

# Feasibility of Heteroresistance Screening on *Escherichia coli* (EC) to Predict the Presence of Inner Colonies (IC) During Fosfomycin Disk Diffusion (DD) Testing

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## REVISED ABSTRACT

**Background:** Growth of IC within the zone of inhibition during fosfomycin DD testing in EC are found, but CLSI and EUCAST interpretation criteria for IC are contradictory. There is a need to determine appropriate methods of interpretation to prevent resistant infections, thus evaluating the susceptibility of IC EC isolates compared to parents can help determine optimal methods for DD testing. A heteroresistance screening test has potential to identify isolates with IC. We sought to determine the feasibility of heteroresistance screening to predict the presence of IC in EC isolates.

**Materials/Methods:** A convenience collection (n = 48) of EC parent isolates underwent fosfomycin DD testing to identify those with IC. Broth microdilution (BMD) testing was performed on all parent and IC (n = 34) to determine minimal inhibitory concentrations (MICs) to establish inclusion criteria for heteroresistance screening. A disk elution test for heteroresistance screening was performed in duplicate on separate days. In tubes of Mueller-Hinton broth, 6 commercial fosfomycin disks (each 200 µg fosfomycin and 50 µg glucose-6-phosphate) were eluted for 90 minutes. One hundred microliters of each bacterial isolate from an overnight culture was suspended in tubes. A positive test was a turbid tube after 72 hours of incubation.

**Results:** The parent isolates (n = 48) had an MIC range of 1 to >256 µg/mL with a median of 4 µg/mL. The subset of IC isolates (n = 34) had an MIC range between ≤8 and >1024 with a median of 128 µg/mL. Nineteen IC isolates met the inclusion criteria for the heteroresistance screening; 5 isolates did not produce IC (MIC range of 1-32 µg/mL) while 14 isolates produced IC (MIC range of 1-16 µg/mL). Of these 19 isolates, 6 (32%) were heteroresistant using the disk elution test. Of those with IC, 4 (29%) were heteroresistant while 2 (40%) isolates without IC were heteroresistant.

**Conclusion:** A heteroresistance screening test did not provide consistent data to predict the presence of IC among this EC collection. A larger isolate set and further studies are needed to understand the feasibility of a heteroresistance screening test and increased resistance in IC resulting from fosfomycin DD testing among EC.

## BACKGROUND

- Contradictions of interpretation for growth of IC within the zone of inhibition during a fosfomycin disk diffusion test between CLSI and EUCAST occur
- Heteroresistance, the presence of subpopulations with increased resistance, has the potential to predict *E.coli* fosfomycin resistance but further work is needed to determine its correlation to resistant IC

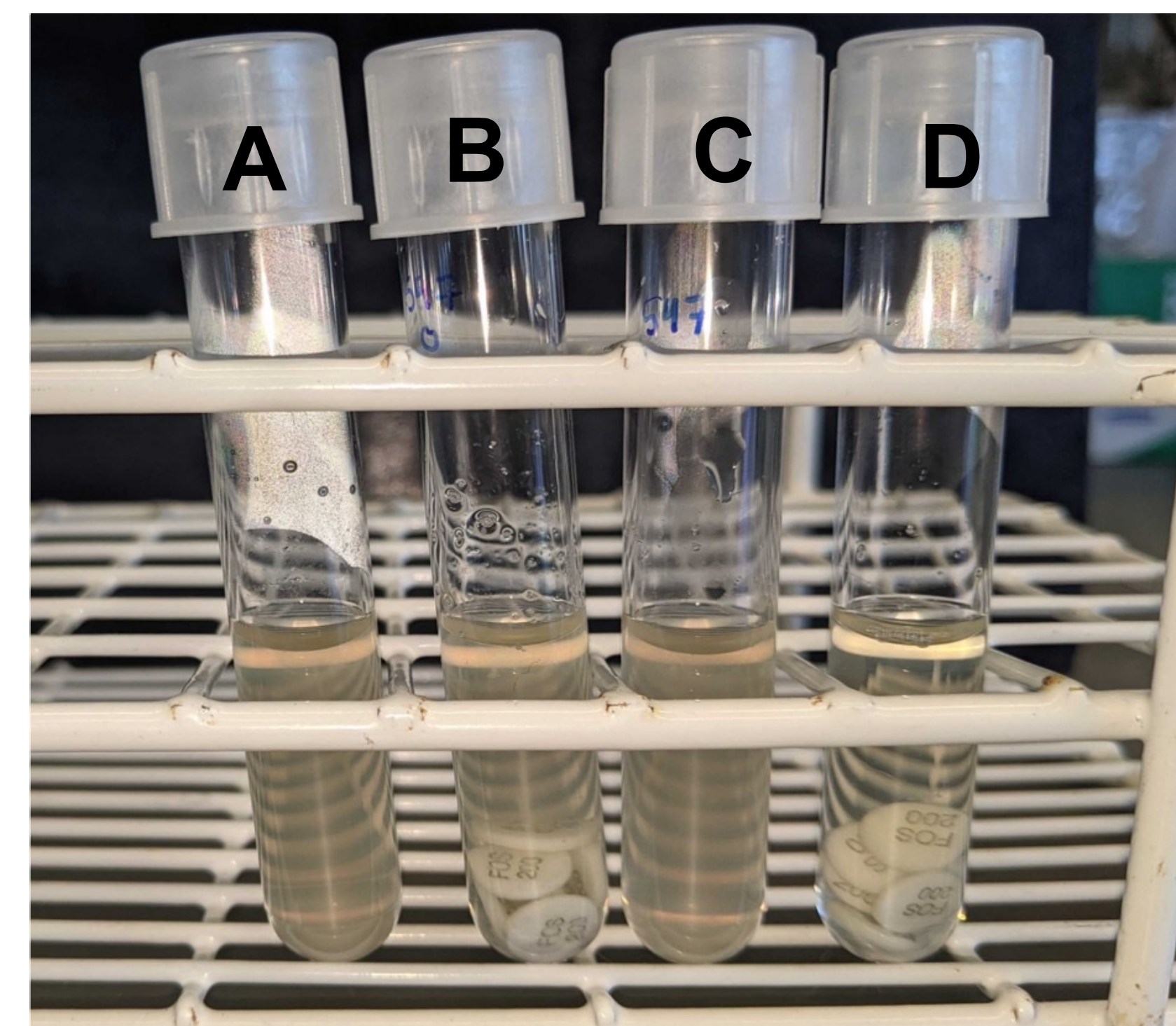
## OBJECTIVE

- Determine the feasibility of heteroresistance screening on clinical *E. coli* isolates to predict the presence of increased resistant IC.

## METHODS

- Disk diffusion test was performed with a commercial fosfomycin disk prior to the disk elution test to identify isolates with IC in the zone of inhibition
- Minimal inhibitory concentration (MIC) values obtained through previous broth microdilution (BMD) experiments were used to establish inclusion criteria
  - Parent MIC of ≤64 µg/mL (susceptible) or 128 µg/mL (intermediate)
  - IC MIC of ≥128 µg/mL
  - A difference of at least 3 dilutions between parent and IC MIC
- Heteroresistance screening was performed on separate days in duplicate using 6 commercial fosfomycin disks (200 µg fosfomycin and 50 µg glucose-6-phosphate) and 1.9 mL of MHB (Figure 1)
- After 90 minutes of elution, 100 µL of an overnight culture was suspended in tubes
- A positive test was a turbid tube after 72 hours of incubation

Figure 1. Heteroresistance screening disk elution test



A. The positive-growth control for tube B. B. A turbid tube after 72 hours of incubation was considered a positive test result indicating heteroresistance. C. The positive-growth control for tube D. D. A clear tube after 72 hours incubation was negative for heteroresistance.

## RESULTS

Table 1. Heteroresistance screening of *E. coli* parent isolates without IC

	Susceptible (S) n (%)	Intermediate (I) n (%)	Resistant (R) n (%)	MIC Range (µg/mL)
Parent (n = 80)	77 (96.2%)	2 (2.5%)	1 (1.3%)	1 to >256
IC (n = 50)	13 (26%)	15 (30%)	22 (44%)	≤8 and >1024

## RESULTS

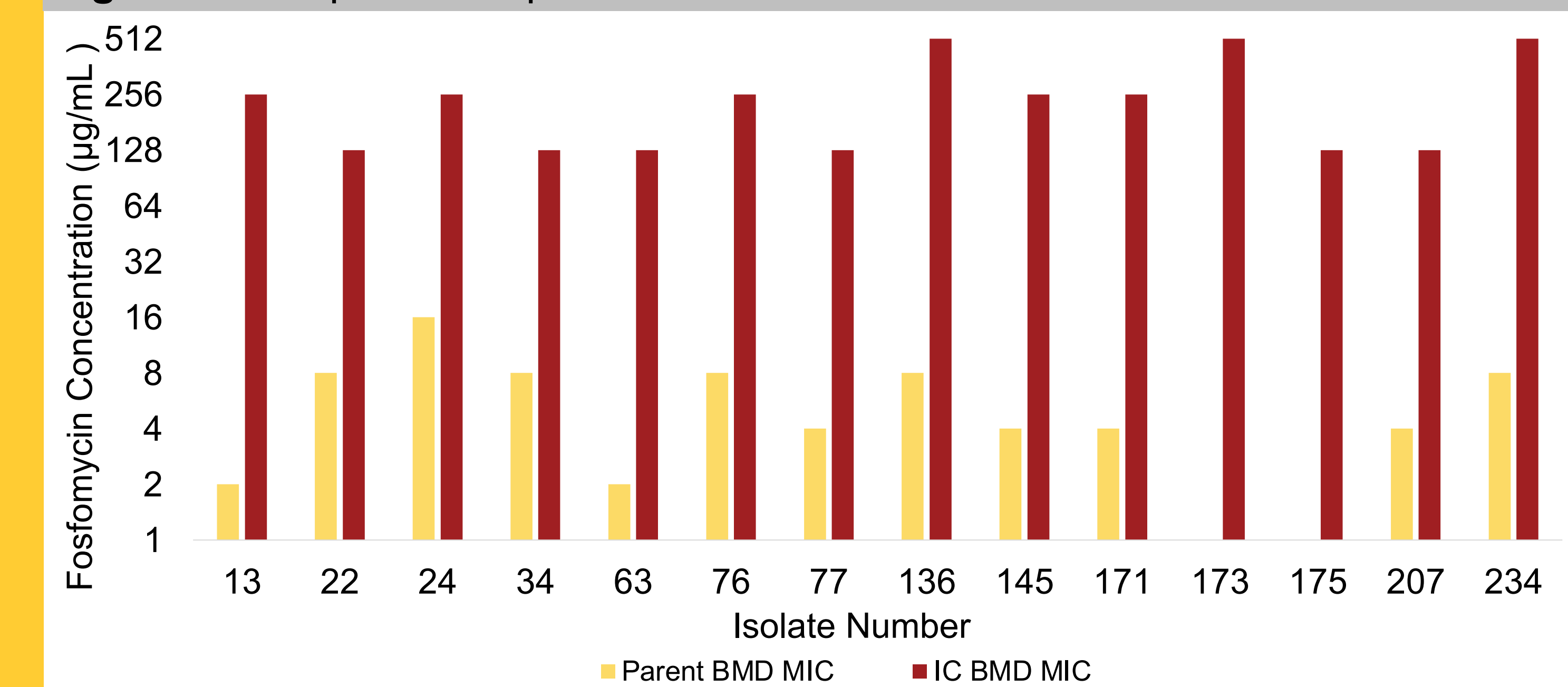
Table 2. Heteroresistance screening of *E. coli* parent isolates without IC

Isolate	Fosfomycin MIC of Parent Isolate (µg/mL)	Heteroresistance Result
4	1	+
238	8	+
292	4	+
526	32	+
607	32	+

Table 3. Heteroresistance screening of *E. coli* parent isolates with IC

Isolate	Number of IC in DD Test	Fosfomycin MIC of IC (µg/mL)	Heteroresistance Result
13	4	256	-
22	14	128	-
24	20	256	+
34	3	128	+
63	2	128	-
76	19	256	-
77	40	128	+
136	15	512	-
145	4	256	-
171	21	256	-
173	5	512	-
175	16	128	+
207	6	128	-
234	13	512	-

Figure 2. Comparison of parent and IC isolates screened for heteroresistance



## CONCLUSIONS

- All parent isolates without IC screened positive for heteroresistance
- The presence of IC did not predict a positive heteroresistance screening, thus no correlation between a positive screening result and the formation of IC in this isolate collection
- Further research is needed to confirm the results of the heteroresistance screening and further understand any potential genetic causes of these inner colonies



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