

# Comparison of fosfomycin (FOF) activity and prevalence of subpopulations between *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) during susceptibility testing

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## REVISED ABSTRACT

**Background:** In the United States, interpretive criteria for FOF are established only for EC, yet those criteria are often extrapolated to KP. Recent studies have highlighted both inferior clinical outcomes after FOF treatment and difficulties in interpretation of inner colony subpopulations, the presence of which may affect clinical efficacy. We sought to compare FOF activity against EC and KP and to determine the prevalence of inner colony subpopulations following disk diffusion (DD) testing of the two species.

**Methods:** A convenience collection of 73 KP and 42 EC isolates from 3 U.S. institutions were included. Minimal inhibitory concentration (MIC) testing was performed in duplicate on separate days using agar dilution (AD) and DD as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines, with application of EC susceptibility ( $\leq 64$ mg/L) breakpoints. The frequency and counts of inner colonies observed during DD testing was calculated, and colonies were subcultured for use in future studies.

**Results:** MIC<sub>50/90</sub> values were 1/16 mg/L and 32/256 mg/L for EC and KP respectively. All EC isolates were considered susceptible and therefore categorical agreement was 100%. The majority of KP isolates were considered susceptible (83.6% with AD and 87.7% with DD) and categorical agreement between the methods was 84.9%. Inner colonies were observed during DD testing in 88.1% of EC isolates and 80.8% of KP isolates during at least one replicate, with 47.6% of EC isolates and 39.7% of KP isolates showing inner colony growth during both DD test replicates. More than 10 inner colonies were observed in 50% of EC isolates compared to 12.3% of KP isolates.

**Conclusions:** KP isolates demonstrated considerably higher FOF MIC values compared to EC, as evidenced by MIC<sub>50/90</sub> values 4-5 dilutions higher than those for EC. The categorical agreement rate was higher among EC than KP, highlighting concerns regarding the practice of extrapolating FOF susceptibility breakpoints for EC to KP. The high frequency of inner colonies observed in DD for both species necessitates further studies to determine best practices for interpreting their relevance, fitness, and resistance in order to identify potential impacts to clinical efficacy of FOF.

## BACKGROUND

- FOF is used as a first-line treatment for uncomplicated urinary tract infections (UTI) in women.
- Lack of CLSI interpretive criteria for FOF against KP indicates the need for further investigation into its use.
- Prior studies have shown a high prevalence of inner colony subpopulations that may affect clinical outcomes.

## OBJECTIVE

- To compare the *in vitro* FOF activity against nationally collected EC and KP isolates.
- To determine prevalence of inner colony subpopulations found during DD testing of EC and KP.

## METHODS

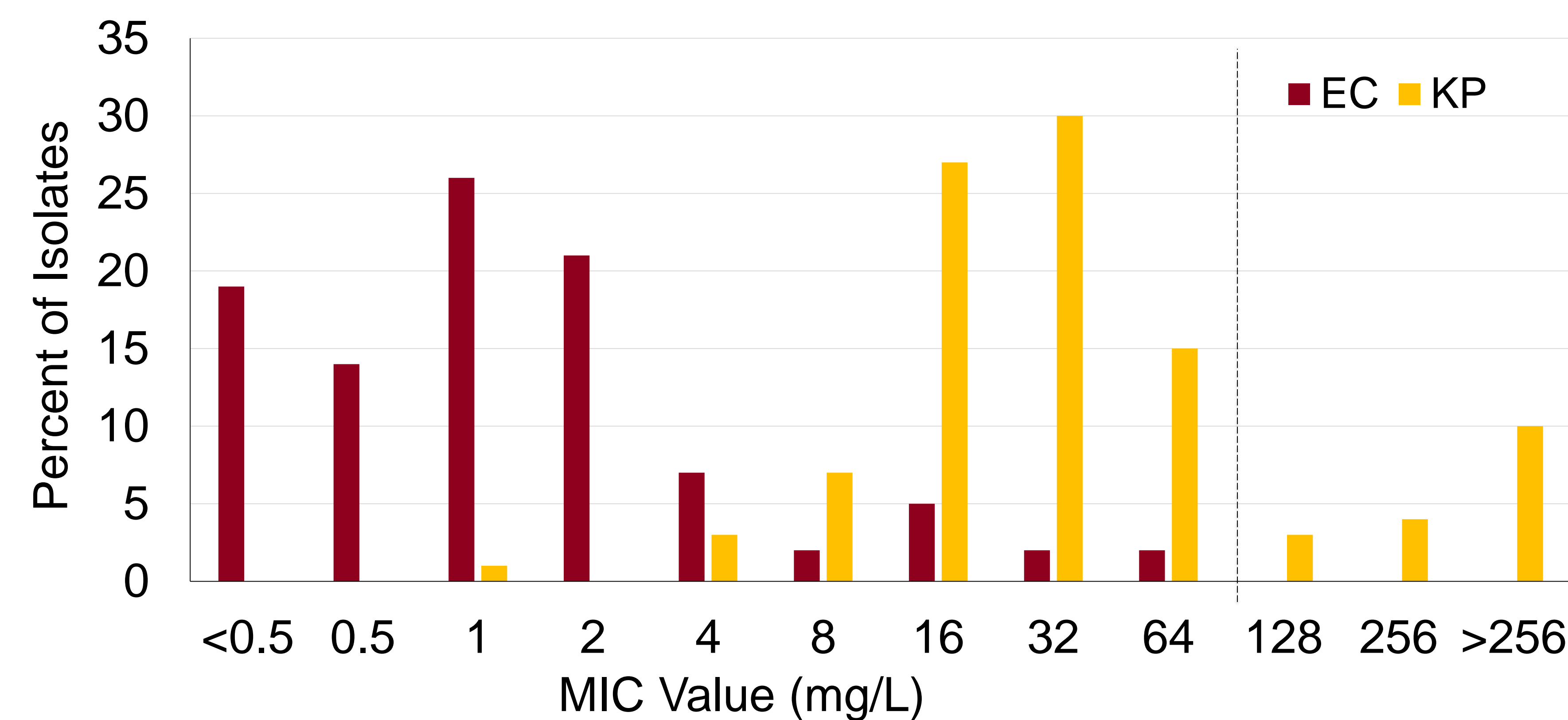
- Convenience sample of 73 KP isolates and 42 EC isolates from Boston, MA (n=35 KP and n=39 EC), Philadelphia, PA (n=31 KP and n=3 EC), and Houston, TX (n=7 KP) were tested
- MICs were determined for each isolate in duplicate on separate days using AD and DD methods as recommended per CLSI
- Susceptibility was determined using CLSI interpretive criteria for FOF against EC as per below:
  - AD (S  $\leq 64$  mg/L, I = 128 mg/L, R  $\geq 256$  mg/L)
  - DD (S  $\geq 16$  mm, I = 13-15 mm, R  $\leq 12$  mm)
- Agreement between methods was calculated using CLSI M23-A3 guidance
- Inner colony counts within the DD zone of inhibition were tabulated

## RESULTS

Table 1. Susceptibility of isolates by species and method

Organism/Method	S n (%)	I n (%)	R n (%)	MIC <sub>50/90</sub> (mg/L)
<b><i>E. coli</i></b> (n = 42)				
AD method	42 (100%)	0 (0%)	0 (0%)	1/16
DD method	42 (100%)	0 (0%)	0 (0%)	-
<b><i>K. pneumoniae</i></b> (n = 73)				
AD method	61 (83.6%)	2 (2.7%)	10 (13.7%)	32/256
DD method	64 (87.7%)	4 (5.5%)	5 (6.8%)	-

Figure 1. AD MIC values by species

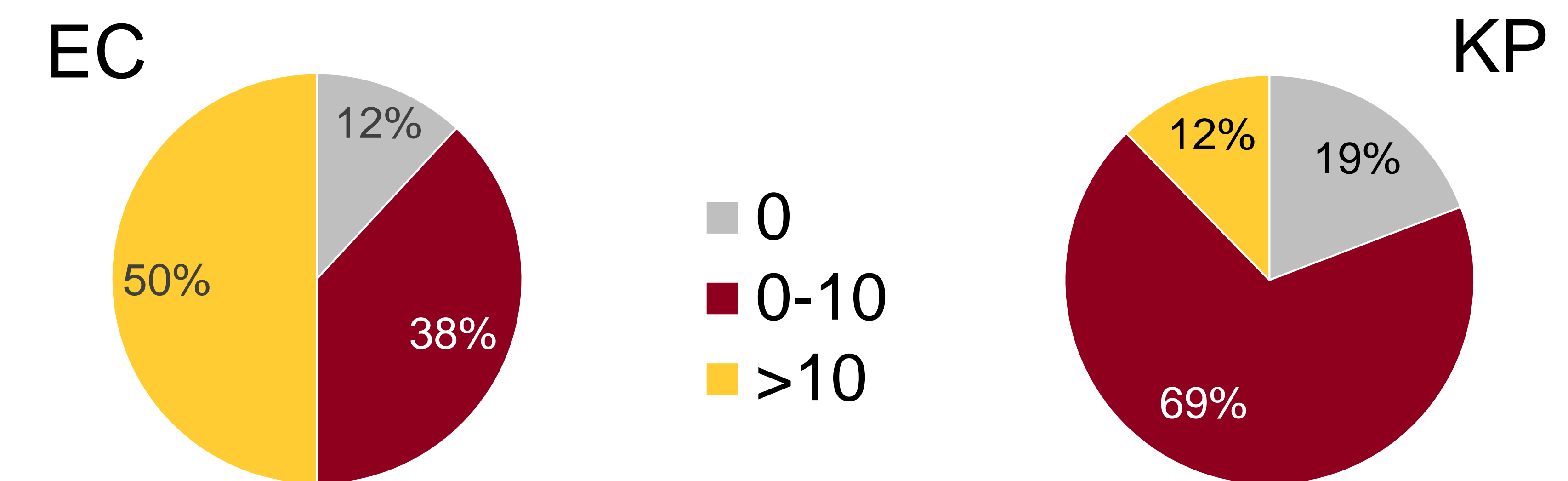


## RESULTS

Table 2. Categorical agreement and error rates

	Categorical Agreement n (%)	Minor Errors n (%)	Major Errors n (%)	Very Major Errors n (%)
EC	42/42 (100%)	-	-	-
KP	62/73 (84.9%)	6/73 (8.2%)	-	5/73 (6.8%)

Figure 2. Inner colony counts during DD testing per species



## CONCLUSIONS

- KP isolates demonstrated considerably higher MIC values for FOF than EC, evidenced by MIC<sub>50/90</sub> values of 1/16 mg/L in EC and 32/256mg/L in KP
- Higher categorical agreement in EC isolates compared to KP highlights concerns regarding the practice of extrapolating interpretive criteria from EC to KP for FOF clinical use
- These concerns indicate a need for further investigation and possible determination of KP-specific FOF breakpoint criteria by CLSI
- High prevalence of inner colony subpopulations in both EC and KP isolates necessitates further study to determine resistance and impact of these colonies through MIC and fitness testing
- Inner colonies found in this study were subcultured and stored for use in future studies
- Current studies in the laboratory are being done to compare MICs between parent and daughter strains, created from the inner colonies
- Clinicians and researchers alike should exercise caution when extrapolating FOF interpretive criteria to KP



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