**Session:** PK/PD to predict emergence of resistance
**Presentation:** O0260
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# Species and baseline resistance are more predictive than fosfomycin MIC for therapeutic success in urinary tract infections

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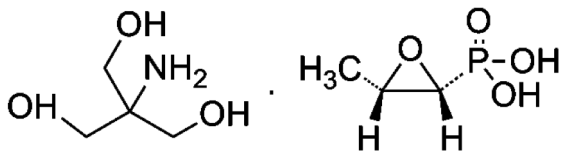
# Research objectives

- Simulate urinary fosfomycin concentrations following a single 3g oral dose using a dynamic bladder infection *in vitro* model
- Compare oral fosfomycin efficacy against ESBL-positive ***E. coli*** and ***K. pneumoniae*** clinical urinary isolates
- Identify baseline isolate characteristics that can predict treatment response

# Fosfomycin tromethamine



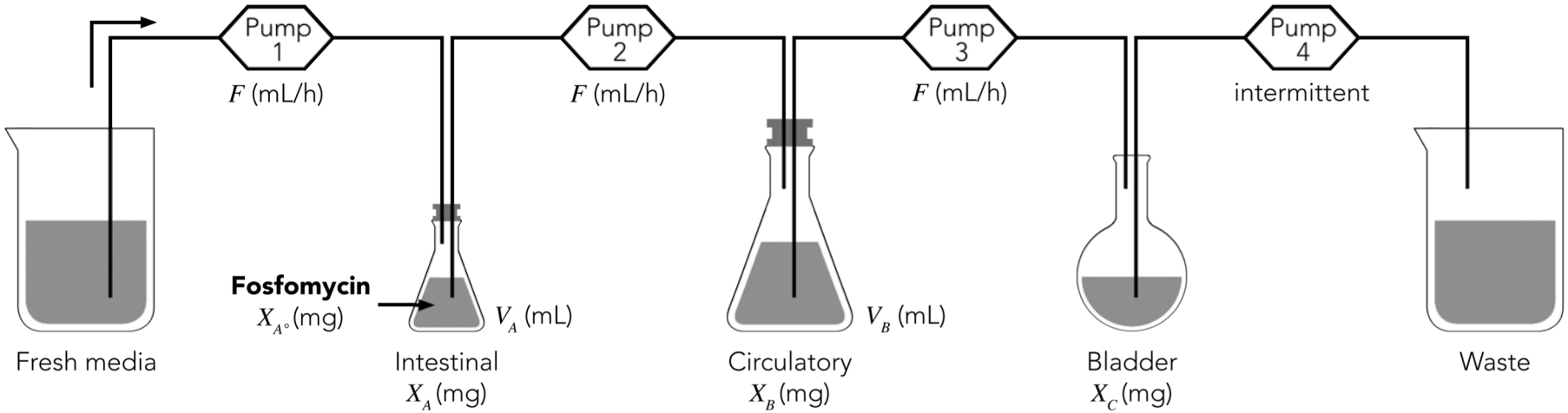

- Indicated for uncomplicated urinary tract infections
  - **Single oral dose** therapy; well tolerated (few side effects)
  - Good activity against MDR-uropathogens (no cross-resistance with other antibiotics)

- Variable clinical response rates reported
  - Older published data reported clinical cure rates: **87 – 93%**
  - Recent clinical trial (Huttner *et al.* JAMA 2018) reported clinical resolution in only **58%**

- Routine susceptibility testing is problematic
  - Gold standard is **agar dilution**; but not widely available
  - Other methods have **poor detection** of resistant isolates and high error rates[1]
  - Different breakpoints: EUCAST (Enterobacterales UTI): S ≤ 32 mg/L, R > 32 mg/L
    CLSI (E. coli UTI only): S ≤ 64 mg/L, R ≥ 256 mg/L

[1] van den Bijllaardt *et al.* JAC 2018

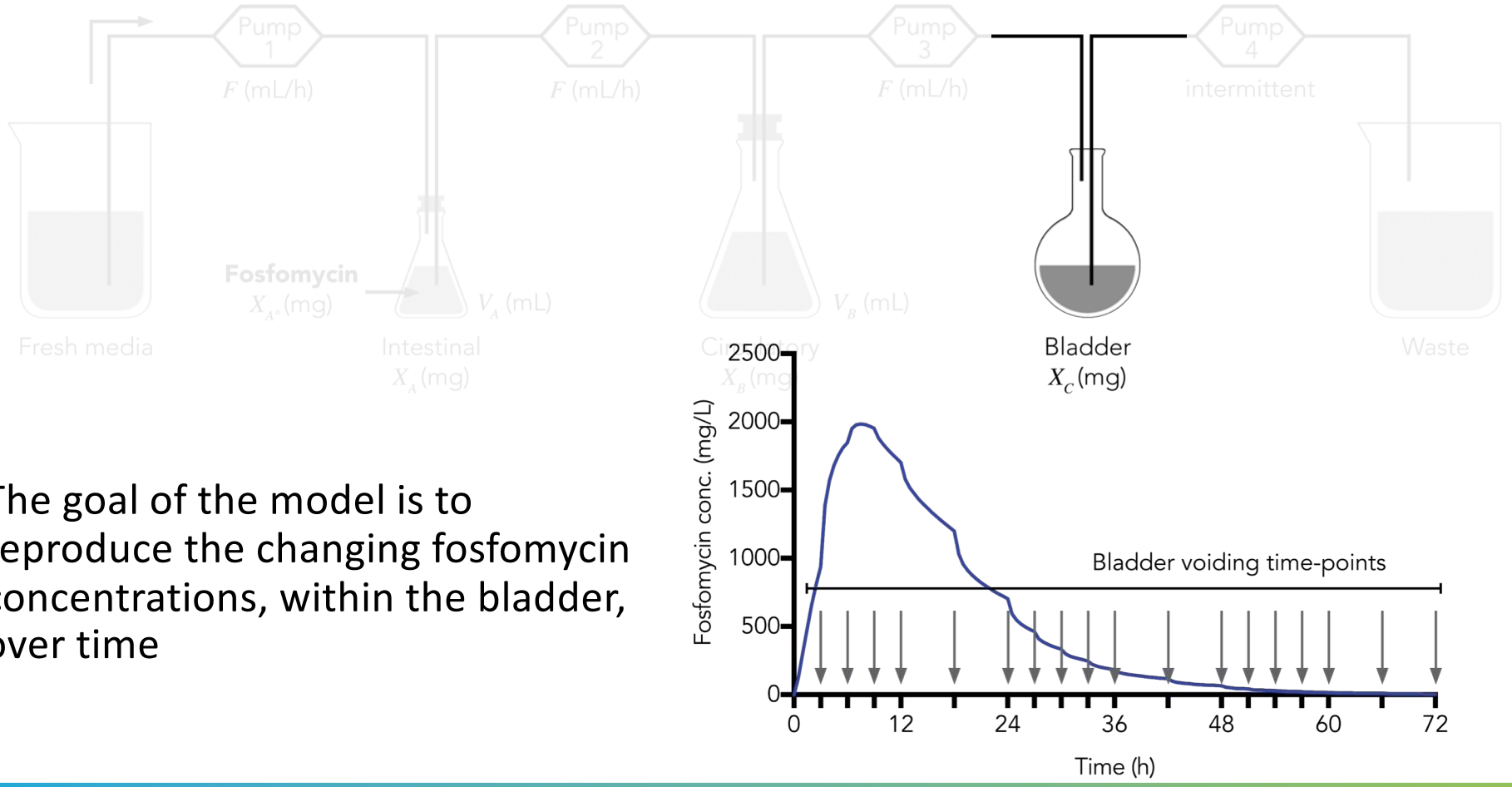
# Bladder infection *in vitro* model




- Simulates dynamic changes in **urinary fosfomycin concentrations** following oral absorption, systemic circulation, and renal excretion
- Average fosfomycin exposures were targeted[2]
  - $C_{max}$ 1982 mg/L (± 1257.4 mg/L); $T_{max}$ 7.5 h (± 4.2 h); remain >128 mg/L for 40 h
  - Despite the large variability seen between subjects

[2] Wijma RA *et al.* Clin Microbiol Infect. 2018

# Bladder infection *in vitro* model




- The goal of the model is to reproduce the changing fosfomycin concentrations, within the bladder, over time

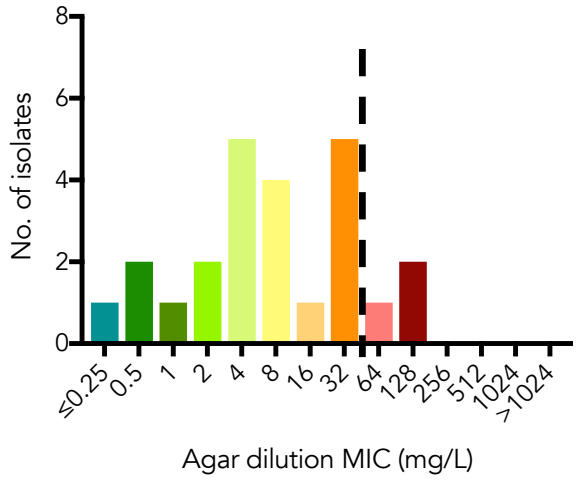
# Bladder infection *in vitro* model

- *In vitro* model simulates normal human urodynamics, but on a reduced 1:16 scale
  - Continuous bladder filling; 6-voids per day; normal post-void residual volume
  - Multiple bladder compartments (*n* = 16) run in parallel
    - Run with Mueller-Hinton broth (MHB) with 25 mg/L G-6-P

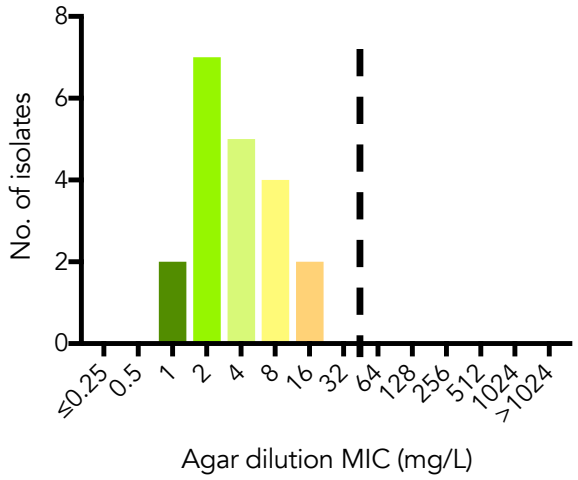
# Test isolates

- 44 clinical isolates
  - 24 *E. coli*; 20 *K. pneumoniae*
  - 42 (95%) ESBL-producing pathogens
  - 38 (86%) originally from a urinary source
- Isolates were selected to represent a range of fosfomycin MIC values
  - 41 (93%) with an MIC ≤ 32 mg/L

***E. coli***

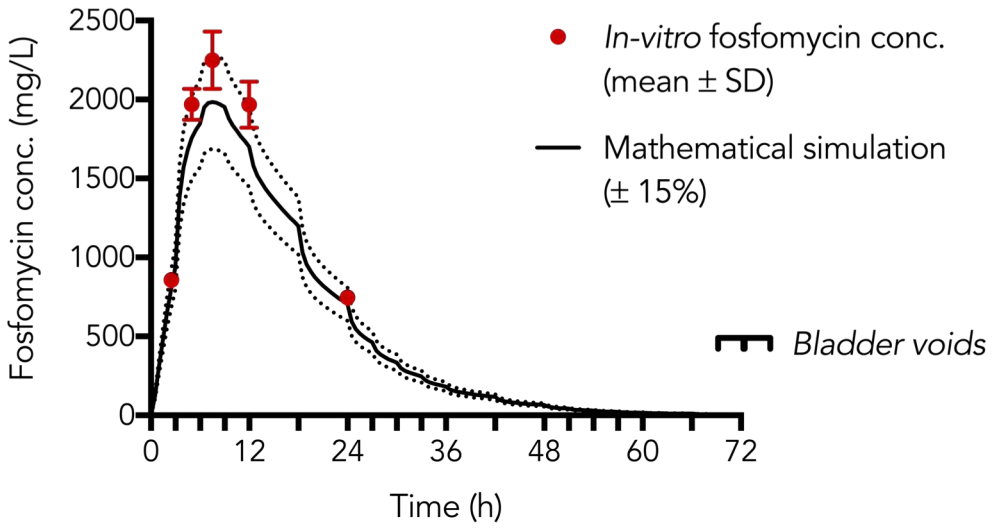


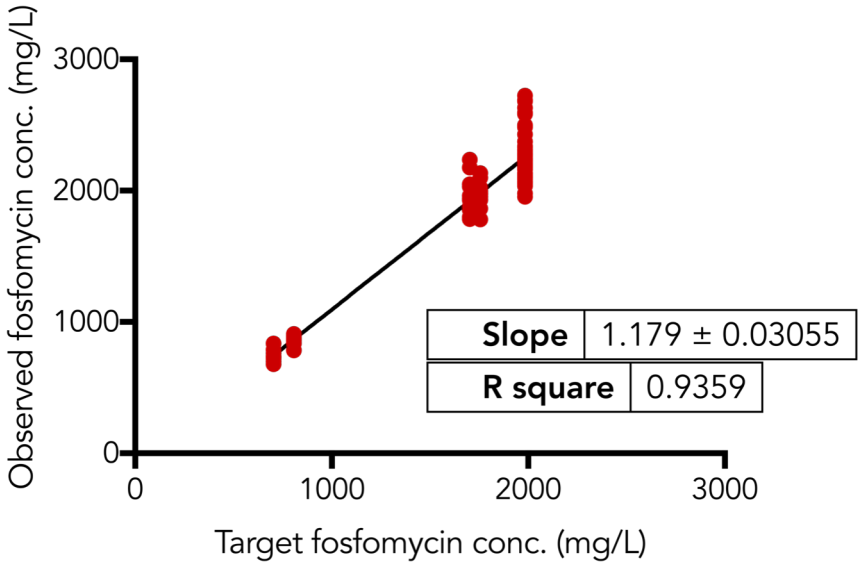

***K. pneumoniae***



# Fosfomycin exposure

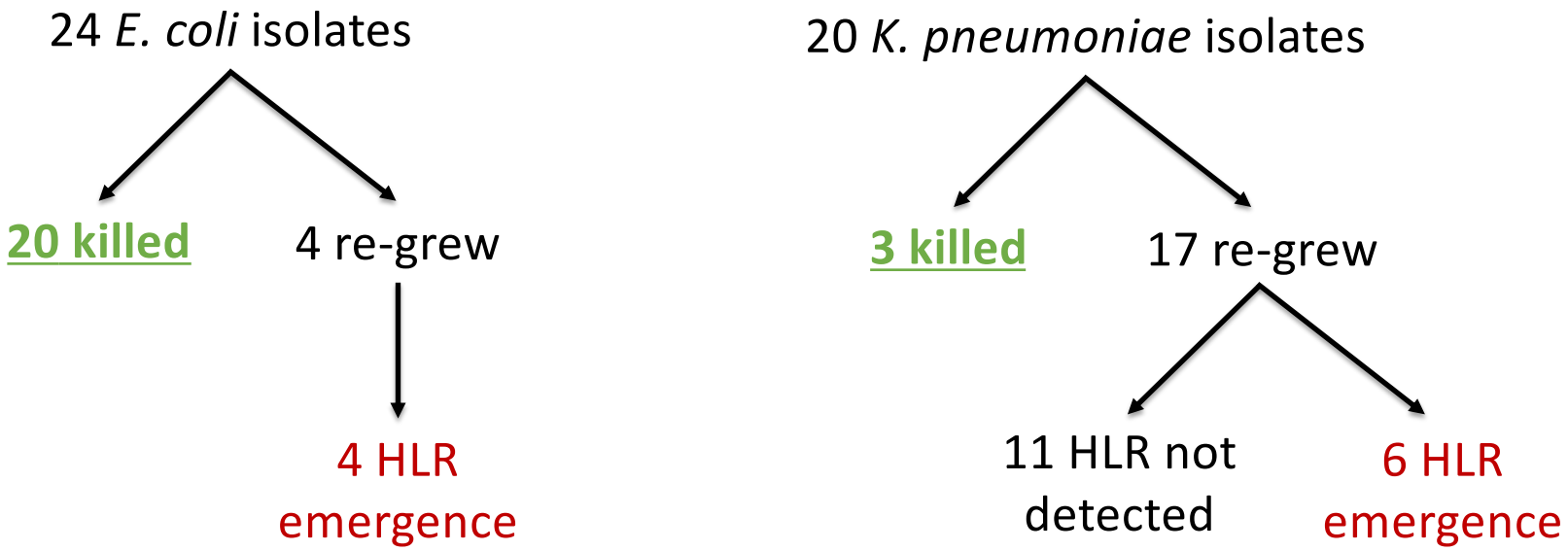
- *In vitro* samples were collected for fosfomycin quantification, measured by LC-MS/MS
- Observed *in vitro* concentrations matched the simulation, with minimal variability between compartments



# Isolate outcome post exposure

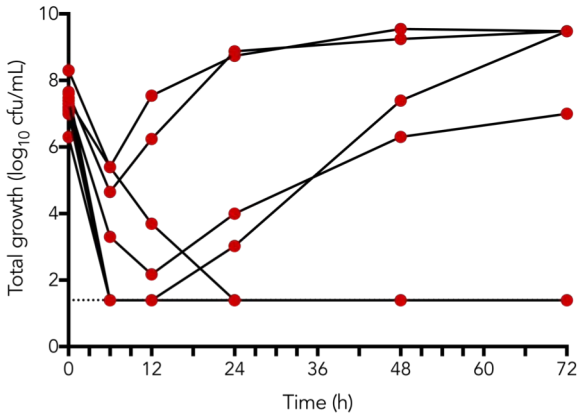
- The final isolate growth was assessed 72 h after fosfomycin administration
  - **Total growth**: quantitative growth on drug-free MHA
  - High-level resistance (**HLR**): quantitative growth on MHA + 512 mg/L fosfomycin




Note: 16-isolates (8 *E. coli*, 8 *K. pneumoniae*) were run in duplicate with concordant PD outcomes

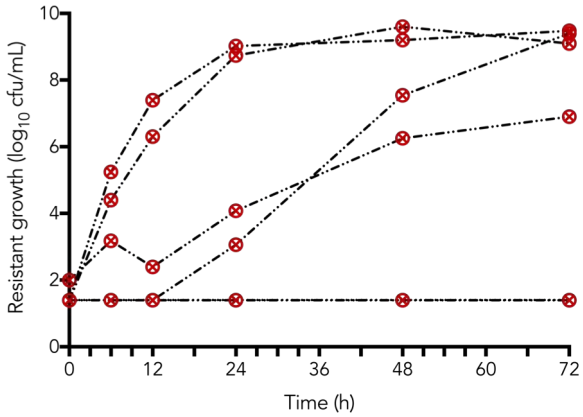
## *E. coli*

Total growth




4/24 isolates re-grew from 24 h onwards

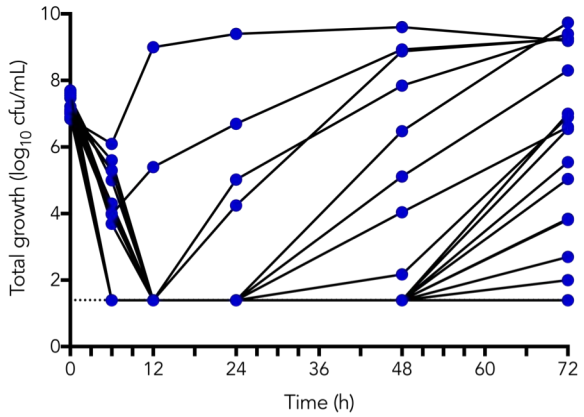
Resistant growth




All that re-grew had population replacement with HLR

## *K. pneumoniae*

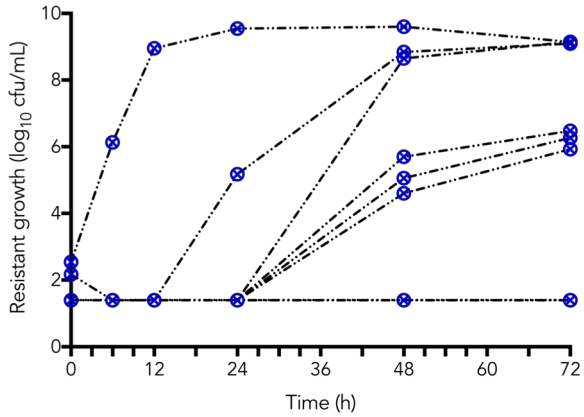
Total growth




17/20 isolates re-grew

*Low-level re-growth; detected at 72 h in several isolates*

Resistant growth




6/17 re-grew with emergence of HLR

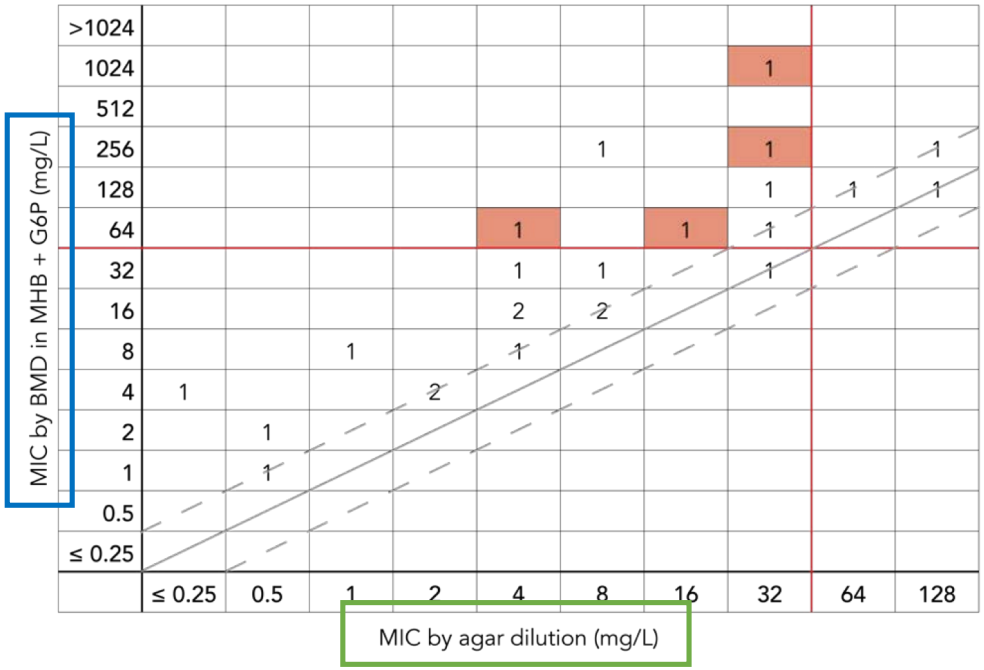
LOD: 1.4 $\log_{10}$ cfu/mL

# Baseline predictors for outcome

- MIC by **agar dilution** (MHA + G6P)
  - Inoculum: 1x $10^4$ cfu/drop
- MIC by **broth microdilution** (MHB + G6P)
  - Inoculum: 5x $10^5$ cfu/well
- **Disk diffusion** (FOT200 Oxoid disk)
  - Inoculum: 0.5 McF density applied as a lawn culture
- Mutant Prevention Concentration (**MPC**) by agar dilution
  - Inoculum: 1x $10^{10}$ cfu/plate
- **Heteroresistance** screen (high-level resistance [HLR])
  - After 18 h drug-free incubation within the bladder infection model
- ***fosA*** gene (fosfomycin inactivating enzyme)
  - Gene detection and quantification of gene expression

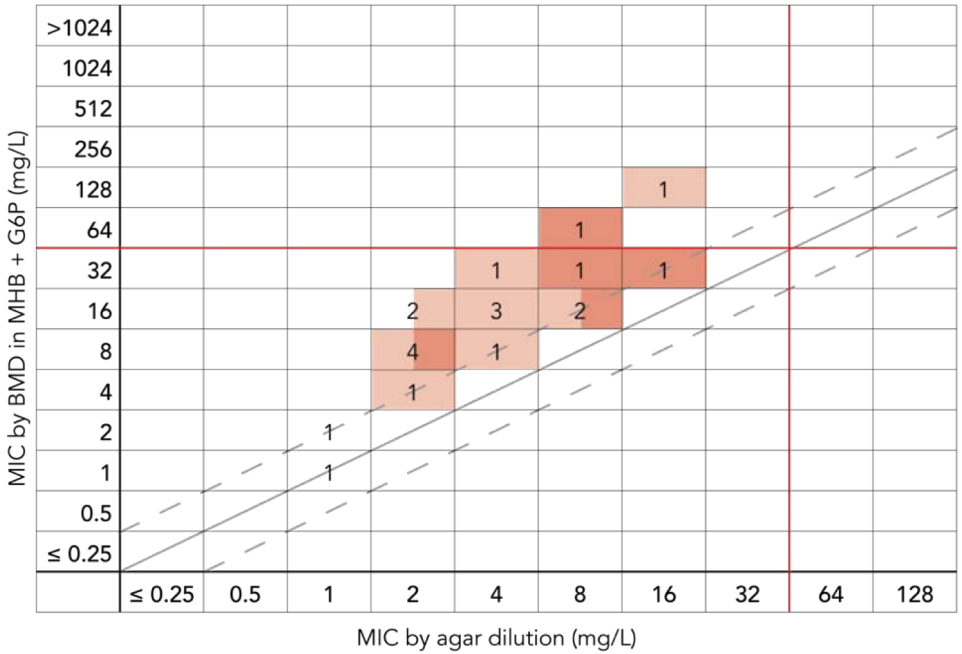
# Broth microdilution MIC (MHB + G6P) *vs.* Agar dilution MIC


***E. coli***




- Agar dilution MIC did not predict re-growth
- BMD MIC values mostly 1 – 3x dilutions higher
- Isolates reliably killed had a BMD MIC ≤ 32 mg/L

***K. pneumoniae***




- Re-growth despite agar dilution MIC 2 – 16 mg/L
- BMD MIC values also 1 – 3x dilutions higher
- Isolates reliably killed had a BMD MIC ≤ 2 mg/L

# Disk diffusion
## *vs.* Agar dilution MIC

Killed
Regrew (no HLR)
Regrew (HLR detected)

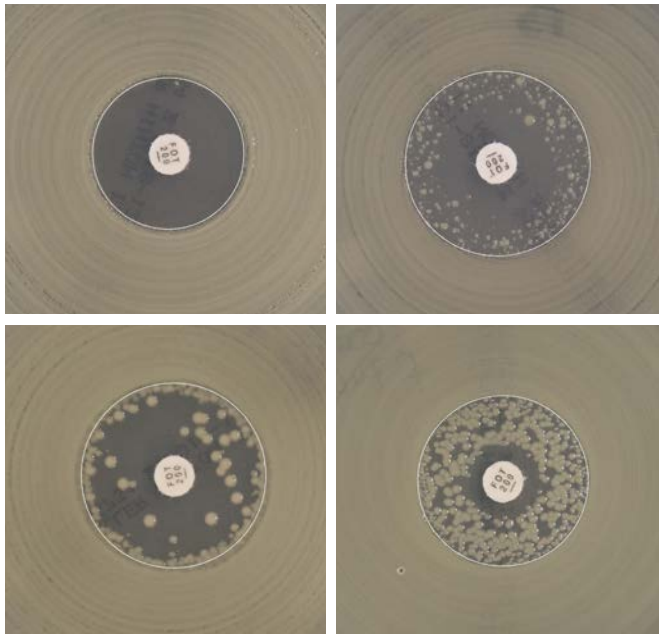
For *E. coli* only
EUCAST: S ≥ 24 mm
CLSI: S ≥ 16 mm

***E. coli***

| Inhibition zone (mm) | ≤ 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 | | | | | | | | | | |
| 19 | | | | | | | | | | |
| 20 | | | | | | | 1 | 1 | | |
| 21 | | | | | | | | | | |
| 22 | | | | | | | | | | |
| 23 | | | | | | | | | | |
| 24 | | | | | | 1 | | | | |
| 25 | | | | | 1 | | | | | |
| 26 | | | | 1 | | | | | 1 | 1 |
| 27 | | | | 1 | | | | 1 | | 1 |
| 28 | | | | | | | | 1 | | |
| 29 | | | | | 1 | | | 2 | | |
| 30 | | | | | | 1 | | | | |
| 31 | | | 1 | | | 1 | | | | |
| 32 | | 1 | | | 2 | | | | | |
| 33 | 1 | | | | | | | | | |
| 34 | | | | | | | | | | |
| 35 | | | | | 1 | 1 | | | | |
| 36 | | 1 | | | | | | | | |

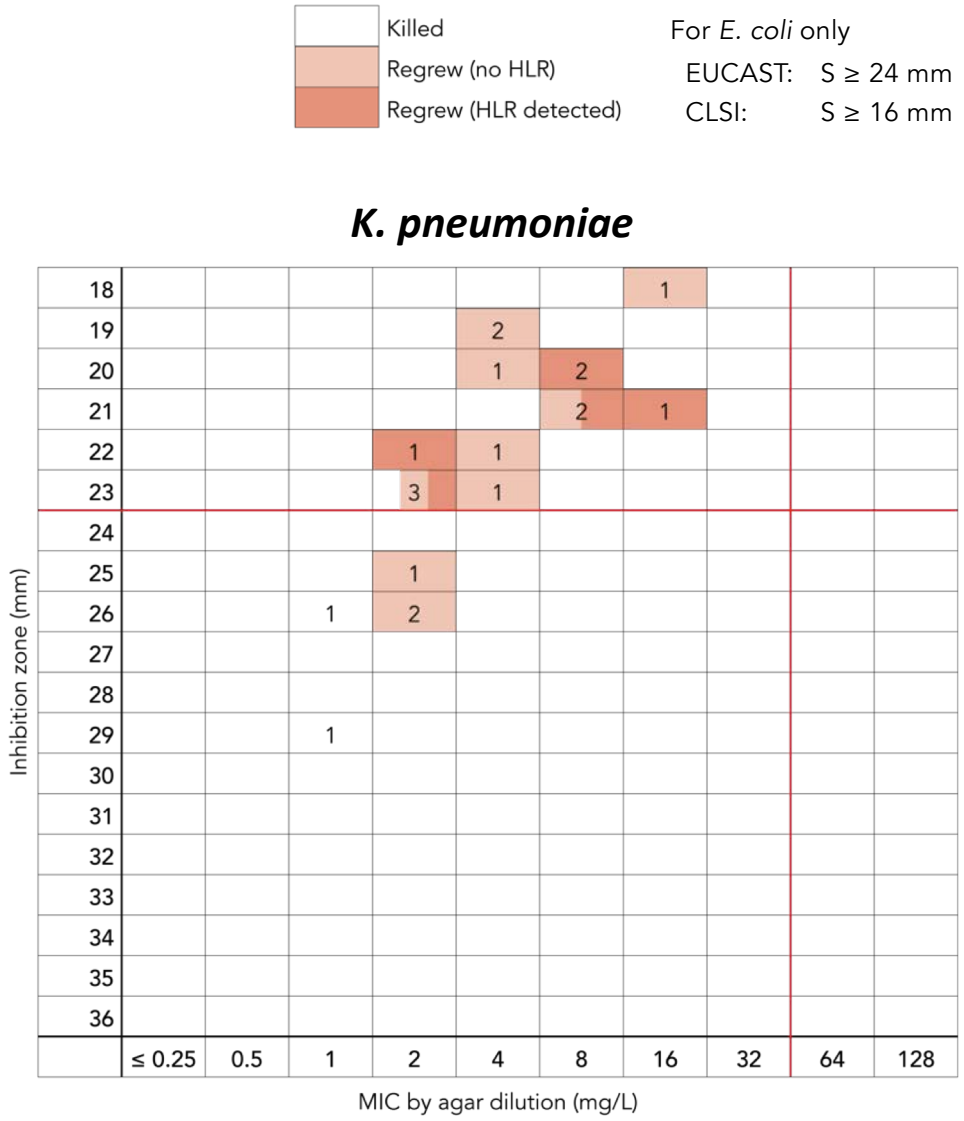
MIC by agar dilution (mg/L)

- The 2-isolates classified as resistant, both re-grew
  - However, all other isolates were classified as susceptible
- Isolates reliably killed had inhibition zone ≥ 28 mm
- However, inhibition zones can be difficult to read



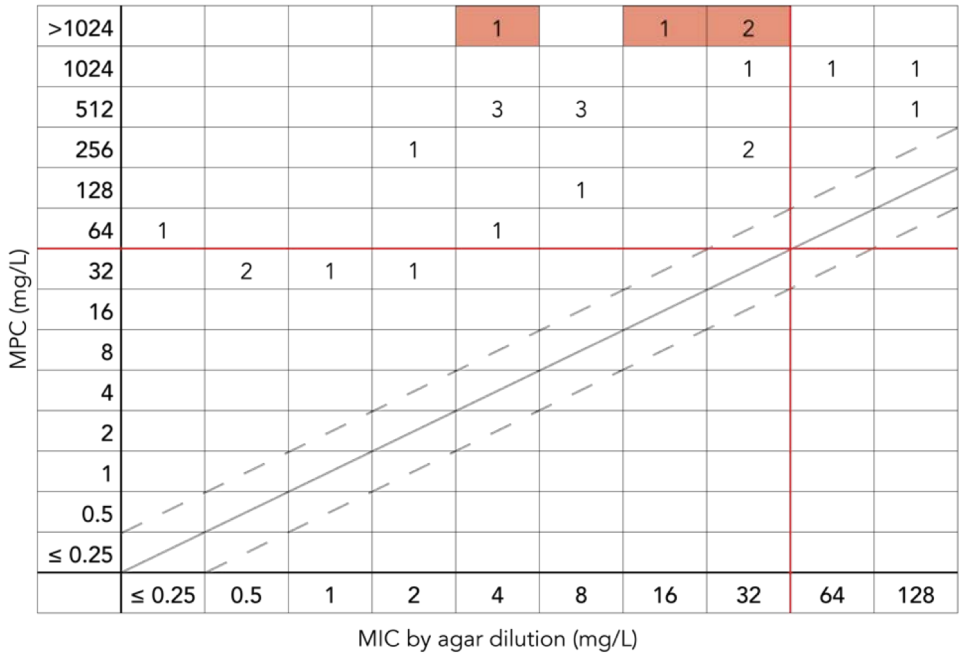
# Disk diffusion
## *vs.* Agar dilution MIC

- All inhibition zones tended to be smaller compared to *E. coli*
- 15 / 20 isolates had a diameter < 24 mm
- Only 1-isolate had a diameter ≥ 28 mm (which was killed)



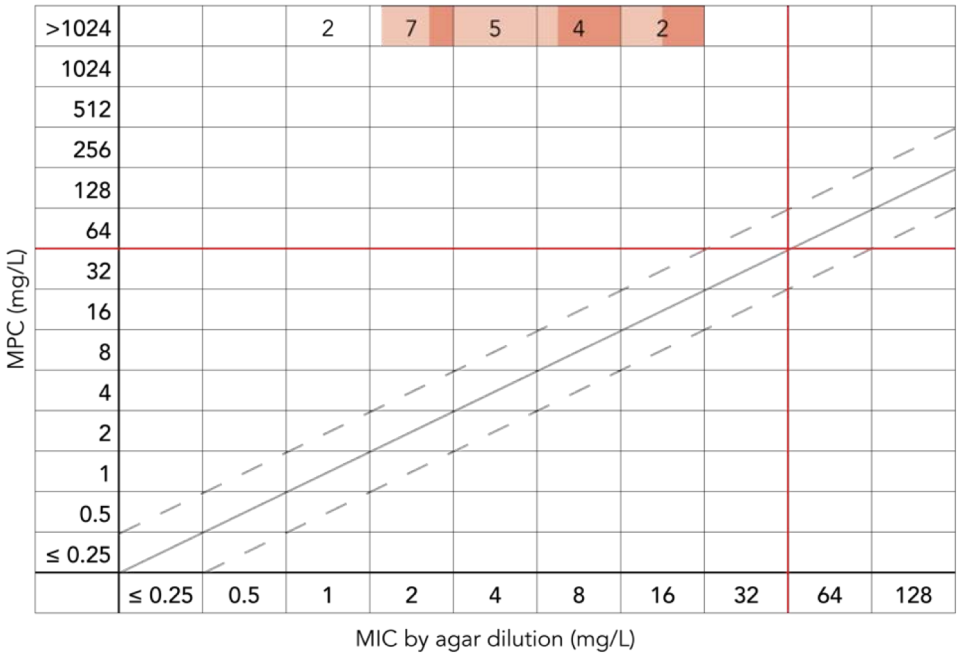
# Mutant Prevention Concentration
## *vs.* Agar dilution MIC


***E. coli***



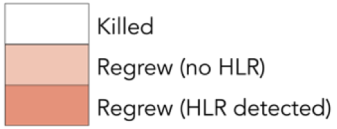

- MPC > 1024 mg/L predicted those isolates that re-grew
  - All other isolates had an MPC 32 – 1024 mg/L

***K. pneumoniae***



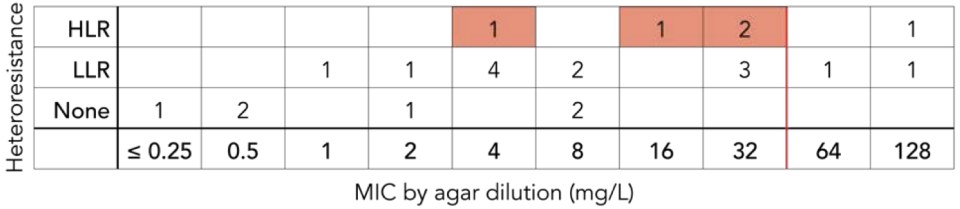

- All isolates had an MPC result > 1024 mg/L
  - Including the 3-isolates that were killed

# Heteroresistance screen
## *vs.* Agar dilution MIC



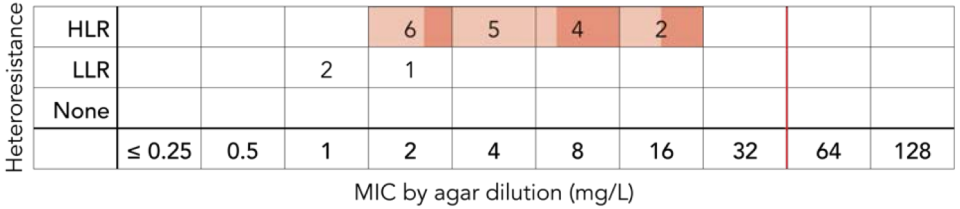

- Dynamic 18 h drug-free control run in the bladder infection *in vitro* model
- Plated onto MHA + 512 mg/L fosfomycin

***E. coli***




- Detection of a HLR subpopulation correlated with re-growth, **except** in only 1-isolate that was killed

***K. pneumoniae***




- All isolates that re-grew had a HLR subpopulation detected
- Those killed did not have HLR detected

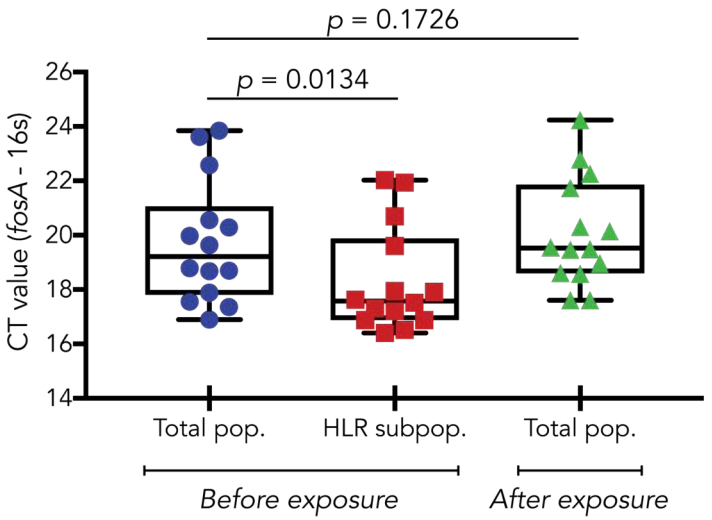
# Enzyme inactivation (*fosA* gene)

***E. coli***

- *fosA* was not detected in any isolate

***K. pneumoniae***

- *fosA* was detected in ALL isolates
  - Including the 3-isolates that were killed
- Gene expression (by RT-qPCR)
  - Greater *fosA* expression in the baseline HLR subpopulation, compared to the total population
  - But no sustained upregulation in the re-growth after fosfomycin exposure



# Conclusions

- *E. coli* and *K. pneumoniae* isolates respond differently after exposure to fosfomycin in a dynamic bladder infection *in vitro* model
- *E. coli* isolates
  - Fosfomycin demonstrated **good activity**, against isolates with a range of MIC values
  - However, failure was related to high-level heteroresistance, which was not identified by the MIC
- *K. pneumoniae* isolates
  - Fosfomycin was **largely ineffective**, regardless of baseline MIC
  - Majority of isolates have a functionally-fit HLR subpopulation, and all have a *fosA* gene
- Overall, fosfomycin MIC appears to be a **poor predictor** for efficacy
  - This challenges the application of clinical breakpoint set for all *Enterobacterales* (by EUCAST)
- Screening for fosfomycin HLR may be more informative
  - Especially, if applied to *E. coli* isolates in conjunction with an existing susceptibility test

# Acknowledgements


- Funding and support through
  - Australian Government Research Training Program Scholarship (NHMRC)
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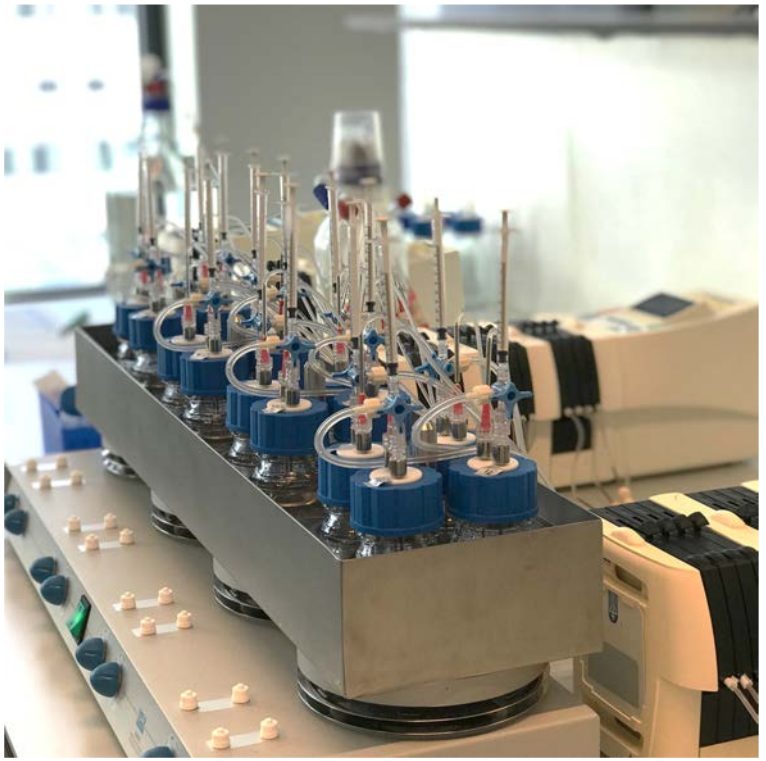
- Technical assistance at Erasmus MC
  - Dept. Microbiology, Research & Development (Carla Roodbol de Goeij)
  - Dept. Experimental Medical Instrumentation (Alex Brouwer and Geert Springeling)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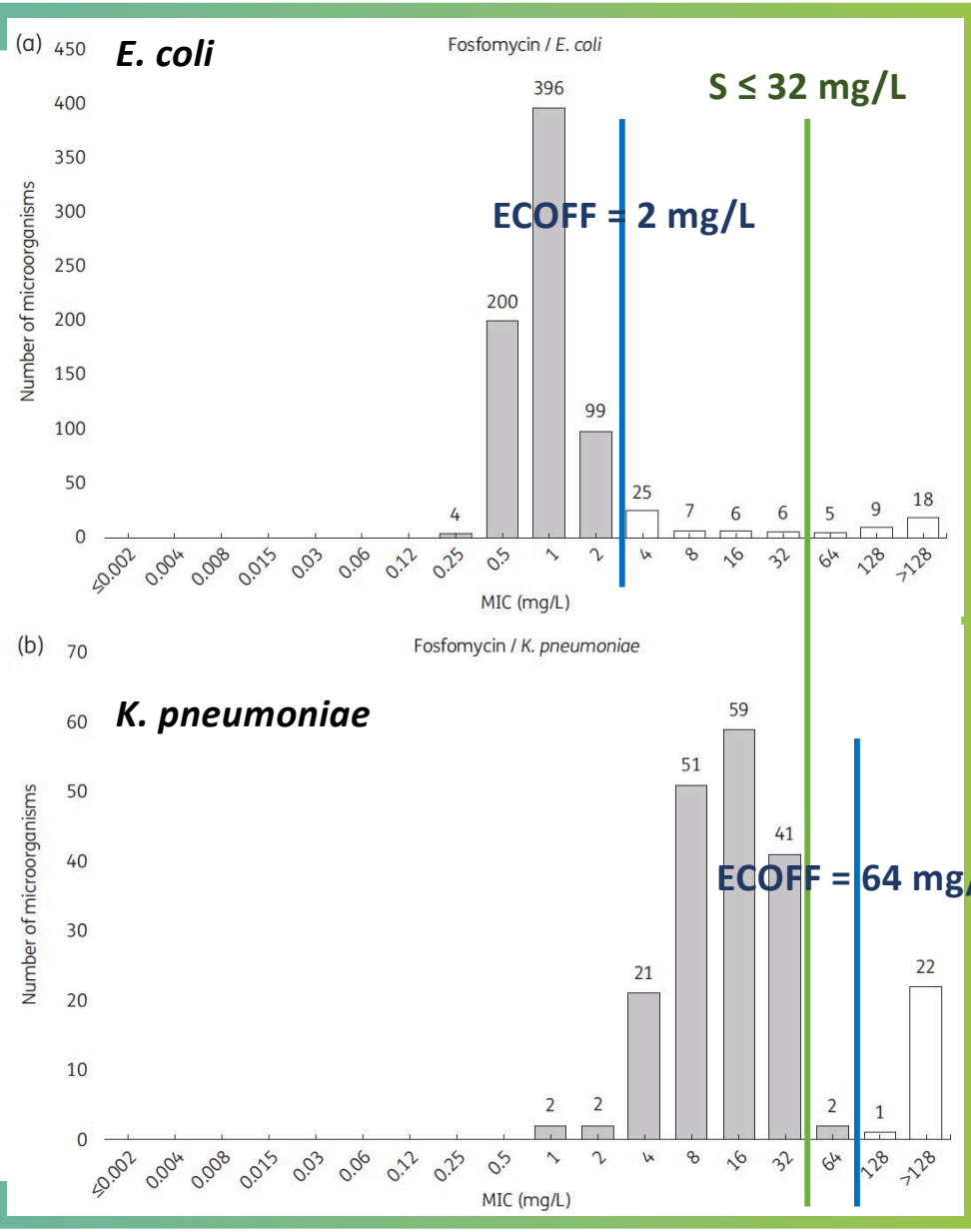
# Extra Slides

# Fosfomycin ECOFF

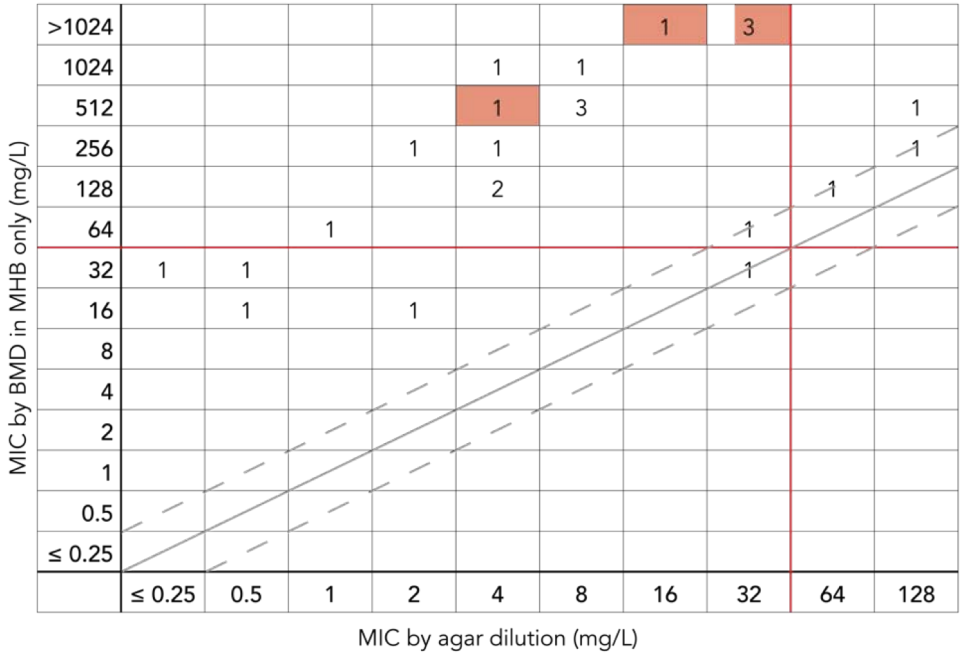
- EUCAST fosfomycin MIC susceptible breakpoint for all *Enterobacterales* is **≤ 32 mg/L**
- However, ECOFF values[1] vary greatly
  - *E. coli* ECOFF: 2 mg/L
  - *K. pneumoniae* ECOFF: 64 mg/L
- NB: if the breakpoint was reduced and applied to *K. pneumoniae*, it would split the wild-type population




[1] van den Bijllaardt W *et al.* J Antimicrob Chemother. 2018

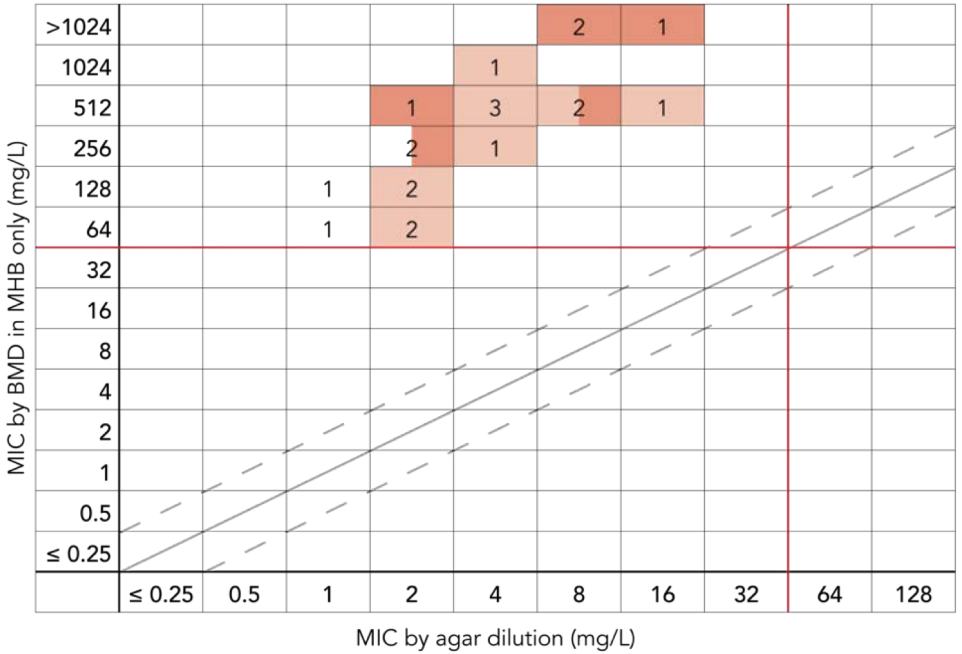
# Broth microdilution MIC (MHB only) *vs.* Agar dilution MIC


***E. coli***




- BMD MIC values were increased without G6P
- **Except** 5 isolates (AD MIC 32 – 128 mg/L)
  - No change, or only a single dilution step rise

***K. pneumoniae***




- All isolates had several dilutions step rise in their BMD MIC value when tested without G6P