

Variations in Agreement and Epidemiological Cutoff Value (ECV) between Fosfomycin (FOF) Agar Dilution and Broth Microdilution Using Standard- and High-Inoculum Protocols for *Klebsiella pneumoniae* (KP)

ABSTRACT

Background: FOF has been used in the treatment of multidrug-resistant (MDR) KP infections despite established susceptibility breakpoints. At present, agar dilution (AD) is considered the reference method for FOF while broth microdilution (BMD) is specifically recommended against despite its convenience over AD. We therefore sought to assess FOF activity against KP, along with essential and categorical agreement between AD and BMD methods to determine if BMD could be used as a reliable testing method.

Materials/Methods: Minimal inhibitory concentration (MIC) values were determined for a convenience collection of 70 KP isolates (59.4% MDR) from three US institutions. MIC testing was conducted in duplicate on separate days using AD and BMD methods; essential and categorical agreement were calculated using AD as the reference method. Fourteen isolates were also analyzed using high-inoculum AD ($10^{5.3-5.9}$ CFU/mL) similar to the BMD method. MIC values were categorized using Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for *Escherichia coli* (≤ 64 mg/L, susceptible). ECVs were determined according to CLSI methodology.

Results: MIC values varied between methods, with MIC₅₀/MIC₉₀ values being 32/256 mg/L for AD and 128/256 mg/L for BMD. Using *E. coli* criteria, susceptible/intermediate/resistant rates were 82.6/2.9/14.5% (AD) and 44.9/21.7/33.3% (BMD). Essential agreement was 44.9% and categorical agreement was 60.8%. When using high-inoculum AD, MIC values were on average three-fold higher compared to standard-inoculum AD, with 10 of the 14 (71.4%) isolates brought into essential agreement with BMD. Calculated ECVs were 128 mg/L for standard-inoculum AD and 1024 mg/L for BMD.

Conclusions: Our collection of KP displayed high MIC values to FOF, in addition to substantial discrepancies between AD and BMD methods. Essential agreement increased with the use of high-inoculum AD testing, which better correlated with BMD results. ECV for BMD was three dilutions higher than that for standard-AD ECV. Based on these results, we recommend further investigation of BMD for FOF testing using a larger isolate collection, along with optimization of currently recommended testing methods. In light of these results, KP-specific breakpoints should also be examined.

METHODS

MIC testing:

- FOF MICs were determined in duplicate, on separate days by 2 methods:
 - AD reference method using MHA supplemented with 25 mg/L glucose-6-phosphate (G-6-P)
 - BMD using MHB supplemented with 25 mg/L G-6-P
- CLSI susceptibility criteria for *E. coli* were used for interpretation:
 - ≤ 64 mg/L (S); 128 mg/L(I); ≥ 256 mg/L (R) for AD and BMD
- High inoculum AD testing (similar to BMD) using $10^{5.3-5.9}$ CFU/mL was conducted for 14 isolates with an MIC discrepancy of ≥ 2 dilutions between AD and BMD results

Evaluation of MIC correlations:

- An evaluation of correlation between methods was performed using CLSI M23-A3 guidance with AD as the reference method
- ECVs were determined according to CLSI methodology

RESULTS

Fig 1. Comparison of S/I/R between BMD and AD testing methods

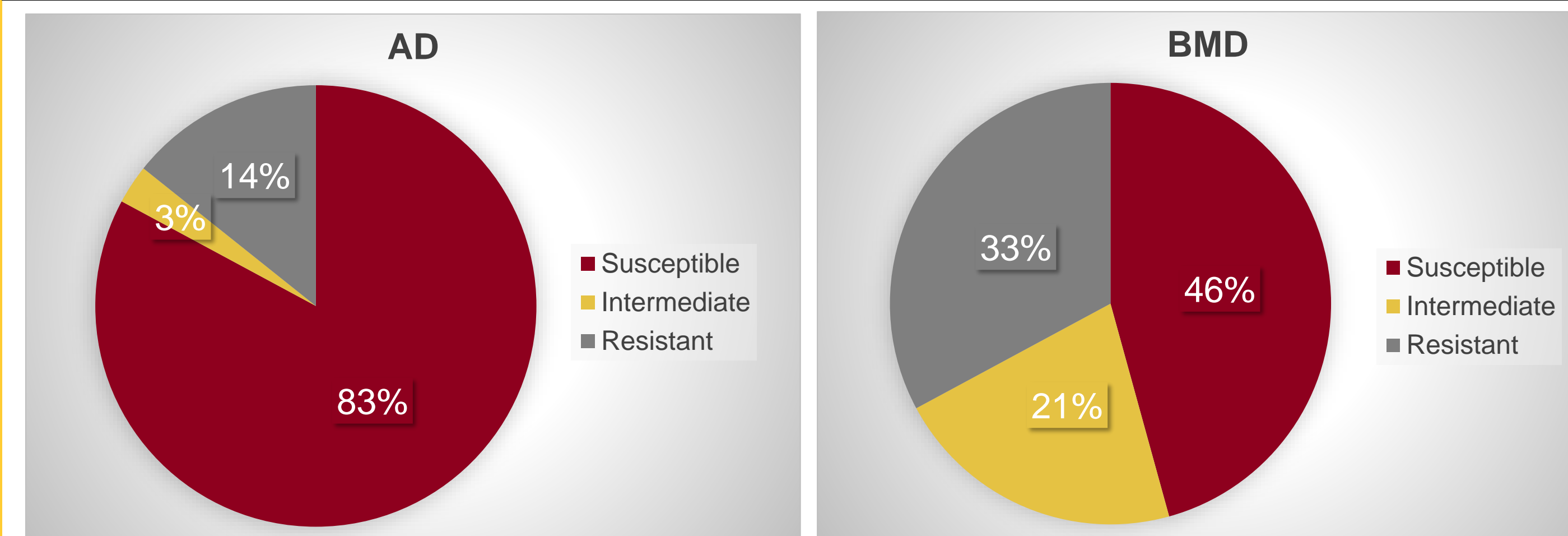


Fig 2. MIC values of 70 isolates of *K. pneumoniae* using BMD and AD methods

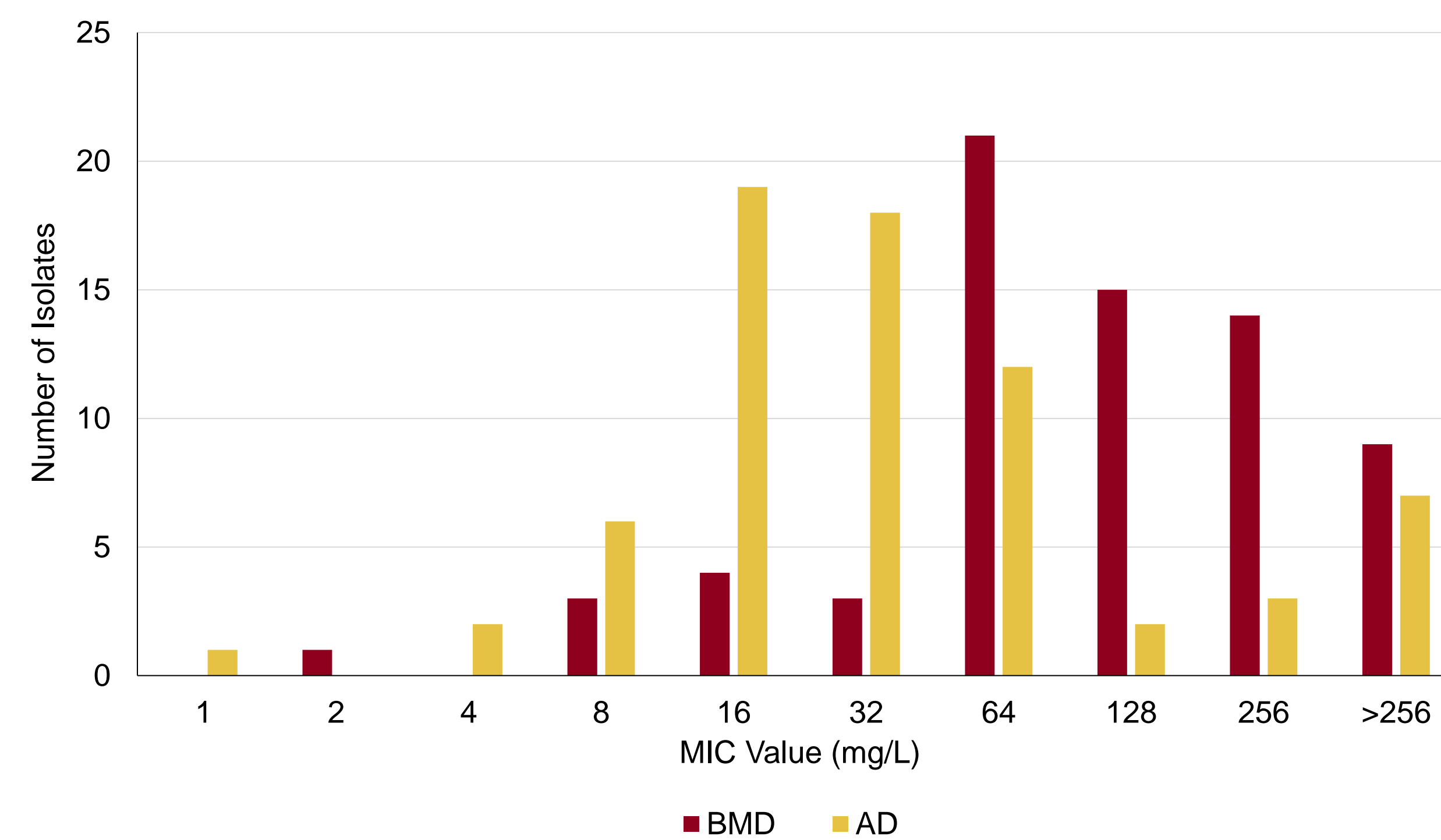
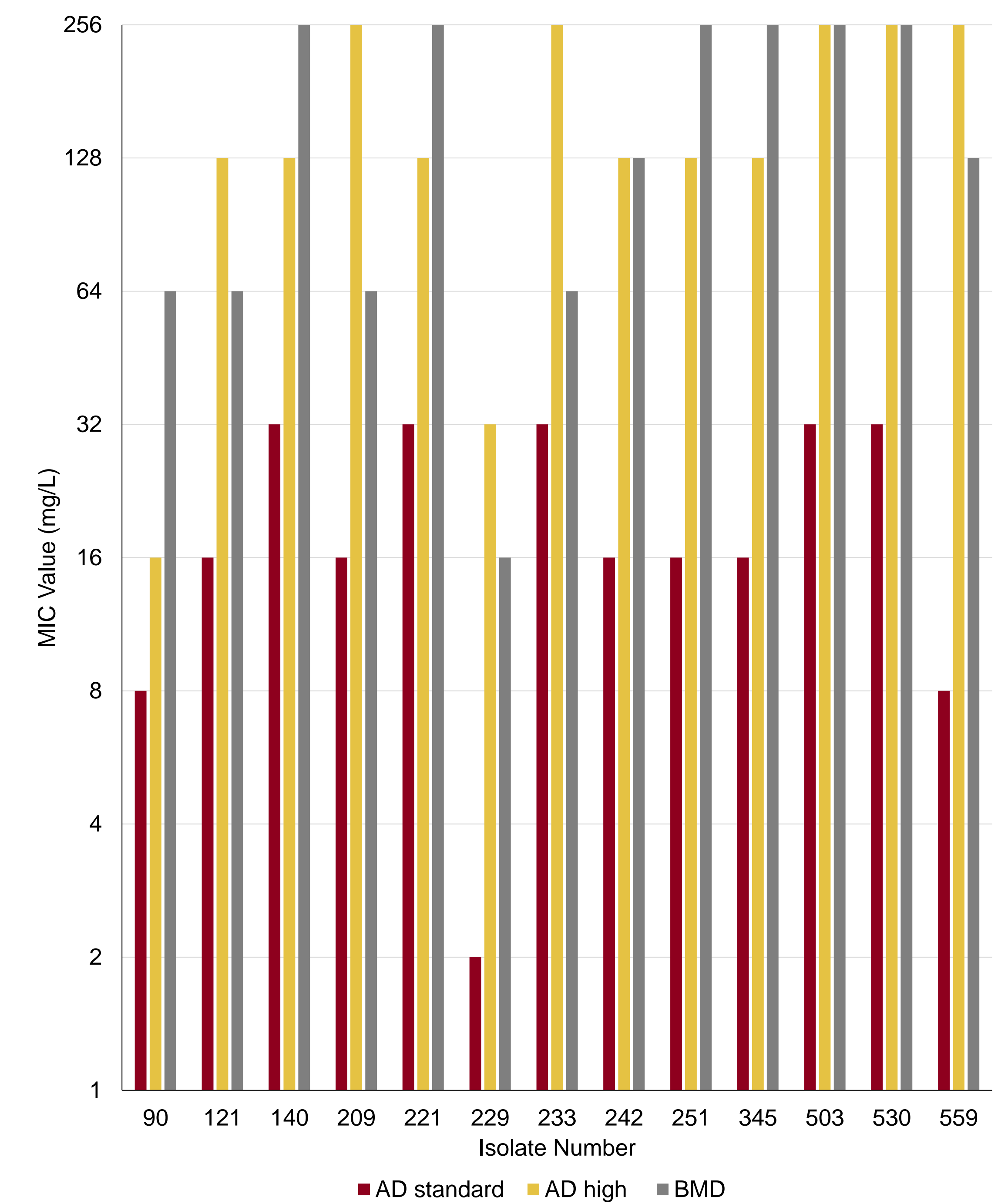


Table 1. Evaluation of MIC correlations

	Essential Agreement (%)	Categorical Agreement (%)	ECV (mg/L)
AD	-	-	128
BMD	44.9	60.8	1024

RESULTS

Fig 3. High-inoculum AD compared to standard AD and BMD



BACKGROUND

- Fosfomycin (FOF) use against urinary tract infections (UTI) has recently undergone a revival due to its activity against MDR-KP; however, there are currently no approved CLSI breakpoints for KP against FOF
- Agar dilution (AD) is the approved reference for FOF susceptibility testing, but it is time- and labor-intensive to employ in a clinical microbiology laboratory
- CLSI specifically recommends against BMD for FOF, but most of these data were presented to CLSI in the 1980's and are not publicly available
- The AD method employs a lower inoculum than BMD, which may be a cause for discrepancies in MIC values between testing methods

OBJECTIVE

- To re-evaluate the FOF agreement of BMD and AD methods against a collection of 70 clinical KP isolates

CONCLUSIONS

- Agar dilution MIC values were typically lower than BMD MIC values. This was evident from S/I/R rates, low essential and categorical agreement, and a 3-dilution difference in ECV seen between methods.
- These values can be brought into agreement by increasing the inoculum of AD to match the inoculum of BMD, with 10 of 14 isolates being brought into essential agreement with BMD
- The variations observed between the susceptibility testing methods leads to more questions about the accuracy of the currently recommended methodology
- We suggest further investigation into the use of BMD for FOF susceptibility testing as well as standardization of the BMD and AD testing methods.
- KP-specific breakpoints should also be investigated since most MIC values tended to cluster near the current *E. coli* breakpoints

