Comparison of inner colony among *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) during fosfomycin disk diffusion (DD) testing

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REVISED ABSTRACT

Background: Recent studies have indicated the presence of IC arising within the zone of inhibition during fosfomycin DD testing. CLSI and EUCAST have contradicting recommendations for interpreting these IC; CLSI recommends considering the presence of IC when interpreting DD results while EUCAST recommends ignoring them. Although DD testing is approved only for use with EC per both organizations, it is often used for testing of non-EC organisms despite neither organization publishing non-EC Enterobacterales breakpoints (BP). We sought to compare the rates of IC production among EC and KP isolates in order to assess the applicability of fosfomycin DD for KP isolates.

Methods: A convenience sample of 157 (80 KP and 77 EC) clinical isolates with varied phenotypic profiles from 3 United States locations were included. Of these, 26% (n = 21) of KP and 27% (n = 21) of EC displayed ESBL phenotypes and 19% (n = 15) of the KP were KPC producing. Fosfomycin susceptibility testing via DD was conducted in duplicate on separate days following interpretations recommended by each organization. Isolates were considered IC-producing if at least 5 IC arose during ≥1 replicate of DD testing.

Results: Overall, 67% (n = 105) of isolates produced IC including 82% (n = 63) of EC and 52.5% (n = 42) of KP (Table 1). Of the EC isolates that produced IC, 94% (n = 59) were susceptible per CLSI and 92% (n = 60) were susceptible per both EUCAST oral and IV BP. In contrast, only 41% (n = 17) of KP isolates with IC were susceptible per CLSI and 2% (n = 1) were susceptible per both EUCAST BP. The presence of IC did not result in significant differences of zone diameters (1-14mm, EC; 2-13mm, KP) when comparing organization procedures.

Conclusions: The BP and IC interpretations from EUCAST and CLSI categorized most EC isolates, including those that produced IC, as susceptible. EUCAST's larger zone measurements and ignoring IC produced near identical results to CLSI. EUCAST's lower BP categorized very few KP as susceptible even when ignoring the presence of IC. CLSI BP captures a more diverse categorization of susceptibility of KP isolates consistent with the diversity of zone measurements. These findings warrant further investigation into the clinical relevance of IC and applicability of fosfomycin DD BP in KP.

BACKGROUND

- Disk diffusion is an approved testing method for fosfomycin susceptibility in EC, however frequent discrete IC mutants have been observed
- CLSI and EUCAST have contradicting recommendations on how IC should be interpreted:
  - EUCAST recommends ignoring all inner colonies
  - CLSI recommends measuring the colony free zone when these inner colonies persist
- DD is often used for non-EC Enterobacterales despite neither organization publishing applicable breakpoints

OBJECTIVE

- Compare the rates of IC production between EC and KP in our clinical isolate collection
- Assess the applicability of DD fosfomycin breakpoints for non-EC Enterobacterales, specifically KP

METHODS

- Convenience sample of clinical *E. coli* (n = 77) and *K. pneumoniae* isolates (n = 80) collected from three locations in the United States
  - 21 *E. coli* and 21 *K. pneumoniae* displayed ESBL phenotypes
  - 15 *K. pneumoniae* were KPC-producing
- Fosfomycin susceptibility testing was conducted in duplicate on separate days and measured following both CLSI and EUCAST interpretation guidelines
- Isolates were considered IC-producing if they had at least 5 discrete inner colonies in ≥1 replicate
- *E. coli* breakpoints from both organizations were utilized to categorize both *E. coli* and *K. pneumoniae* (extrapolating them for use in *K. pneumoniae)*

Table 1. CLSI and EUCAST breakpoints applied to *E. coli* and extrapolated for *K. pneumoniae*

	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
CLSI (*E. coli* only)	≥16	13-15	≤12
EUCAST oral (*E. coli* only)	≥24	--	<24
EUCAST IV (*E. coli* only)	≥21	--	<21

RESULTS

- 82% (63/77) of *E. coli* isolates produced ≥5 inner colonies in at least one replicate
  - 94-95% of these 63 IC-producing isolates were susceptible
- 52.5% (42/80) of *K. pneumoniae* isolates produced ≥5 inner colonies in at least one replicate
  - 2-41% of these 42 IC-producing isolates were susceptible

Table 2. Production of IC by susceptibility categorization based on three sets of fosfomycin breakpoints

	Susceptible		Intermediate		Resistant	
	n (%)	IC n/N (%)	n (%)	IC n/N (%)	n (%)	IC n/N (%)
CLSI oral breakpoints						
***E. coli* (n = 77)**	73 (94)	59/63 (94)	3 (4)	3/63 (5)	1 (1)	1/63 (2)
***K. pneumoniae* (n = 80)**	35 (44)	17/42 (41)	20 (25)	12/42 (29)	25 (31)	13/42 (31)
EUCAST oral breakpoints						
***E. coli* (n = 77)**	72 (94)	60/63 (95)	NA	NA	5 (6)	3/63 (5)
***K. pneumoniae* (n = 80)**	2 (3)	1/42 (2)	NA	NA	78 (98)	41/42 (98)
EUCAST IV breakpoints						
***E. coli* (n = 77)**	72 (94)	60/63(95)	NA	NA	5 (6)	3/63 (5)
***K. pneumoniae* (n = 80)**	17 (21)	1/42 (2)	NA	NA	63 (79)	41/42 (98)

RESULTS

Figure 1. Zone diameter measurements based on CLSI interpretations

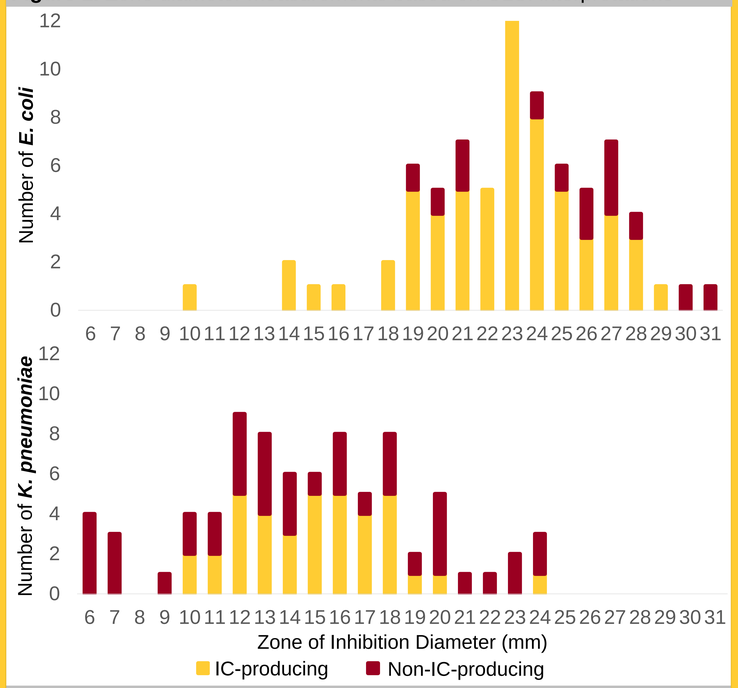
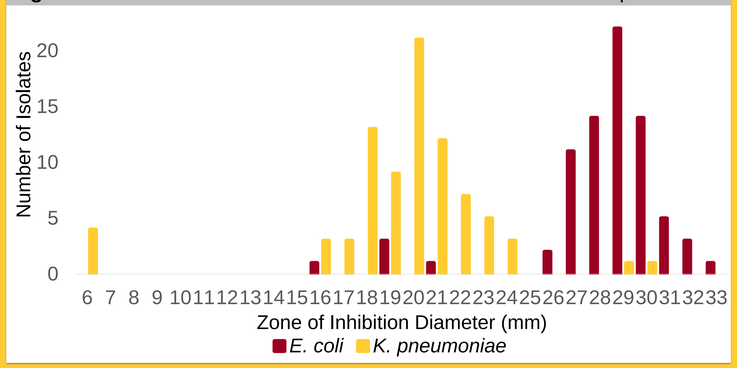


Figure 2. Zone diameter measurements based on EUCAST interpretations



CONCLUSIONS

- Breakpoints and IC interpretations from both CLSI and EUCAST categorize nearly all *E. coli* isolates, including those that produced inner colonies, as susceptible
  - Ignoring inner colonies with a larger zone of inhibition diameter (EUCAST) produced near identical results to a smaller zone diameter when considering inner colonies (CLSI)
- EUCAST categorized very few *K. pneumoniae* isolates as susceptible even when ignoring the presence of inner colonies
  - CLSI breakpoints demonstrated a more diverse categorization of susceptibility more in line with the diversity of zone diameters
- Further investigation is warranted to determine the clinical relevance of IC and the applicability of fosfomycin DD breakpoints in *K. pneumoniae*

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