

# Evaluation of Ceftolozane-Tazobactam MIC Test Strip Compared to Broth Microdilution MIC for *Enterobacteriaceae* and *Pseudomonas aeruginosa*

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## Abstract

**Background:** Ceftolozane-tazobactam (C-T) is a cephalosporin - beta lactamase inhibitor combination antimicrobial agent that was approved by the Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis and in combination with metronidazole for the treatment of complicated intra-abdominal infections (cIAI). This study was performed to evaluate the performance of a newly developed gradient strip, the ceftolozane-tazobactam MIC Test Strip (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to a broth microdilution method against indicated Gram negative isolates.

**Methods:** The study isolates (50 *Enterobacteriaceae* [21 *E. coli*, 13 *K. pneumoniae*, 9 *E. cloacae*, 4 *P. mirabilis* and 3 *K. oxytoca*] and 53 *P. aeruginosa*) were recent clinical isolates primarily from indicated sources and chosen to include a wide range of C-T MIC results (0.12-4 to >32-4 µg/mL). Each isolate was tested for C-T MIC by broth microdilution (BMD; LSI prepared frozen panels) and by C-T MTS on 100 mm Mueller Hinton agar (MHA) plates (Becton Dickinson, Sparks, MD). QC strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603) were tested on 9 days with MHA from 2 suppliers (Becton Dickinson and Hardy Diagnostics, Santa Maria, CA) and results compared to CLSI expected ranges.

**Results:** As shown in the table, C-T MTS and BMD results were within +/- one doubling dilution (essential agreement) for 94.0% of *Enterobacteriaceae* and 96.2% of *P. aeruginosa*. Quality control results were within CLSI established ranges, with exception of one outlier result for *E. coli* ATCC 35218 on Hardy MHA.

Organism	Dilution difference of ceftolozane-tazobactam MTS-BMD (n)							Total
	-3	-2	-1	0	1	2	OS>	
Enterobacteriaceae	1	2	11	16	14	0	6	50
<i>P. aeruginosa</i>	2		6	12	28		5	53

OS: Off-scale (> highest concentration tested)

**Conclusion:** This initial evaluation of the C-T MTS showed good correlation to BMD MIC. Further testing with additional isolates at multiple sites and with media from multiple manufacturers is warranted.

## Introduction

- Ceftolozane-tazobactam (C-T) is a combination product consisting of an antibacterial drug combination of a novel cephalosporin ceftolozane, which has activity against *P. aeruginosa*, and tazobactam, an established beta-lactamase inhibitor. C-T is indicated for the treatment of complicated intra-abdominal infection and complicated urinary tract infection, including pyelonephritis and is in phase 3 trials for nosocomial pneumonia.
- Liofilchem (Roseto degli Abruzzi, Italy) has developed a ceftolozane-tazobactam MIC test strip. The Liofilchem MIC test strip is a quantitative agar-based diffusion assay for determining the minimum inhibitory concentration (MIC) and is available for a variety of different antimicrobial agents.
- This study was performed to compare the ceftolozane-tazobactam MTS MIC to broth microdilution MIC for the indicated Gram negative organisms: *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

## References:

- Clinical and Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 10<sup>th</sup> ed. Approved standard, CLSI M7-10, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2016 Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard - 26<sup>th</sup> Edition. CLSI document M100-26 Wayne, PA.
- http://www.liofilchem.net/en/mov\_mic\_test\_strip.php

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## Methods

### Study Strains (challenge set\* including recent clinical isolates from a variety of sources in US):

Bacterial Species	Ceftolozane/Tazobactam BMD Reference MIC (µg/mL) (Tazobactam at 4 µg/mL)										Total n
	0.12	0.25	0.5	1	2	4	8	16	32	>32	
<i>E. coli</i>		1	1	3	5	4	4	1	1	1	21
<i>E. cloacae</i>					1	2		1	3	2	9
<i>K. oxytoca</i>	1						1	1			2
<i>K. pneumoniae</i>		2	2	1	1	2				4	13
<i>P. mirabilis</i>			2	1	1						4
<i>P. aeruginosa</i>			5	7	8	9	7	8	3	6	53
All Isolates	1	3	10	12	16	17	12	11	8	13	102

- \*42% of Enterobacteriaceae with reference MIC results of 2-8 µg/mL (at S-I-R breakpoint concentrations)
- \*45% of *P. aeruginosa* with reference MIC results of 4-16 µg/mL (at S-I-R breakpoint concentrations)

QC strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603)

### MIC methods:

- Each isolate was tested once at Laboratory Specialists, Inc. by broth microdilution according to CLSI method (1) with frozen panels containing ceftolozane-tazobactam concentrations of 0.06-32 µg/mL and by ceftolozane-tazobactam MTS (3) containing concentrations of 0.016 -256 µg/mL (Liofilchem, Roseto degli Abruzzi, Italy) on 100 mm Mueller Hinton Agar II plates [Becton Dickinson (BD) Sparks, MD].
- Quality control strains were tested in triplicate on 2 days using 2 manufacturer's MHA (BD and Hardy Diagnostics (Santa Maria, CA) and results compared to CLSI expected ranges (2)
- MTS results were rounded up to next doubling dilution for analysis. MIC results were interpreted according to FDA/CLSI breakpoints (2)

## Results

- Quality Control (Table 1): Ceftolozane MTS MIC results for all 4 QC strains were within the CLSI expected ranges with exception of 1 result for *E. coli* ATCC 35218 on Hardy MHA.
- Enterobacteriaceae (Figure 1): Ceftolozane-tazobactam MTS MIC results were within +/- one doubling dilution for 47/50 isolates. For 3 *E. coli* MTS MIC results were 2-3 dilutions lower than the BMD MIC. Category agreement was 84.0% (14% minor errors and 5.6% very major errors (attributed to 1 *E. coli*).
- Pseudomonas aeruginosa* (Figure 2): Ceftolozane-tazobactam MTS MIC results were within +/- one doubling dilution for 51/53 isolates. For 2 isolates MTS MIC results were ≥3 dilutions lower than the BMD MIC. Category agreement was 71.7% (26.4% minor errors and 5.9% very major errors (attributed to 1 isolate).

Table 1. C-T MTS Quality Control Results by Media

QC strain	MHA	C-T MIC (µg/mL)									
		0.06	0.12	0.19	0.25	0.38	0.5	0.75	1	1.5	2
<i>E. coli</i> ATCC 25923	BD				1	3	2				
	Hardy	1	2	1	2						
<i>P. aeruginosa</i> ATCC 27853	BD					1	4	1			
	Hardy					1	4				
<i>E. coli</i> ATCC 35218	BD		1	5							
	Hardy	1	1	3	1						
<i>K. pneumoniae</i> ATCC 700603	BD							1	4	1	
	Hardy							1	4	1	

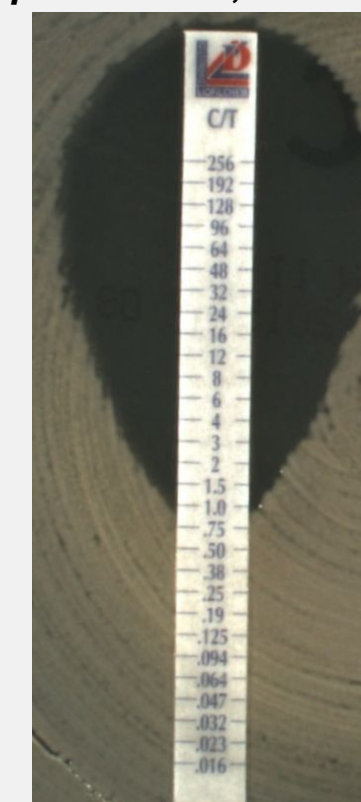
Expected QC range is shown in shaded box

Ceftolozane-Tazobactam MTS photographs of select study isolates (MIC in µg/mL)

*E. coli*, MIC = 2 µg/mL



*K. pneumoniae*, MIC = 1 µg/mL



*P. aeruginosa*, MIC = 0.5

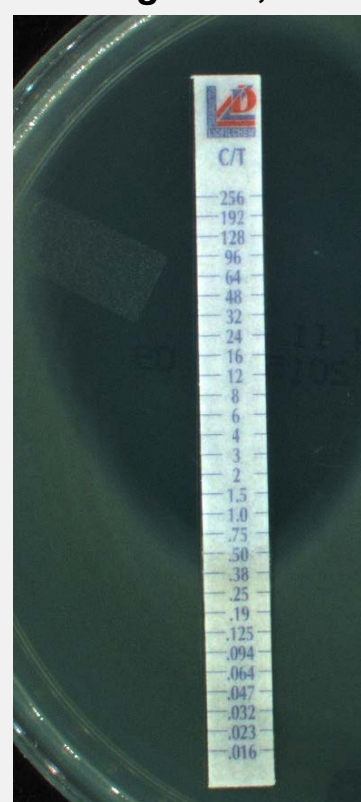
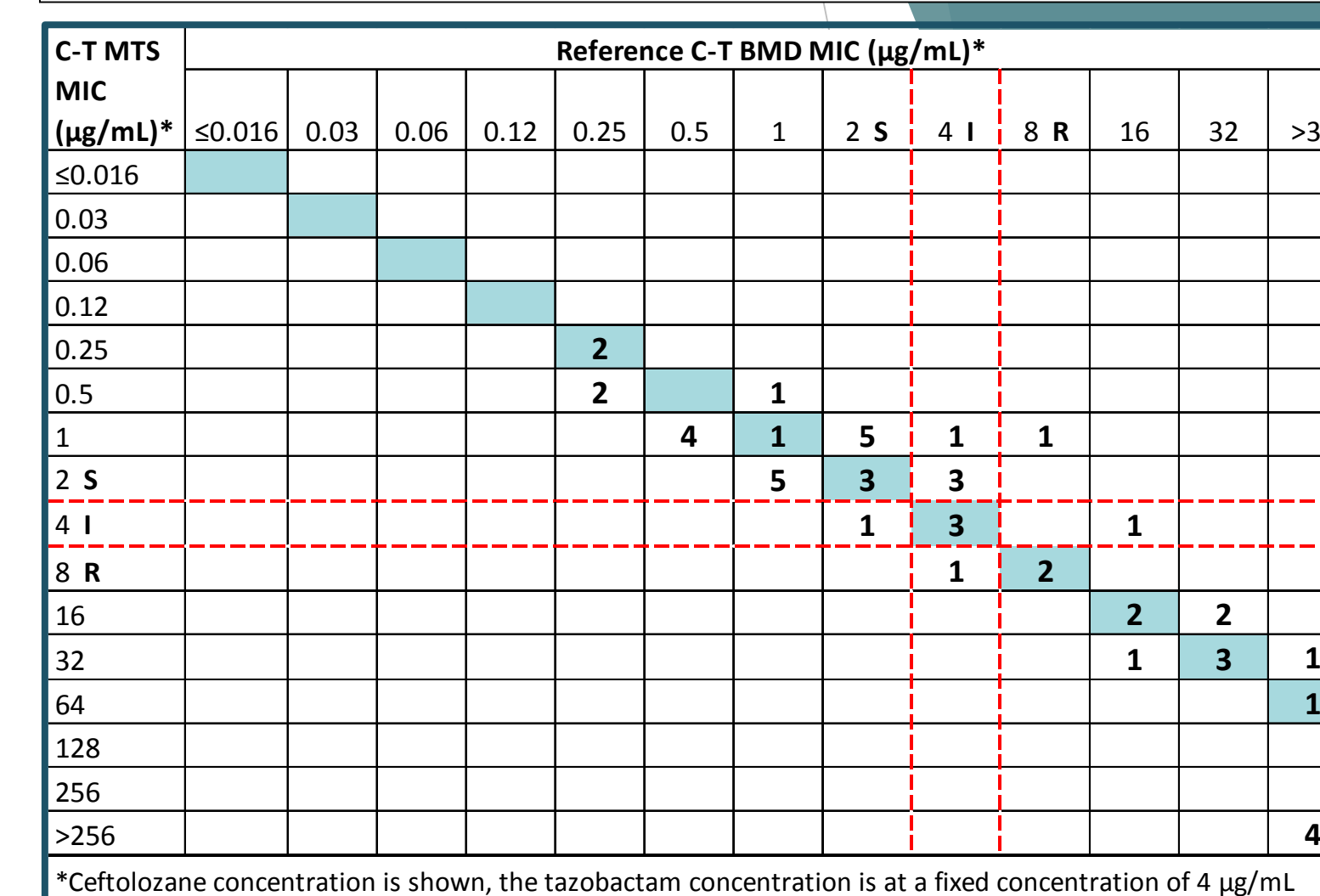


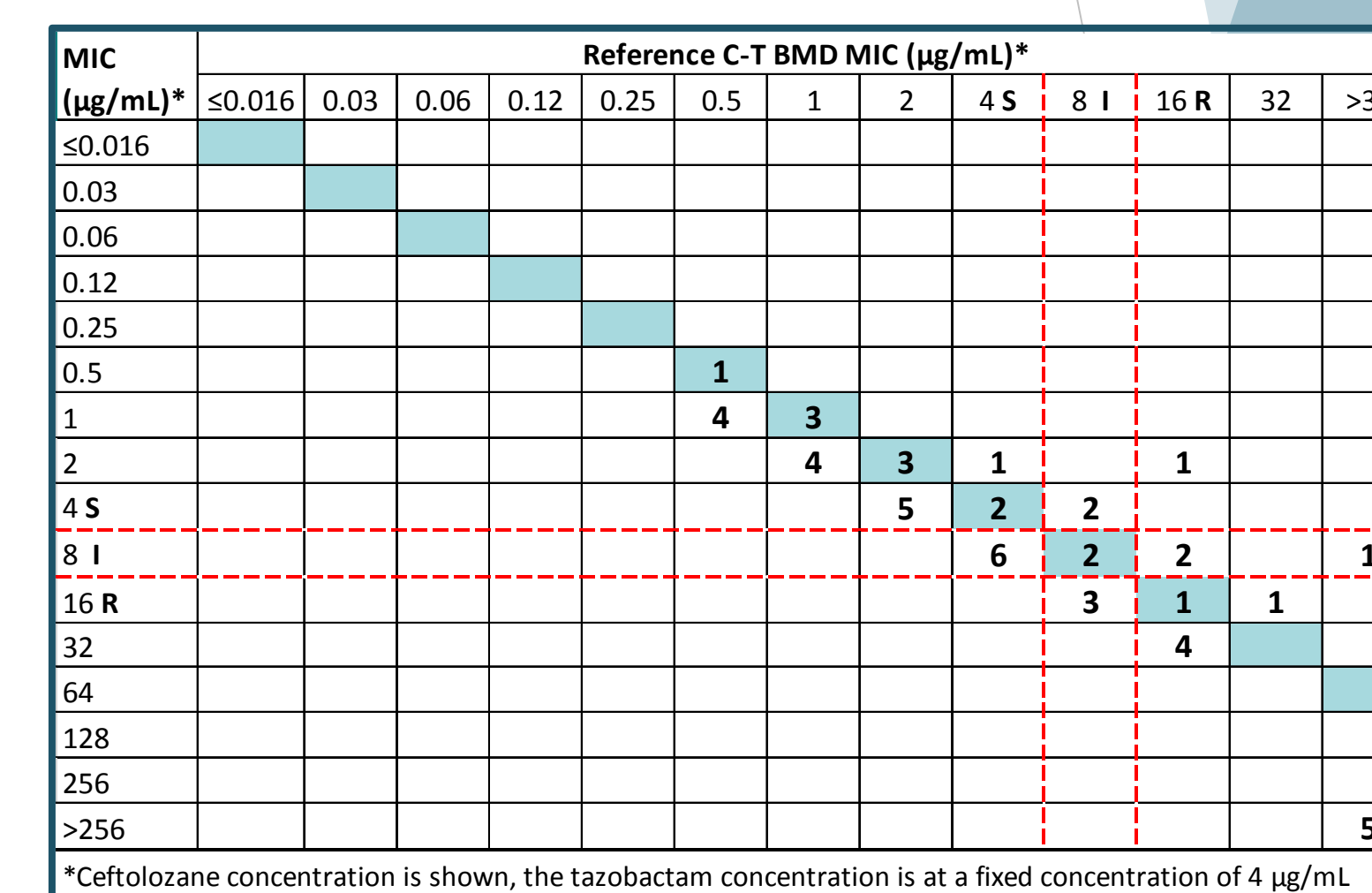
Figure 1. Scatterplot of MTS MIC to BMD MIC for 50 Enterobacteriaceae



Evaluation		
Overall EA	47/50	94.0%
EA (evaluable results)	41/44	93.2%
Category Agreement	42/50	84.0%
Category Errors	Minor	7/50 14.0%
	Major	0/24 0.0%
	Very Major	1/18 5.6%

EA - essential agreement (within +/- 1 dilution of reference MIC)

Figure 2. Scatterplot of MTS MIC to BMD MIC for 53 *P. aeruginosa*



Evaluation		
Overall EA	51/53	96.2%
EA (evaluable results)	46/47	97.9%
Category Agreement	33/42	71.7%
Category Errors	Minor	14/53 26.4%
	Major	0/29 0.0%
	Very Major	1/17 5.9%

EA - essential agreement (within +/- 1 dilution of reference MIC)

## Conclusions

- The ceftolozane-tazobactam MTS against *Enterobacteriaceae* and *P. aeruginosa* performed similar to BMD testing.
- With exception of 2 strains with lower MTS MICs, there was a tendency for higher MTS MIC results compared to BMD MIC results for *P. aeruginosa*.
- Additional testing with more isolates, at multiple sites and with multiple MHA is recommended for further validation. Since the completion of this study, data from a multi-site evaluation was included in a 510(k) submission and is currently under review by FDA.