftolozane-tazobactam susceptible and resistant population, the 2011-2012 European Union and United States surveillance data was analyzed for meropenem-resistant *K. pneumoniae*, a phenotypic marker for KPC-producing isolates (see figure below). The distribution falls clearly to the right of the susceptible breakpoint.

Figure 21: Percent of Meropenem-resistant *Klebsiella pneumoniae* Inhibited at Each MIC (mcg/mL) of Ceftolozane-Tazobactam

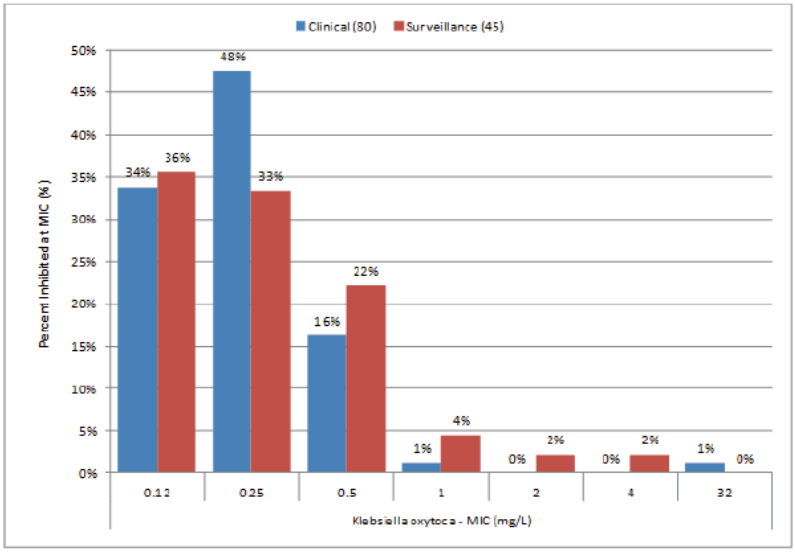


MIC=minimum inhibitory concentration
Note: Meropenem-resistant *K. pneumoniae* N=100
Data from Ceftolozane/Tazobactam 2011-2012 European Union and United States Surveillance
Meropenem non-susceptible is used as a marker for KPC producing *K. pneumoniae*
Source: Farrell, et al. [46]

Klebsiella oxytoca

For the 125 isolates of *K. oxytoca*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=80) was similar to that of the surveillance study isolates (n=45) (see figure below). The majority of the isolates fell between the range of 0.12 to 0.5 mcg/mL. The clinical and surveillance isolates had identical MIC50 and MIC90 values of 0.25 and 0.5 mcg/mL, respectively.

Figure 22: Percentage of Isolates Inhibited at Each MIC (mg/L of Ceftolozane-Tazobactam Against *Klebsiella oxytoca*

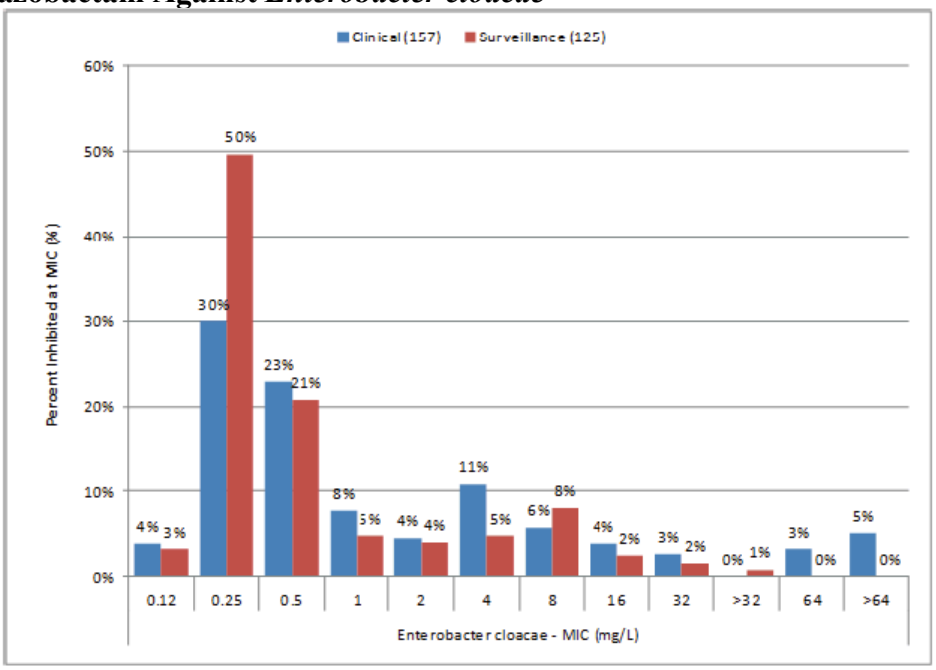


MIC=minimum inhibitory concentration
Note: Total *K. oxytoca* N=125
Source: M5.3.5.4\CXA.060.MC

Enterobacter cloacae

For the 282 isolates of *E. cloacae*, the overall distribution of ceftolozane-tazobactam for clinical trial isolates (n=157) was similar to that of the surveillance study isolates (n=125) (see figure below). The majority of the isolates fell between the range of 0.25 to 0.5 mcg/mL. However, a somewhat higher percentage of the clinical isolates were at the higher end of the distribution. This resulted in clinical isolate MIC50/MIC90 values of 0.5 and 32 mcg/mL while surveillance isolate MIC50 /MIC90 values were 0.5 and 8 mcg/mL.

Figure 23: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Enterobacter cloacae*

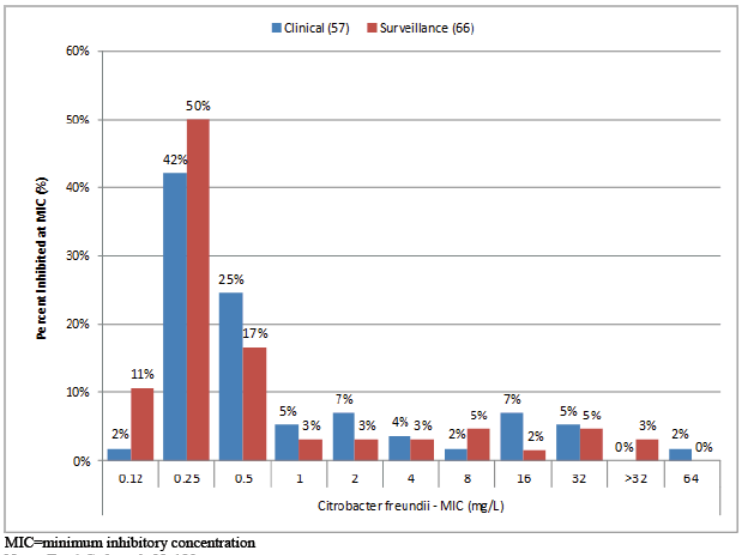


MIC=minimum inhibitory concentration
Note: Total *E. cloacae* N=282
Source: M5.3.5.4\CXA.060.MC

Citrobacter freundii

For the 123 isolates of *C. freundii*, the overall distribution of ceftolozane-tazobactam for clinical trial isolates (n=57) was similar to that of the surveillance study isolates (n=66) (see figure below). The majority of the isolates fell between the range of 0.25 to 0.5 mcg/mL. However, a slightly higher percentage of the clinical isolates were at the higher end of the distribution. This resulted in clinical isolate MIC50/MIC90 values of 0.5 and 16 mcg/mL while surveillance isolate MIC50 /MIC90 values were 0.25 and 8 mcg/mL.

Figure 24: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Citrobacter freundii*

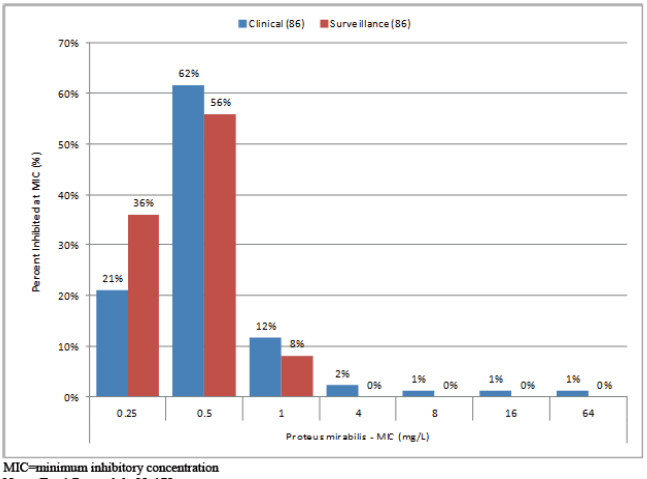


MIC=minimum inhibitory concentration
Note: Total *C. freundii* N=123
Source: M5.3.5.4\CXA.060.MC

Proteus mirabilis

For the 172 isolates of *P. mirabilis*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=86) was similar to that of the surveillance study isolates (n=86) (see figure below). The majority of the isolates fell between the range of 0.25 to 0.5 mcg/mL. The clinical isolate MIC50/MIC90 values of 0.5 and 1 mcg/mL while surveillance isolate MIC50 /MIC90 values were 0.5 and 0.5 mcg/mL.

Figure 25: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-tazobactam Against *Proteus mirabilis*

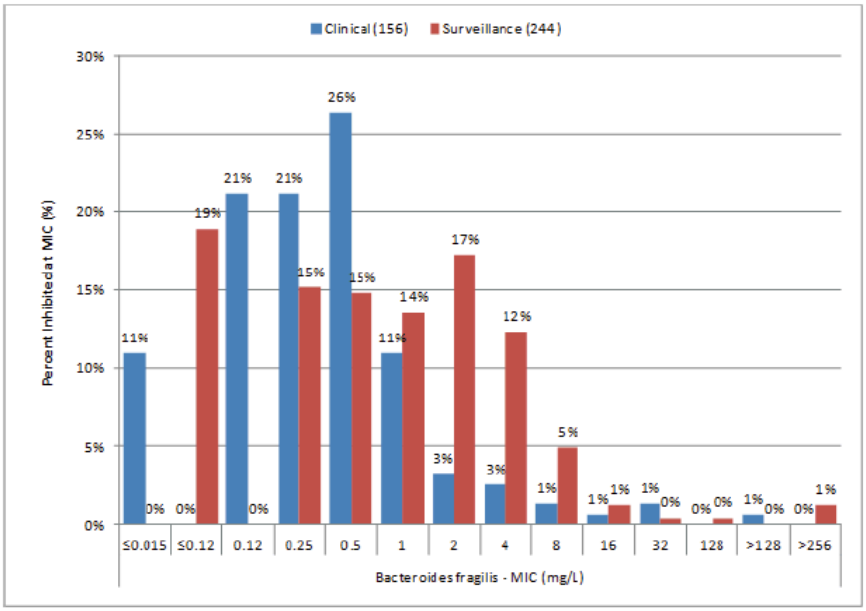


MIC=minimum inhibitory concentration
Note: Total *P. mirabilis* N=172
Source: M5.3.5.4\CXA.060.MC

Bacteroides fragilis

For the 400 isolates of *B. fragilis*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=156) were less similar to that of the Tufts University surveillance isolates (n=244) (see the figure below) than was found with other species in these studies. The majority of the clinical isolates fell between the range of 0.12 to 0.5 mcg/mL while the majority of the Tufts University isolates fell between a wider range of MIC values (0.25 to 4 mcg/mL). This distribution resulted in MIC50/MIC90 values of 0.25 and 1 mcg/mL for the clinical isolates while the MIC50 /MIC90 for the surveillance isolates were 1 and 4 mcg/mL.

Figure 26: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-tazobactam Against *Bacteroides fragilis*

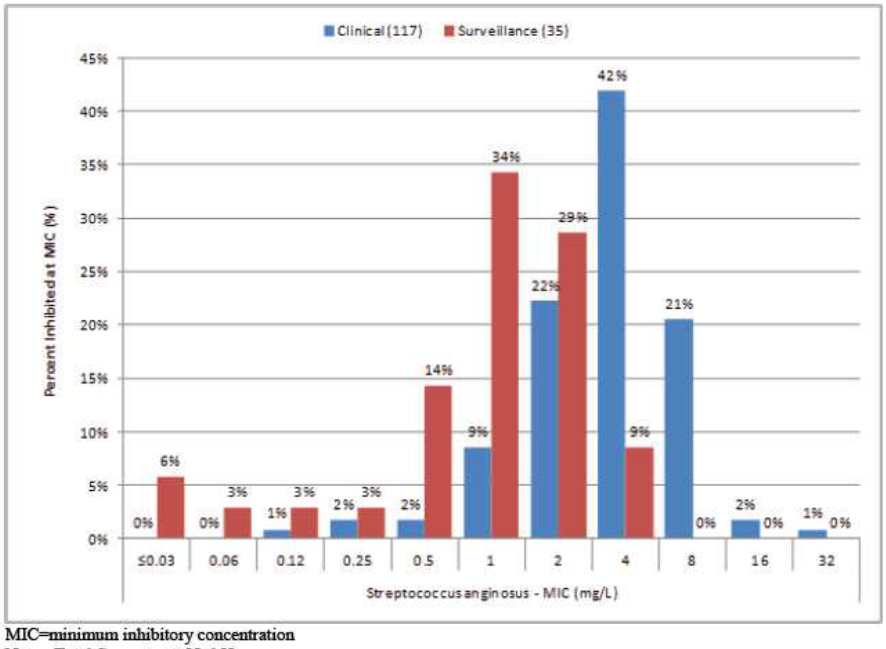


MIC=minimum inhibitory concentration
Note: Total *B. fragilis* N=400
Source: M5.3.5.4\CXA.060.MC

Streptococcus anginosus

For the 152 isolates of *Streptococcus anginosus*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=117) were less similar to that of the surveillance isolates (n=35) (see the figure below) than was found with other species in these studies. The majority of the clinical isolates fell between the range of 2 to 8 mcg/mL while the majority of the surveillance isolates fell between a range 0.5 to 2 mcg/mL. This distribution resulted in MIC50/MIC90 values of 4 and 8 mcg/mL for the clinical isolates while the MIC50 /MIC90 for the surveillance isolates were 1 and 2 mcg/mL.

Figure 27: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Streptococcus anginosus*

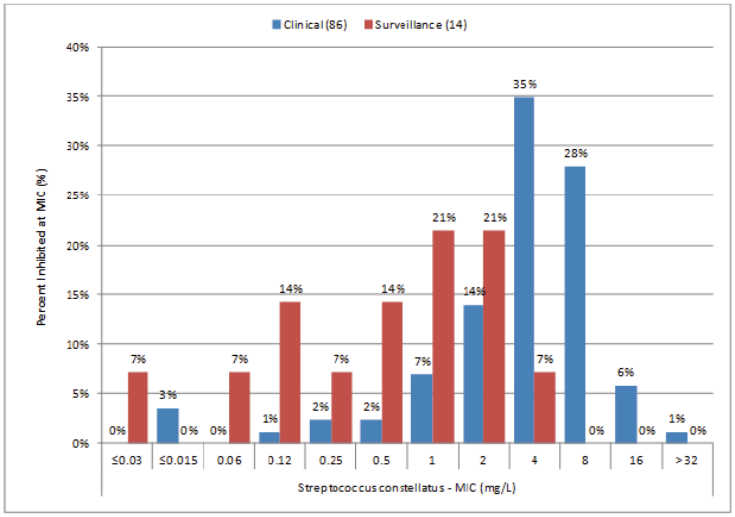


MIC=minimum inhibitory concentration
Note: Total *S. anginosis* N=152
Source: M5.3.5.4\CXA.060.MC

Streptococcus constellatus

For the 100 isolates of *S. constellatus*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=86) was similar to that of the surveillance study isolates (n=14) (see figure below). The majority of the isolates fell between the range of 1 to 8 mcg/mL. However, a somewhat higher percentage of the clinical isolates were at the higher end of the distribution. This resulted in clinical isolate MIC50/MIC90 values of 4 and 8 mcg/mL while surveillance isolate MIC50/MIC90 values were 0.5 and 2 mcg/mL.

Figure 28: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Streptococcus constellatus*

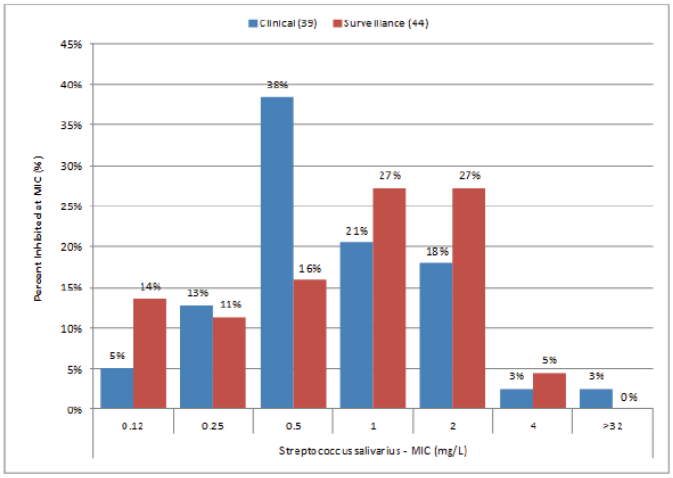


MIC=minimum inhibitory concentration
Note: Total *S. constellatus* N=100
Source: M5.3.5.4\CXA.060.MC

Streptococcus salivarius

For the 83 isolates of *S. salivarius*, the distribution of ceftolozane-tazobactam for clinical trial isolates (n=39) was similar to that of the surveillance study isolates (n=44) (see figure below). The majority of the isolates fell between the range of 0.5 to 2 mcg/mL. The clinical and surveillance isolates had nearly identical MIC50 and MIC90 values. The clinical isolate MIC50/MIC90 values were 0.5 and 2 mcg/mL while surveillance isolate MIC50 /MIC90 values were 1 and 2 mcg/mL.

Figure 29: Percentage of Isolates Inhibited at Each MIC (mg/L) of Ceftolozane-Tazobactam Against *Streptococcus salivarius*



MIC=minimum inhibitory concentration
Note: Total *S. salivarius* N=83
Source: M5.3.5.4\CXA.060.MC

NDA 206829 DATE REVIEW COMPLETED: 9-26-14
Ceftolozane-Tazobactam

Conclusions
For the overall population of Enterobacteriaceae and for *E. coli*, *K. oxytoca,* and *S. salivarius* the clinical isolate MIC distribution matched the surveillance MIC distribution. For *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *C. freundii*, *E. cloacae*, *S. anginosus,* and *S. constellatus*, a higher percentage of the clinical isolates were at the higher end of the distribution than the surveillance isolates. This may be because the clinical isolates came from sicker patients than surveillance isolates or because some of the clinical isolates came from clinical trial sites in parts of the world where higher rates of Gram-negative resistance are seen (such as Eastern Europe). For *Bacteroides fragilis* isolates, the clinical trial and surveillance isolates showed a somewhat different distribution, with MICs lower for the clinical isolates. This may be because the surveillance isolates came from a single lab at Tufts University and were not chosen according to standard surveillance practices.

SCATTER PLOTS RELATED TO MIC AND DISC DIFFUSION METHODS
Correlation Between Broth Microdilution and Kirby-Bauer Disk Diffusion
Two studies were performed to establish the MIC and zone diameter correlation for ceftolozane-tazobactam (CXA.028.MC, CXA.076.MC). Testing was performed by [redacted] (b) (4)) in accordance with CLSI document M23-A3. Analysis was done to determine the best fit and lowest error rate using the provisional breakpoints.

In the [redacted] (b) (4) study, more than 400 Enterobacteriaceae (including ESBL producers and ceftazidime resistant isolates), more than 200 *P. aeruginosa* (including MDR and ceftazidime-resistant strains), and more than 200 gram-positive species were analyzed. Each isolate was concurrently tested by broth microdilution and disk diffusion methodology. The preliminary ceftolozane-tazobactam disk and broth microdilution breakpoints were provided to [redacted] (b) (4) by Cubist Pharmaceuticals, Inc. for use in the analyses. Disk zone versus broth MIC scatterplots were generated and error rate analyses were performed to identify the lowest error rate breakpoints. Results are summarized in the table below.

Reviewer's Comment
The Applicant explained that strains that showed error rates that exceeded CLSI guidelines for acceptable discrepancy rates were most likely due to the small number of strains within a particular MIC range.

Table 118: Kirby-Bauer Disk Versus Broth Microdilution Correlation for Ceftolozane-Tazobactam

Strain	N	Preliminary Broth Microdilution Breakpoints Applied for Error Rate Analysis (µg/mL)	Preliminary Disk Breakpoints Applied to Error Rate Analysis (mm)	No. of Major or Very Major Errors	No. of Minor Errors	Correlation Coefficient
P. aeruginosa	254	≤8 [S], 16 [I], ≥32 [R]	≥14 [S], 9-13 [I], ≤8 [R]	0	6	0.66
E. coli	152	≤8 [S], 16 [I], ≥32 [R]	≥13 [S], 10-12 [I], ≤9 [R]	1[a]	1	0.67
K. pneumoniae	101	≤8 [S], 16 [I], ≥32 [R]	≥13 [S], 10-12 [I], ≤9 [R]	1[a]	7[a]	0.76
S. pneumoniae	130	≤8 [S], 16 [I], ≥32 [R]	≥13 [S], 10-12 [I], ≤9 [R]	0	9	0.88

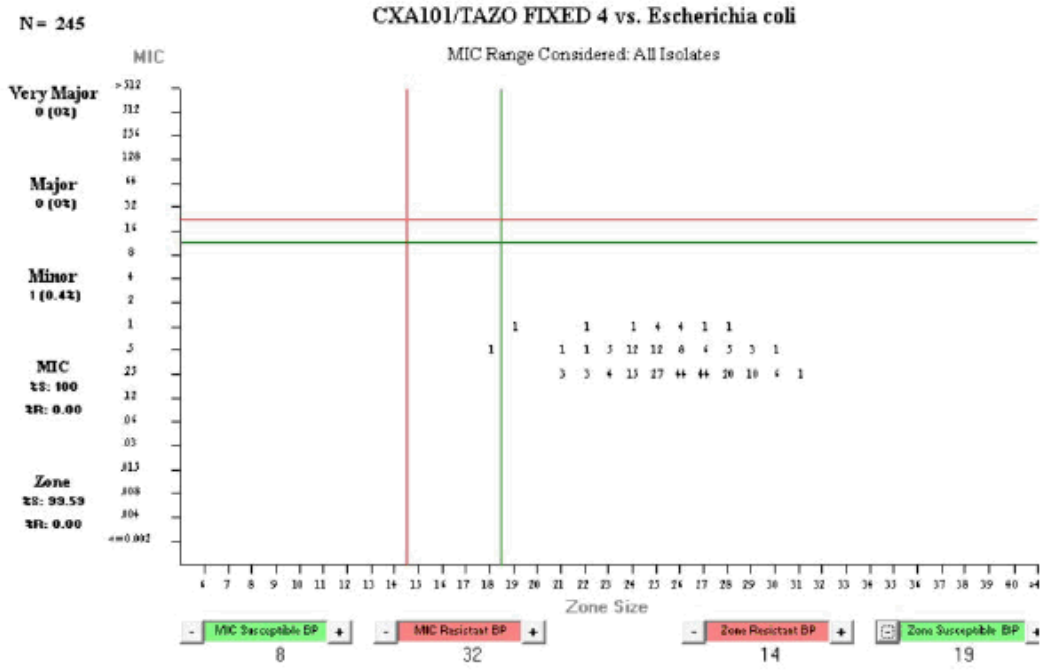
CLSI=Clinical and Laboratory Standards Institute; I=intermediate; R=resistant; S=susceptible

[a] Exceeded CLSI recommended guidelines for discrepancy rates: Very major error: ≥I+2 2.0%; I+1 to I-1 10%; ≤I-2 NA; Major ≥I+2 NA; I+1 to I-1 10%, ≤I-2 2.0%; Minor ≥I+2 5.0%, I+1 to I-1 40%, ≤I-2 5.0%.

Source: M5.3.5.4\CXA.028.MC

A collection of 245 clinical isolates of *E. coli* producing CTX-M-14 and CTX-M-15-type ESBLs were tested with both broth microdilution and Kirby-Bauer disk diffusion methods. Only 1 minor error was encountered using 2 different sets of ceftolozane-tazobactam proposed breakpoints which is well within CLSI M23 document guidance for acceptable discordance rates (see figures below). All of the isolates were susceptible by MIC (MIC values ≤ 1 mcg/mL) and had zone sizes ≥18 mm.

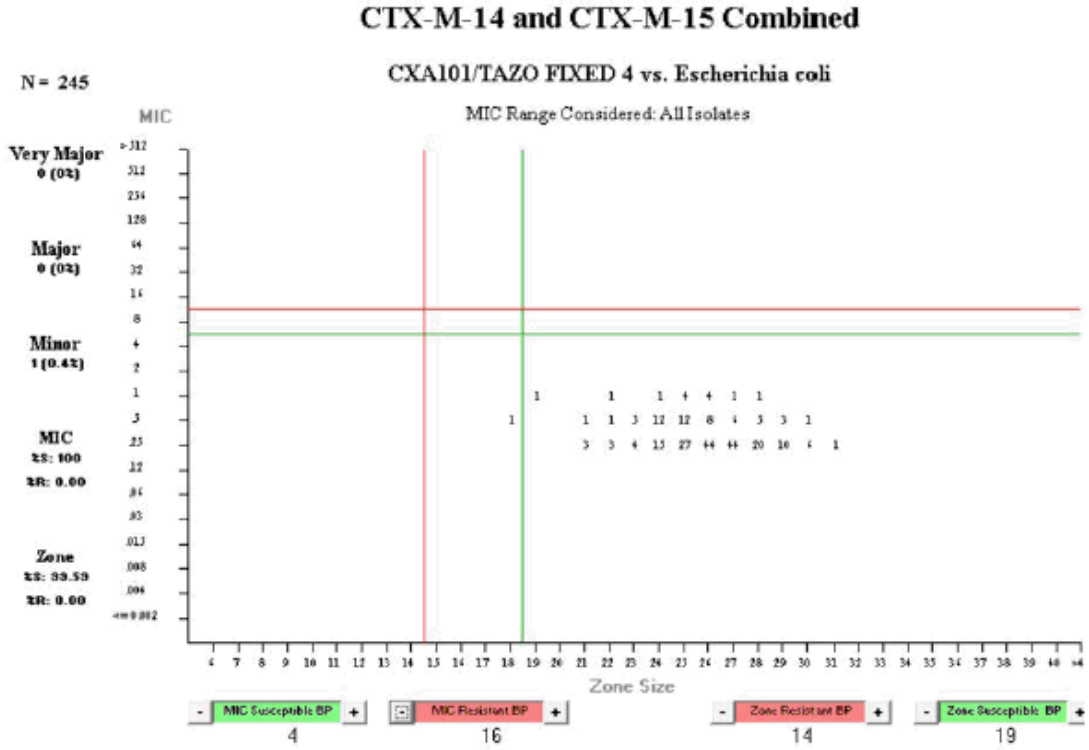
Figure 30: Ceftolozane-Tazobactam Broth Microdilution versus Disk Zone Diameter Against CTX-M-14 and CTX-M-15 *Escherichia coli* (n-245) Using Ceftolozane-Tazobactam MIC (mcg/mL) Breakpoints(≤S|I| ≥R: ≤8|16|≥32) and Disk Diameter (mm) Breakpoints (≤S|I| ≥R: ≥19|15-18|≤14)



Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancis (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/= I+2	0	0 (0%)	NA	0 (0%)
I+1 to I-1	0	0 (0%)	0 (0%)	0 (0%)
</= I-2	245	NA	0 (0%)	1 (0.4%)
All Isolates	245	0 (0%)	0 (0%)	1 (0.4%)

Figure 31: Ceftolozane-Tazobactam Broth Microdilution versus Disk Zone Diameter Against CTX-M-14 and CTX-M-15 *Escherichia coli* (n-245) Using Ceftolozane-Tazobactam MIC (mcg/mL) Breakpoints(≤S|I| ≥R: ≤4|8|≥16) and Disk Diameter (mm) Breakpoints (≤S|I| ≥R: ≥19|15-18|≤14)



Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/= I+2	0	0 (0%)	NA	0 (0%)
I+1 to I-1	0	0 (0%)	0 (0%)	0 (0%)
</= I-2	245	NA	0 (0%)	1 (0.4%)
All Isolates	245	0 (0%)	0 (0%)	1 (0.4%)

CXA101=ceftolozane; I=intermediate; MIC=minimum inhibitory concentration; R=resistant; S=susceptible; TAZO=tazobactam
Source: M5.3.5.4\CXA.061.MC

Study CXA.061.MC evaluated the correlation of broth microdilution MIC values to disk zone diameters for ceftolozane-tazobactam and comparators, meropenem and levofloxacin. This study was conducted within the appropriate CLSI guidelines as defined in CLSI documents M23-A3 using the error rate bounded analysis methodology and presented in scattergram graphic format Two sets of tentative microdilution susceptibility breakpoints, ≤8, 16, and 32 and ≤4, 8, 16 µg/mL, were provided by Cubist for evaluation against data from two clinical trial data sets, cIAI and cUTI, alone and in

combination. The broth microdilution MICs and disk zone diameters for *P. aeruginosa* and Enterobacteriaceae gave concordance results for the very major, major, and minor rates for ceftolozane-tazobactam that were well within the CLSI M23 targets. One isolate of viridans streptococci resulted in an unacceptable very major error rate for this group of organisms using the breakpoints of ≤4, 8, and ≥16 µg/mL. However, using the breakpoints of ≤8, 16, and ≥32 µg/mL, the viridans streptococci error rates were within the CLSI M23 targets.

Data were analyzed for six target species: *Escherichia coli, Klebsiella pneumoniae*, *Klebsiella oxytoca, Enterobacter cloacae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The viridans streptococci: *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus salivarius* were analyzed as a combined viridans group Table 3. Four species: *Citrobacter braakii*, *Citrobacter freundii*, *Morganella morganii*, and *Serratia marcescens* were not analyzed individually but were included in the analysis of the Enterobacteriaceae group.

Analyses were performed on the combined datasets and each of the cIAI and cUTI clinical data sets individually at the tentative provisional MIC susceptible, intermediate, and resistant breakpoints of ≤8, 16, and ≥32 µg/mL. Each analysis was repeated at MIC susceptible, intermediate, and resistant breakpoints of ≤4, 8, and ≥16 µg/mL. The three species of viridans streptococci were collected only in the cIAI study.

Reviewer's Comment
The scatterplots for the combined studies are below. Final conclusions from these scatterplots are deferred due to pending discussions within the Agency related to breakpoint analysis. Further discussion may be in a subsequent separate review.

Reviewer's Comment
Scatterplots of zone diameter and MIC correlations related to recent surveillance studies were not located in the submission. The Applicant may be asked to provide this information and it may be discussed in an addendum to this review.

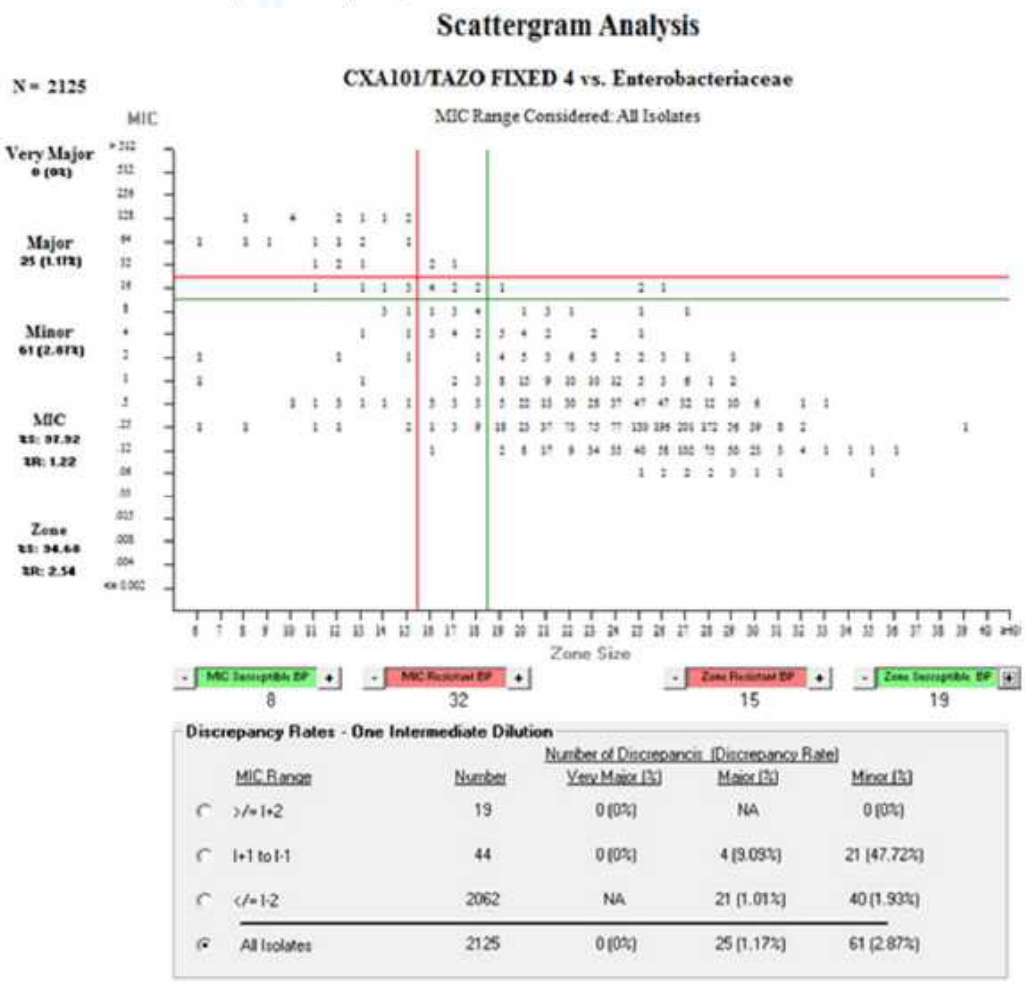
Table 119: Organisms Used in Analysis by Clinical Study and CLSI family group and species

Organism Family	Organism	cIAI	cUTI	Combined Totals
Enterobacteriaceae	*Citrobacter braakii**	13		13
	*Citrobacter freundii**	37	8	45
	Enterobacter cloacae	92	23	115
	Escherichia coli	888	706	1594
	Klebsiella oxytoca	66	10	76
	Klebsiella pneumoniae	116	69	185
	*Morganella morganii**	12	3	15
	Proteus mirabilis	34	36	70
	*Serratia marcescens**	5	7	12
***Enterobacteriaceae* Total**		**1263**	**862**	**2125**
Non-Fermenters	*Pseudomonas aeruginosa*	107	32	139
Non-Fermenters Total		**107**	**32**	**139**
Viridans streptococci	*Streptococcus anginosus**	114		114
	*Streptococcus constellatus**	83		83
	*Streptococcus salivarius**	39		39
Viridans streptococci Total		**236**		**236**
Grand Total		**1606**	**894**	**2500**

* Analyzed aggregately with the organism family; not graphed separately.

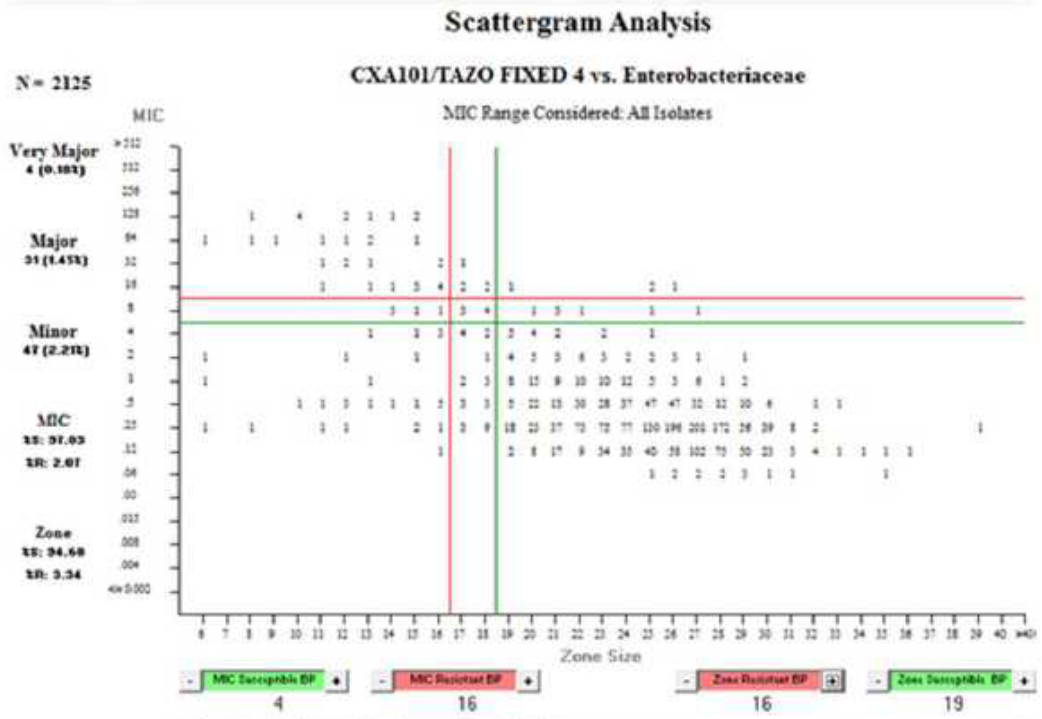
Source: This submission. Study CXA.061.MC.

Figure 32: Ceftolozane-Tazobactam Broth Microdilution versus Disk Zone Diameter against Enterobacteriaceae from Combined Clinical Studies (n=2125)- MIC Breakpoints ≤8|16|≥32



Source: This submission. Study CXA.061.MC.

Figure 33: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against Enterobacteriaceae from Combined Clinical Studies (n=2125)- MIC Breakpoints ≤4|8|≥16

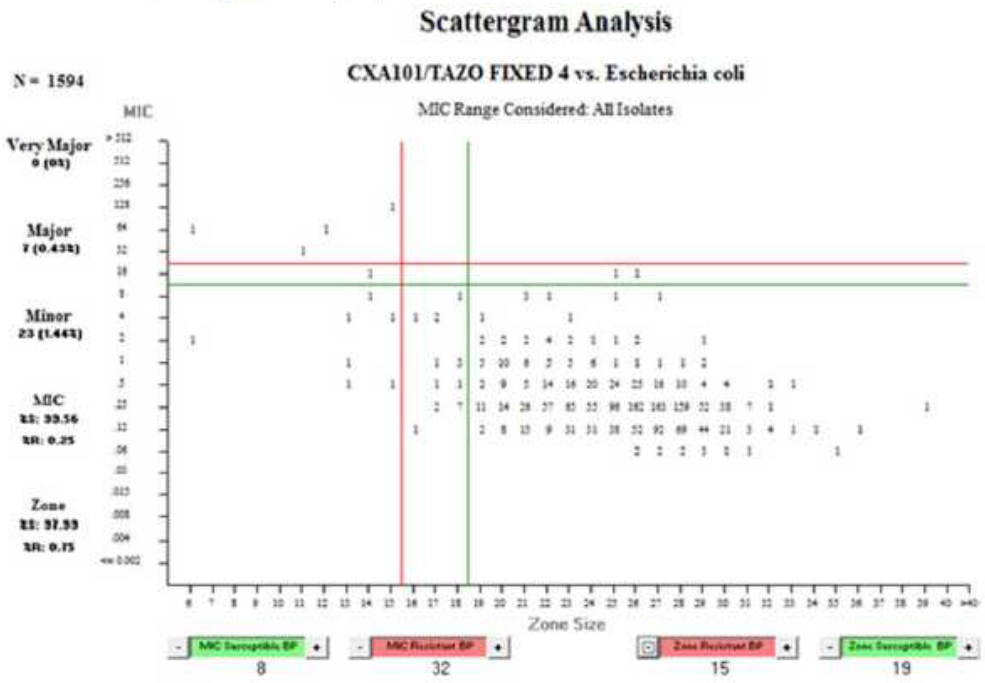


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	26	0 (0%)	NA	1 (3.84%)
I+1 to I-1	62	4 (6.45%)	5 (8.06%)	22 (35.48%)
</=I-2	2037	NA	26 (1.27%)	24 (1.17%)
All Isolates	2125	4 (0.18%)	31 (1.45%)	47 (2.21%)

Source: This submission. Study CXA.061.MC.

Figure 34: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Escherichia coli* from Combined Clinical Studies (n=1594)-MIC Breakpoints ≤8|16|≥32

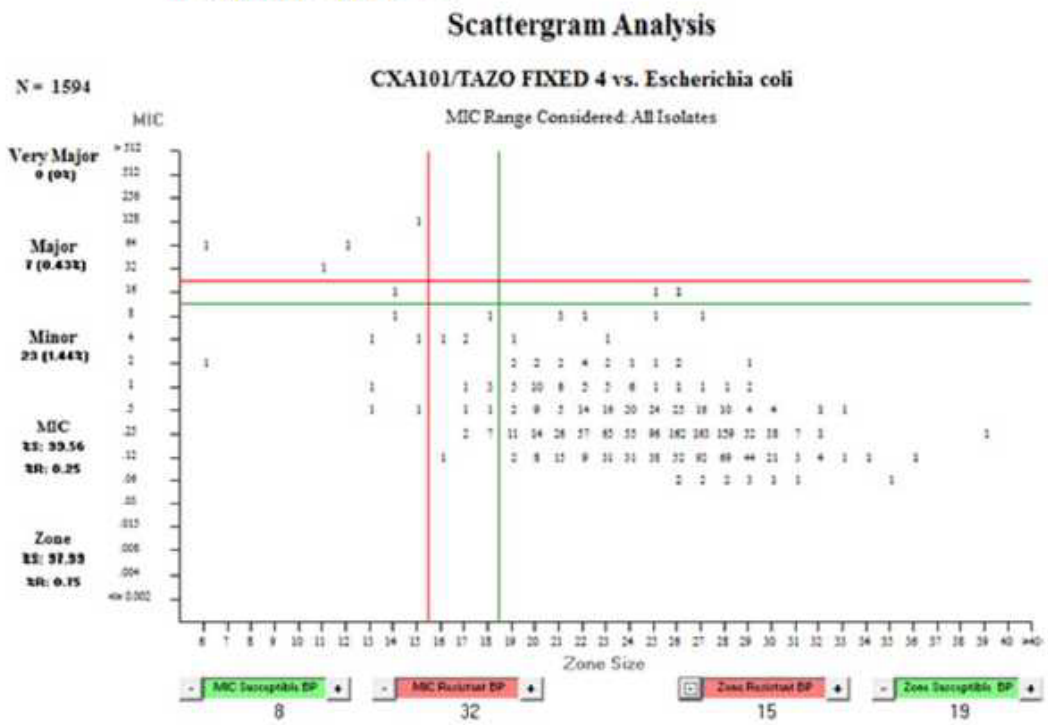


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/= I+2	3	0 (0%)	NA	0 (0%)
I+1 to I-1	12	0 (0%)	1 (8.33%)	4 (33.33%)
</= I-2	1579	NA	6 (0.37%)	19 (1.2%)
All Isolates	1594	0 (0%)	7 (0.43%)	23 (1.44%)

Source: This submission. Study CXA.061.MC.

Figure 35: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Escherichia coli* from Combined Clinical Studies (n=1594)-MIC Breakpoints ≤4|8|≥16

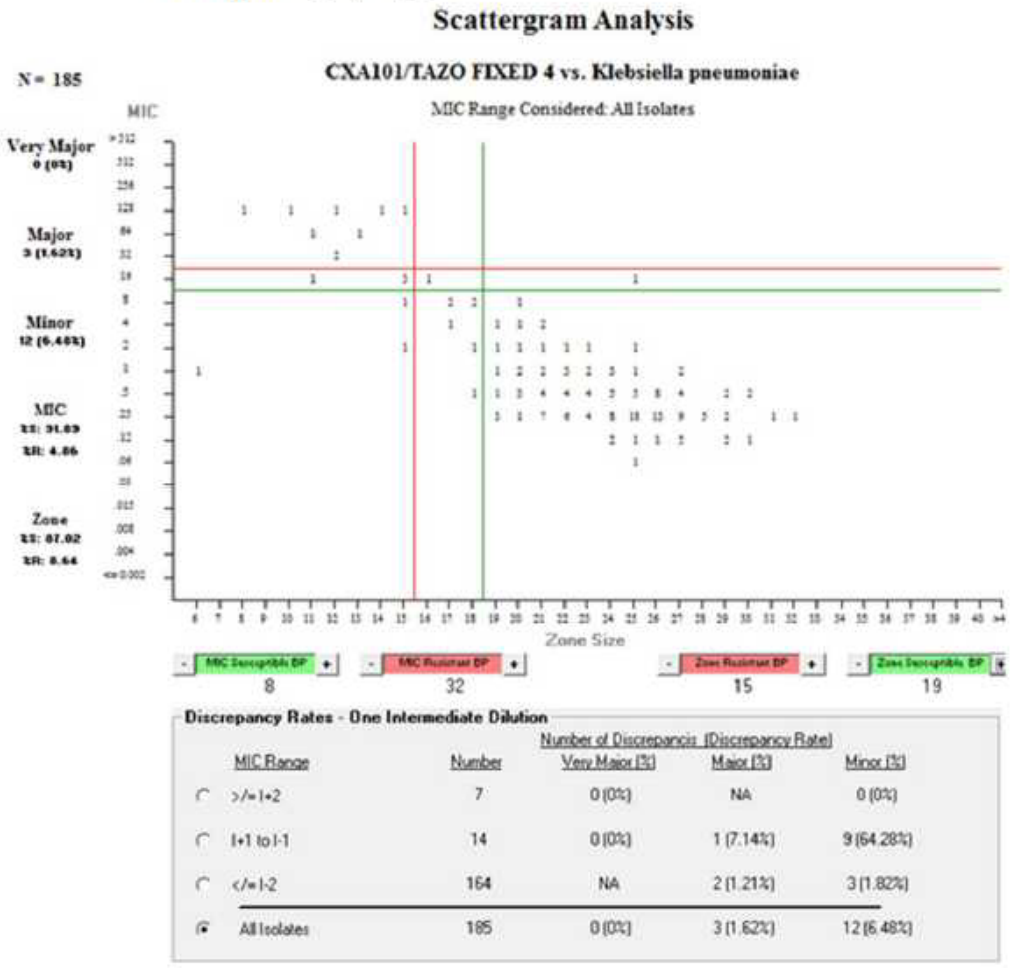


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/= I+2	3	0 (0%)	NA	0 (0%)
I+1 to I-1	12	0 (0%)	1 (8.33%)	4 (33.33%)
</= I-2	1579	NA	6 (0.37%)	19 (1.2%)
All Isolates	1594	0 (0%)	7 (0.43%)	23 (1.44%)

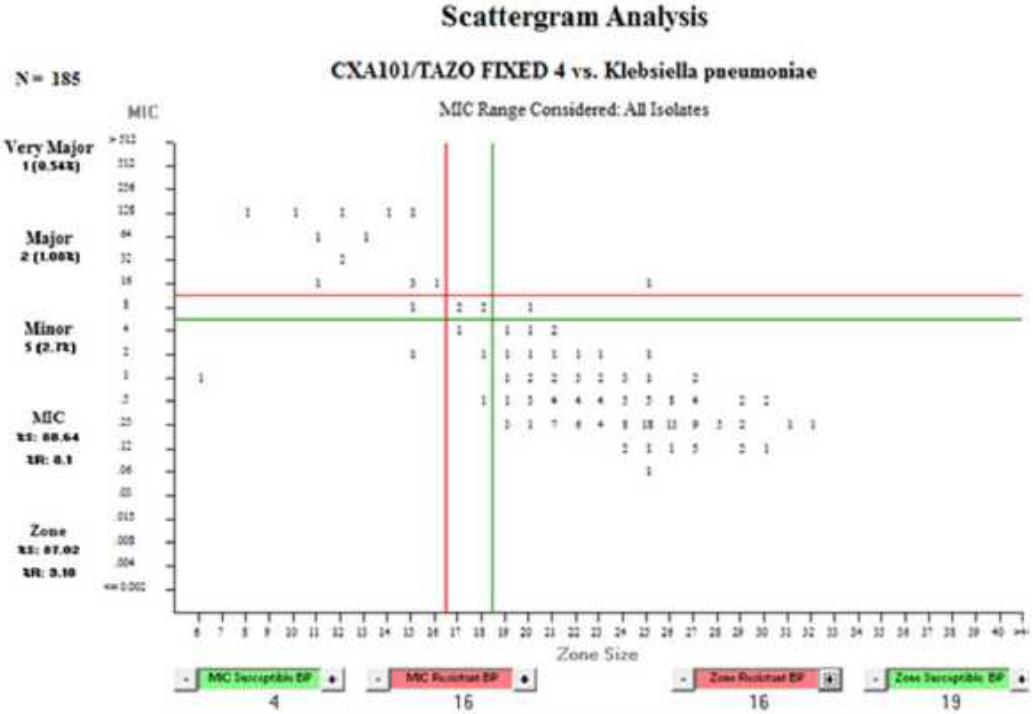
Source: This submission. Study CXA.061.MC.

Figure 36: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Klebsiella pneumoniae* from Combined Clinical Studies (n=185)- MIC Breakpoints ≤8|16|≥32



Source: This submission. Study CXA.061.MC.

Figure 37: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against Enterobacteriaceae from Combined Clinical Studies (n=2125)- MIC Breakpoints ≤4|8|≥16

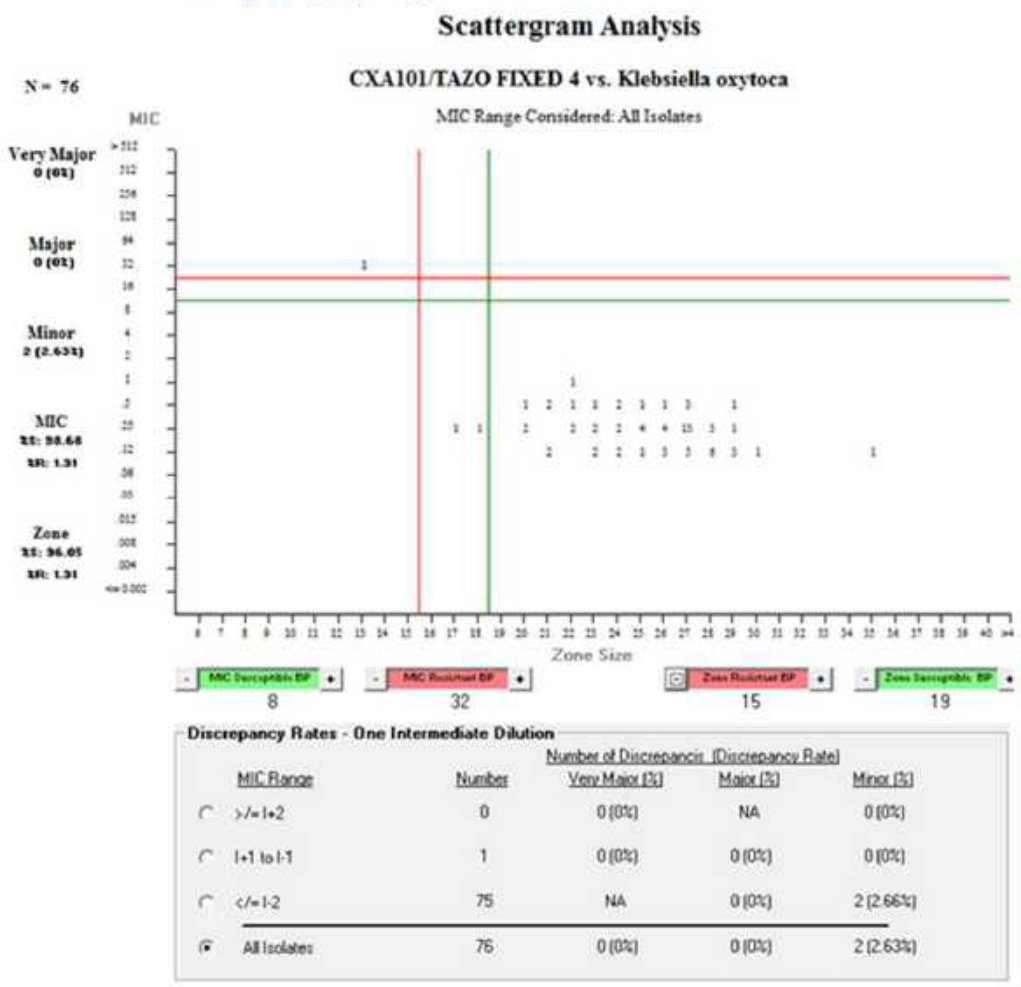


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/= I+2	9	0 (0%)	NA	0 (0%)
I+1 to I-1	17	1 (5.88%)	0 (0%)	3 (17.64%)
</= I-2	159	NA	2 (1.25%)	2 (1.25%)
All Isolates	185	1 (0.54%)	2 (1.08%)	5 (2.7%)

Source: This submission. Study CXA.061.MC.

Figure 38: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Klebsiella oxytoca* from Combined Clinical Studies (n=76)-MIC Breakpoints ≤8|16|≥32

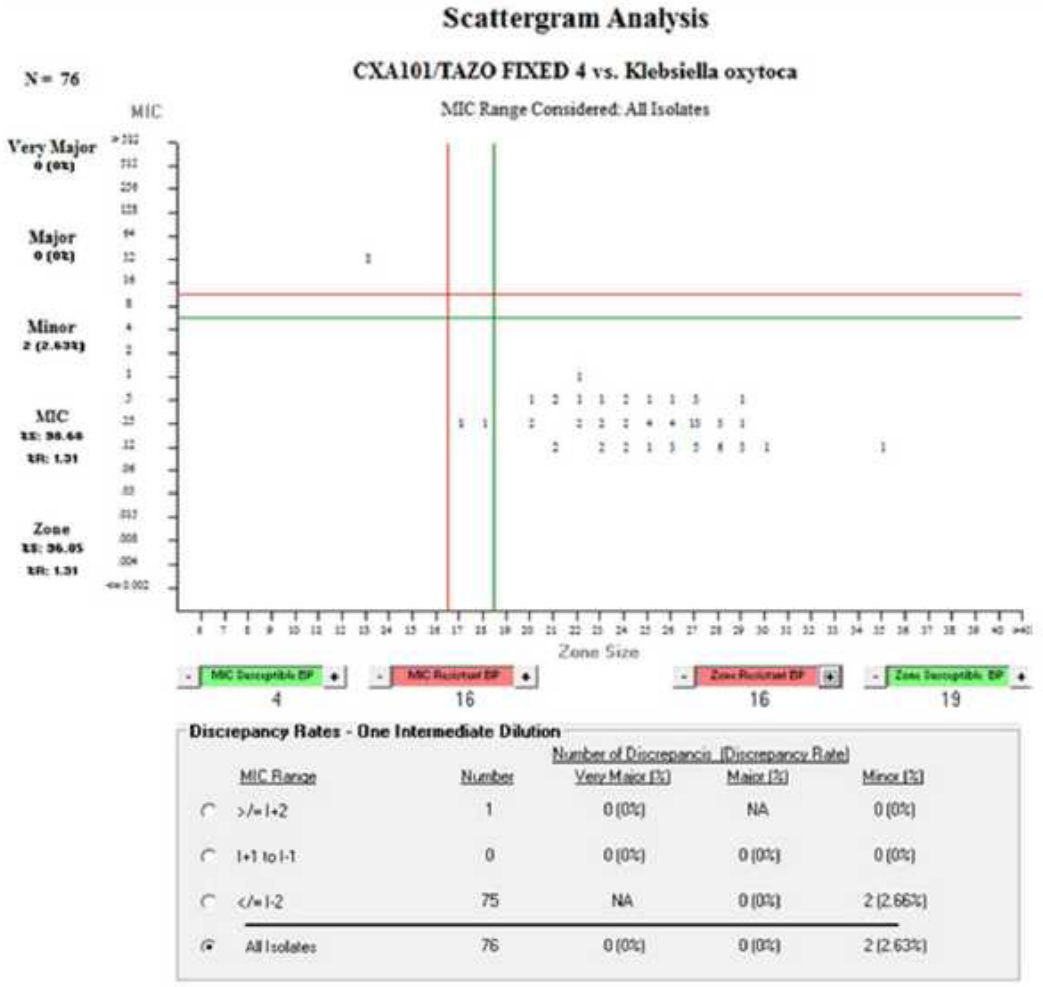


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	0	0 (0%)	NA	0 (0%)
I+1 to I-1	1	0 (0%)	0 (0%)	0 (0%)
</=I-2	75	NA	0 (0%)	2 (2.66%)
All Isolates	76	0 (0%)	0 (0%)	2 (2.63%)

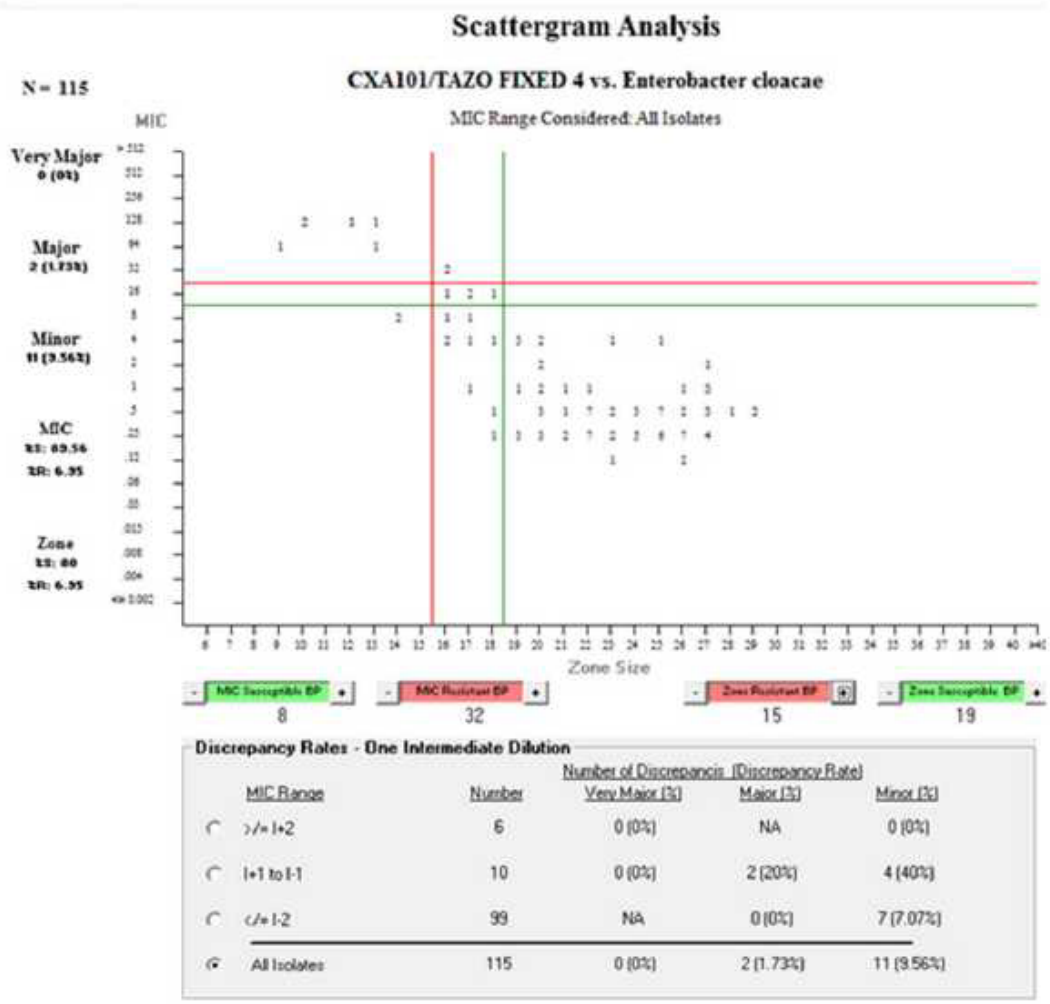
Source: This submission. Study CXA.061.MC.

Figure 39: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Klebsiella oxytoca* from Combined Clinical Studies (n=76)-MIC Breakpoints ≤4|8|≥16



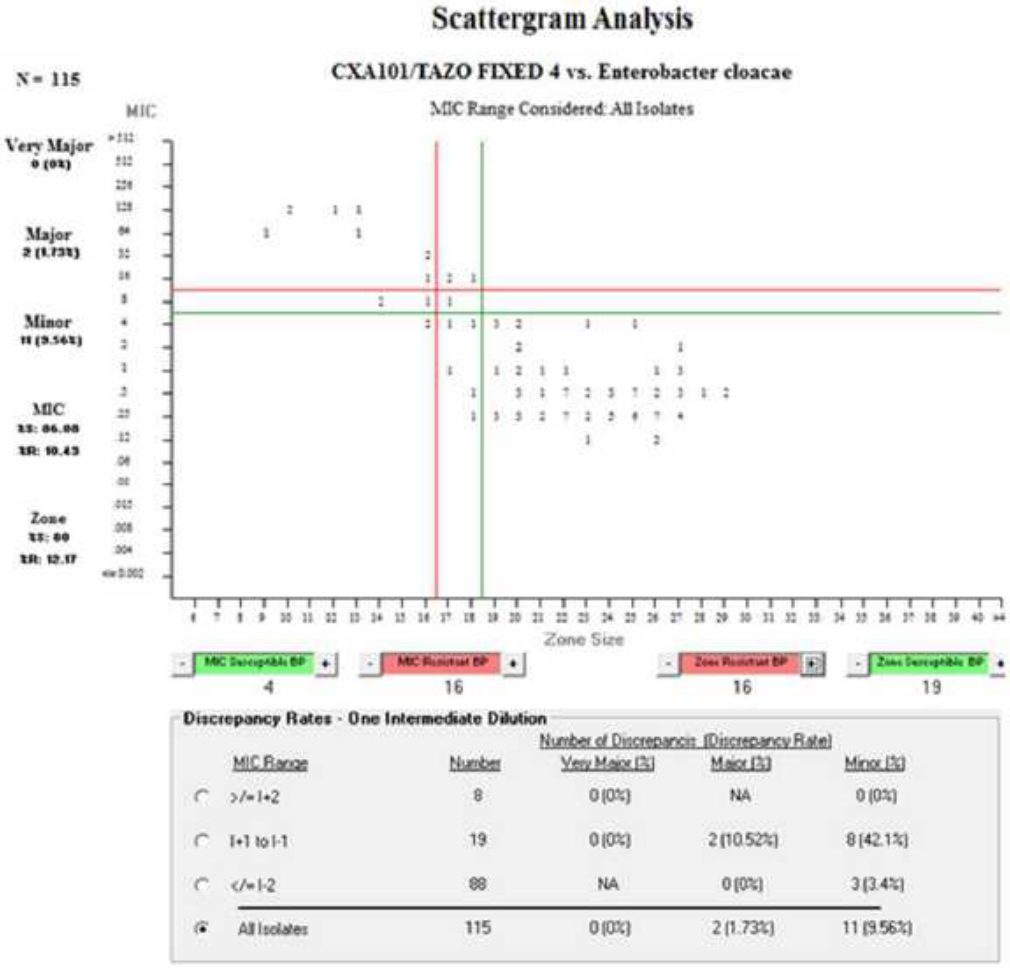
Source: This submission. Study CXA.061.MC.

Figure 40: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Enterobacter cloacae* from Combined Clinical Studies (n=115)- MIC Breakpoints ≤8|16|≥32



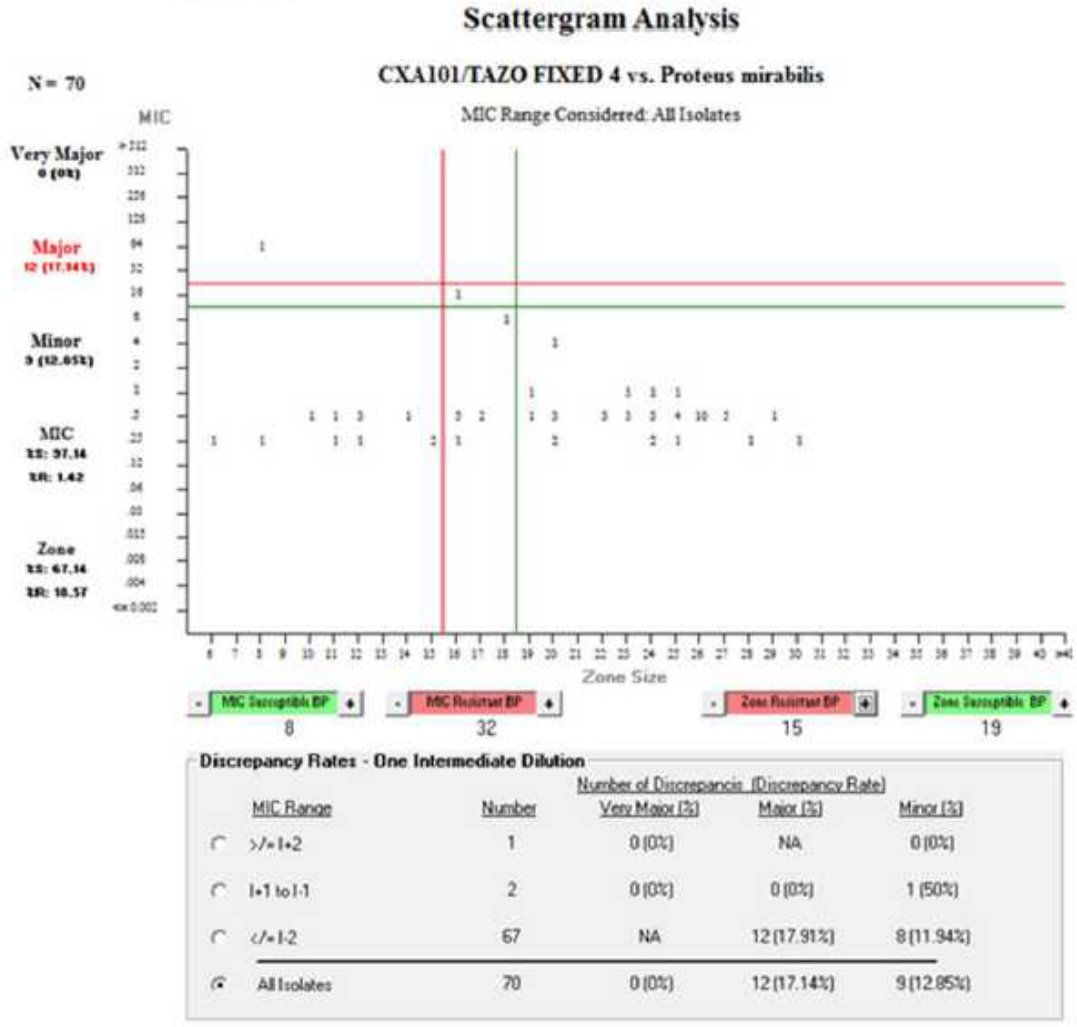
Source: This submission. Study CXA.061.MC.

Figure 41: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Enterobacter cloacae* from Combined Clinical Studies (n=115)- MIC Breakpoints ≤4|8|≥16



Source: This submission. Study CXA.061.MC.

Figure 42: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Proteus mirabilis* from Combined Clinical Studies (n=70)-MIC Breakpoints ≤8|16|≥32

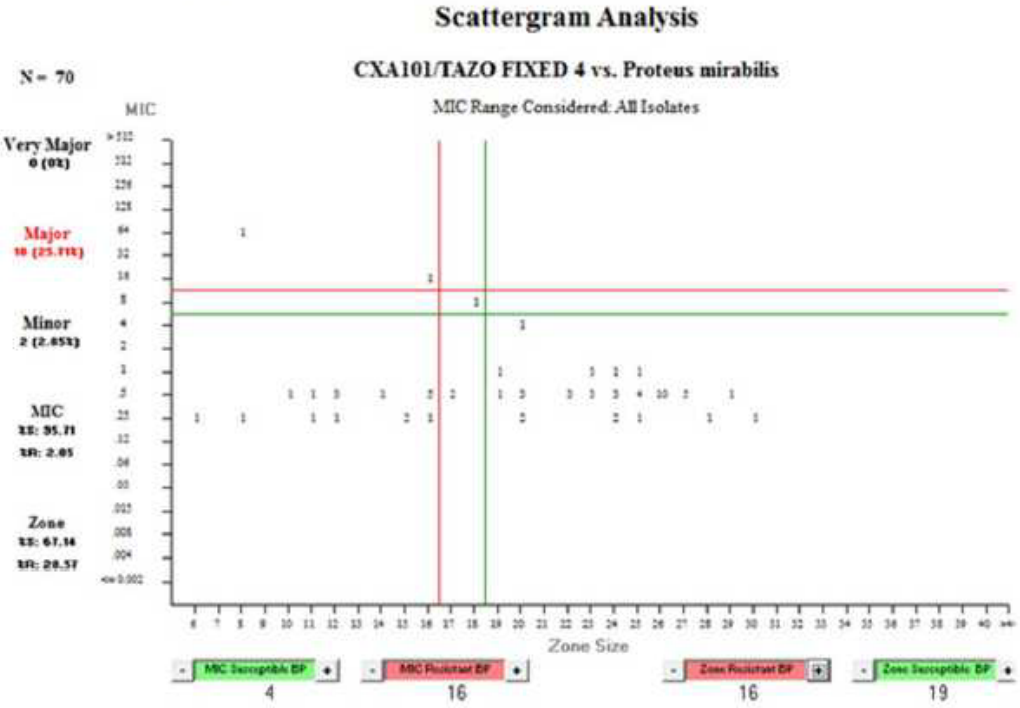


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	1	0 (0%)	NA	0 (0%)
I+1 to I-1	2	0 (0%)	0 (0%)	1 (50%)
</=I-2	67	NA	12 (17.91%)	8 (11.94%)
All Isolates	70	0 (0%)	12 (17.14%)	9 (12.85%)

Source: This submission. Study CXA.061.MC.

Figure 43: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Proteus mirabilis* from Combined Clinical Studies (n=70)-MIC Breakpoints ≤4|8|≥16



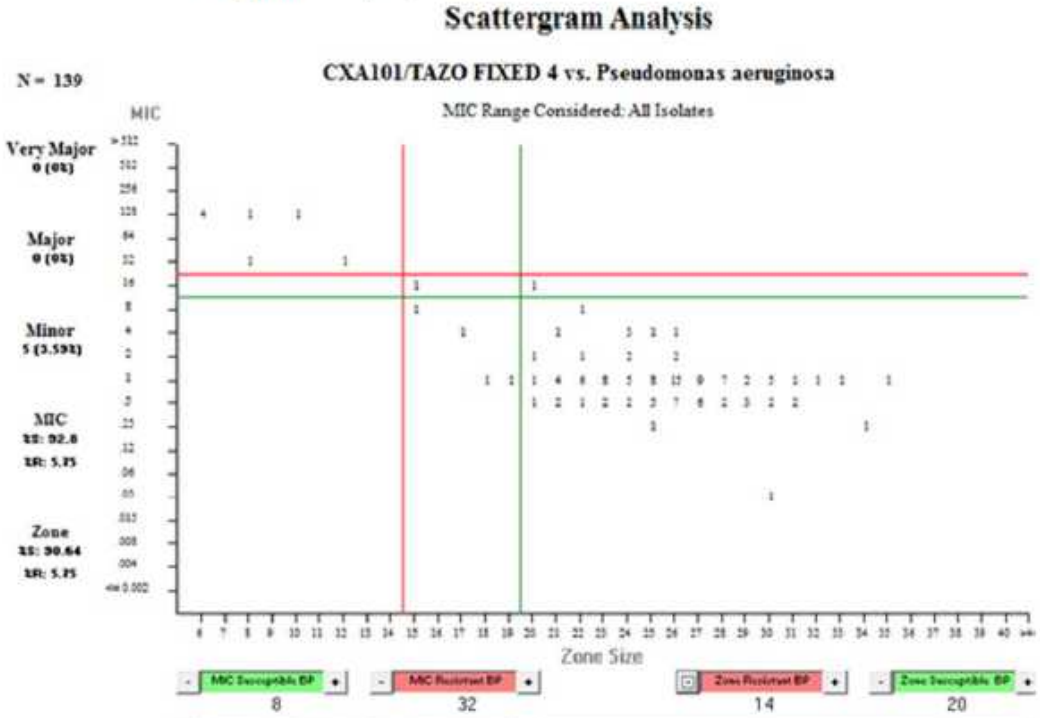
Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	1	0 (0%)	NA	0 (0%)
I+1 to I-1	3	0 (0%)	0 (0%)	0 (0%)
</=I-2	66	NA	18 (27.27%)	2 (3.03%)
All Isolates	70	0 (0%)	18 (25.71%)	2 (2.85%)

Source: This submission. Study CXA.061.MC.

Figure 44: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Pseudomonas aeruginosa* from Combined Clinical Studies (n=139)-MIC Breakpoints ≤8|16|≥32

Figure 13: Ceftolozane/Tazobactam Broth Microdilution vs Disk Zone Diameter against *Pseudomonas aeruginosa* from Combined Clinical Studies, (n=139) - MIC breakpoints ≤8 | 16 | ≥32.

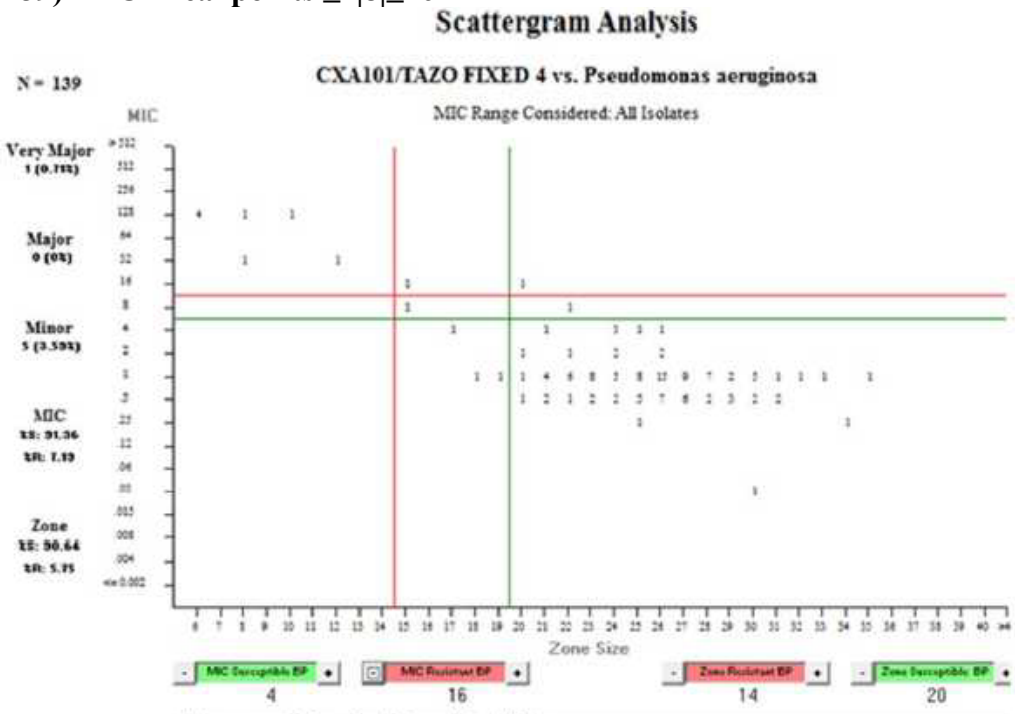


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	6	0 (0%)	NA	0 (0%)
I+1 to I-1	6	0 (0%)	0 (0%)	2 (33.33%)
</=I-2	127	NA	0 (0%)	3 (2.36%)
All Isolates	139	0 (0%)	0 (0%)	5 (3.59%)

Source: This submission. Study CXA.061.MC.

Figure 45: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against *Pseudomonas aeruginosa* from Combined Clinical Studies (n=139)-MIC Breakpoints ≤4|8|≥16

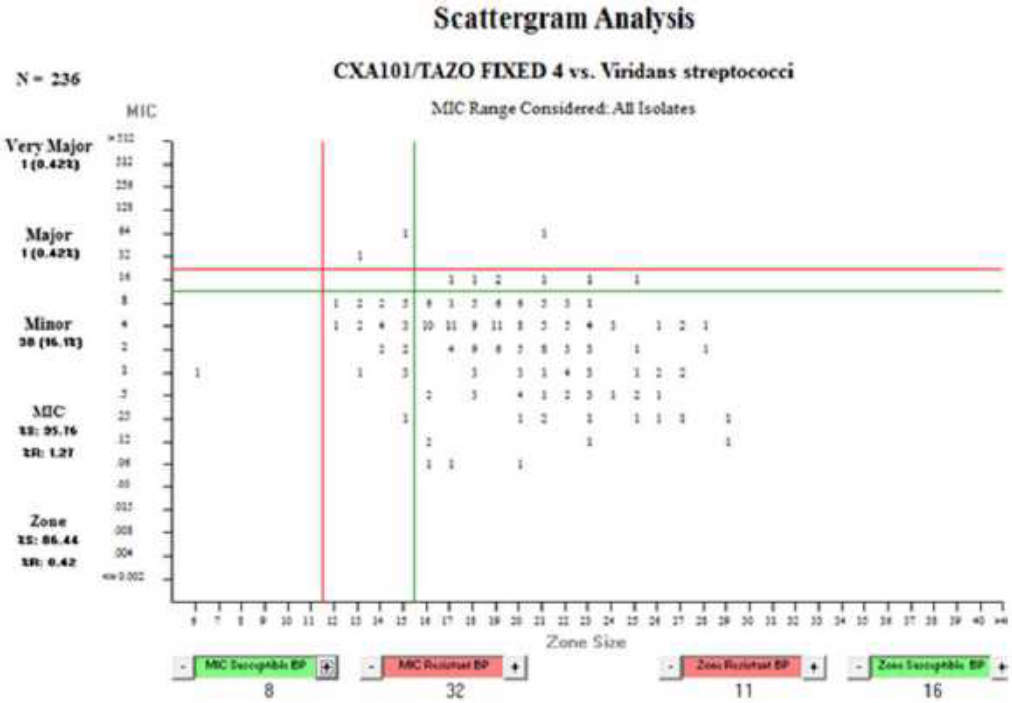


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=1+2	8	0 (0%)	NA	0 (0%)
1+1 to 1-1	11	1 (9.09%)	0 (0%)	3 (27.27%)
</=1-2	120	NA	0 (0%)	2 (1.66%)
All Isolates	139	1 (0.71%)	0 (0%)	5 (3.59%)

Source: This submission. Study CXA.061.MC.

Figure 46: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against all Viridans Streptococci from Combined Clinical Studies (n=236)-MIC Breakpoints ≤8|16|≥32

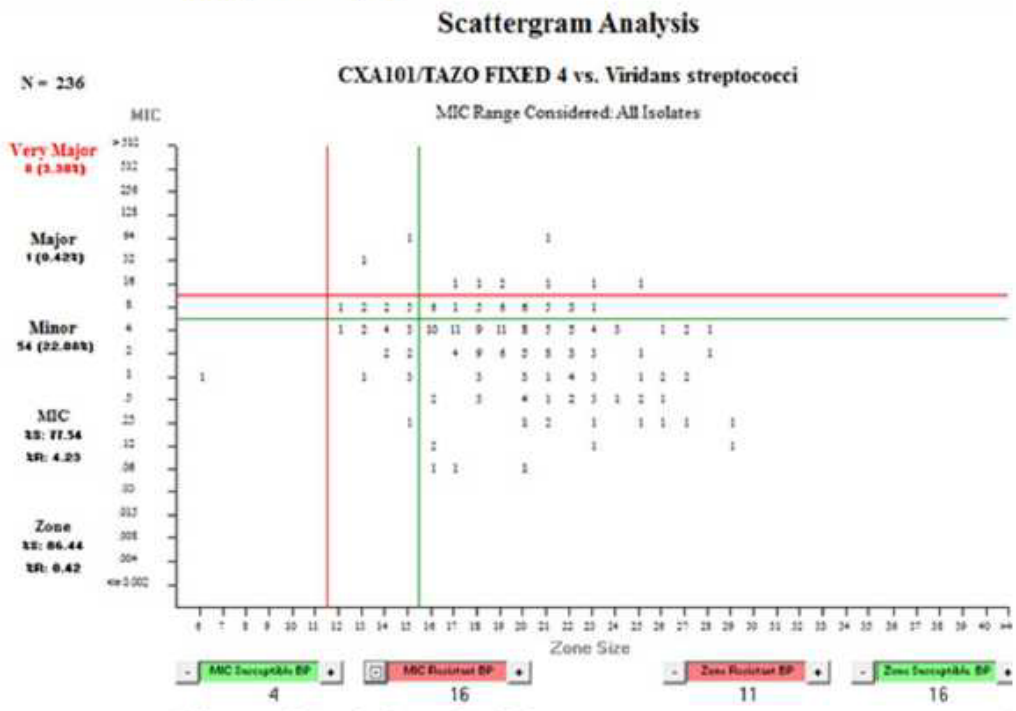


Discrepancy Rates - One Intermediate Dilution

MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=1+2	2	1 (50%)	NA	1 (50%)
1+1 to 1-1	51	0 (0%)	0 (0%)	18 (35.29%)
</=1-2	183	NA	1 (0.54%)	19 (10.38%)
All Isolates	236	1 (0.42%)	1 (0.42%)	38 (16.1%)

Source: This submission. Study CXA.061.MC.

Figure 47: Ceftolozane-tazobactam Broth Microdilutions Versus Disk Zone Diameter Against all Viridans Streptococci from Combined Clinical Studies (n=2125)-MIC Breakpoints ≤4|8|≥16



Discrepancy Rates - One Intermediate Dilution

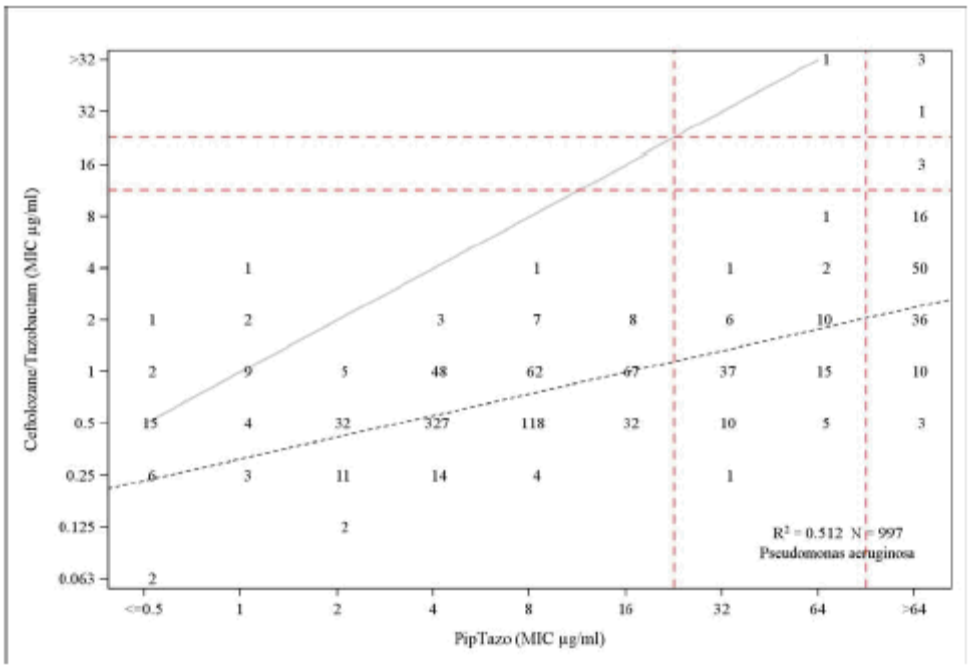
MIC Range	Number	Number of Discrepancies (Discrepancy Rate) Very Major (%)	Major (%)	Minor (%)
>/=I+2	3	1 (33.33%)	NA	2 (66.66%)
I+1 to I-1	130	7 (5.38%)	0 (0%)	43 (33.07%)
</=I-2	103	NA	1 (0.97%)	9 (8.73%)
All Isolates	236	8 (3.38%)	1 (0.42%)	54 (22.88%)

Source: This submission. Study CXA.061.MC.

MIC to MIC Correlation Results

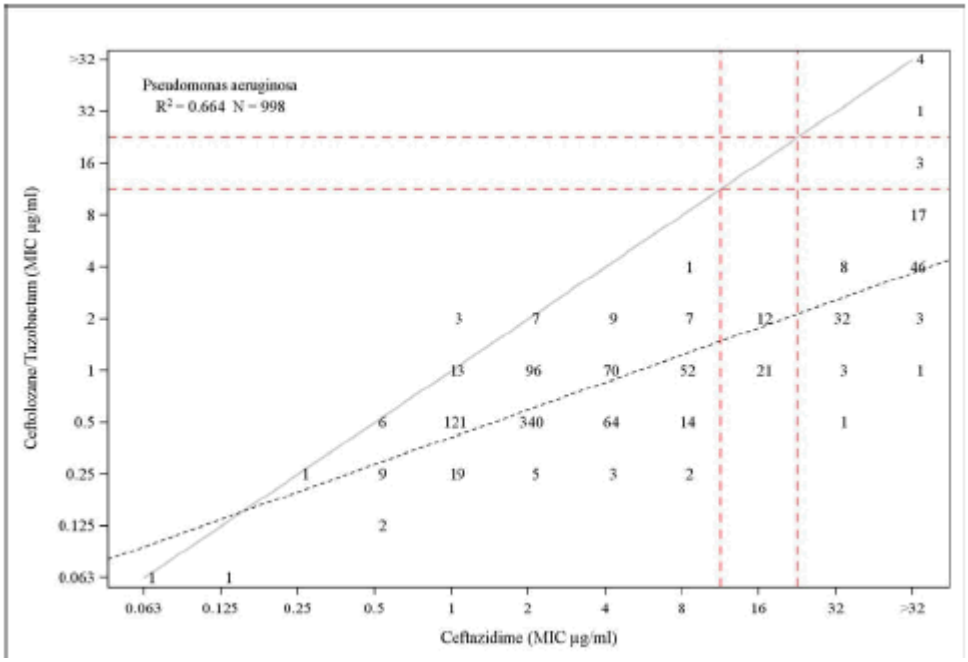
For the each following figures, the solid line is the identity line and the dotted line is the regression line of the graph. The red lines depict error bound analysis for each graph. These lines were chosen using proposed sensitive, intermediate and resistant susceptibility breakpoints for ceftolozane-tazobactam and 2013 CLSI susceptibility breakpoints for comparator antibiotics.

Figure 48: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Piperacillin-Tazobactam, in *Pseudomonas aeruginosa* isolates using 2012 US Surveillance Data



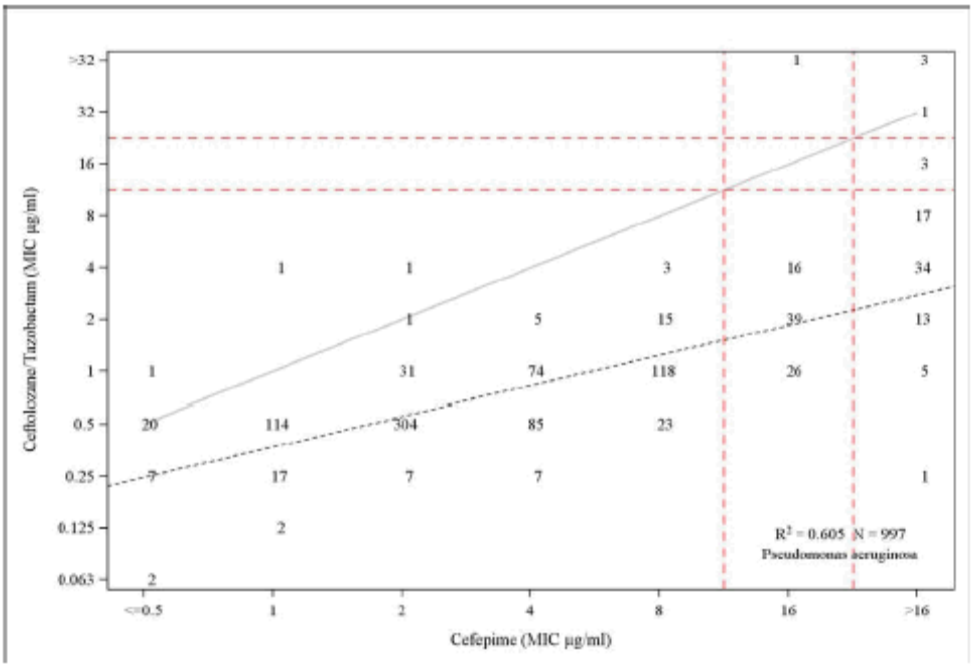
Source: This submission. Study CXA.049.MC

Figure 49: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Ceftazidime, in *Pseudomonas aeruginosa* isolates using 2012 US Surveillance Data



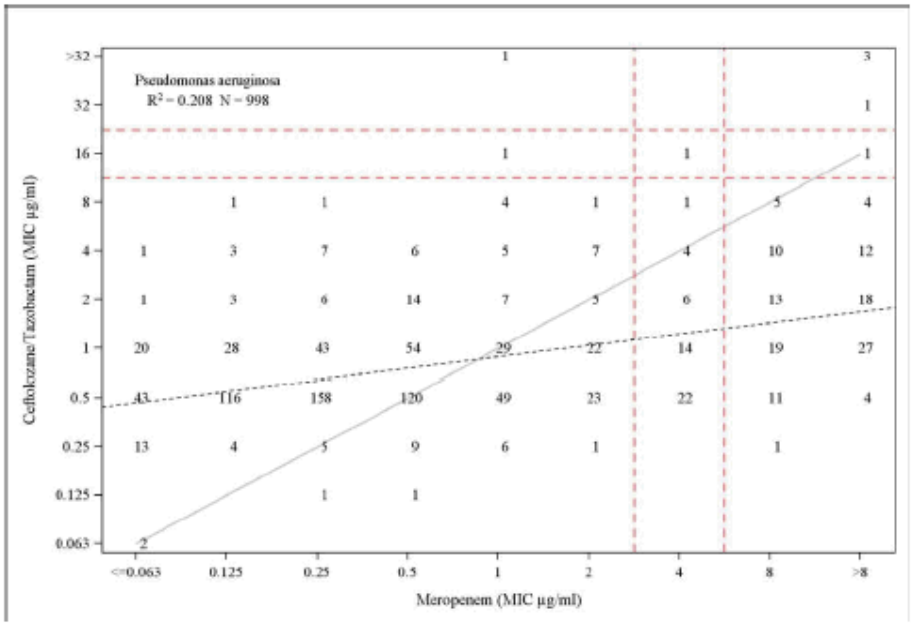
Source: This submission. Study CXA.049.MC

Figure 50: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Cefepime, in *Pseudomonas aeruginosa* isolates using 2012 US Surveillance Data



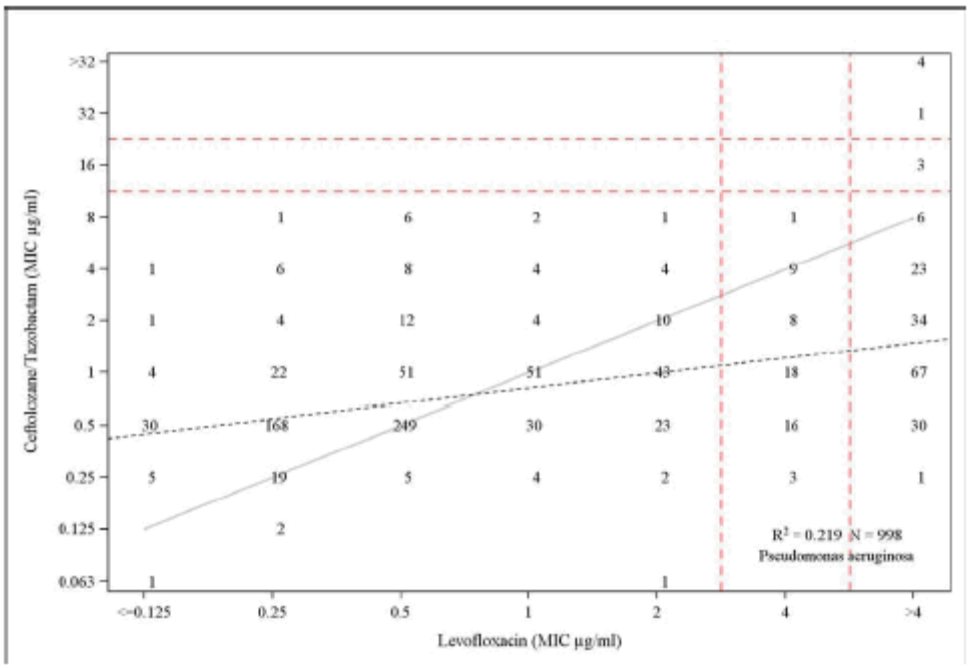
Source: This submission. Study CXA.049.MC

Figure 51: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Meropenem, in *Pseudomonas aeruginosa* isolates using 2012 US Surveillance Data



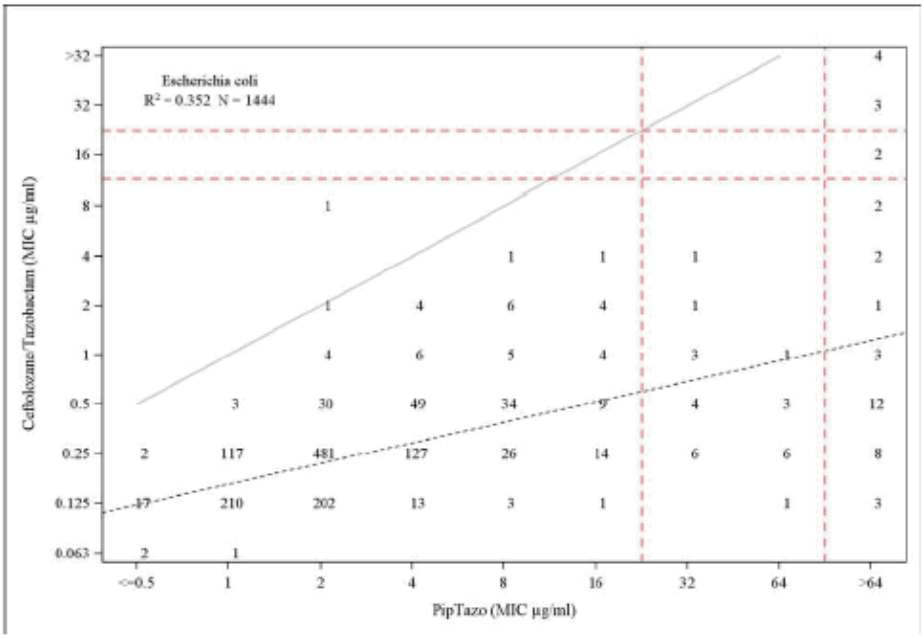
Source: This submission. Study CXA.049.MC

Figure 52: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Levofloxacin, in *Pseudomonas aeruginosa* isolates using 2012 US Surveillance Data



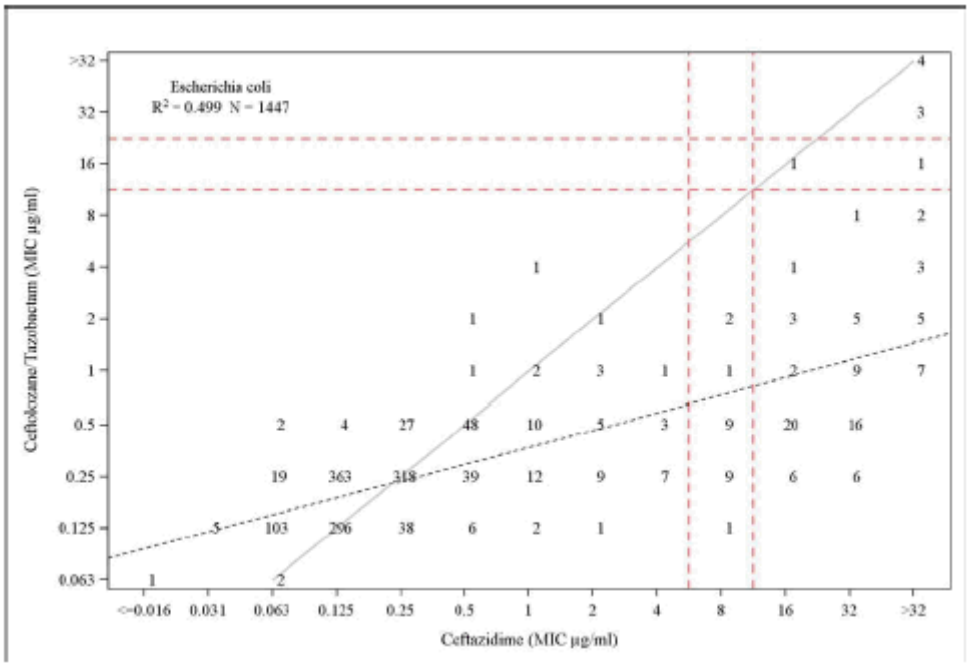
Source: This submission. Study CXA.049.MC

Figure 53: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Piperacillin-Tazobactam, in *Escherichia coli* isolates using 2012 US Surveillance Data



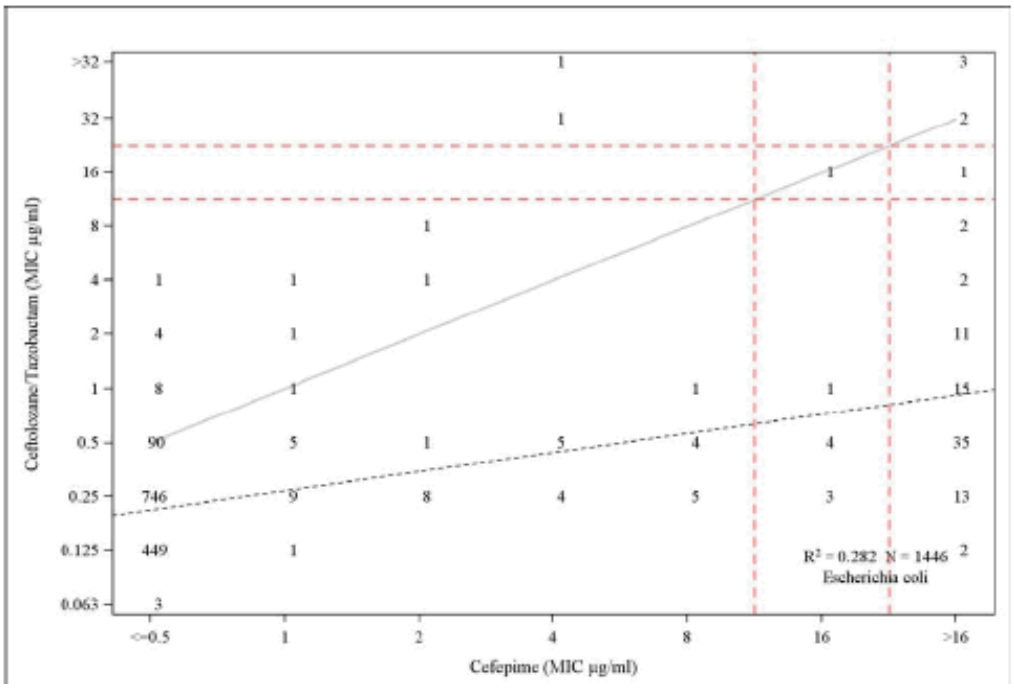
Source: This submission. Study CXA.049.MC

Figure 54: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Ceftazidime, in *Escherichia coli* isolates using 2012 US Surveillance Data



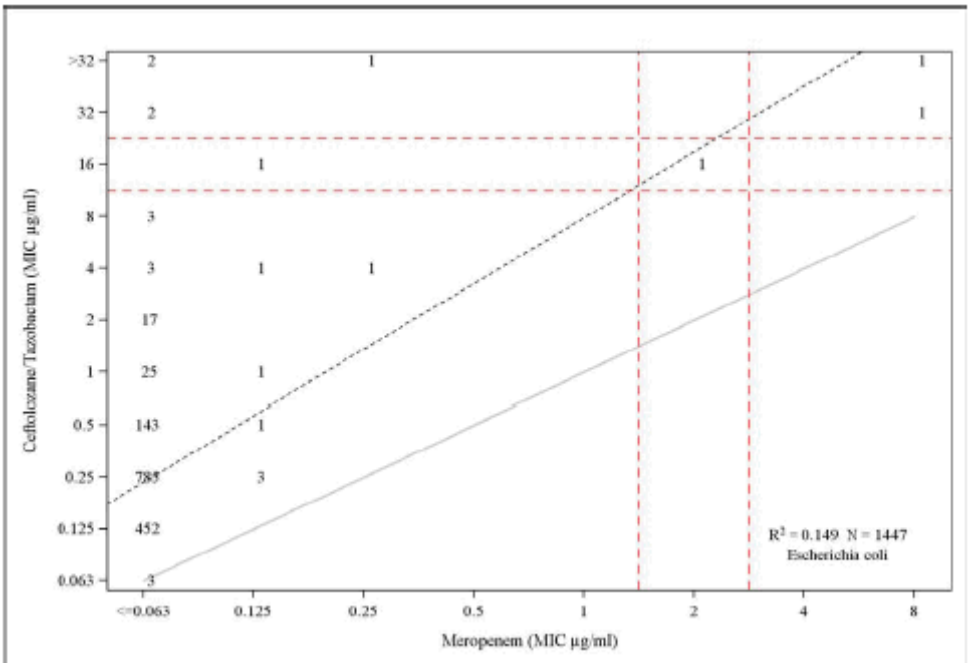
Source: This submission. Study CXA.049.MC

Figure 55: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Cefepime, in *Escherichia coli* isolates using 2012 US Surveillance Data



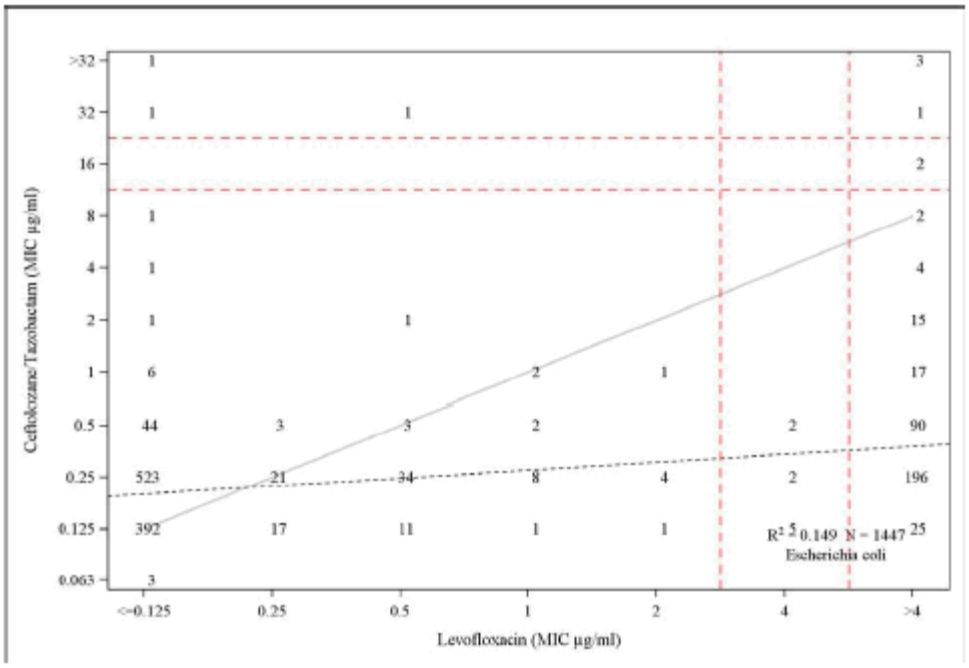
Source: This submission. Study CXA.049.MC

Figure 56: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Meropenem, in *Escherichia coli* isolates using 2012 US Surveillance Data



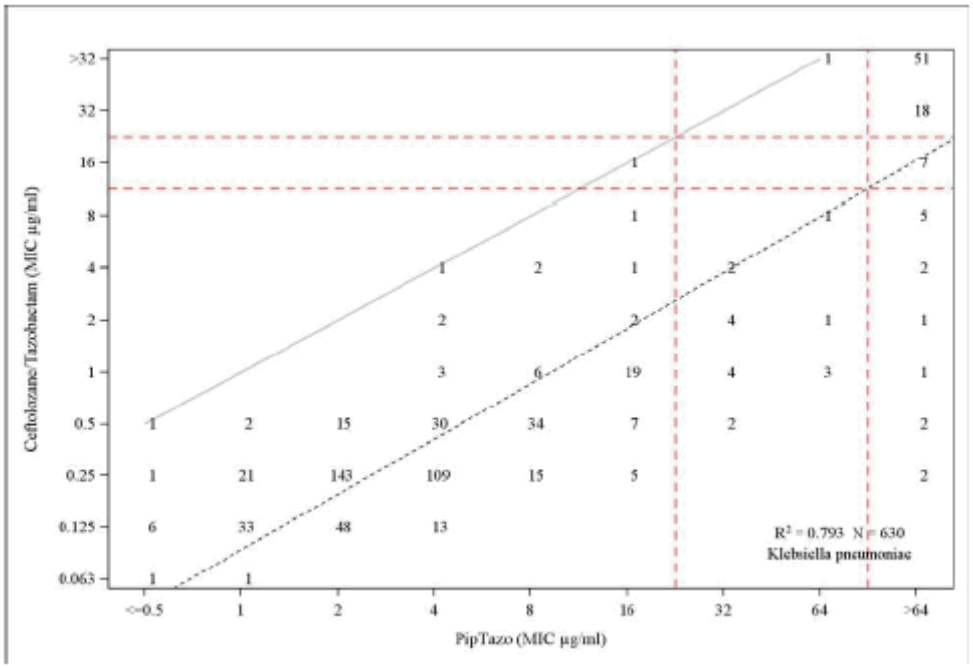
Source: This submission. Study CXA.049.MC

Figure 57: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Levofloxacin, in *Escherichia coli* isolates using 2012 US Surveillance Data



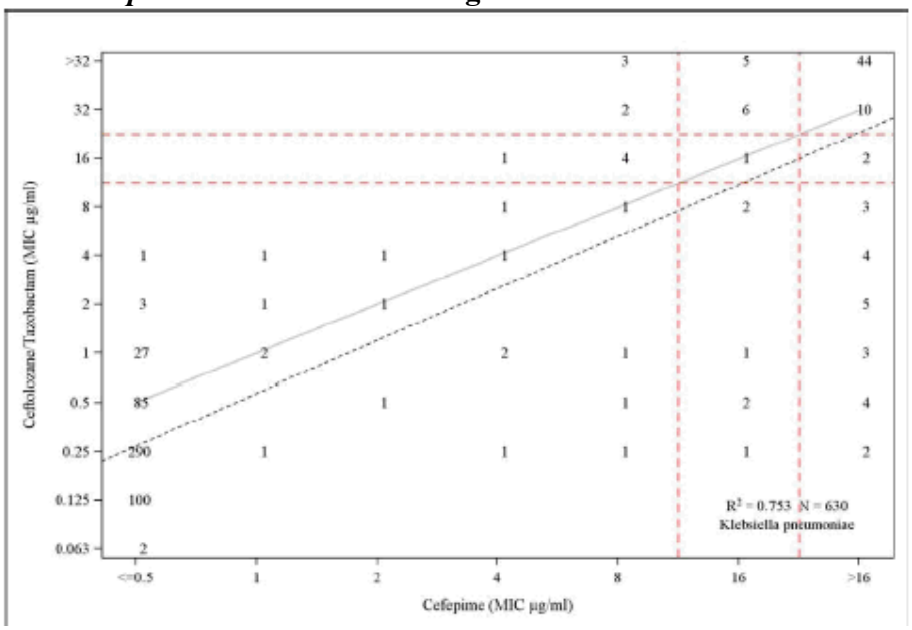
Source: This submission. Study CXA.049.MC

Figure 58: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Piperacillin-Tazobactam, in *Klebsiella pneumoniae* isolates using 2012 US Surveillance Data



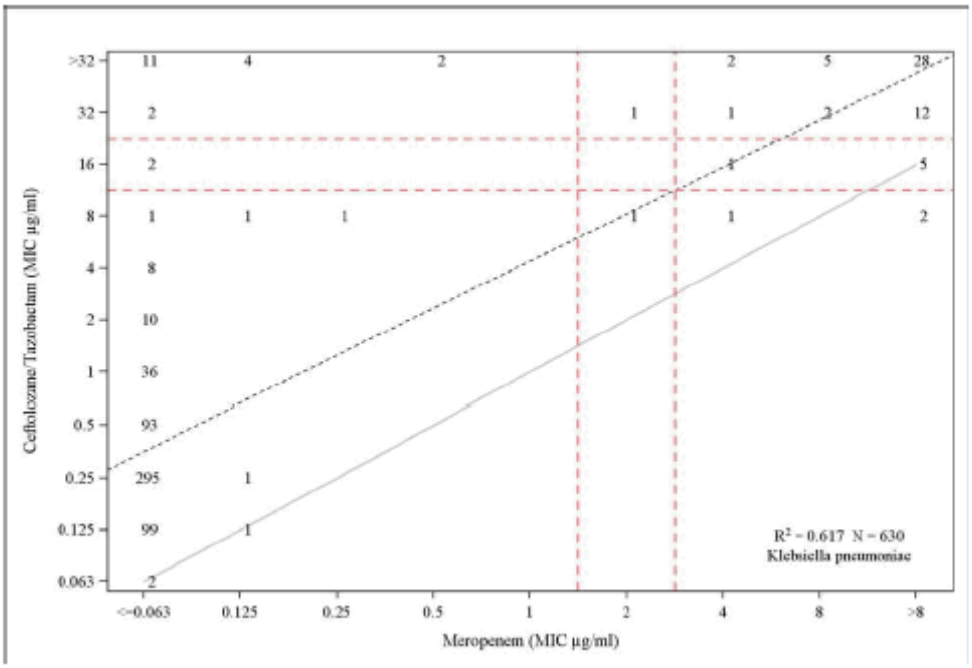
Source: This submission. Study CXA.049.MC

Figure 59: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Cefepime, in *Klebsiella pneumoniae* isolates using 2012 US Surveillance Data



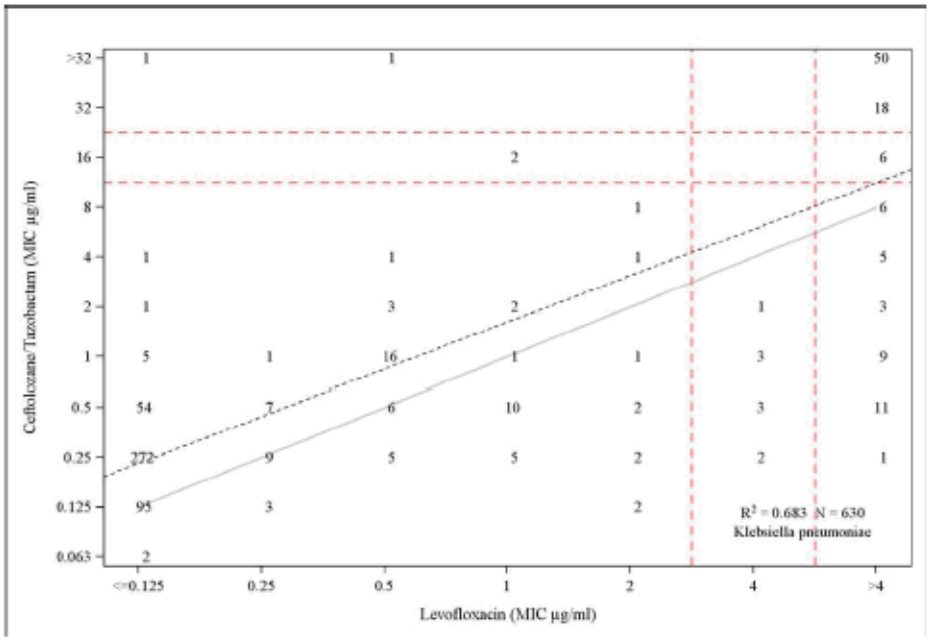
Source: This submission. Study CXA.049.MC

Figure 60: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Meropenem, in *Klebsiella pneumoniae* isolates using 2012 US Surveillance Data



Source: This submission. Study CXA.049.MC

Figure 61: MIC to MIC Correlation: Ceftolozane-Tazobactam, to Levofloxacin, in *Klebsiella pneumoniae* isolates using 2012 US Surveillance Data



Source: This submission. Study CXA.049.MC

This report summarizes ceftolozane-tazobactam susceptibility data from 2008-2012 through global surveillance studies for ceftolozane-tazobactam and comparator antibiotics. Overall, the MIC90 values remained constant for ceftolozane-tazobactam when the data are analyzed by year of surveillance and organism. The surveillance MIC data did not show a potential for cross-resistance between ceftolozane-tazobactam and marketed comparator antibiotics. The highest R^2 value was seen between ceftolozane-tazobactam and piperacillin-tazobactam in *Klebsiella pneumoniae* isolates (R^2=.793) but this is not considered significant.

Sensititre™ MIC Panel Development

A dried Sensititre 18-24 hour susceptibility (MIC) plate for ceftolozane-tazobactam was developed and tested by (b) (4) (b) (4)). Equivalency with the standard CLSI broth microdilution method was established using 200 challenge and clinical isolates, as well as the ATCC QC strains (CXA.008.MC). Essential agreements were calculated using the ± 1 log2 dilution standard comparison. The essential agreement rate for ceftolozane-tazobactam was 99.1% before repeat testing, and 100% after repeat testing. Additionally, a method for manufacturing the dried panel with ceftolozane-tazobactam for auto-read was developed (CXA.019.MC).

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Etest

[redacted] (b) (4)) developed a ceftolozane-tazobactam combination Etest strip for MIC determinations over the range of 0.016/4 to 256/4 mcg/mL (CXA.030.MC). Initial studies were performed on multiple prototypes to determine the adequate dissolution conditions for the strip, performance of the strip against the QC strains and a larger strain collection. Additionally, the impact of inoculum density, pH of the medium, and initial strip stability were evaluated. Testing of the Etest strips for all species studied, with the exception of *Acinetobacter baumannii*, were similar to broth microdilution testing (within ± 1dilution). The Etest reading was determined to be clear and the ellipses were sharp. There was no impact of the inoculum on results at either the standard conditions (0.4 to 0.6 McFarland) or at extreme conditions (0.3 to 1 McFarland). Additionally, there was no impact on strip performance dependent on the medium pH. Finally, the ceftolozane-tazobactam Etest strip is stable for shipping and storage. Additional long-term stability studies are ongoing.

In brief, 106 strains were tested by Etest and compared to broth microdilution. The results are displayed in the table below. Errors were classified as very major, i.e., false susceptible result; major, i.e., false resistant result; and minor, i.e., one system reporting an intermediate result and the other reporting a susceptible or resistant result. For the ceftolozane-tazobactam Etest strip, there were 0% major errors, 5.8% minor errors with no bias, and 5.9% very major errors. The very major errors resulted from a single strain of *A. baumannii* (a wild-type strain) and 1 strain of *Haemophilus influenzae,* which previously gave susceptible results on broth microdilution. For *A. baumannii* the discrepant results were primarily observed when the MIC values were high and the ellipse was often deformed. It is suggested that the Etest should not be used for *A. baumannii;* however, against all other pathogen species studied, the strip performed well. Additional studies are planned to evaluate the performance against more clinical isolates as well as the reproducibility in multiple laboratories.

Reviewer's Comment

Testing of the Etest strips for all species studied, with the exception of *Acinetobacter baumannii*, were similar to broth microdilution testing (within ± 1dilution). It is suggested that the Etest should not be used for *A. baumannii.*

Table: 120: Performance of the Etest Prototype Compared to Broth Microdilution

Phenotype	Strains (N)	Essential Agreement	Percent (%) Essential Agreement
Streptococcus spp.	24	21/24	87.5
Acinetobacter spp.	16	10/16	62.5
Haemophilus influenzae	13	12/13	92.3
Pseudomonas aeruginosa	19	18/19	94.7
Enterobacteriaceae	31	29/31	93.5
Total	103[a]	90/103	87.3

Essential agreements were calculated as ± 1 $\log_2$ dilution with broth microdilution result
[a] 106 strains tested; 3 strains did not grow
Source: M5.3.5.4\CXA.030.MC

Conclusions
Susceptibility testing of ceftolozane and ceftolozane-tazobactam has been performed using broth microdilution and agar dilution assays following CLSI methodology. The MIC values were generally consistent among different laboratories and the addition of tazobactam appears to have no effect on the reliability of microbiological test methods. A disk potency study was conducted and the data were supportive of a 30/10 mcg ceftolozane-tazobactam Kirby-Bauer disk, which resulted in zone diameters that can likely differentiate susceptible and resistant isolates. Standard methodologies and QC studies were used to identify QC ranges, which were approved by the CLSI for both broth microdilution, agar dilution (anaerobes only), and Kirby-Bauer disk diffusion testing for a variety of relevant QC organisms. Based on the known chemical and microbiological properties of ceftolozane and tazobactam, susceptibility testing is not likely to present any major technical issues.

Breakpoint Analysis
Reviewer's Comment
Discussions with clinical pharmacology of breakpoints are still ongoing within the Agency. Results of these discussions, as they pertain to clinical microbiology, will be filed as an addendum to this review.

Reviewer's Comment
In microbiology Section 12.4 of the ceftolozane-tazobactam labeling below (list of microorganisms for complicated intra-abdominal infection, under gram-negative anaerobes), (b) (4) It is recommended that these organisms are not listed in the labeling. See Agency's proposed labeling below.

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Reviewer's Comment
The Agency's currently proposed Microbiology (section 12.4) and References (section 15.0) of the ceftolozane-tazobactam package insert is below. Since discussions are still ongoing within the Agency, this does not represent the final version of the labeling.

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

and community-acquired pathogens. These reports should aid the physician in selecting an antibacterial drug product for treatment.

Dilution Techniques

Quantitative methods are used to determine antimicrobial MICs. Ceftolozane-tazobactam susceptibility testing is performed with a fixed 4 ug/mL concentration of tazobactam. These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds. The MICs should be determined using a standardized test method (broth, and/or agar).[1] The MIC values should be interpreted according to the criteria in Table 1.

Diffusion Techniques

Quantitative methods that require measurement of zone diameters can also provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. The zone size provides an estimate of the susceptibility of bacteria to antimicrobial compounds. The zone size should be determined using a standardized test method.[2] This procedure uses paper disks impregnated with 30 mcg of ceftolozane and 10 mcg of tazobactam to test the susceptibility of microorganisms to ceftolozane/tazobactam. Results should be interpreted according to the criteria in Table 1.

Table 3: Susceptibility Interpretive Criteria for ZERBAXA™

Pathogen and Isolate Source	**Minimum Inhibitory Concentrations (mcg/mL)**			**Disk Diffusion Zone Diameter (mm)**		
	S	I	R	**S**	I	R
Enterobacteriaceae	≤8.0	16.0	≥32.0	≥19	16-18	≤15
Pseudomonas aeruginosa	≤8.0	16.0	≥32.0	≥20	15-19	≤14
Streptococcus anginosus Streptococcus constellatus and Streptococcus salivarius	≤8.0	16.0	≥32.0	≥16	12-15	≤11
Anaerobes	≤8.0	16.0	≥32.0	NA	NA	NA

S = susceptible, I = intermediate, R = resistant

Anaerobic Techniques:

For anaerobic bacteria, the susceptibility to ceftolozane-tazobactam can be determined by standardized test method.[3] The MIC values obtained should be interpreted according to criteria provided in Table 1.

A report of "Susceptible" indicates that the antimicrobial is likely to inhibit growth of the pathogen if the antimicrobial compound reaches the concentration at the infection site necessary to inhibit growth of the pathogen. A report of "Intermediate" indicates that the

result should be considered equivocal, and if the microorganism is not fully susceptible to alternative clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where the drug is physiologically concentrated. This category also provides a buffer zone that prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the antimicrobial is not likely to inhibit growth of the pathogen if the antimicrobial compound reaches the concentrations usually achievable at the infection site; other therapy should be selected.

Quality Control

Standardized susceptibility test procedures require the use of laboratory controls to monitor and ensure the accuracy and precision of supplies and reagents used in the assay, and the techniques of the individuals performing the test. Standard ceftolozane-tazobactam powder should provide the following range of MIC values provided in Table 2. For the diffusion technique using the 30 mcg ceftolozane / 10 mcg tazobactam disk the criteria provided in Table 2 should be achieved. [4]

Table 4: Acceptable Quality Control Ranges for Susceptibility Testing

Quality Control Organism	Minimum Inhibitory Concentrations (mcg/mL)	Disk Diffusion Zone Diameters (mm)
Escherichia coli ATCC 25922	0.12-0.5	24-32
Escherichia coli ATCC 35218	0.06-0.25	25-31
Pseudomonas aeruginosa ATCC 27853	0.25-1	25-31
Haemophilus influenzae ATCC 49247	0.5-2	23-29
Klebsiella pneumoniae ATCC 700603	0.5-2	17-25
Streptococcus pneumoniae ATCC 49619	0.25 – 1	21-29
Bacteroides fragilis ATCC 25285 (agar and	0.12-1	Not Applicable

Quality Control Organism	Minimum Inhibitory Concentrations (mcg/mL)	Disk Diffusion Zone Diameters (mm)
broth)		
Bacteroides thetaiotaomicron ATCC 29741 (agar)	16-128	Not Applicable
Bacteroides thetaiotaomicron ATCC 29741 (broth)	16-64	Not Applicable

ATCC = American Type Culture Collection

15 REFERENCES

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RECOMMENDATIONS

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

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Study References
Ceftolozane-tazobactam studies referenced in the NDA and in this review are shown in the Table below:

Table 121: Ceftolozane-Tazobactam Microbiology Studies

Study Number	Description/Title
CRE060041	In vivo antibacterial activity of FR264205
CRE060042	In vitro antibacterial activity of FR264205
CXA.003.MC	Quality control limits for broth microdilution susceptibility tests of CXA-101/tazobactam against *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, Haemophilus influenzae ATCC 49247, *Bacteriodes fragilis* ATCC 25285 and *Bacterioides thetaiotaomicron* ATCC 29741 and agar dilution susceptibility tests against *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 297541
CXA.004.MC	Quality control ranges for disk diffusion susceptibility tests of CXA-101/tazobactam against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, Escherichia coli ATCC 25922, *Escherichia coli* ATCC 35218 on Mueller-Hinton Agar and Haemophilus Test Medium, *Klebsiella pneumoniae* ATCC 700603 Pseudomonas aeruginosa ATCC 27853, *Haemophilus influenzae* ATCC 49247 using CLSI Methodology and *Haemophilus influenzae* NCTC 8468 Using EUCAST Methodology
CXA.006.MC	In vitro susceptibility testing – impact of test method parameters on the activity of CXA-201
CXA.007.MC	Development of a method for manufacturing Sensititre 'dry' MIC plates containing CXA-101/TAZ4 or CXA-101
CXA.008.MC	An equivalency study of the commercially prepared dried MIC plate [Test] compared with the CLSI broth microdilution reference method for CXA and comparator antimicrobials
CXA.009.MC	In vivo comparison of CXA-101 (FR264205) with and without tazobactam versus piperacillin-tazobactam using human simulated exposures against phenotypically diverse Gram-negative organisms
CXA.010.MC	Effect of CXA-201 in combination with metronidazole at varying concentrations against *Escherichia coli* and *Klebsiella pneumoniae* in a checkerboard MIC assay under aerobic, microaerophilic and anaerobic conditions
CXA.011.MC	Effect of different concentrations of surfactant on the MIC of CXA-101/201 against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*

Study Number	Description/Title
CXA.012.MC	Acquisition of resistance in *P. aeruginosa* in the presence of CXA-101, ceftazidime or meropenem by serial passage
CXA.013.MC	Resistance incidence of CXA-101, ceftazidime or meropenem in *Pseudomonas aeruginosa*
CXA.014.MC	Susceptibility of *P. aeruginosa* and *A. baumannii* efflux mutants to ceftolozane and ceftolozane + 4μg/ml tazobactam (TOL/TAZ).
CXA.015.MC	PBPs profile and binding affinities of CXA-101, ceftazidime and imipenem of pan-beta-lactam resistant *Pseudomonas aeruginosa* clinical strains
CXA.016.MC	Quality control limits For microdilution susceptibility tests of CXA-201 against *S. pneumoniae* ATCC 49619
CXA.017.MC	Surveillance of ceftolozane/tazobactam antimicrobial activity when tested against Gram-negative organisms and streptococci isolated in Europe (2011). To determine the mechanisms responsible for elevated ceftolozane/tazobactam MIC values among selected *Pseudomonas aeruginosa* isolates from a European surveillance study.
CXA.018.MC	CANWARD 2011: Study A National Population Based Surveillance System, Assessing the Prevalence of Antimicrobial Resistance in Pathogens Associated with Respiratory, Skin and Soft Tissue, Urinary and Bacteremic Infections in Hospitalized Patients in Canada
CXA.019.MC	Ceftolozane/Tazobactam for auto-read
CXA.020.MC	Analysis of the antibacterial interactions between ceftolozane/tazobactam and other antimicrobial agents by determining fractional inhibitory concentrations (FIC) and time kill kinetics
CXA.021.MC	Potential role of extended-spectrum AmpCs in decreased susceptibility to ceftolozane (previously CXA-101) among AmpC-derepressed *P. aeruginosa*.
CXA.022.MC	Surveillance of ceftolozane/tazobactam antimicrobial activity when tested against Gram-negative organism and *Streptococci* Isolated in the USA (2011)
CXA.023.MC	In vitro activity of ceftolozane in combination with tazobactam when tested against ESBL-producing Enterobacteriaceae and various other selected Gram-negative *bacilli*
CXA.024.MC	In vitro dynamics and mechanisms of resistance development to CXA-201 and comparators in *P. aeruginosa*
CXA.025.MC	Evaluation of the in vivo inoculum effect of CXA 201 in a murine thigh model
CXA.026.MC	Evaluation of the in vitro activity of CXA-201 against a broad spectrum of recent clinical anaerobic isolates with an emphasis on *Bacteroides fragilis* group

Study Number	Description/Title
CXA.027.MC	Disk mass study to confirm appropriate mass or potency of ceftolozane/tazobactam (CXA-201) for EUCAST disk diffusion testing
CXA.028.MC	Disk versus broth correlation study for ceftolozane/tazobactam (CXA-201)
CXA.030.MC	Report of September Results of Etest CXA201 (=Etest C/T 256)
CXA.031.MC	Determination of resistance incidence (RI) frequency of ceftolozane and comparators to *Pseudomonas aeruginosa* PA01
CXA.033.MC	In vitro activity of ceftolozane/tazobactam against 952 Pseudomonas aeruginosa clinical isolates including piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, meropenem, ciprofloxacin, gentamicin or colistin resistant as well as multi-drug resistant (MDR) strains obtained from Canadian hospitals
CXA.039.MC	Pharmacokinetic-pharmacodynamic evaluation of CXA-201 against multi-drug resistant Gram-negative organisms in an in vitro hollow fiber model
CXA.040.MC	Pharmacodynamics of CXA-101 plus tazobactam (CXA-201) studied in an in vitro pharmacokinetic model of infection
CXA.041.MC	Assessment of the in vitro post β-Lactamase inhibitor effect (PBLIE) of tazobactam when associated with ceftolozane and tested against well characterized ESBL-producing strains
CXA.042.MC	Assessment of pharmacokinetics and protein binding of ceftolozane and tazobactam in neutropenic mice.
CXA.043.MC	Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an in vitro infection model
CXA.044.MC	Optimizing CXA-201 cell kill and preventing *Pseudomonas* resistance emergence in an in vitro infection model
CXA.045.MC	Validation of the tazobactam pharmacokinetic-pharmacodynamic driver when used in combination with CXA-101 versus three clinical strains of Klebsiella pneumoniae in an in vitro infection model
CXA.047.MC	Relationship Between Ceftolozane/Tazobactam Exposure and Drug-Resistance Amplification in a Hollow-Fiber Infection Model
CXA.048.MC	Surveillance of ceftolozane/tazobactam Antimicrobial Activity when tested Against Gram-Negative Organism and *Streptococci* Isolated in the USA (2012)
CXA.049.MC	Surveillance of ceftolozane/tazobactam antimicrobial activity when tested against Gram-Negative organisms and *streptococci* isolated in the USA (2008, 2011,2012), EU, Canada and the Britain (2011, 2012)
CXA.050.MC	Enzyme kinetics for tazobactam versus TEM-1, CTX-M-14, and CTX-M-15 Beta-Lactamase Enzymes
CXA.051.MC	Evaluation of the in vitro activity of ceftolozane/tazobactam against recent Gram-positive clinical isolates from the United States and worldwide.

Study Number	Description/Title
CXA.052.MC	EUCAST Laboratory for Antimicrobial Susceptibility Testing: MIC and zone diameter correlate study for ceftolozane-tazobactam to establish EUCAST breakpoints
CXA.053.MC	Stability of ceftolozane/tazobactam in broth medium
CXA.054.MC	Surveillance of Ceftolozane/Tazobactam Antimicrobial Activity when tested Against Gram-Negative Organism and Streptococci Isolated in Europe (2012)
CXA.055.MC	Efficacy of ceftolozane in a murine model of *Pseudomonas aeruginosa* acute pneumonia: in vivo antimicrobial activity and impact on host inflammatory response
CXA.057.MC	In vitro susceptibility of ceftolozane and ceftolozane/tazobactam and selected comparators against clinical isolates of *Escherichia coli* producing CTX-M-14 type ESBLs
CXA.060.MC	Comparison of ceftolozane/tazobactam annual global surveillance data (2011-2012) to baseline pathogens from clinical studies CXA-cUTI-10-04 and CXA-cUTI-10-05
CXA.061.MC	Analysis of ceftolozane/tazobactam broth microdilution MIC vs disk zone diameter against target species from cIAI and cUTI clinical studies
CXA.062.MC	CXA-cUTI-10-04 and CXA-cUTI-10-05 Central Microbiology Laboratory Summary of Antimicrobial Susceptibility Testing and Quality Control
CXA.063.MC	CXA-cUTI-10-08 and CXA-cUTI-10-09 central microbiology laboratory summary of antimicrobial susceptibility testing and quality control
CXA.065.MC	Summary of Supplemental Molecular Characterization at (b) (4) for CXA-cUTI-10-04 and CXA-cUTI-10-05 Clinical Studies
CXA.066.MC	Summary of Supplemental Molecular Characterization at (b) (4) for CXA-cIAI-10-08 and CXA-cIAI-10-09 Clinical Studies
CXA.067.MC	Summary of antimicrobial susceptibility testing of non-*B. fragilis* group anaerobic isolates from clinical studies CXA-cIAI-10-08 and CXA-cIAI-10-09
CXA.068.MC	Decreased Susceptibility to Ceftolozane/Tazobactam on Therapy in CXA-cUTI-10-04 and CXA-cUTI-10-05: Identification of Genetic Determinants that confer Non-Susceptibility to Ceftolozane/Tazobactam
CXA.072.MC	CXA-201 and Comparison β-lactams: Propensities to Select Less Susceptible Mutants
CXA.073.MC	Determination of pharmacodynamic target for ceftolozane against *Streptococcus pneumoniae* in the neutropenic thigh infection model
CXA.075.MC	CANWARD 2012: Study A National Population Based Surveillance System, Assessing the Prevalence of Antimicrobial Resistance in Pathogens Associated with Respiratory, Skin and Soft Tissue, Urinary and Bacteremic Infections in Hospitalized Patients in Canada

Study Number	Description/Title
CXA.076.MC	In vitro susceptibility of ceftolozane and ceftolozane/tazobactam and selected comparators against clinical isolates of *Escherichia coli* producing CTX-M-15 type ESBLs
CXA.078.MC	Phenotypic and genotypic characterization of *Klebsiella pneumonia* clinical isolates to be utilized for pharmacodynamics modeling studies
CXA.079.MC	Construction of Recombinant Vectors Transcribing Different Levels of blaCTX-M-15 mRNA to be Utilized in an Isogenic Background for Pharmacodynamic Modeling studies
CXA.080.MC	Affinity of the ceftolozane to the penicillin binding proteins of Pseudomonas aeruginosa and *Escherichia coli*
CXA.081.MC	Stability of ceftolozane and tazobactam in water and saline at various temperatures and time points
CXA.082.MC	In vitro activity of ceftolozane alone and in combination with tazobactam against extended spectrum β-lactamase harbouring Enterobacteriaceae
CXA.083.MC	Ceftolozane/tazobactam: pharmcodynamics of ceftolozane combined with tazobactam in a neutropenic mouse thigh model
CXA.084.MC	In vitro dynamics and mechanisms of resistance development to CXA-201 and comparators in *P. aeruginosa*-Part II
CXA.085.MC	The Pharmacodynamic Activities of CXA-101: Addendum to Report CXA101-M-004-Analysis of 2-Log Kill Data
CXA-101-M-001	Characterizing the activity of CXA-101: susceptibility by broth microdilution/agar dilution, effect of testing conditions on activity, and assessment of cidality by timekill kinetics/MBC analysis
CXA-101-M-002	Spectrum of activity and potency of CXA 101 combined with a Beta lactamase inhibitor
CXA-101-M-004	The pharmacodynamic activites of the CXA-101
CXA101-M-006	Evaluation of appropriate disk mass for testing CXA101 alone and in combination with tazobactam against clinically relevant pathogens
CXA101-M-007	Activity of the new antipsuedomonal cephalosporin CXA-101 against *P. aeruginosa* isolates from chronically infected cystic fibrosis patients
CXA101-M-010	Activity of the new antipseudomonal cephalosporin CXA-101 against carbapenam resistant *P. aeruginosa* isolates from a large multi-center study in Spain
CXA101-M-011	Activity of the new cephalosporin CXA-101 against beta lactam and fluoroquinolone resistant *Pseudomonas aeruginosa* from intensive care unit patients
CXA101-M-012	Activity of the new cephalosporin CXA-101 against biofilm of relevant *P. aeruginosa* pheontypes in cystic fibrosis chronic lung infection: mucoid and hypermutable strains

Study Number	Description/Title
CXA101-M-013	Affinity of the new cephalosporin CXA-101 to penicillin binding proteins of *Pseudomonas aeruginosa*
CXA-101-M-014	Activity of CXA-101 against *Pseudomonas aeruginosa* beta lactam resistant mechanisms: ampD, ampDh2, ampDh3, dacB and oprD mutations
CXA201-M-001	Chequerboard titrations of cephalosporin CXA-101 and tazobactam vs. beta lactamase producing Enterobacteriaceae
CXA201-M-002	In vitro activity of CXA-101 plus tazobactam against genotypically characterized extended spectrum B-lactamase producing *E. coli* and *K. pneumoniae*
CXA201-M-003	Surveillance of CXA-101/tazobactam against target Gram-negative pathogens and *Streptococci*
CXA201-M-004	In vitro potency of CXA-101, a novel cephalosporin, against resistant phenotypes of *Pseudomonas aeruginosa*, including multi-drug resistant isolates
CXA201-M-006	In vitro assessment of an investigational cephalosporin, CXA-101, in combination with tazobactam, against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* strains exhibiting different resistant phenotypes
CXA201-M-009	Spectrum of activity and potency of CXA-101 combined with tazobactam tested against *Pseudomonas aerginosa*
CXA201-M-010	50% Effective dose (ED50) determination of CXA-101 alone or in combination with tazobactam for treating experimental peritonitis in mice due to extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strains: comparison with ceftazidime and piperacillin/tazobactam
CXA201-M-013	CXA201-M-013 (CXA.005.MC) - In vitro antibacterial activity of CXA-101 against *P. aeruginosa* with characterized mechanisms of resistance
CXA201-M-014	CXA201-M-014 - 50% Effective dose (ED50) determination of CXA-101 alone or in combination with tazobactam for treating experimental peritonitis in mice due to extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* strains: comparison with ceftazidime and piperacillin/tazobactam
CX-BD-001	Activity of cephalosporin CXA-101 (FR264205) against *Pseudomonas aeruginosa* and Burkholderia cepacia group strains and isolates.

DIVISION OF ANTI-INFECTIVE PRODUCTS
CLINICAL MICROBIOLOGY REVIEW

NDA 206829 DATE REVIEW COMPLETED: 9-26-14
Ceftolozane-Tazobactam

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Clinical Microbiology Reviewer, DAIP

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Clinical Microbiology Team Leader
26 September 2014

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

/s/

KERIAN K GRANDE ROCHE
09/26/2014

KERRY SNOW
09/26/2014

Product Quality Microbiology Review

15 September 2014

NDA: 206829

Drug Product Name
Proprietary: ZERBAXA
Non-proprietary: ceftolozane/tazobactam

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
19 APR 2014	21 APR 2014	21 APR 2014	24 APR 2014
08 JUL 2014	08 JUL 2014	N/A	N/A
25 JUL 2014	25 JUL 2014	N/A	N/A
25 AUG 2014	25 AUG 2014	N/A	N/A
27 AUG 2014	27 AUG 2014	N/A	N/A

Applicant/Sponsor
Name: Cubist Pharmaceuticals, Inc.
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Representative: Charles Miller
Telephone: 781-240-7676

Name of Reviewer: Erika Pfeiler, Ph.D.

Conclusion: Recommended for Approval

Product Quality Microbiology Data Sheet

A. 1. TYPE OF SUBMISSION: 505(b)(2)

2. SUBMISSION PROVIDES FOR: Initial marketing of a sterile drug product

3. MANUFACTURING SITE: (b) (4)

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:
Sterile lyophilized powder
1.5 g (1 g ceftolozane, 0.5 g tazobactam)
Intravenous infusion

5. METHOD(S) OF STERILIZATION: N/A, drug product manufacturer (b) (4) fills sterile drug substance powders.

6. PHARMACOLOGICAL CATEGORY: Treatment of complicated urinary tract infections (cUTI) and complicated intra-abdominal infections (cIAI)

B. SUPPORTING/RELATED DOCUMENTS:
Microbiology Review 1 of DMF (b) (4) DARRTS Date 15 September 2014
Microbiology Review 1 of DMF (b) (4) DARRTS Date 11 September 2014
Microbiology Review 1 of DMF (b) (4) DARRTS Date 11 September 2014
Microbiology Review 12 of DMF (b) (4) DARRTS Date 11 September 2014.

C. REMARKS: N/A

filename: N206829R1.doc

Executive Summary

I. Recommendations

A. Recommendation on Approvability - Recommended for Approval

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

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – Product is (b) (4) filled at the manufacturing site.

B. Brief Description of Microbiology Deficiencies – N/A

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

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

III. Administrative

A. Reviewer's Signature _____

Erika Pfeiler, Ph.D.
Microbiologist

B. Endorsement Block_____

Stephen Langille, Ph.D.
Senior Review Microbiologist

C. CC Block
N/A

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

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/s/

ERIKA A PFEILER
09/15/2014

STEPHEN E LANGILLE
09/15/2014

CLINICAL Microbiology: 45-Day Meeting NDA Checklist for Filing
NDA: 206829 Zerbaxa (ceftolozane/tazobactam).
Received: 04/21/14
Due: 12/21/14
Clinical Microbiology Reviewer: Avery Goodwin, Ph.D

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

No.	Item	Yes	No	Comments
1	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	✓		
2	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA indexed, paginated, and/or linked in a manner to allow substantive review to begin?	✓		
3	Is the clinical microbiology information (preclinical/nonclinical and clinical) in different sections of the NDA legible so that substantive review can begin?	✓		
4	On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/ isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	✓		
5	Has the applicant submitted draft provisional breakpoint and interpretive criteria, along with quality control (QC) parameters, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA studies, and in a manner to allow substantive review to begin?	✓		
6	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	✓		
7	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	✓		
8	Has the applicant submitted the clinical microbiology datasets in a format which intends to correlate baseline pathogen with clinical and microbiologic outcomes exhibited by relevant pathogens isolated from test of cure or end of treatment?	✓		
9	Has the applicant submitted a clinical microbiology dataset in a format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or	✓		

CLINICAL Microbiology: 45-Day Meeting NDA Checklist for Filing
NDA: 206829 Zerbaxa (ceftolozane/tazobactam).
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	genotype (such as mutations) of the baseline relevant pathogen with clinical and microbiologic outcome as exhibited by relevant pathogens isolated from test of cure or end of treatment?			
10	Has the applicant used standardized methods or if non-standardized methods were used has the applicant included full details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	✓		
11	Is the clinical microbiology draft labeling consistent with 21 CFR Parts 201, 314, 601 and current Divisional policy.	✓		
12	FROM A CLINICAL MICROBIOLOGY PERSPECTIVE, IS THIS NDA FILEABLE? **IF NO, GIVE REASONS BELOW.**	✓		

Application Type: PDUFA V Application / Priority with QIDP designation / 8-month review clock

INDICATIONS: Complicated Intra-abdominal infections; Complicated Urinary Tract Infections including pyelonephritis.

Any Additional Clinical Microbiology Comments: There are no additional comments

Avery Goodwin, Ph.D.
Reviewing Clinical Microbiologist
DAIP

Kerry Snow, MS.
Team Leader Clinical Microbiology
DAIP

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/s/

AVERY C GOODWIN
06/12/2014

KERRY SNOW
06/12/2014

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 206829 **Applicant:** Cubist Pharmaceuticals, Inc. **Letter Date:** 19 April 2014

Drug Name: ZERBAXA (proposed) **NDA Type:** 505(b)(2) **Stamp Date:** 21 April 2014

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

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

Additional Comments:
This application describes a sterile powder for reconstitution which is followed by intravenous infusion. This is a rolling submission, with the applicable CMC and quality microbiology information submitted in April 2014. Prior to this submission, a series of meetings were held between FDA and the applicant regarding [redacted] (b) (4) at the drug product manufacturing site. FDA agreed to file the application prior to this facility being ready for inspection, and prior to performance of [redacted] (b) (4) qualifying media fills. As part of this agreement, the applicant committed to submit data following completion of the fills in August 2014.
Letters of Authorization for Drug Master Files [redacted] (b) (4) were provided in the application, as these provide supporting information for the NDA review.

Erika Pfeiler, Ph.D. Date
Microbiologist

Stephen Langille, Ph.D. Date
Senior Review Microbiologist

The following information requests should be conveyed to the applicant in the 74-day letter.

Sterile Ceftolozane Drug Substance

1. Your application describes the manufacturing process for the sterile ceftolozane drug substance, and states that a commercial filling batch size will be (b) (4) Provide a description of the planned manufacturing campaign for the drug substance, including the maximum campaign duration.

2. Your application provides stability information for the non-sterile ceftolozane drug substance, but does not provide information for the sterile drug substance. Provide a stability summary, post-approval stability protocol and commitment, and stability data for the sterile ceftolozane drug substance.

Drug Product

1. Your application describes microbiological spiking studies to support post-dilution hold time of the drug product in 0.9% saline or D5W. The study methods that you describe are adequate; however, the study duration of your 2-8°C samples is 14 days to support a (b) (4) day post-dilution hold period. Typical hold time studies use a hold time that is 2-3 times longer than the proposed holding period listed on the product label. You may wish to repeat the 2-8°C hold study for a minimum of 20 days; alternatively, your labeling should state a maximum holding time of 7 days. Please note that this comment only applies to the product held at 2-8°C.

2. A description of the requalification studies for the (b) (4) equipment was provided in your application, and states that the expiration date for one set of biological indicators was August 2013, when the study was performed in October 2013 (Section 5.3.4.4). Clarify the expiration date of your biological indicators.

3. Your application describes the (b) (4) drug substance powders. Provide a list of equipment used for drug product filling, including auxiliary equipment used for (b) (4)). List the location of each piece of equipment in your facility.

4. Your application states that the (b) (4) are requalified once every three years. Typically, requalification is performed on an annual basis. Provide a rationale for your schedule of requalification for this equipment.

5. Your application describes a requalification study for the (b) (4) Provide the date on which this study was conducted.

6. Your application describes media fill simulations that use sterile (b) (4) to simulate powder filling, which is immediately followed by the in-line addition of (b) (4). Provide a description of how equipment used in media fill simulations is different from equipment used to fill drug product.

7. Your application describes methods for media fill simulations, but you do not state your planned requalification schedule. Provide your proposed schedule of media fill simulations.

8. The description of your facility's media fills states that acceptance criteria are considered to be met if the number [redacted] (b) (4) is in agreement with limits listed in Table 68 (Section 5.4.2). This criterion is unacceptable, [redacted] (b) (4) even though they represent a worst-case for media fill failure. You should revise your media fill acceptance criteria to account for all filled and incubated vials.

9. You perform growth promotion testing as a part of your media fill simulations. Confirm that this testing is performed at the same incubation temperature as used for media fill test vials.

10. We acknowledge that you plan to submit data from media fills to qualify [redacted] (b) (4) no later than August 25, 2014. Further, we acknowledge the summaries of previous media fill activities that you provided in your application. Provide a summary description of the media fill study that you plan to conduct following completion of the manufacturing [redacted] (b) (4). Include the number of fills, the duration of fills, a summary of proposed interventions, and proposed acceptance criteria.

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/s/

ERIKA A PFEILER
06/02/2014

STEPHEN E LANGILLE
06/02/2014