TITLE: OPTIMIZATION OF THE METHOD OF DILUTION IN AGAR FOR PHOSPHYMICINE FOR USE IN CLINICAL MICROBIOLOGY ROUTINE

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ABSTRACT:

The fosfomycin susceptibility test is a challenge for all clinical microbiology laboratories, since the reference method when testing this antimicrobial is dilution in agar. The broth microdilution and disk-diffusion methods are validated only for *Escherichia coli*, but there is a demand for susceptibility evaluation for other genera and multiresistant bacterial species detected in cases of urinary tract infections. The routine use of agar dilution is limited by the difficulty in preparing large numbers of plates containing different concentrations of fosfomycin, since the range of MICs acceptable for the control strain *E. coli* ATCC 25922 (0.5 to 2.0 mg / L) differs from the sensitivity cutoff (32 mg / L) from BrCAST-EUCAST. The objective of this study was to identify and characterize a strain of *Klebsiella pneumoniae* that presented a fosfomycin MIC of 16 mg / L or 32 mg / L, in order to allow the use of only four different concentrations for susceptibility in clinical microbiology routine. Method: Mueller-Hinton II (Becton-Dickinson) cation adjusted agar plates containing 25 mg / L glucose-6-phosphate and fosfomycin concentrations of 0.25 mg / L to 64 mg / L were prepared. Mueller-Hinton II agar plates containing only 25 mg / L of glucose-6-phosphate were also produced. A set of 20 strains of *K. pneumoniae* previously analyzed using Etest® was used. Suspensions with a turbidity equivalent to the 0.5 standard of the McFarland scale were prepared from recent growth obtained on CPS ID3 agar (bioMérieux). The suspensions were diluted 1/10 in cation-adjusted Mueller-Hinton broth and then the suspensions were applied to the surface of the plates using a Steers replicator. The plates were incubated for about 16 to 20 hours in ambient air prior to visual reading. Once the strain with MIC of 16 or 32 mg / L was detected, replicate tests were performed for four different days. Results: Three strains among 20 tested had a MIC value of 32 mg / L and were selected for replicate testing. Replicate tests showed stability of MIC values obtained for strain xxxxxxx ranging from 16 mg / L to 32 mg / L. Conclusions: The *K. pneumoniae* strain E7222941 can be used as a reference strain for quality control of routine fosfomycin susceptibility tests, allowing the use of only four distinct concentrations (8 to 64 mg / L) and a growth control plate, representing a saving of 55% in consumables compared to the procedure using *E. coli* strain ATCC 25922.

Keywords: fosfomycin, agar dilution, quality control

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