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Susceptibility of Multiresistant Gram-Negative Bacteria to Fosfomycin and Performance of Different Susceptibility Testing Methods

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Fosfomycin may be a treatment option for multiresistant Gram-negative bacteria. This study compared susceptibility methods using 94 multiresistant clinical isolates. With agar dilution (AD), susceptibilities were 81%, 7%, 96%, and 100% (CLSI) and 0%, 0%, 96%, and 30% (EUCAST), respectively, for *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter* spp. Categorical agreement between Etest and AD for *Enterobacteriaceae* and *A. baumannii* was ≥80%. Disk diffusion was adequate only for *Enterobacter*. CLSI criteria for urine may be adequate for systemic infections.

Multidrug Gram-negative bacilli are a worldwide threat (1, 2). Furthermore, the availability of new antimicrobials has not matched new patterns of resistance (3). Tigecycline and aminoglycosides are treatment options; however, tigecycline has low plasma and urinary levels and aminoglycosides have significant adverse effects. Moreover, resistance to these antibiotics has increased (4–6).

Fosfomycin, derived from phosphonic acid (7, 8), is chemically unrelated to other antimicrobials and used to treat community-acquired urinary tract infections (8–10). With the spread of multiresistance, fosfomycin is a potential option, although experience with intravenous fosfomycin is scarce (11, 12).

In addition, breakpoints are not well defined by American (CLSI) or European (EUCAST) standards. By CLSI guidelines, only disk diffusion (DD) and agar dilution (AD) for urinary isolates of *Escherichia coli* and *Enterococcus faecalis* are approved methods and broth microdilution should not be used (13). EUCAST recommends agar dilution or broth microdilution (14).

Our study compared methods to evaluate the susceptibility to fosfomycin of Gram-negative multiresistant clinical isolates.

Blood, urine, and respiratory isolates of hospitalized patients were obtained from 2 teaching hospitals in Brazil (2010 to 2013). One isolate per patient was included: there were 42 *Acinetobacter baumannii* isolates, 15 *Pseudomonas aeruginosa* isolates, 27 *Klebsiella pneumoniae* isolates, and 10 *Enterobacter* species isolates, all resistant to carbapenems by microdilution.

A. baumannii strains were previously studied for resistance genes: all studied strains carried $bla_{\text{OXA-51}}$ and *carO*, 25 carried $bla_{\text{OXA-148}}$, 7 carried $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-143}}$, 2 carried bla_{IMP}, 1 carried bla_{IMP} and $bla_{\text{OXA-143}}$, and 1 carried only $bla_{\text{OXA-23}}$ (15). All *K. pneumoniae* strains carried bla_{KPC} (16).

Three susceptibility methods were used, and these were DD, AD, and Etest. AD used fosfomycin disodium salt (Sigma-Aldrich Laboratories, St. Louis, MO, USA) and Mueller-Hinton agar (MHA) containing 25 μg/ml of glucose-6-phosphate (G6P) (13). DD used MHA with 50 μg of G6P with 50-μg (Oxoid, Basingstoke, United Kingdom) and 200-μg (Cefar, São Paulo, Brazil)

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TABLE 1 Antimicrobial activity of fosfomycin against 94 multiresistant Gram-negative bacillus clinical isolates[a]

Microorganism	No. of isolates	Method	MIC (μg/ml)			% of isolates				
						CLSI			EUCAST	
			MIC_{50}	MIC_{90}	Range	S	I	R	S	R
A. baumannii	42	AD	64	128	64–128	81	19	0	0	100
		ET	48	96	32->1,024	90	2	8	31	69
P. aeruginosa	15	AD	256	256	64–256	7	13	80	0	100
		ET	128	128	48–256	33	60	7	0	100
K. pneumoniae	27	AD	16	32	2–256	96	0	4	96	4
		ET	48	256	0.5->1,024	85	4	11	48	52
Enterobacter spp.	10	AD	64	64	8–64	100	0	0	30	70
		ET	12	128	8–128	80	20	0	70	30

[a] AD, agar dilution; ET, Etest; S, susceptible; I, intermediate susceptibility; R, resistant; MIC_{50}, MIC that inhibited 50% of isolates; MIC_{90}, MIC that inhibited 90% of isolates; CLSI, Clinical and Laboratory Standards Institute interpretative criteria (susceptible, MIC of ≤64 μg/ml; intermediate, MIC of 128 μg/ml; resistant, MIC of ≥256 μg/ml); EUCAST, European Committee on Antimicrobial Susceptibility interpretative criteria (susceptible, MIC of ≤32 μg/ml; resistant, MIC of >32 μg/ml).

TABLE 2 Comparison of disk diffusion using the 200-μg disk with agar dilution against fosfomycin for 94 multiresistant Gram-negative bacillus clinical isolates[a]

Microorganism	No. of isolates	% of isolates with agreement or errors							
		CLSI				EUCAST			
		CA	m	M	VM	CA	m	M	VM
A. baumannii	42	86	14	0	0	0	5	0	95
P. aeruginosa	15	7	13	0	80	0	0	0	100
K. pneumoniae	27	96	0	0	4	96	0	0	4
Enterobacter spp.	10	100	0	0	0	30	0	0	70

[a] CA, categorical agreement; m, minor error; M, major error; VM, very major error; CLSI, Clinical and Laboratory Standards Institute interpretative criteria (susceptible, MIC of ≤64 μg/ml or zone of ≥16 mm; intermediate, MIC of 128 μg/ml or zone of 13 to 15 mm; resistant, MIC of ≥256 μg/ml or zone of ≤12 mm); EUCAST, European Committee on Antimicrobial Susceptibility interpretative criteria (no criteria for DD; susceptible, MIC of ≤32 μg/ml; resistant, MIC of >32 μg/ml).

fosfomycin disks. Fosfomycin Etest strips (supplemented with G6P) (bioMérieux, Hazelwood, MO, USA) were used for determination of MICs. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were controls (13). Experiments were performed in duplicate on separate days. Results were read by two observers plus a third, in case of nonagreement.

Isolates were classified according to CLSI breakpoints (susceptible, MIC of ≤64 μg/ml or zone of ≥16 mm [200-μg disk]; intermediate, MIC of 128 μg/ml or zone of 13 to 15 mm; resistant, MIC of ≥256 μg/ml or zone of ≤12 mm) and EUCAST breakpoints (no criteria for DD; susceptible, MIC of ≤32 μg/ml; resistant, MIC of >32 μg/ml) (13, 14). DD and Etest results were compared with AD results. Categorical agreement was defined as results within the same susceptibility category (17). Errors were ranked as very major errors, major errors, and minor errors (17, 18).

AD and Etest results and interpretation according to EUCAST and CLSI standards are shown in Table 1.

Median zone diameters using the 200-μg disk were 21 mm, 20 mm, 25 mm, and 25 mm for *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter*, respectively. For the 50-μg disk, median diameters were 11 mm, 3 mm, 15 mm, and 18 mm, respectively. Using CLSI criteria, susceptibility to *A. baumannii* was 95% (5% intermediate). All *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter* isolates were considered susceptible.

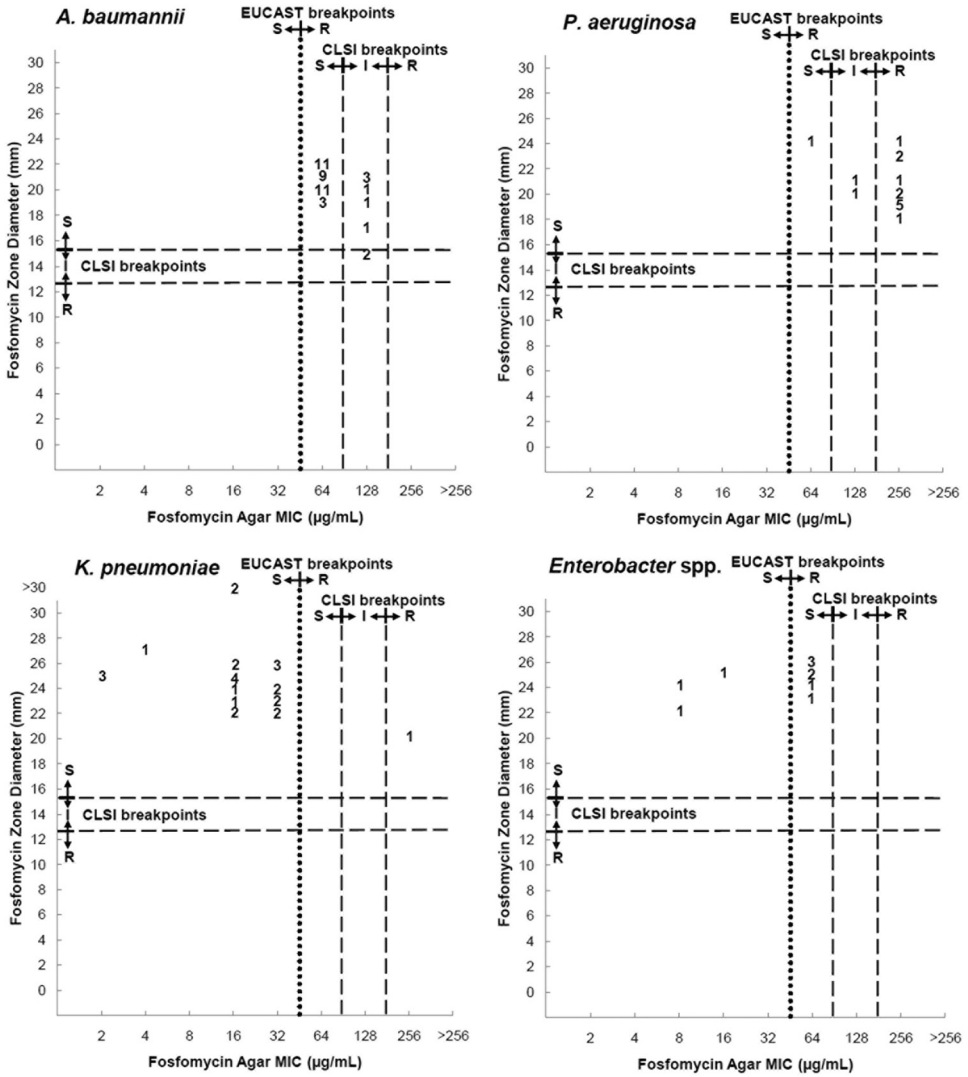

FIG 1 Scattergram results for 200-μg fosfomycin disk zone diameters and agar dilution MICs. S, susceptible; I, intermediate susceptibility; R, resistant.

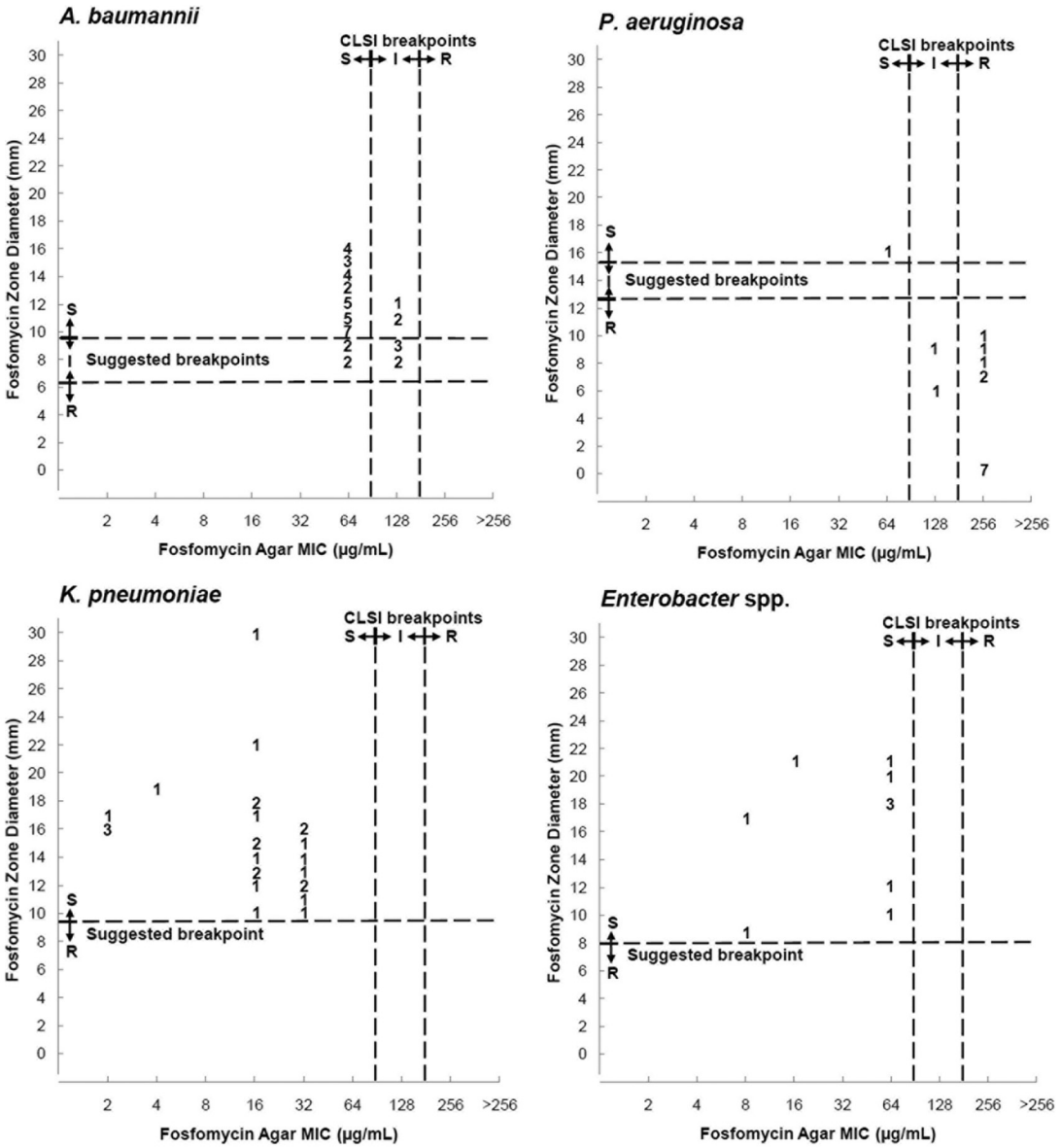

FIG 2 Scattergram results for 50-μg fosfomycin disk zone diameters and agar dilution MICs interpreted according to CLSI breakpoints. We included suggested disk diffusion breakpoints. S, susceptible; I, intermediate susceptibility; R, resistant.

Table 2 and Fig. 1 to 3 compare results of DD with AD, and in the figures, we suggest breakpoints for the 50-μg DD method.

Agreement (≤2-fold dilutions) between Etest and AD results was high for *A. baumannii* (98%) and *P. aeruginosa* (93%). For *K. pneumoniae* and *Enterobacter*, differences of >2 dilutions occurred frequently (30% and 50%, respectively).

Table 3 compares Etest and AD. The best performance occurred for *P. aeruginosa* using EUCAST breakpoints. Very major errors occurred only with EUCAST breakpoints.

In short, fosfomycin presented activity against carbapenem-resistant Gram-negative bacilli and may be a treatment alternative. Fosfomycin MICs were lower for *Enterobacteriaceae* than for nonfermenters. In another study, most isolates of *Enterobacteriaceae* presented MICs of ≤64 μg/ml, similar to our results, but the MICs for *P. aeruginosa* and *A. baumannii* were lower than in our findings (19).

Although our MICs may be considered high, we cannot conclude without clinical trials that therapeutic failure is the predictable outcome. In pharmacokinetic studies, peak concentrations varied from 132.1 ± 31.8 mg/liter to 350.2 ± 124.69 mg/liter when 20 to 100 mg/kg (body weight) of fosfomycin was infused (20–23). It has not been established if fosfomycin activity is concentration dependent or time dependent.

Agar dilution is considered the gold standard, but breakpoints are not clear. Our main interest is to use fosfomycin against systemic infections. Based on predicted serum levels of fosfomycin, it would seem that EUCAST breakpoints are very stringent. Probably CLSI breakpoints, defined for urinary tract infections (13), would be applicable to systemic infections.

To consider a susceptibility test adequate, CLSI recommends that it obtain <10% minor error, <3% major error, and <1.5% very major error rates (17, 18). Performance of DD (200-μg disk)

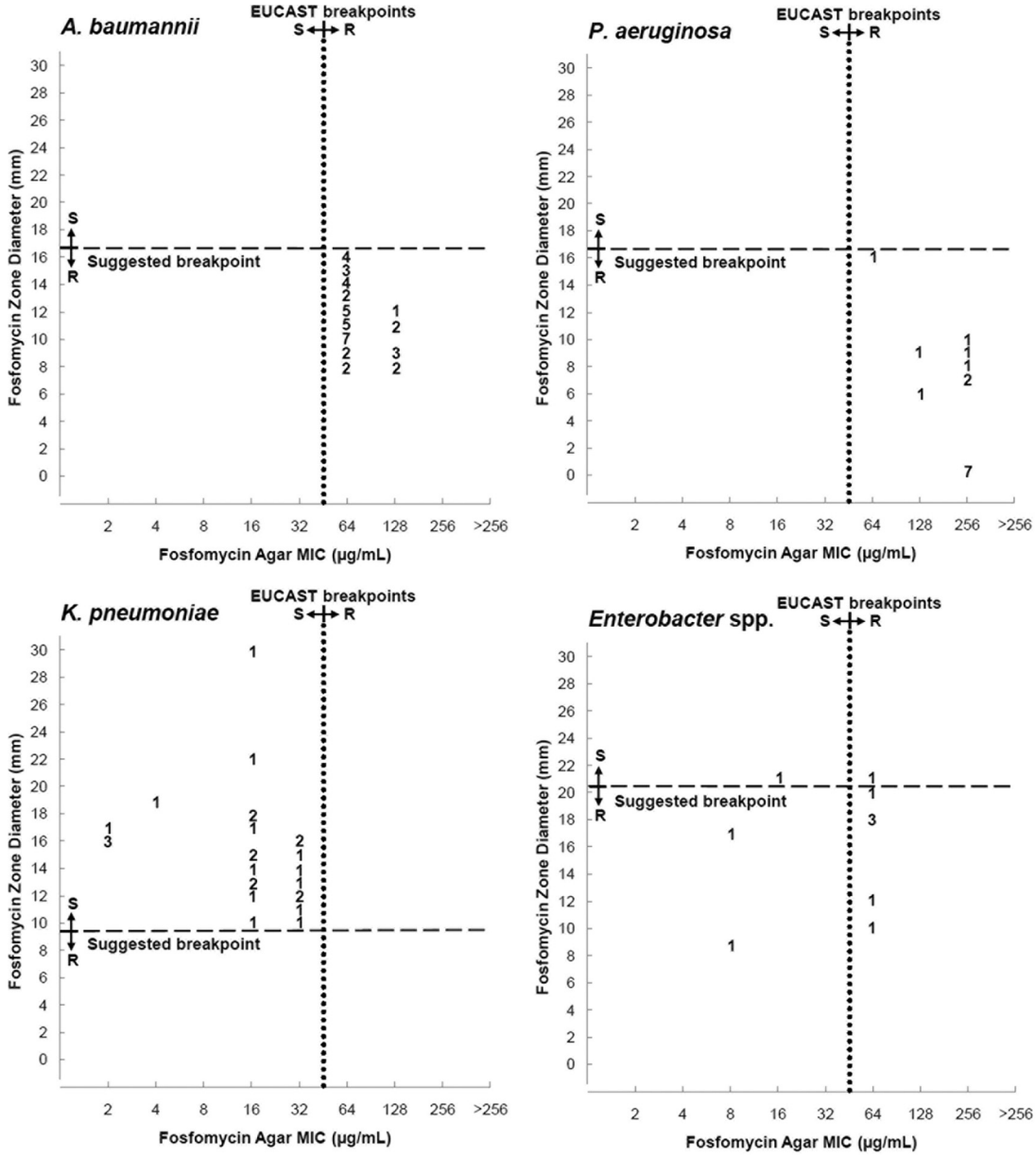

FIG 3 Scattergram results for 50-μg fosfomycin disk zone diameters and agar dilution MICs interpreted according to EUCAST breakpoints. We included suggested disk diffusion breakpoints. S, susceptible; I, intermediate susceptibility; R, resistant.

TABLE 3 Categorization of Etest errors when compared with agar dilution method for fosfomycin for 94 multiresistant Gram-negative bacillus clinical isolates[a]

Microorganism	% of isolates with agreement or errors							
	CLSI				EUCAST			
	CA	m	M	VM	CA	m	M	VM
A. baumannii	81	17	2	0	69	0	0	31
P. aeruginosa	53	34	13	0	100	0	0	0
K. pneumoniae	89	4	7	0	52	0	48	0
Enterobacter spp.	80	20	0	0	40	0	10	50

[a] CA, categorical agreement; m, minor error; M, major error; VM, very major error; CLSI, Clinical and Laboratory Standards Institute interpretative criteria; EUCAST, European Committee on Antimicrobial Susceptibility interpretative criteria.

was adequate for *Enterobacter*, although only 10 isolates were tested. For *K. pneumoniae*, despite high categorical concordance, there was a 4% rate of very major errors, and for *A. baumannii*, the rate of minor errors was 14%. Performance for *P. aeruginosa* was very poor. CLSI breakpoints, in another study, were not appropriate for *K. pneumoniae*, *Enterobacter cloacae*, and *P. aeruginosa* (19). We proposed breakpoints for DD using 50-μg disks; however, these were different for each microorganism.

Despite low agreement between Etest and AD for *K. pneumoniae* and *Enterobacter*, categorical agreement was frequent. For *P. aeruginosa*, the opposite occurred and results for *A. baumannii* were more uniform. Etest performed very poorly for *P. aeruginosa*, as already reported recently (24). For *A. baumannii* and *Enterobacter*, there were unacceptable proportions of minor errors, and for *K. pneumoniae*, there were unacceptable major errors.

Our study has limitations, including small numbers of isolates, as only carbapenem-resistant clinical isolates were used, and similar MICs, making it difficult to analyze the performance of the methods. On the other hand, the 42 isolates of *A. baumannii* used in this study were previously evaluated and belonged to 10 different clusters and 21 profiles (15). Moreover, although *K. pneumoniae* isolates were not typed, isolates that circulate in Brazil belong to several different strains (25). These facts minimize the possibility that our results refer to only one or very few clones.

In conclusion, fosfomycin presented activity against multiresistant microorganisms. It is possible that CLSI interpretative criteria for urine may be adequate for systemic infections. DD using 200 μg presented a medium performance for *A. baumannii* and *Enterobacteriaceae* and very poor performance for *P. aeruginosa*. Etest performed poorly.

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