ASM Microbe 2017
Saturday - 411

Correlation of Reference Agar Dilution MIC Values and Kirby-Bauer Disk Diffusion Testing for Fosfomycin against Gram-Positive and Gram-Negative Bacteria

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Abstract

Background: Intravenous ZTI-01 (fosfomycin; FOS) is under US development to treat hospitalized patients with complicated urinary tract infections (cUTI). FOS is an active bactericidal agent that targets early cell wall synthesis inhibition and has *in vitro* activity against gram-negative (GN) and -positive (GP) bacteria, including MDR organisms. CLSI interpretive criteria, based on the oral formulation, exist only for urinary tract isolates of *Enterococcus faecalis* (EF) and *Escherichia coli* (EC) for agar dilution (AD; 25 mg/L glucose-6-phosphate [G6P] supplementation) and disk diffusion (DD) methods.

Methods: A total of 938 GN and GP isolates collected in US medical centers were tested against FOS by AD (25 mg/L [G6P] supplementation) and DD (FOS, 200 µg/50 µg G6P). Interpretive discrepancy rates occurring between disk diffusion results and MIC values were calculated. For analysis purposes, the current CLSI interpretive criteria for EF/EC were applied to all organism groups. No major (ME) or very major (VME) errors occurred when applying interpretive criteria to test results of *Staphylococcus aureus* and coagulase-negative staphylococci. Minor errors (MIE) for coagulase-negative staphylococci were 12.5% in the I+1 to I-1 range; 2.9% MIE overall. For EF, there were no ME/VME and 2.6% MIE (I+1 to I-1); 1.5% MIE overall. ME of 3.9% and 27.5% MIE occurred in the I+1 to I-1 range for *E. faecium*; 3.5% and 24.1% overall. Error rates were high for β-haemolytic streptococci at 23.8% ME and 23.8% MIE overall. The ME and MIE rates for *Enterobacteriaceae* were 10% (1/10) and 30% (3/10) for the I+1 to I-1 range; total error rates were 0.28% and 2.0%, respectively. EC had no ME/VME and only 1 MIE (0.9%). Error rates were high for *Pseudomonas aeruginosa* (PA) and *Acinetobacter baumannii* (AB).

Conclusions: The current CLSI MIC and DD interpretive criteria for EC and EF performed well in the correlation of MIC and disk zone diameters. Other enterics and the staphylococci also performed well in the correlation when applying CLSI breakpoints for EC/EF. β-haemolytic streptococci, PA, and AB did not perform well. The adequacy of breakpoints to be used for FOS, currently being studied at a dose of 6g q 8 hr, will need to account for the significantly higher plasma and urine concentrations obtained after IV administration compared to the approved 3g oral dosage with limited bioavailability. Results from the Phase 2/3 cUTI trial, including correlation of MIC and DD testing results, will be important in determining the appropriateness of the FDA current breakpoints.

Introduction

- Fosfomycin has been used in an intravenous and an oral form to treat a variety of infections, including urinary tract, respiratory tract, and skin and skin structure
- In the United States, fosfomycin is licensed as an oral dosage form (3 grams) taken once daily for use in treating uncomplicated urinary tract infections
- The bioavailability of the oral formulation is limited, ~37% of the intravenous form
- Fosfomycin inhibits bacterial cell wall production at an early stage, unique from all other bacterial cell wall inhibitors, by covalently binding to MurA, the first step in cell wall synthesis
- Fosfomycin is primarily transported into the bacterial cell by active transport via 2 transport systems, a glycerol-3-phosphate and a hexose-6-phosphate transport system
- Impaired function of the uptake systems is one mechanism for fosfomycin resistance
- As the expression of the full level of antimicrobial activity requires the functioning of at least one active transport system, glucose-6-phosphate at 25 mg/L should be included in the susceptibility test system used to test fosfomycin
- The additive glucose-6-phosphate enhances uptake into only the hexose-6-phosphate transporter (1 of the 2 active transport systems in *Enterobactericeae* but that which is notably lacking in other species, such as *Pseudomonas* spp.)
- In this study, we evaluated the activity of fosfomycin against a collection of gram-positive and gram-negative bacteria and compared the MIC and disk diffusion testing results using existing US-FDA interpretative criteria for comparative purposes

Materials and Methods

- A total of 938 gram-negative and gram-positive aerobic recent clinical isolates (>90% collected during 2015) as part of the global SENTRY Antimicrobial Surveillance Program were tested
- Fosfomycin MIC testing was performed by agar dilution using Mueller-Hinton agar supplemented with 25 mg/L glucose-6-phosphate, following CLSI recommendations
  - For comparative assessments, CLSI MIC interpretive criteria were utilized which are S/I/R, ≤64/128/≥256 mg/L, and apply only to *Escherichia coli* and *Enterococcus faecalis* from urinary tract infections (CLSI (M100-S27 [2017])
- Antimicrobial susceptibility disk testing was performed following CLSI recommendations using the 200 µg fosfomycin/50 µg glucose-6-phosphate disk (CLSI (M100-S27 [2017])
  - CLSI disk zone diameter interpretive criteria are S/I/R, ≥16/13-15/≤12 mm
- For analysis purposes, these interpretive criteria were applied to other organisms tested
- Interpretive discrepancy rates occurring between the disk zone diameter and MIC result were calculated according to CLSI guidelines (M23-ed 4; 2016)

Results

- *Staphylococcus aureus*
  - Susceptibility, as measured by both agar and disk, was identical at 100.0% (Tables 1 and 2)
  - When correlating disk and MIC values, there were no major or minor errors (Table 3)
- Coagulase-negative staphylococci
  - Susceptibility by agar dilution was 91.2% compared to disk diffusion at 89.2% (Tables 1 and 2)
  - When correlating disk and MIC values, there were no major errors and 12.5% minor errors in the I+1 to I-1 category (Table 3)
- *Enterococcus faecalis*
  - Susceptibility by agar dilution was 100.0% compared to disk diffusion at 98.5% (Tables 1 and 2)
  - When correlating disk and MIC values, there were no major errors and 2.6% minor errors in the I+1 to I-1 category (Table 3; Figure 1)
- *Enterococcus faecium*
  - Susceptibility by agar dilution was 84.5% compared to disk diffusion at 70.7% (Tables 1 and 2)
  - When correlating disk and MIC values, there were 2 major errors (3.9%) and 27.5% minor errors in the I+1 to I-1 category (Table 3)
- *β-haemolytic streptococci*
  - Susceptibility by agar dilution was 100.0% compared to disk diffusion at 52.5% (Tables 1 and 2)
  - When correlating disk and MIC values, there were many major errors (81.8% in the I+1 to I-1 range;16.7% in the ≤I-2 range), 18.2% minor errors in the I+1 to I-1 range, and 24.4% minor errors in the ≤I-2 range (Table 3)
  - Total major errors and total minor errors each were 23.8% (Table 3)
- *Enterobacteriaceae*
  - Susceptibility by agar dilution was 97.8% compared to disk diffusion at 96.4% (Tables 1 and 2)
  - When correlating disk and MIC values, there was 1 major error (10.0%; a *Klebsiella pneumoniae* isolate) and 3 (30.0%) minor errors (Table 4) in the I+1 to I-1 range
  - In the ≤I-2 range, there were 4 (1.2%) minor errors (Table 4)
  - Total major error rate was 0.3% and total minor error rate was 2.0% (Table 4; Figure 2)
- *Pseudomonas aeruginosa*
  - Susceptibility by agar dilution was 80.0% compared to disk diffusion at 38.1% (Tables 1 and 2)
  - When correlating disk and MIC values, there were 23 (34.3%) major errors and 34 (50.8%) minor errors (Table 4) in the I+1 to I-1 range
  - In the ≤I-2 range, there were 3 (8.3%) minor errors (Table 4)
  - Total major error rate was 21.9% and total minor error rate was 35.2% (Table 4)
- *Acinetobacter baumannii* species complex
  - Susceptibility by agar dilution was 1.9% compared to disk diffusion at 5.8% (Tables 1 and 2)
  - When correlating disk and MIC values, there were no major errors and 51 (49.5%) minor errors (Table 4) in the I+1 to I-1 range
  - Total minor error rate was 49.0% (Table 4)

Table 1 Summary of fosfomycin agar (with 25 mg/L glucose-6-phosphate) activity

		MIC (mg/L)		CLSI		
Organism	**N**	**50%**	**90%**	**%S**	**%I**	**%R**
Staphylococcus aureus	111	4	8	(100.0)	(0.0)	(0.0)
Coagulase-negative staphylococci	34	4	64	(91.2)	(2.9)	(5.9)
Enterococcus faecalis	66	64	64	100.0	0.0	0.0
Enterococcus faecium	58	64	128	(84.5)	(15.5)	(0.0)
β-haemolytic streptococci	101	16	64	(100.0)	(0.0)	(0.0)
Enterobacteriaceae	359	4	16	(97.8)	(0.8)	(2.4)
Escherichia coli	117	0.5	1	100.0	0.0	0.0
Klebsiella pneumoniae	106	8	16	(97.2)	(0.0)	(2.8)
Pseudomonas aeruginosa	105	64	128	(80.0)	(15.2)	(4.8)
Acinetobacter baumannii-calcoaceticus species complex	104	128	256	(1.9)	(79.8)	(19.3)

Table 2 Summary of disk activity (200 µg fosfomycin/50 µg glucose-6-phosphate)

		CLSI[a]		
Organism	**N**	**%S**	**%I**	**%R**
Staphylococcus aureus	111	(100.0)	(0.0)	(0.0)
Coagulase-negative staphylococci	34	(89.2)	(5.9)	(5.9)
Enterococcus faecalis	66	98.5	1.5	0.0
Enterococcus faecium	58	(70.7)	(22.4)	(6.9)
β-haemolytic streptococci	101	(52.5)	(23.7)	(23.8)
Enterobacteriaceae	359	(96.4)	(1.1)	(2.5)
Escherichia coli	117	99.1	0.9	0.0
Klebsiella pneumoniae	106	(96.2)	(0.0)	(3.8)
Pseudomonas aeruginosa	105	(38.1)	(20.0)	(41.9)
Acinetobacter baumannii-calcoaceticus species complex	104	(5.8)	(30.7)	(63.5)

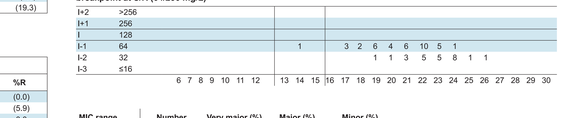
Table 3 Error rates for gram-positive organisms

Organism	**MIC range**	**Number**	**Very major (%)**	**Major (%)**	**Minor (%)**
Staphylococcus aureus	≥I+2	0	0	N/A	0
	I+1 to I-1	1	0	0	0
	≤I-2	110	N/A	0	0
	Total	111	0	0	0
Coagulase-negative staphylococci	≥I+2	2	0	N/A	0
	I+1 to I-1	8	0	0	1 (12.5)
	≤I-2	24	N/A	0	0
	Total	34	0	0	1 (2.94)
Enterococcus faecalis	≥I+2	0	0	N/A	0
	I+1 to I-1	38	0	0	1 (2.63)
	≤I-2	28	N/A	0	0
	Total	66	0	0	1 (1.52)
Enterococcus faecium	≥I+2	0	0	N/A	0
	I+1 to I-1	51	0	2 (3.92)	14 (27.45)
	≤I-2	7	N/A	0	0
	Total	58	0	2 (3.45)	14 (24.14)
B-haemolytic streptococcus	≥I+2	0	0	N/A	0
	I+1 to I-1	11	0	9 (81.82)	2 (18.18)
	≤I-2	90	N/A	15 (16.67)	22 (24.44)
	Total	101	0	24 (23.76)	24 (23.76)

Table 4 Error rates for gram-negative organisms

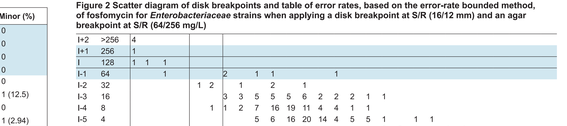
Organism	**MIC range**	**Number**	**Very major (%)**	**Major (%)**	**Minor (%)**
Enterobacteriaceae	≥I+2	4	0	N/A	0
	I+1 to I-1	10	0	1 (10.0)	3 (30.0)
	≤I-2	345	N/A	0	4 (1.16)
	Total	359	0	1 (0.28)	7 (1.95)
Escherichia coli	≥I+2	0	0	N/A	0
	I+1 to I-1	1	0	0	0
	≤I-2	116	N/A	0	1 (0.86)
	Total	117	0	0	1 (0.85)
Klebsiella pneumoniae	≥I+2	3	0	N/A	0
	I+1 to I-1	1	0	1 (100.0)	0
	≤I-2	102	N/A	0	0
	Total	106	0	1 (0.94)	0
Pseudomonas aeruginosa	≥I+2	2	0	N/A	0
	I+1 to I-1	67	0	23 (34.33)	34 (50.75)
	≤I-2	36	N/A	0	3 (8.33)
	Total	105	0	23 (21.9)	37 (35.24)
Acinetobacter baumannii	≥I+2	1	0	N/A	0
	I+1 to I-1	103	0	0	51 (49.51)
	≤I-2	0	N/A	0	0
	Total	104	0	0	51 (49.04)

Figure 1 Scatter diagram of disk breakpoints and table of error rates, based on the error-rate bounded method, of fosfomycin for *Enterococcus faecalis* strains when applying a disk breakpoint at S/R (16/12 mm) and an agar breakpoint at S/R (64/256 mg/L)



MIC range	**Number**	**Very major (%)**	**Major (%)**	**Minor (%)**
≥I+2	0	0	N/A	0
I+1 to I-1	38	0	0	1 (2.63)
≤I-2	28	N/A	0	0
Total	**66**	**0**	**0**	**1 (1.52)**

Figure 2 Scatter diagram of disk breakpoints and table of error rates, based on the error-rate bounded method, of fosfomycin for *Enterobacteriaceae* strains when applying a disk breakpoint at S/R (16/12 mm) and an agar breakpoint at S/R (64/256 mg/L)



MIC range	**Number**	**Very major (%)**	**Major (%)**	**Minor (%)**
≥I+2	4	0	N/A	0
I+1 to I-1	10	0	1 (10.0)	3 (30.0)
≤I-2	345	N/A	0	4 (1.16)
Total	**359**	**0**	**1 (0.28)**	**7 (1.95)**

Conclusions

- The current CLSI MIC and disk zone diameter breakpoints correlate very well for *Enterococcus faecalis* and *Escherichia coli*, which are the 2 organisms with CLSI breakpoints (urinary isolates only)
- When applying the existing CLSI breakpoints for *E. faecalis* and *E. coli* to other organisms for analysis purposes, the comparative susceptibility of *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterobacteriaceae* were very close, and the MIC/disk zone diameter correlations were very good
- β-haemolytic streptococci, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* species complex all performed poorly in the correlation exercise
- When considering interpretive breakpoints for any agent, the dosage regimen and route of administration are a critical component to assess adequacy of achieving therapeutic (i.e. bactericidal) concentrations
- The upcoming results of the Phase 2/3 complicated urinary tract infection trial for ZTI-01, (IV fosfomycin; ZEUS NCT02753946) including correlation with MIC and disk zone diameter results, will be important in determining the appropriateness of the exisitng CLSI breakpoints for fosfomycin from an intravenous regimen in which 6 grams of fosfomycin given 3 times daily are administered

Acknowledgements

The authors wish to thank the following staff members at JMI Laboratories (North Liberty, Iowa, USA): A. Watters, M. Janechek, J. Oberholser, J.E. Schuchert, and J.M. Streit for technical support. This study was performed by JMI Laboratories and supported by Zavante Therapeutics, which included funding for services related to preparing this poster.

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