

Effect of Various Testing Parameters and the Activity of CXA-101 by Broth Microdilution

F1-357

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Abstract

Background: CXA-101 (CXA) is an investigational cephalosporin with potent *in vitro* activity against *Pseudomonas aeruginosa* (PA). It is important to understand the impact of a variety of *in vitro* testing conditions on the *in vitro* activity of antibiotics. This study addresses the effect of human serum, pH, inoculum size, divalent cation concentration, and testing media on the activity of CXA against select pathogens.

Methods: Two isolates each of *Staphylococcus aureus* (SA), *Streptococcus pneumoniae* (SP), *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), and PA were tested for MIC by broth microdilution against CXA and ceftazidime (CTZ) under standard conditions (CLSI M100-S18, M7-A7). The effects of varied pH (pH 5, 6, 8), human serum (10 and 50%), inocula (5x10⁶ and 5x10⁸ CFU/ml), divalent cations (Ca²⁺ at 50 mg/ml), and media (cation-adjusted Mueller-Hinton Broth [CAMHB], CAMHB + lysed horse blood [LHB], and *Haemophilus* testing medium [HTM]) on the activity of CXA was evaluated.

Results: Human serum (20 or 50%) had little to no effect on the activity of CXA overall though a drop in CXA MICs was noted against one PA isolate (4-fold) and one EC isolate (2 to 8-fold).

Altering the inoculum concentrations to 5x10⁸ CFU/ml and 5x10⁶ CFU/ml did not affect the overall activity of CXA. CAMHB + LHB or HTM did not affect the activity of CXA against SA, PA, EC, and KP. Testing SP with HTM did not affect CXA MICs, while testing SP in CAMHB alone either did not support growth of the isolate or resulted in lower MICs by 2-fold for CXA, which is likely due to poor growth of the organism in the absence of LHB. Testing at 50 mg Ca²⁺ did not affect the activity of CXA against any of the isolates tested. pH did not significantly affect the activity of CXA overall, though higher MICs for CXA were noted at low pH (pH 5) against EC and KP.

Conclusion: Variations in pH, inoculum size, testing medium, divalent cation concentration, or human serum concentration had little to no effect on the antimicrobial activity of CXA against the gram-positive and -negative pathogens tested.

Background

CXA-101 is a new cephalosporin that is currently under development by Calixa Therapeutics, Inc. CXA-101 has a broad spectrum of *in vitro* activity consistent with that of the third-generation cephalosporins and is particularly potent against *Pseudomonas aeruginosa*. It is important to understand the effect of various testing conditions on the *in vitro* activity of this new antibiotic. This study addresses the effect of human serum, pH, inoculum size, divalent cation concentration, and testing media on the activity of CXA-101 against representative pathogens.

Materials & Methods

The MIC of CXA-101 and ceftazidime was determined by broth microdilution for two isolates each of *S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* under standard conditions according to CLSI M100-S18 and M7-A7. Concurrently, the effect of varied pH, human serum, inocula, divalent cations (Ca²⁺), and media on the activity of CXA-101 was evaluated. The testing conditions were as follows:

pH	Testing Conditions		
	5.0	6.0	8.0
Human Serum	20%	50%	
Inocula	5x10 ⁶ CFU/ml	5x10 ⁸ CFU/ml	
Cation (Ca ²⁺)	50 µg/ml		
Media	Mueller-Hinton (MHB) with Lysed Horse Blood (LHB)	Haemophilus Test Medium (HTM)	

All test parameters were evaluated for isolates tested in parallel under standard conditions (pH of 7.2-7.4, a 0% serum concentration, inocula concentration of 5x10⁶ CFU/ml, cation (Ca²⁺) concentration of 20-25 µg/ml, and Mueller-Hinton broth (excluding *S. pneumoniae* where MHB + LHB was the standard media).

Table 1. MICs of CXA-101 and ceftazidime of all isolates tested

Organism	Isolate ID	CXA-101		Ceftazidime	
		MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	CLSI*
<i>S. aureus</i>	1731625	32	4		S
	1731646	32	2		S
	1731673	1	2	NA	
<i>S. pneumoniae</i>	1731713	2	1	NA	
	1732281	0.25	0.25	S	
<i>E. coli</i>	1732283	0.25	0.12	S	
<i>K. pneumoniae</i>	1732569	0.5	0.25	S	
<i>P. aeruginosa</i>	1732369	0.5	0.25	S	
<i>P. aeruginosa</i>	1731884	1	2	S	
<i>P. aeruginosa</i>	1731923	1	2	S	

*S, susceptible; NA, not applicable

CXA-101 was most active against the gram-negative organisms, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (MICs ≤1 µg/ml) and was also active against *S. pneumoniae* (MIC ranging 1-2 µg/ml). CXA-101 was least active against *S. aureus* with an MIC of 32 µg/ml.

Table 2. CXA-101 and ceftazidime MIC (µg/ml) results from the effects of pH

Organisms	Isolate ID	Replicate	MIC (µg/ml)						
			pH 7.2-7.4		pH 5.0		pH 8.0		
<i>S. aureus</i>	1731646	CXA-101	32	32	16	32	32	16	32
	1731626	CXA-101	32	32	8	4	8	8	8
	1731673	CXA-101	32	32	8	4	8	8	8
<i>S. pneumoniae</i>	1731713	CXA-101	0.12	0.12	NO*	NO	0.25	0.25	0.12
	1732281	CXA-101	0.25	0.25	NO	NO	0.12	0.12	0.25
	1732283	CXA-101	2	2	NO	NO	2	2	1
<i>P. aeruginosa</i>	1731884	CXA-101	1	1	1	1	1	1	1
	1731923	CXA-101	1	1	1	1	1	1	0.5
	1732281	CXA-101	1	1	1	1	1	1	1
<i>E. coli</i>	1732283	CXA-101	0.5	0.5	2	2	1	0.25	0.25
	1732283	CXA-101	0.12	0.25	0.5	1	0.25	0.5	0.12
	1732283	CXA-101	0.12	0.25	0.5	1	0.25	0.5	0.12
<i>K. pneumoniae</i>	1732369	CXA-101	0.25	0.5	1	1	0.5	0.5	0.5
	1732369	CXA-101	0.25	0.5	1	1	0.5	0.5	0.5
	1732366	CXA-101	0.25	0.25	1	1	0.5	0.5	0.25

*Standard pH according to CLSI

NO, no growth

At pH 5.0, 6.0 and 8.0, the activity of CXA-101 and ceftazidime showed no change or only a one doubling dilution change in MIC relative to those observed under standard conditions (pH 7.2-7.4). Higher MICs for CXA-101 were noted at a low pH of 5 against *E. coli* and *K. pneumoniae*. The same effect was noted to occur with ceftazidime against these isolates. At a pH of 5.0, *S. pneumoniae* showed no growth.

Table 3. CXA-101 and ceftazidime MIC (µg/ml) results from the effects of human serum

Organisms	Isolate ID	Replicate	MIC (µg/ml)					
			0% Serum		20% Serum		50% Serum	
<i>S. aureus</i>	1731646	CXA-101	32	32	16	16	16	16
	1731626	CXA-101	32	32	8	4	8	4
	1731673	CXA-101	32	32	32	32	16	16
<i>S. pneumoniae</i>	1731713	CXA-101	0.12	0.12	0.12	0.12	0.12	0.12
	1732281	CXA-101	0.25	0.25	0.25	0.25	0.25	0.25
	1732283	CXA-101	2	2	1	1	1	1
<i>P. aeruginosa</i>	1731884	CXA-101	1	1	0.5	0.5	0.5	0.5
	1731923	CXA-101	1	1	0.25	0.25	0.12	0.12
	1732281	CXA-101	2	2	0.5	0.5	0.25	0.25
<i>E. coli</i>	1732281	CXA-101	0.5	0.5	0.25	0.25	0.12	0.12
	1732283	CXA-101	0.12	0.25	0.12	0.12	0.12	0.12
	1732283	CXA-101	0.12	0.25	0.12	0.12	0.06	0.12
<i>K. pneumoniae</i>	1732369	CXA-101	0.25	0.5	0.25	0.25	0.25	0.25
	1732369	CXA-101	0.25	0.5	0.12	0.12	0.12	0.12
	1732366	CXA-101	0.25	0.25	0.25	0.25	0.12	0.25

*Standard concentration according to CLSI

20% and 50% human serum had little to no effect on the activity of CXA-101 and ceftazidime overall, though a drop in CXA-101 MICs was noted against one *P. aeruginosa* isolate (4-fold) and one *E. coli* isolate (2 to 8-fold).

Results

Table 4. CXA-101 and ceftazidime MIC (µg/ml) results from the effects of inocula concentrations

Organisms	Isolate ID	Agent	MIC (µg/ml)					
			Inoculum 5x10 ⁶		Inoculum 5x10 ⁸		Inoculum 5x10 ⁶	
<i>S. aureus</i>	1731646	CXA-101	32	32	32	16	32	32
	1731626	CXA-101	8	8	4	4	8	8
	1731673	CXA-101	32	32	16	32	32	32
<i>S. pneumoniae</i>	1731713	CXA-101	0.12	0.12	0.12	0.12	0.12	0.25
	1732281	CXA-101	0.25	0.25	0.25	0.25	0.25	0.25
	1732283	CXA-101	2	2	1	1	2	2
<i>P. aeruginosa</i>	1731884	CXA-101	1	1	1	1	1	1
	1731923	CXA-101	1	1	1	1	1	1
	1732281	CXA-101	2	2	2	2	4	4
<i>E. coli</i>	1732281	CXA-101	0.5	0.5	0.25	0.25	0.5	1
	1732283	CXA-101	0.12	0.25	0.25	0.12	0.25	0.25
	1732283	CXA-101	0.12	0.25	0.12	0.12	0.25	0.25
<i>K. pneumoniae</i>	1732369	CXA-101	0.25	0.5	0.25	0.25	0.5	0.5
	1732366	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5
	1732366	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5

*Inocula are in CFU/ml

*Standard inocula according to CLSI

Overall, inocula concentrations of 5x10⁶ CFU/ml and 5x10⁸ CFU/ml did not affect CXA-101 MIC, with either no change in MIC or only a doubling dilution decrease in MIC compared to the standard (5x10⁶ CFU/ml).

Similarly, little to no change in ceftazidime MIC (identical or within one doubling dilution of standard inoculum) were observed at the two test conditions relative to the standard inoculum with the exception of one *P. aeruginosa* at inoculum concentrations of 5x10⁶ CFU/ml where MICs dropped from 4 µg/ml (standard inoculum) to 1 µg/ml.

Table 5. CXA-101 and ceftazidime MIC (µg/ml) results from different testing media

Organisms	Isolate ID	Agent	MIC (µg/ml)					
			MHB*		MHB+LHB		HTM*	
<i>S. aureus</i>	1731646	CXA-101	32	32	32	32	16	16
	1731626	CXA-101	32	32	8	8	4	4
	1731673	CXA-101	32	32	32	32	16	16
<i>S. pneumoniae</i>	1731713	CXA-101	0.06	0.06	0.12	0.12	0.12	0.12
	1732281	CXA-101	0.06	0.06	0.25	0.25	0.12	0.12
	1732283	CXA-101	NO*	NO*	2	2	0.25	0.25
<i>P. aeruginosa</i>	1731884	CXA-101	1	1	1	1	1	1
	1731923	CXA-101	1	1	0.5	0.5	0.5	0.5
	1732281	CXA-101	2	2	2	2	4	4
<i>E. coli</i>	1732281	CXA-101	0.5	0.5	0.25	0.25	0.5	2
	1732283	CXA-101	0.25	0.25	0.5	0.5	0.5	0.5
	1732283	CXA-101	0.12	0.25	0.25	0.12	0.25	0.25
<i>K. pneumoniae</i>	1732369	CXA-101	0.25	0.5	0.25	0.25	0.5	0.5
	1732366	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5
	1732366	CXA-101	0.25	0.25	0.25	0.25	0.5	0.5

*MHB, Mueller-Hinton Broth; standard media for *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* according to CLSI

*MHB+LHB, Mueller-Hinton Broth+Lysed Horse Blood, standard media for *S. pneumoniae* only according to CLSI

*HTM, *Haemophilus* Test Medium

NO, no growth

Cation-adjusted Mueller-Hinton broth with lysed horse blood or *Haemophilus* Test Medium did not affect the activity of CXA-101 and ceftazidime against *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*.

Testing *S. pneumoniae* with *Haemophilus* Test Medium did not affect CXA-101 MICs against isolate 1731713 but showed an 8-fold decrease in MIC against isolate 1731673, while testing *S. pneumoniae* in cation-adjusted Mueller-Hinton broth alone did not support growth of the isolate or resulted in lower MICs by 2-fold for CXA-101, which is likely due to poor growth of the organism in the absence of LHB.

Table 6. CXA-101 and ceftazidime MIC (µg/ml) results from the effects of divalent cation (Ca²⁺) concentrations

Organisms	Isolate ID	Agent	MIC (µg/ml)			
			Calcium 20-25 µg/ml		Calcium 50 µg/ml	
<i>S. aureus</i>	1731646	CXA-101	32	32	32	32
	1731626	CXA-101	8	8	8	8
	1731673	CXA-101	32	32	32	32
<i>S. pneumoniae</i>	1731713	CXA-101	0.12	0.12	0.12	0.12
	1732281	CXA-101	0.25	0.25	0.25	0.25
	1732283	CXA-101	2	2	2	2
<i>P. aeruginosa</i>	1731884	CXA-101	1	1	1	1
	1731923	CXA-101	1	1	1	1
	1732281	CXA-101	2	2	2	2
<i>E. coli</i>	1732281	CXA-101	0.5	0.5	0.25	0.25
	1732283	CXA-101	0.12	0.25	0.25	0.25
	1732283	CXA-101	0.12	0.25	0.12	0.25
<i>K. pneumoniae</i>	1732369	CXA-101	0.25	0.5	0.5	0.5
	1732366	CXA-101	0.25	0.25	0.25	0.25
	1732366	CXA-101	0.25	0.25	0.25	0.25

*Standard concentration according to CLSI

Testing at 50 mg/L Ca²⁺ did not affect the activity of CXA-101 or ceftazidime against any of the isolates tested, 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).

Conclusions

Overall, CXA-101 MICs for the evaluated organisms under standard CLSI testing conditions were similar (ident