

Comparison of Fosfomycin Susceptibility Testing Methods

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Introduction

Fosfomycin is a phosphoenolpyruvate analogue that inhibits bacterial cell wall synthesis. It remains a convenient oral option for uncomplicated cystitis and intravenous administration may be considered for salvage therapy of non-urinary tract infections, in combination with other agents.

Agar dilution is the only approved MIC method as per CLSI, with minimum inhibitory concentration (MIC) and minimum inhibitory diameter (MID) interpretive criteria for urinary isolates of *Escherichia coli* and *Enterococcus faecalis*¹. *In vitro* susceptibility testing requires glucose-6-phosphate supplementation for fosfomycin entry via the hexose phosphate transport system². We assessed accuracy of current in-use testing methods against agar dilution.

Materials and Methods

Liofilchem fosfomycin agar dilution, bioMérieux fosfomycin ETEST, bioMérieux VITEK 2 AST-N356 (for *E. coli*), and Thermo Fisher Scientific Oxoid fosfomycin disk diffusion were performed for 60 urinary isolates of *E. coli*, *E. faecalis* and *Enterococcus faecium*. Results were interpreted with CLSI M100 S35, extrapolating *E. faecalis* breakpoints to *E. faecium*.

With agar dilution as the reference method, the following parameters were assessed:

- Essential agreement (EA): % of test results with MIC within one doubling dilution of the reference method
- Categorical agreement (CA): % of test results with the same clinical interpretation as the reference method
- Minor error (mE) rates: % of test results where one method yields an intermediate while the other yields a susceptible or resistant result
- Major error (ME) rates (false-resistant results): where the reference method yields a susceptible result while the other yields a resistant result. This is expressed as a % of susceptible isolates.
- Very major error (VME) rates (false-susceptible results): where the reference method yields a resistant result while the other yields a susceptible result. This is expressed as a % of resistant isolates.

In-house acceptance criteria for the various parameters are EA ≥90%, CA ≥90%, mE ≤10%, ME ≤3% and VME ≤3%.

Results

Taking agar dilution as the reference standard:

- ETEST
 - EA = (49/60) **81.7%**
 - CA = (45/60) **75.0%**
 - mE rates = (10/60) **16.7%**
 - ME rates = (4/37) **10.8%**
 - VME rates (1/14) **7.1%**
- VITEK 2
 - EA = (28/30) **93.3%**
 - CA = (23/30) **76.7%**
 - mE rates = (5/30) **16.7%**
 - ME rates = (1/15) **6.7%**
 - VME rates = (1/10) **10.0%**
- Disk Diffusion
 - CA = (39/60) **65.0%**
 - mE rates = (9/60) **15.0%**
 - ME rates = (11/37) **29.7%**
 - VME rates = (1/14) **7.1%**

ETEST, VITEK 2, and disk diffusion demonstrated unacceptable performance across most parameters for fosfomycin susceptibilities.

Conclusion

Discordance with agar dilution was observed across all methodologies for fosfomycin susceptibilities and agar dilution has since been adopted in-house as the only method for clinical diagnostics.

Reliable testing is crucial not only for patient management, but for robust data collection in order to define epidemiological cutoff values and establish clinical breakpoints for more drug-bug combinations.

Organism	Agar Dilution			E TEST			Disk Diffusion			VITEK 2			
	MIC (µg/ml)		MIC (µg/ml)		CA	EA	MID (mm)		CA	MIC (µg/ml)		CA	EA
<i>E. coli</i>	≤0.25	S	0.19	S	Y	Y	6	R	ME	≤16	S	Y	Y
<i>E. coli</i>	≤0.25	S	0.5	S	Y	Y	24	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.19	S	Y	Y	27	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.19	S	Y	Y	16	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.5	S	Y	Y	24	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.5	S	Y	Y	23	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.5	S	Y	Y	20	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.5	S	Y	Y	13	I	mE	≥256	R	ME	N
<i>E. coli</i>	0.5	S	0.75	S	Y	Y	26	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.75	S	Y	Y	24	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	0.75	S	Y	Y	22	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	1	S	Y	Y	21	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	1	S	Y	Y	6	R	ME	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	4	S	Y	N	22	S	Y	≤16	S	Y	Y
<i>E. coli</i>	0.5	S	≥512	R	ME	N	6	R	ME	≤16	S	Y	Y
<i>E. coli</i>	128	I	256	R	mE	Y	12	R	mE	≥256	R	mE	Y
<i>E. coli</i>	128	I	256	R	mE	Y	6	R	mE	≥256	R	mE	Y
<i>E. coli</i>	128	I	256	R	mE	Y	6	R	mE	≥256	R	mE	Y
<i>E. coli</i>	128	I	≥512	R	mE	N	6	R	mE	≥256	R	mE	Y
<i>E. coli</i>	128	I	≥512	R	mE	N	6	R	mE	≥256	R	mE	Y
<i>E. coli</i>	256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	>256	R	≥512	R	Y	Y	10	R	Y	≥256	R	Y	Y
<i>E. coli</i>	>256	R	≥512	R	Y	Y	6	R	Y	≤16	S	VME	N
<i>E. coli</i>	>256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	>256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	>256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. coli</i>	>256	R	≥512	R	Y	Y	6	R	Y	≥256	R	Y	Y
<i>E. faecalis</i>	32	S	32	S	Y	Y	22	S	Y				
<i>E. faecalis</i>	32	S	32	S	Y	Y	21	S	Y				
<i>E. faecalis</i>	32	S	32	S	Y	Y	20	S	Y				
<i>E. faecalis</i>	32	S	32	S	Y	Y	19	S	Y				
<i>E. faecalis</i>	32	S	32	S	Y	Y	18	S	Y				
<i>E. faecalis</i>	32	S	32	S	Y	Y	9	R	ME				
<i>E. faecalis</i>	32	S	48	S	Y	Y	18	S	Y				
<i>E. faecalis</i>	32	S	48	S	Y	Y	10	R	ME				
<i>E. faecalis</i>	64	S	64	S	Y	Y	20	S	Y				
<i>E. faecalis</i>	64	S	96	I	mE	Y	11	R	ME				
<i>E. faecalis</i>	64	S	128	I	mE	Y	10	R	ME				
<i>E. faecalis</i>	64	S	256	R	ME	N	18	S	Y				
<i>E. faecalis</i>	128	I	≥512	R	mE	N	9	R	mE				
<i>E. faecalis</i>	128	I	≥512	R	mE	N	6	R	mE				
<i>E. faecalis</i>	256	R	≥512	R	Y	Y	7	R	Y				
<i>E. faecalis</i>	256	R	≥512	R	Y	Y	6	R	Y				
<i>E. faecalis</i>	>256	R	≥512	R	Y	Y	6	R	Y				
<i>E. faecalis</i> (vancomycin resistant)	64	S	64	S	Y	Y	11	R	ME				
<i>E. faecium</i>	32	S	24	S	Y	Y	20	S	Y				
<i>E. faecium</i>	32	S	32	S	Y	Y	17	S	Y				
<i>E. faecium</i>	32	S	48	S	Y	Y	16	S	Y				
<i>E. faecium</i>	64	S	48	S	Y	Y	16	S	Y				
<i>E. faecium</i>	64	S	64	S	Y	Y	6	R	ME				
<i>E. faecium</i>	64	S	192	R	ME	N	17	S	Y				
<i>E. faecium</i>	64	S	192	R	ME	N	12	R	ME				
<i>E. faecium</i>	128	I	128	I	Y	Y	13	I	Y				
<i>E. faecium</i>	128	I	128	I	Y	Y	6	R	mE				
<i>E. Faecium</i>	>256	R	64	S	VME	N	16	S	VME				
<i>E. faecium</i> (vancomycin resistant)	32	S	96	I	mE	N	20	S	Y				
<i>E. faecium</i> (vancomycin resistant)	64	S	64	S	Y	Y	12	R	ME				

Table I: Discrepancies across all testing methods for urinary isolates of *E. coli* and *Enterococci* spp. as compared to the agar dilution reference method for fosfomycin susceptibility testing.

References

1. CLSI. *M100: Performance standards for antimicrobial susceptibility testing*. 35th edition
2. Detter GA *et al.* *Comparative study of fosfomycin activity in Mueller–Hinton media and in tissues*. J. Antimicrob. Chemother. 1983. 11(6): 517-524