

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

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UPDATED ABSTRACT

Background: The presence of IC within the zone of inhibition during disk diffusion (DD) testing in *E. coli* have been noted, but CLSI and EUCAST interpretation criteria for IC are contradictory. Because of the presence of resistant IC, there is a need to determine the most appropriate method of interpretation to prevent a potential increase of resistant infections, thus screening test and increased resistance in IC in *E. coli*. Evaluating the susceptibility of IC *E. coli* isolates in comparison to the corresponding parent isolates will help determine a more optimal method for DD testing. A heteroresistance (subpopulations with increased resistance) 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 clinical *E. coli* isolates.

Materials/Methods: A convenience collection (n=46) of *E. coli* parent isolates underwent DD testing to isolate those with IC. Broth microdilution (BMD) testing was performed on all isolates and their IC, when present (n=40), to determine minimal inhibitory concentrations (MICs) and to establish inclusion criteria for the heteroresistance screening. All parent isolates with MICs of ≤ 64 $\mu\text{g/mL}$ and IC isolates with MICs of ≥ 128 $\mu\text{g/mL}$ were chosen for heteroresistance screening. A difference of at least 3 dilutions between parent and IC isolate MIC established the inclusion criteria. 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 μL of each bacterial isolate from an overnight culture was suspended in the tubes. A positive test was a turbid tube after 72 hours of incubation.

Results: The parent isolates (n=46) had an MIC range between 1 and > 256 $\mu\text{g/mL}$ with a median of 4 $\mu\text{g/mL}$. The subset of IC isolates (n=34/40) tested to date had an MIC range between ≤ 8 and > 1024 with a median of 128 $\mu\text{g/mL}$. Nineteen IC isolates met the inclusion criteria for the heteroresistance screening. Of the 19 isolates with heteroresistance screening completed to date, 14 isolates produced IC (MIC range of 1-16 $\mu\text{g/mL}$) and 5 isolates did not produce IC (MIC range of 1-32 $\mu\text{g/mL}$). Of these 19 isolates, 9 (47.3%) were heteroresistant using the disk elution test. Of those with IC, 4 (21.1%) were heteroresistant while 5 (100%) isolates without IC were heteroresistant.

Conclusion: A heteroresistance screening test did not provide consistent data to predict the presence of IC among this *E. coli* collection. A larger isolate set and further studies are needed to understand the feasibility of a heteroresistance

INTRODUCTION

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

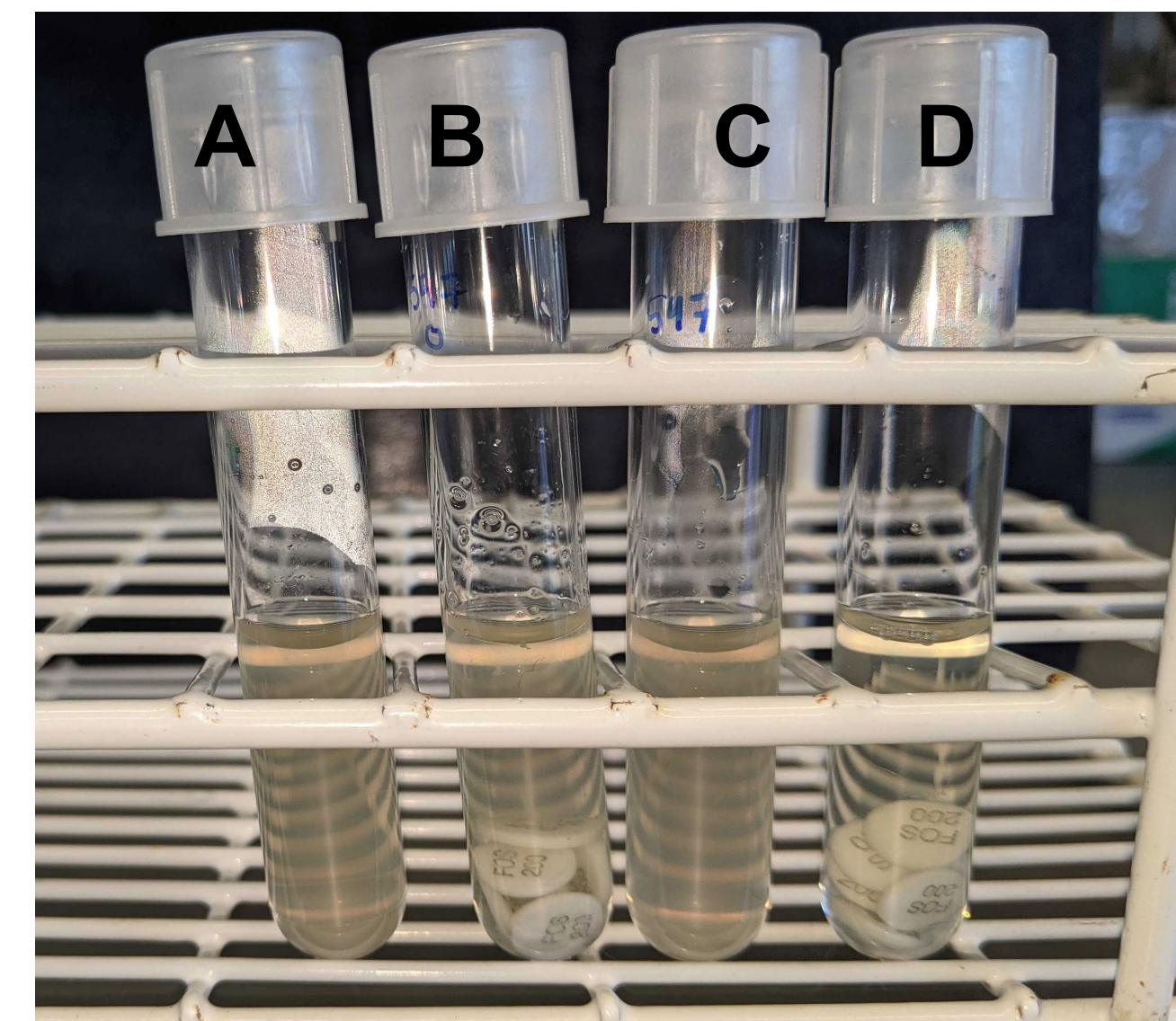
OBJECTIVE

- 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 concentrations (MIC) obtained through previous broth microdilution (BMD) experiments were used to establish inclusion criteria
 - Parent MIC of ≤ 64 $\mu\text{g/mL}$ (susceptible) or 128 $\mu\text{g/mL}$ (intermediate)
 - IC MIC of ≥ 128 $\mu\text{g/mL}$
 - 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 isolate B. B. A turbid tube after 72 hours of incubation was considered a positive test result indicating heteroresistance. C. The positive-growth control for isolate 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
Parent (n=46)	44 (95.7%)	2 (4.3%)	0 (0%)	1 to > 256
IC (n=34)	13 (38.2%)	10 (29.4%)	11 (32.4%)	≤ 8 and > 1024

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

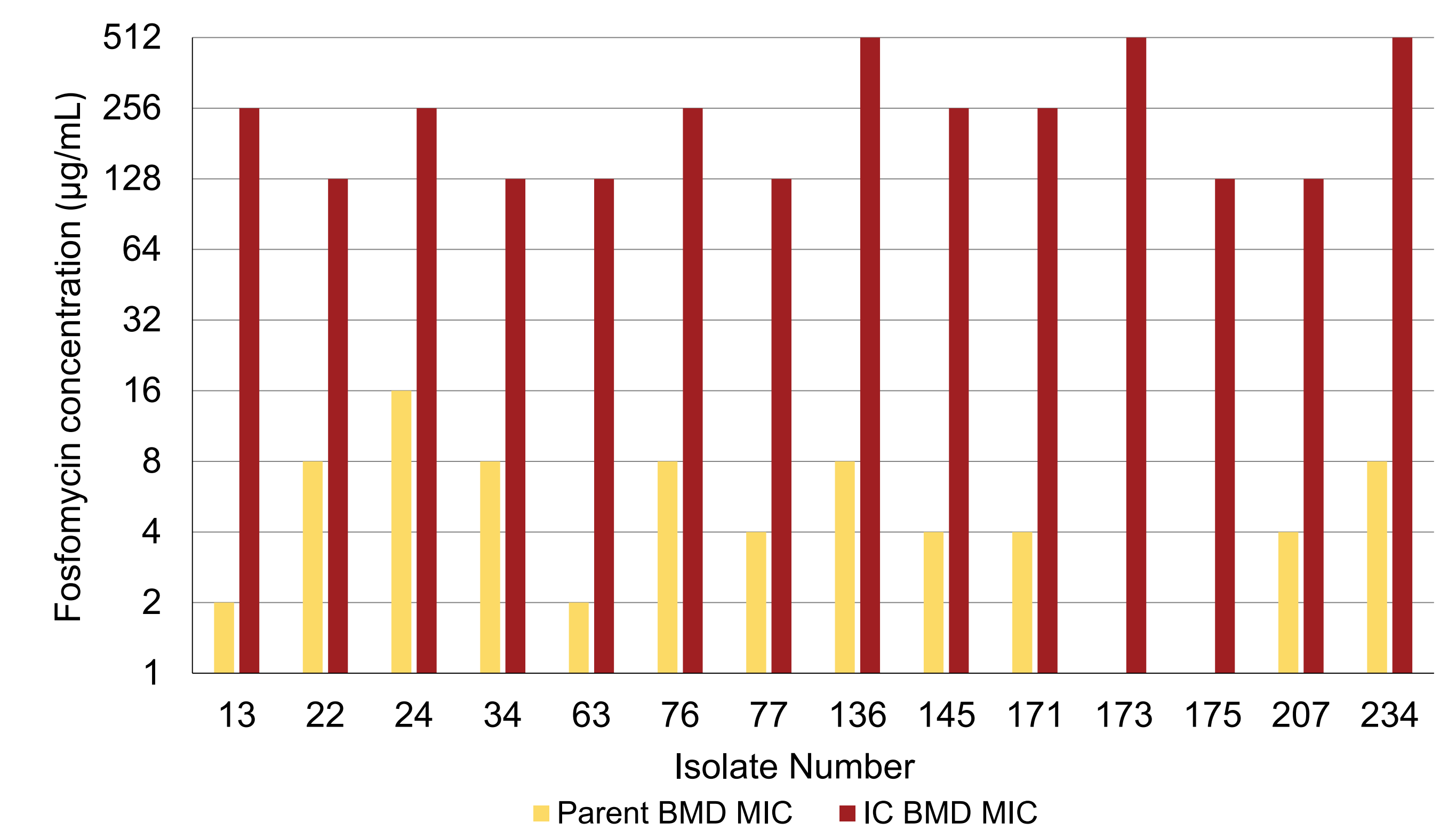
Isolate	Fosfomycin MIC of parent isolate ($\mu\text{g/mL}$)	Heteroresistance result
4	1	+
238	8	+
292	4	+
526	32	+
607	32	+

RESULTS

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

Isolate	Number of IC in DD testing	Fosfomycin MIC of IC ($\mu\text{g/mL}$)	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.



CONCLUSION

- All parent isolates without IC screened positive for heteroresistance.
- A heteroresistance screening did not indicate a positive screening for all resistant IC isolates thus there is no correlation between a positive heteroresistance screening and resistant IC formation in this collection.
- We suggest further studies are needed to understand the presence of increase resistant IC in *E. coli*.

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