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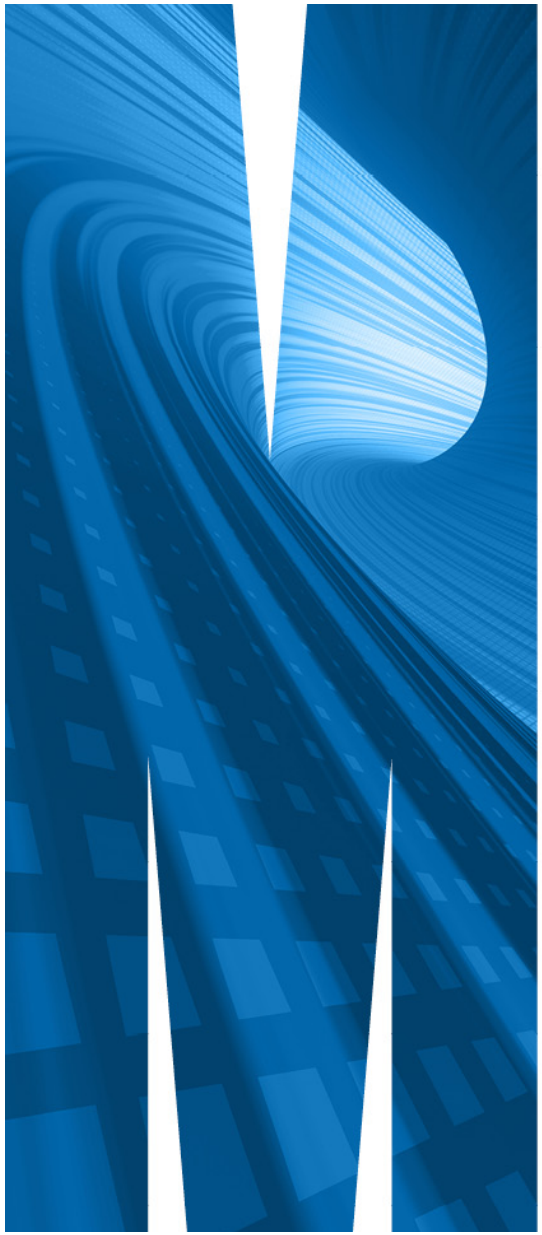
FOSFOMYCIN FRUSTRATIONS

Dr. Iain J. Abbott

26th February 2020

EUCAST Workshop, ASA 2020



Questions that need answering

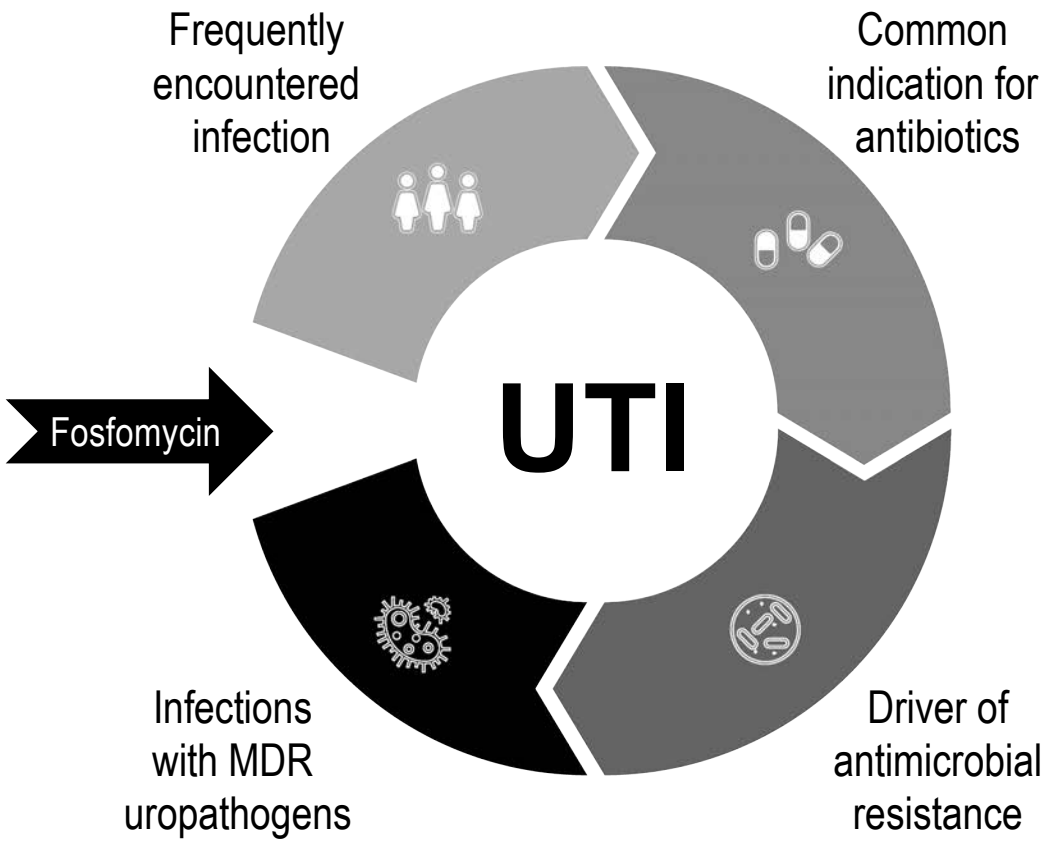
Where does fosfomycin fit in the world of microbiology & infectious diseases?

- What are the target pathogens?
  - Gram-negative: *E. coli* only? All Enterobacterales? *Pseudomonas aeruginosa*?
  - Gram-positive: *Enterococcus faecalis* & *E. faecium* (inc. VRE)? *Staphylococcus aureus*?
- What are the clinical indications?
  - Strictly only for “uncomplicated UTIs”?
  - Or include ”infections originating from the urinary tract” (including pyelonephritis and BSIs)?
  - Infections outside of the urinary tract (e.g. prostatitis, MDR infections)?
- Oral and intravenous formulations
  - Oral: Is a single 3g dose sufficient? Should multiple doses be given? What dosing frequency?
  - IV: When to use? What dose? Monotherapy *vs.* combination?
- How can the diagnostic laboratory confidently report susceptibility?
  - Should clinical breakpoints change?
  - Is the gold standard MIC measurement the best predictor for clinical success?

Urinary tract infections

Among the most commonly reported infections

- Affecting 150 million people each year
- >1:10 women report having had a UTI in the past 12 months
- *E. coli* accounts for ≃ 70%
- Resistance in *E. coli* (AURA 2019)
  - Trimethoprim: 24.1%
  - Nitrofurantoin: 1.1%
  - Amox.-clav.: 13.5%
  - 3G Cef: 7.8%
  - Quinolones: 10%



Flores-Mireles AL *et al.* **Nat Rev Microbiol** (2015)

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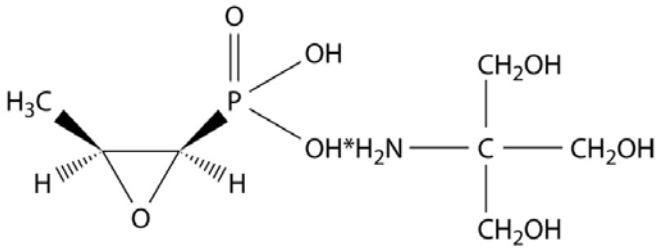
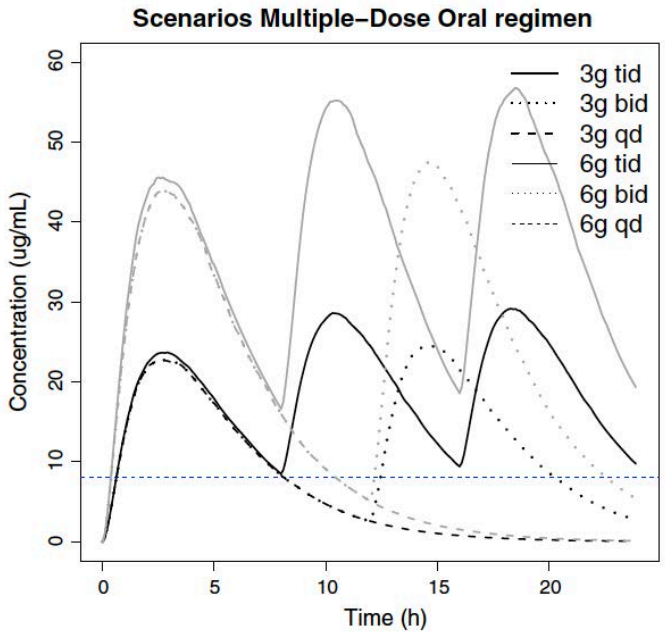
Fosfomycin tromethamine

Oral formulation



- Unique structure, low molecular weight, no cross-resistance
  - Fosfomycin inhibits bacterial cell wall synthesis
  - *In vitro* activity in MDR pathogens (ESBL + CRE, VRE, MRSA, ± PAE)
- First-line for uncomplicated UTIs in EU and US guidelines
  - **Single 3g** oral dose (well tolerated; but ↑ diarrhoea with repeat daily dosing)
  - PK: Urinary conc. 100x higher than serum
    - C_{max} 1000 – 2000 mg/L, *c/w* 20 mg/L in serum
- Variable clinical response rates reported
  - Old data report **clinical cure** rates: 87 – 93%
  - Clinical trial: clinical resolution in **58%** (c/w 70% nitrofurantoin)



JAMA | Original Investigation

Effect of 5-Day Nitrofurantoin vs Single-Dose Fosfomycin on Clinical Resolution of Uncomplicated Lower Urinary Tract Infection in Women

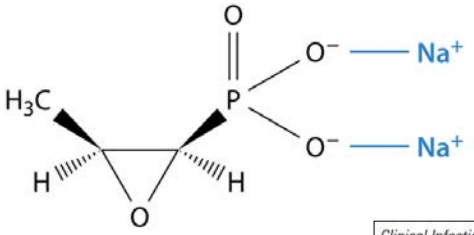
A Randomized Clinical Trial

Ortiz Zacarias NV. **Pharmacol Res Perspect** (2018)

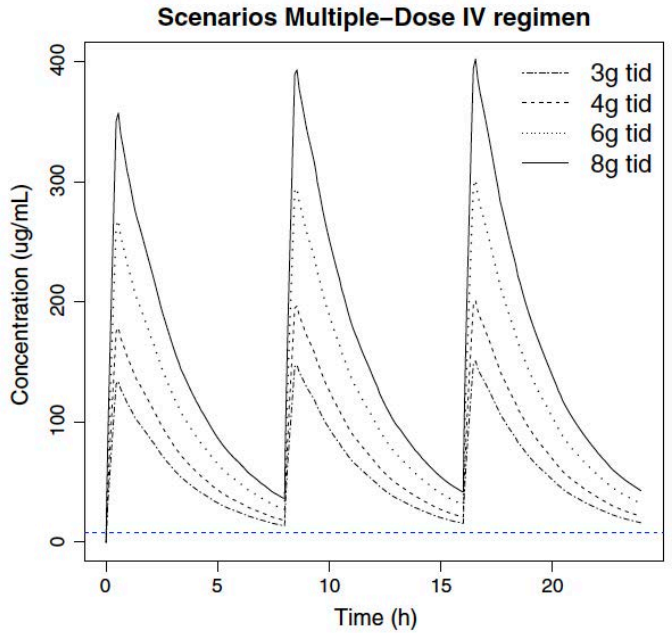
Huttner A. **JAMA** (2018)

Fosfomycin disodium

Intravenous formulation



- Dose: 4 – 8g q8h
  - Well tolerated (beware excess sodium) + good tissue penetration
  - ZEUS study: FOS 6g q8h *vs.* TZP 4.5g q8h (non-inferiority in cUTI)
- Plasma PK (8g dose)
  - C_{max} 370 mg/L; $AUC_{0-\infty}$ 1000 mg.h/L; Renal excretion (unchanged) 80%
  - PK/PD: *f*AUC/MIC; ± time-dependence related to emergence of resistance
- Emergence of resistance
  - Biggest challenge to monotherapy
  - Combinations/synergy reported against MDR pathogens
    - Beta-lactams (inc. MER, CAZ-AVI), quinolones, colistin, aminoglycosides, daptomycin



Kay KS. **Clin Infect Dis** (2019)

Ortiz Zacarias NV. **Pharmacol Res Perspect** (2018)

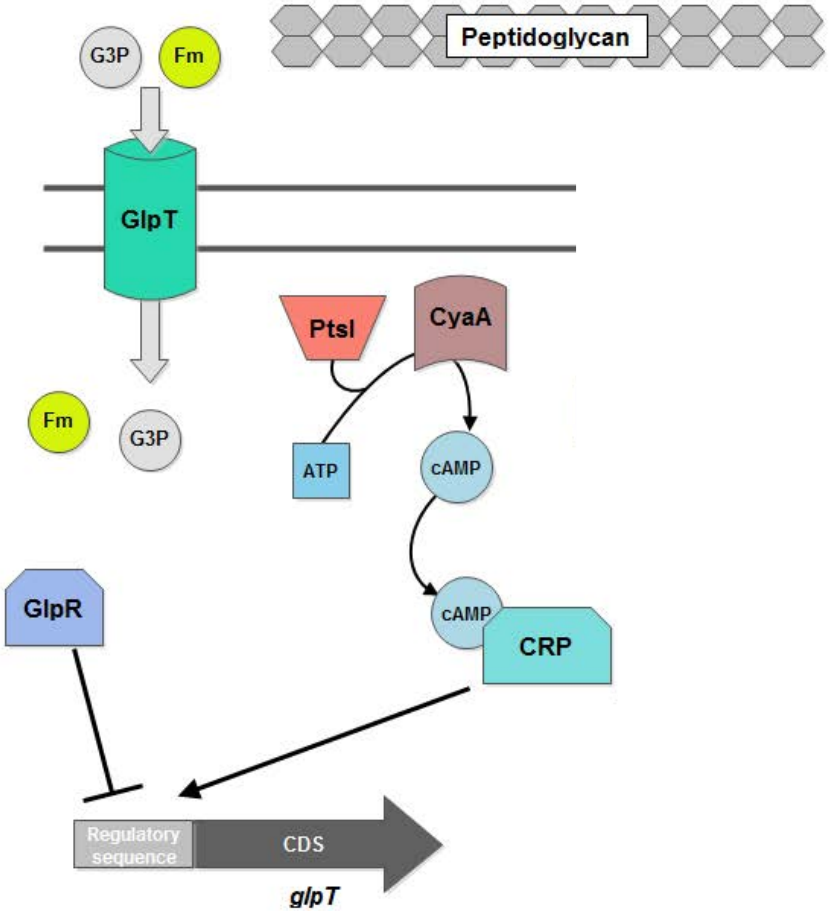
Grabein B. **Clin Microbiol Infect** (2017)

Mechanisms of resistance

Fm **Fosfomycin**

Prevent entry

- **GlpT (G3P)**
- UhpT



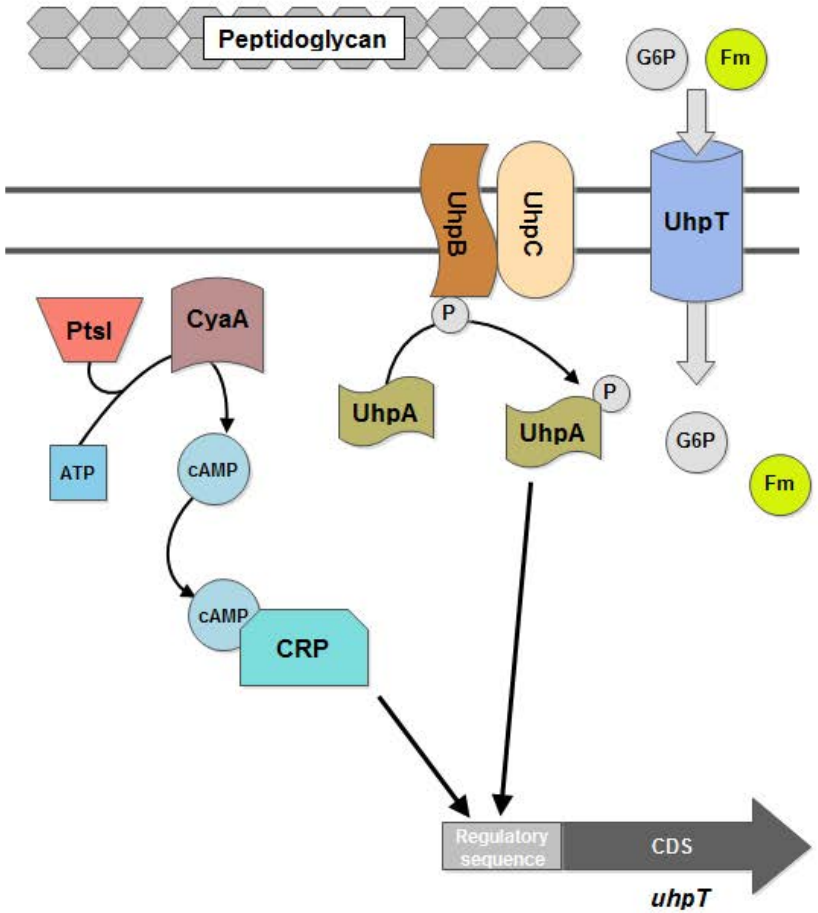
glycerol-3-phosphate (G3P)

Mechanisms of resistance

Fm **Fosfomycin**

Prevent entry

- GlpT
- **UhpT (G6P)**



glucose-6-phosphate (G6P)

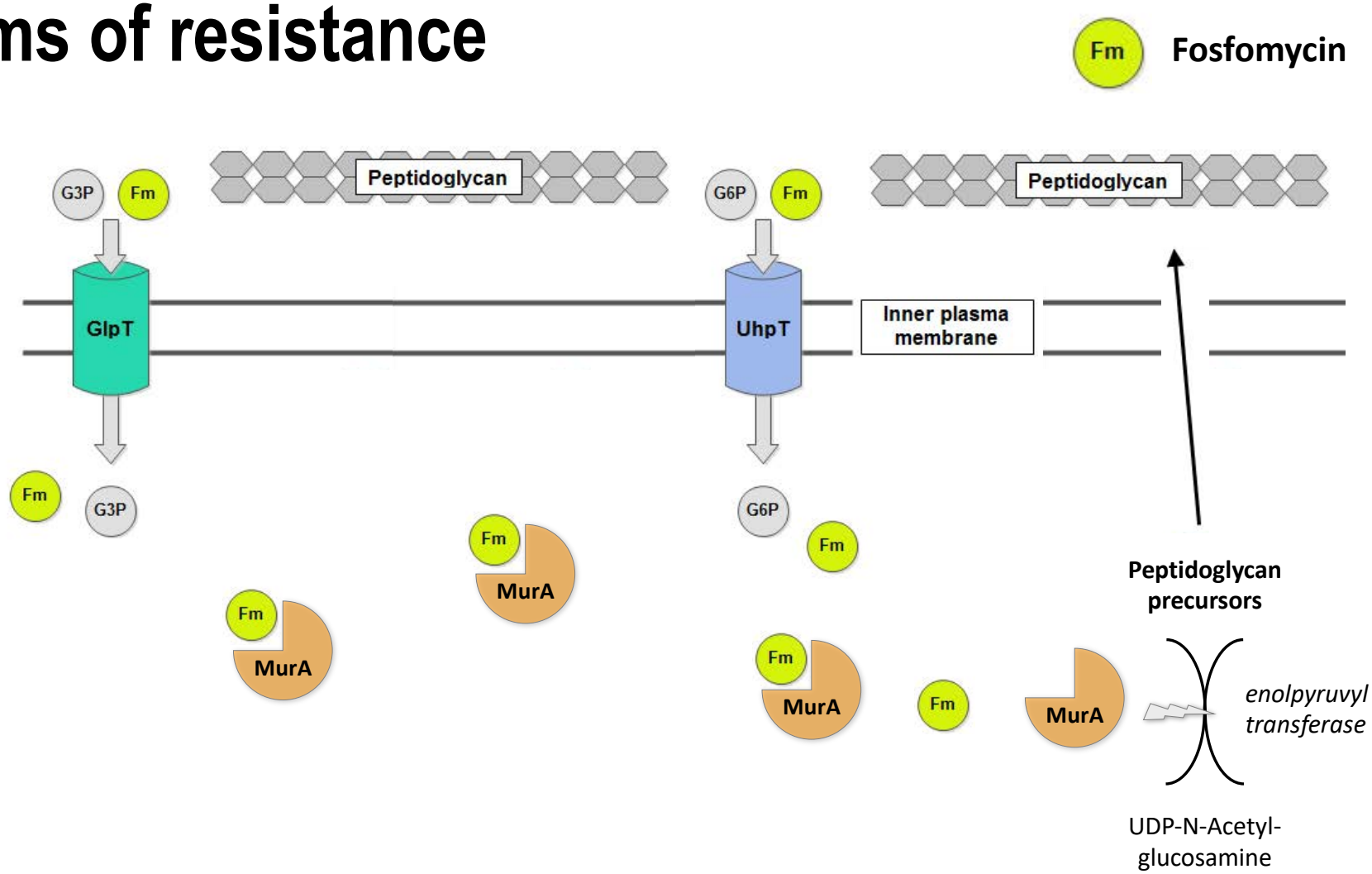
Mechanisms of resistance

Prevent entry

- GlpT
- UhpT

Affect binding

- **MurA**



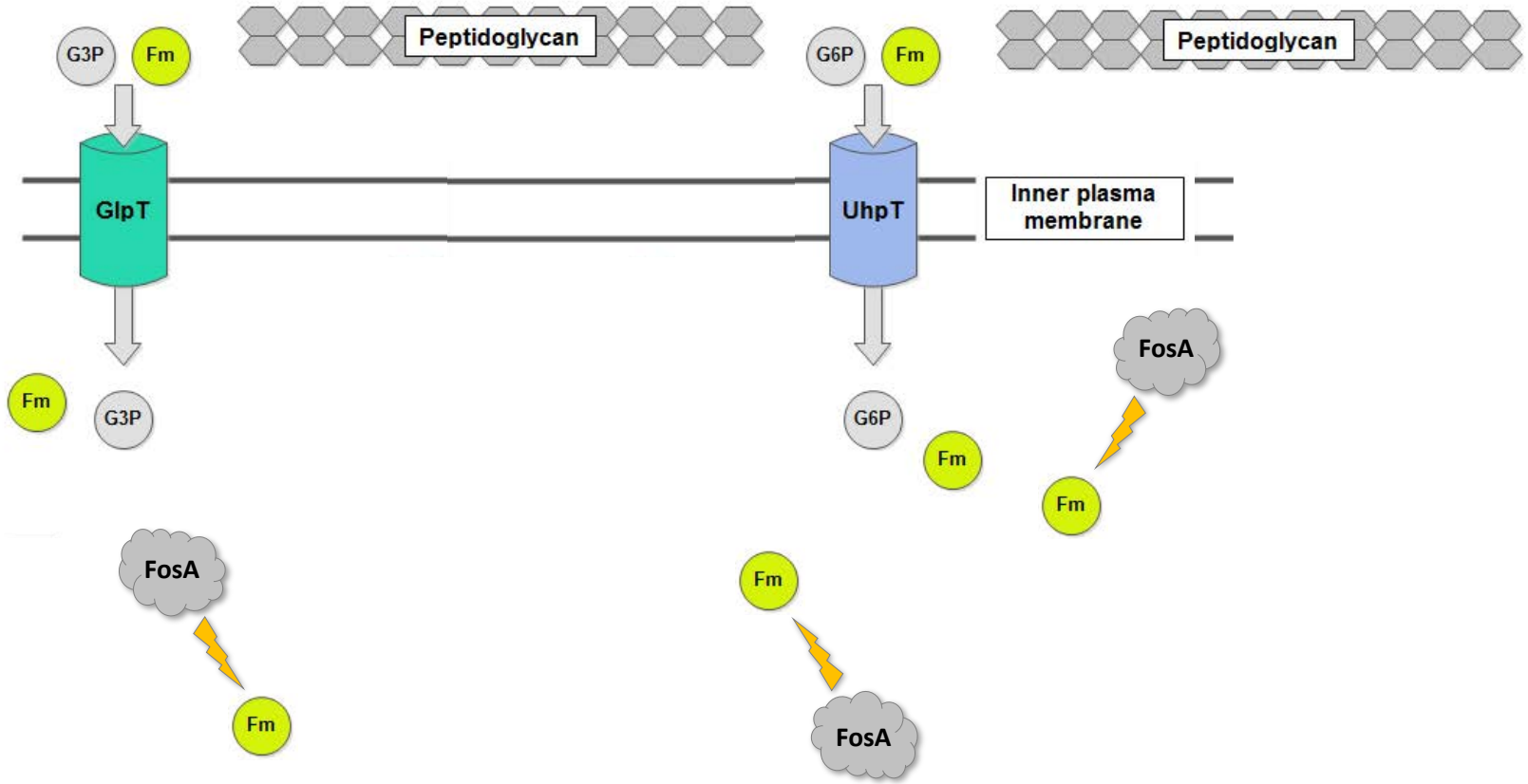
Mechanisms of resistance

Fm Fosfomycin

Prevent entry
- GlpT
- UhpT

Affect binding
- MurA

Inactivation
- **FosA**



Gene location: Plasmid (in *E. coli*) or chromosomal (in *K. pneumoniae* & *P. aeruginosa*)

Susceptibility discrepancies

Some of the people who have recently published their “fosfomycin frustrations”

- Diez-Aguilar M. Is a New Standard Needed for Diffusion Methods for In Vitro Susceptibility Testing of Fosfomycin against Pseudomonas aeruginosa? *Antimicrob Agents Chemother* **2016**; 60: 1158-61.
- Ballestero-Tellez M. Role of inoculum and mutant frequency on fosfomycin MIC discrepancies by agar dilution and broth microdilution methods in Enterobacteriaceae. *Clin Microbiol Infect* **2017**; 23: 325-31.
- van den Bijllaardt W. Susceptibility of ESBL Escherichia coli and Klebsiella pneumoniae to fosfomycin in the Netherlands and comparison of several testing methods including Etest, MIC test strip, Vitek2, Phoenix and disc diffusion. *J Antimicrob Chemother* **2018**; 73: 2380-7.
- Elliott ZS. The Role of fosA in Challenges with Fosfomycin Susceptibility Testing of Multispecies Klebsiella pneumoniae Carbapenemase-Producing Clinical Isolates. *J Clin Microbiol* **2019**; 57.
- Cottell JL. Experiences in fosfomycin susceptibility testing and resistance mechanism determination in Escherichia coli from urinary tract infections in the UK. *J Med Microbiol* **2019**; 68: 161-8.
- Mojica MF. Performance of disk diffusion and broth microdilution for fosfomycin susceptibility testing of multi-drug resistant clinical isolates of Enterobacterales and Pseudomonas aeruginosa. *J Glob Antimicrob Resist* **2020**.

Susceptibility testing

General comments

- Agar dilution (AD) is the only approved MIC method
  - Broth microdilution (BMD) is **not** recommended
  - But AD is not widely available (or practical)
- Other methods (Gradient strip, DD, Vitek2/Phoenix) have poor concordance with AD
  - Approx. major error rates: 20%, 13%, 15%, respectively
  - Uncertain impact of underlying **heteroresistance** in discrepancies
- Requirement for 25 mg/L glucose-6-phosphate (G6P) supplement in media (and disks/strips)
  - **No physiological** reason for supplementation
  - Enhances fosfomycin activity by **promoting** fosfomycin uptake via the UhpT transporter
  - However, enhancement of activity **does not** uniformly occur in all Enterobacterales
  - **No enhancement** in *Pseudomonas* or *Enterococcus* spp. (both lack the UhpT transporter)
- Differences in breakpoints and DD interpretation advice between CLSI and EUCAST

Susceptibility testing

EUCAST vs. CLSI

EUCAST

- Oral and iv breakpoints
- MIC breakpoints apply to Enterobacterales (oral + iv) and *Staphylococcus* (iv only)
- Disk diffusion breakpoints only for *E. coli*
- *Pseudomonas*:
  - Wild-type isolates (ECOFF: MIC 128 mg/L; zone diameter 12 mm) treated with fosfomycin in combination with other agents
- **MIC**: S ≤ 32 mg/L R > 32 mg/L
- **DD**: S ≥ 24 mm R < 24 mm
  - Ignore all colonies and read the outer zone edge

CLSI

- Oral only
- Disk diffusion and MIC breakpoints apply only to *E. coli* and *E. faecalis* urinary isolates
  - "Should **not** be extrapolated to other species of Enterobacterales"
- *Pseudomonas*:
  - No breakpoints provided
- **MIC**: S ≤ 64 µg/mL R ≥ 256 µg/mL
- **DD**: S ≥ 16 mm R < 12 mm
  - Measure the colony-free inner zone

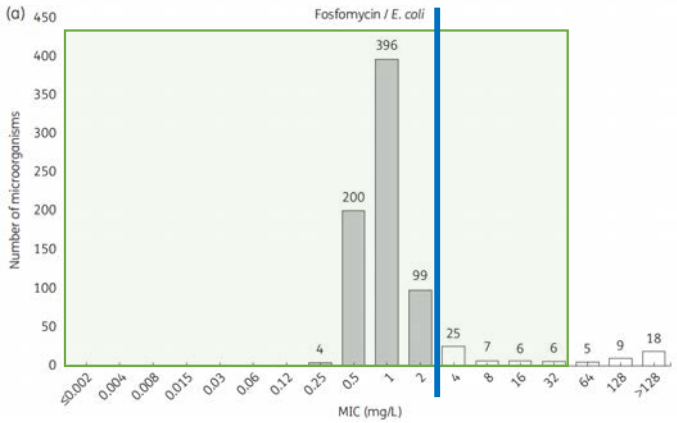
Wild-type distributions

Enterobacterales

EUCAST (Enterobacterales): S ≤ 32 mg/L

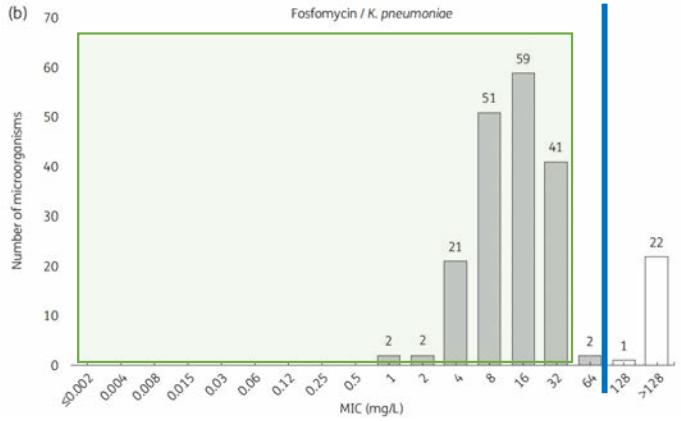
E. coli

ECOFF = 2 mg/L



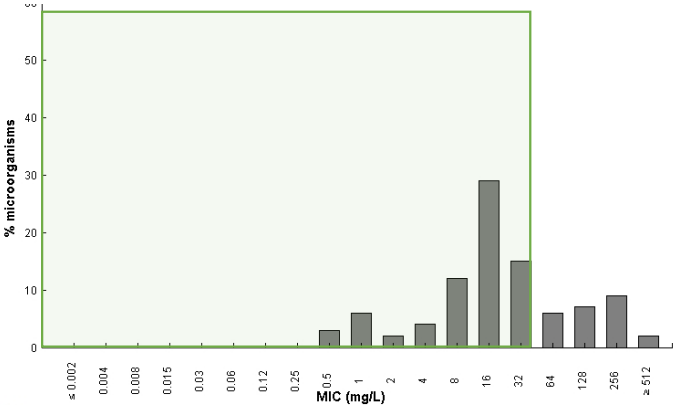
K. pneumoniae

ECOFF = 64 mg/L



***Enterobacter* spp.**

ECOFF = ??



van den Bijllaardt W. **J Antimicrob Chemother** (2018)

https://mic.eucast.org/Eucast2/

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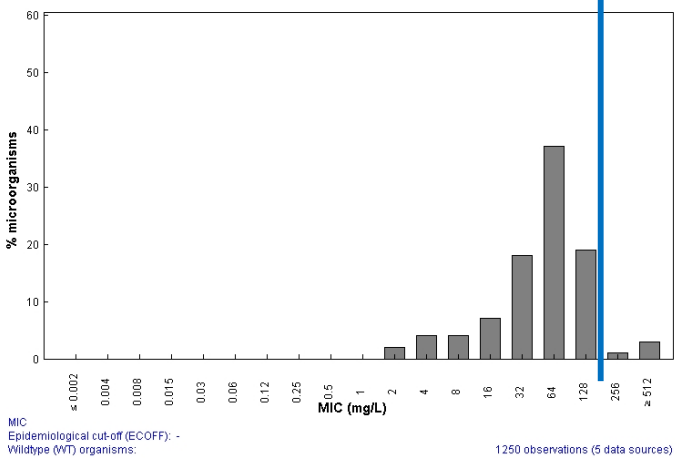
Wild-type distributions

Pseudomonas spp. & *Enterococcus* spp.

CLSI (*E. faecalis*): S ≤ 32 mg/L

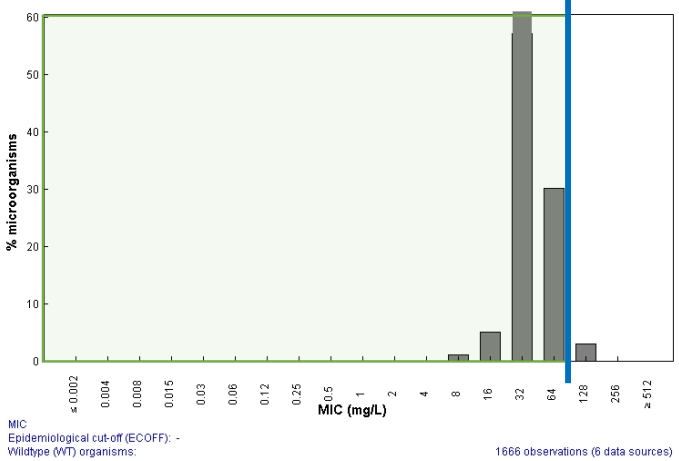
P. aeruginosa

ECOFF = 128 mg/L



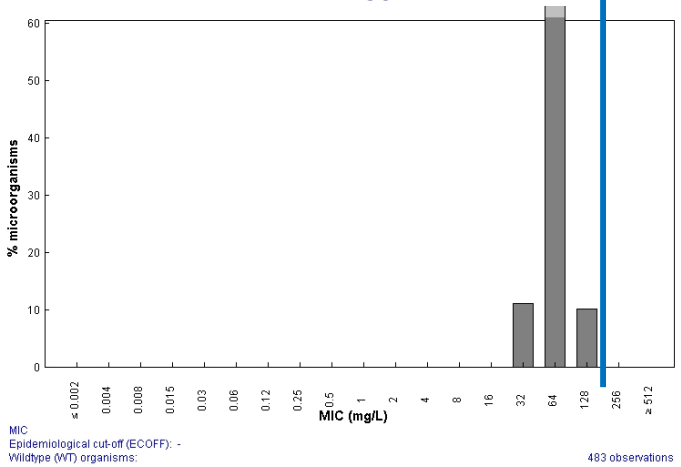
E. faecalis

MIC_{90} = 64 mg/L



E. faecium

MIC_{90} = 128 mg/L



https://mic.eucast.org/Eucast2/

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MIC measurement

Agar dilution (AD) *vs.* Broth microdilution (BMD)

- Important inoculum differences between MIC methods
  - Differences in measured MICs in part due to the enrichment of the starting inoculum with resistant subpopulations
- Poor correlation of AD MIC and efficacy
  - Especially for *K. pneumoniae*
  - Does not identify isolates (inc. *E. coli*) that have a baseline resistant subpopulation
  - Better relationship demonstrated with *Pseudomonas* and *Enterococcus* spp.
- Urinary bactericidal titers (UBTs) values do not correlate with MICs determined in the presence of G6P
  - An assessment of *ex vivo* urinary bactericidal activity

Agar dilution method

Cultures adjusted to the 0.5 McF standard contain 1.5 x 10^8 CFU/mL with most species, and **the final inoculum required is 10^4 CFU** per spot of 5-8mm.

Broth microdilution method

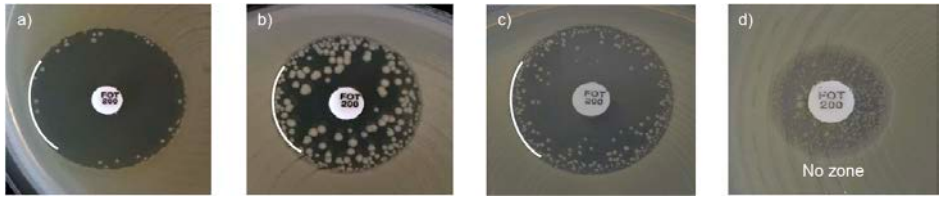
The final test concentration of bacteria in each well is approx. 5 x 10^5 CFU/mL (range 2 to 8 x 10^5 CFU/mL).

Ballestero-Tellez M. **Clin Microbiol Infect** (2017)
Seroy JT. IJ. **J Antimicrob Chemother** (2016)
Abbott IJ. **J Antimicrob Chemother** (2019)
Ballestero-Tellez M. **J Antimicrob Chemother** (2017)
Wenzler E. **Antimicrob Agents Chemo** (2020)
Wijma RA. Int J Antimicrob Agents (2019)

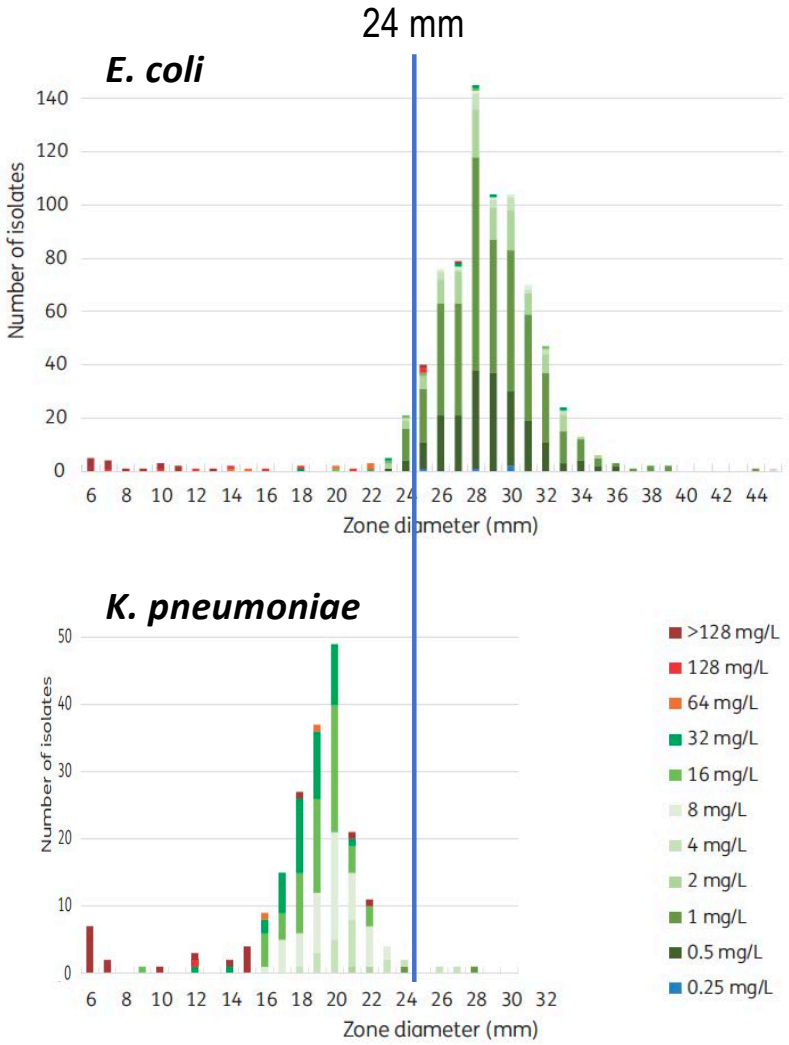
Disk diffusion

E. coli vs. *K. pneumoniae*

- *E. coli* isolates tend to have larger inhibitions zones compared with *K. pneumoniae* isolates
  - Even in isolates with the same agar dilution MIC
- Can be difficult to read due to the presence of colonies within the inhibition zone
  - EUCAST – **ignore** colonies
  - CLSI – do **not** ignore colonies



- Mojica et al. (2020) concluded (for Enterobacterales):
  - "best performance of DD is achieved when read as indicated by EUCAST, but interpreted according the CLSI breakpoints"



FOT200 disk (200mg FOS + 50mg G6P)
EUCAST (*E. coli*): S ≥ 24 mm, R < 24 mm

Mojica MF. **J Glob Antimicrob Resist** (2020)
van den Bijllaardt W. **J Antimicrob Chemother** (2018)

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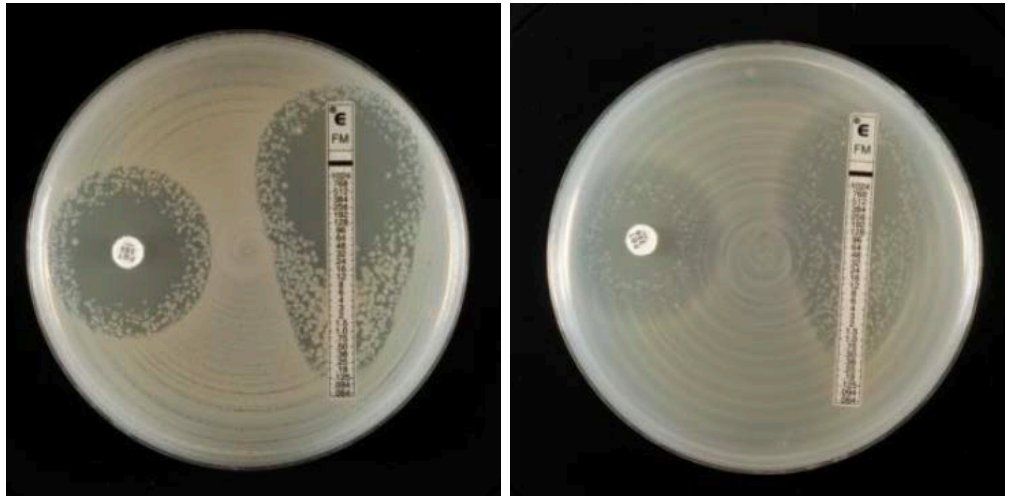
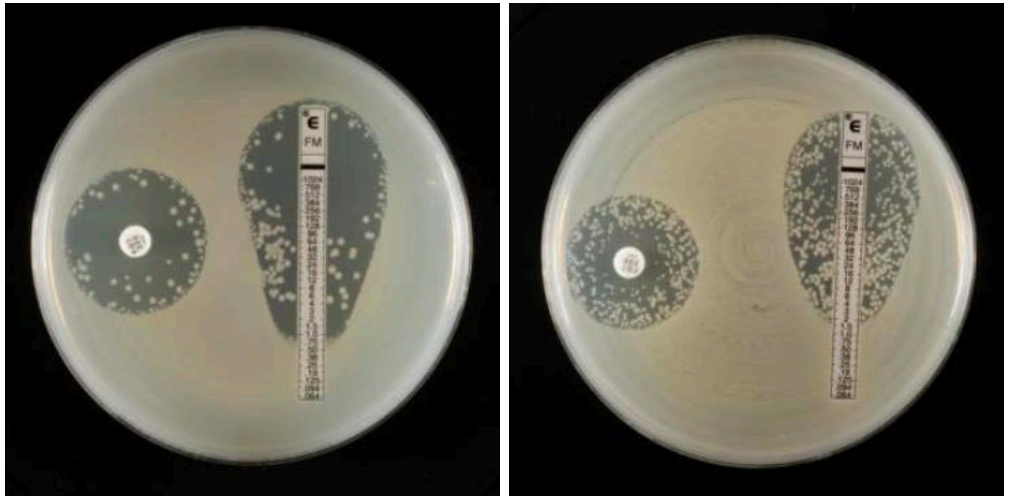
Disk diffusion & gradient strip

In relation to WGS results

Fosfomycin resistance genes NOT likely



Fosfomycin resistance genes likely



- Inner colonies in *E. coli* are relatively infrequent (3% of isolates tested; 1/3 repeat)
- Mostly accounted for with mutations which confer fitness cost to the bacteria

Automated AST systems

E.g. Vitek2, Phoenix

- Broth microdilution several manufacturers include fosfomycin on their AST panels
  - Despite these methodologies are essentially variations of BMD, which is not recommended fosfomycin AST
- Limited concentration ranges tested
  - E.g. Vitek2 AST-N344 card (Netherlands): 16 – 128 mg/L
- Vitek2 + Phoenix testing compared to agar dilution
  - Limited by few resistant isolates included
    - *E. coli*: Categorical agreement - 99 + 99.5%; Very major error rate - 18.8% + 12.5%
    - *K. pneumoniae*: CA: 94.5% + 93% ; VME – 16.0% + 12%

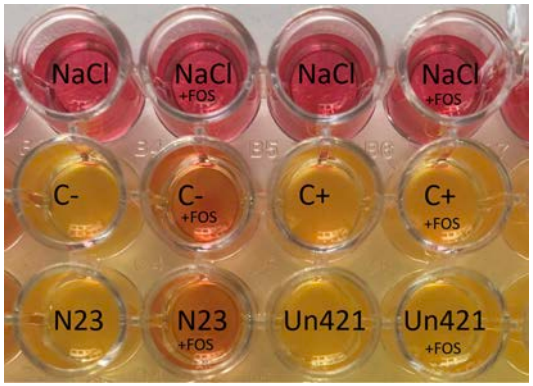
van den Bijllaardt W. **J Antimicrob Chemother** (2018)

Detection of resistance

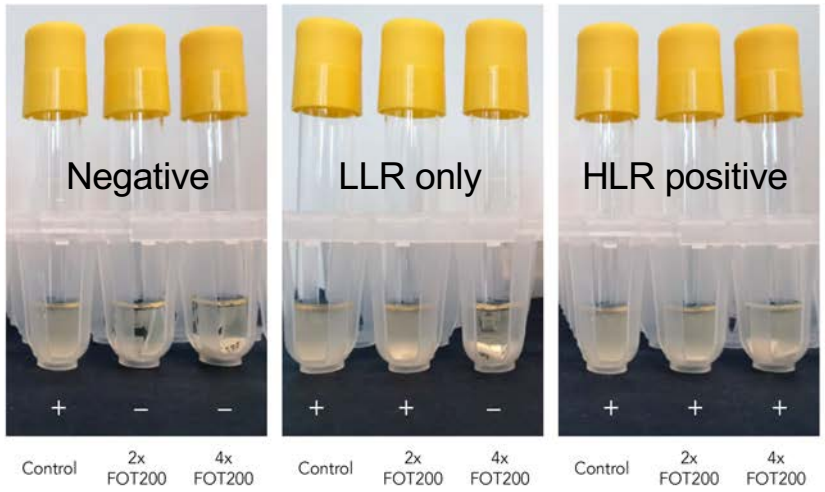
Heteroresistance with a high-level resistance subpopulation

- Potentially clinically-relevant fosfomycin heteroresistance is **not detected** by agar dilution MIC
  - Less common in *E. coli* isolates
  - Very common in *K. pneumoniae* + *P. aeruginosa*
  - Not detected in *Enterococcus* spp.
- Heteroresistance identification can be onerous
  - Population analysis profile (PAP)
  - Mutant prevention concentration (MPC)
  - Mutation rate vs. mutant frequency
- Novel approaches
  - 2.0 McF inoculum, overnight cultures -> disk/gradient strips
  - Rapid fosfomycin NP/*E. coli* test
  - Disk elution screen (4x FOT200 disks added to MHB)

Rapid fosfomycin NP/*E.coli*



Disk elution screen



LLR – low level resistance
HLR – high level resistance

Nordmann P. **J Clin Microbiol** (2019)

Abbott IJ. **J Antimicrob Chemother** (2019)

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Site specific breakpoints

Importance of urinary pharmacokinetics (PK)

- Marked **variability** in fosfomycin urinary fosfomycin concentrations:

Segre G. (Eur Urol 1987)	C_{max} 2895 ± 842 mg/L	by 4 h
Wenzler E. (AAC 2017)	C_{max} 1040 ± 868 mg/L	by 4 h
Wijma RA. (CMI 2018)	C_{max} 1982 ± 1257 mg/L	by 7.5 h

- Important human **behavioural variables** can greatly impact upon urinary antimicrobial concentrations:

  Time of administration; Fluid intake; Urine output; Voiding pattern

- This is in addition to the more **predictable PK variants**:

  Absorption; Distribution; Elimination (renal function)

Site specific breakpoints

Importance of urinary pharmacodynamics (PD)

- Nutritional factors are **less available** *in vivo* compared to laboratory media
- Urine is a complex and relatively **harsh environment** for bacterial growth
  - Nutritionally deplete and naturally antimicrobial
  - Hypertonicity, low pH, low oxygen content, high nitrites + urea
- Standard laboratory media will **not reflect** bacterial growth kinetics in urine
  - In UTIs, bacterial doubling time is a critical
    - Needs to be quicker than the rate of dilution by urine production and intermittent voiding
  - Urine chemistry (and pH) can impact upon antimicrobial activity
  - Urine contains only negligible amounts (0.2 mg/L) of glucose-6-phosphate
- However, working with human urine is largely **impractical**
  - Marked variability, short shelf-life, onerous sterilisation, ethical + safety considerations

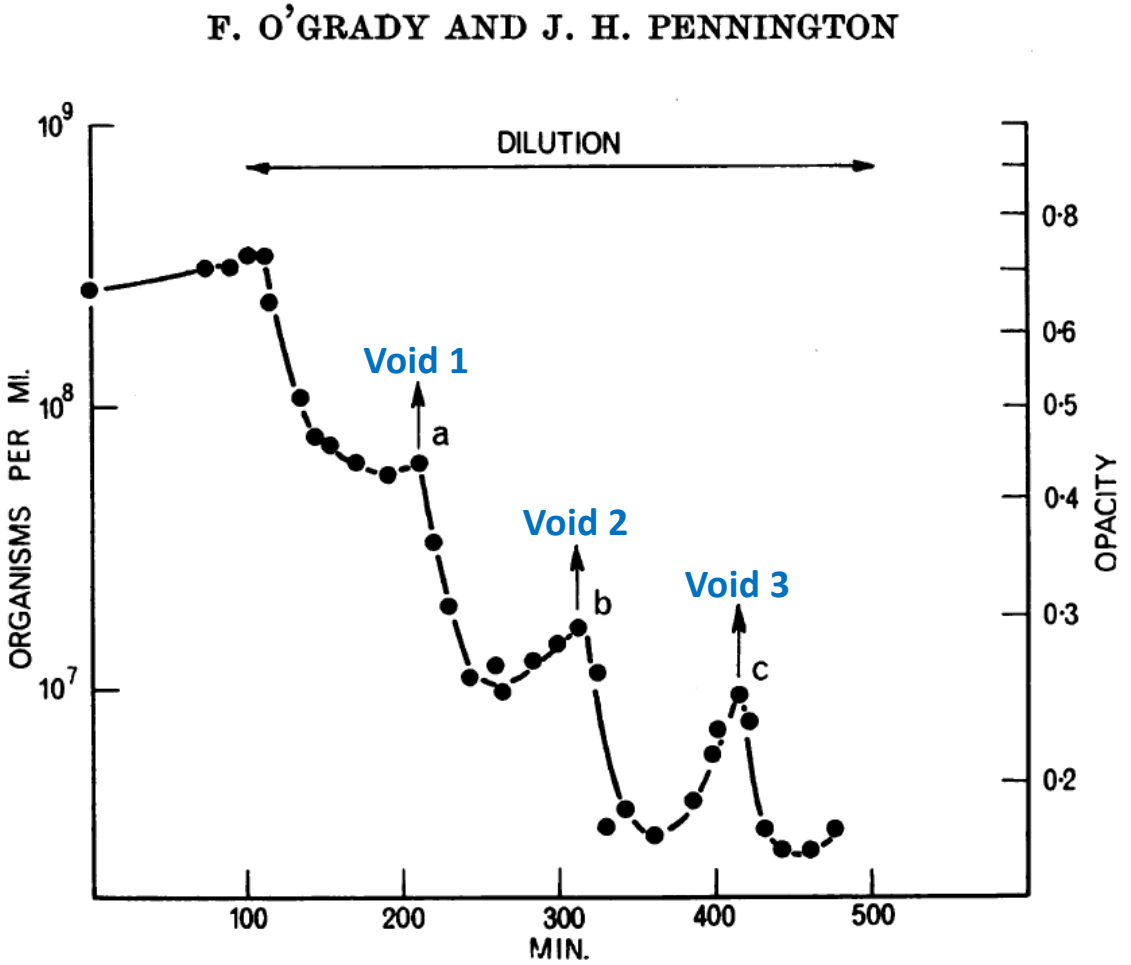
Site specific breakpoints

Importance of bladder urodynamics

- The same urodynamic characteristics (high urinary output, large volume voids) that can **lower** urinary antimicrobial exposures can equally **increase** bacterial clearance

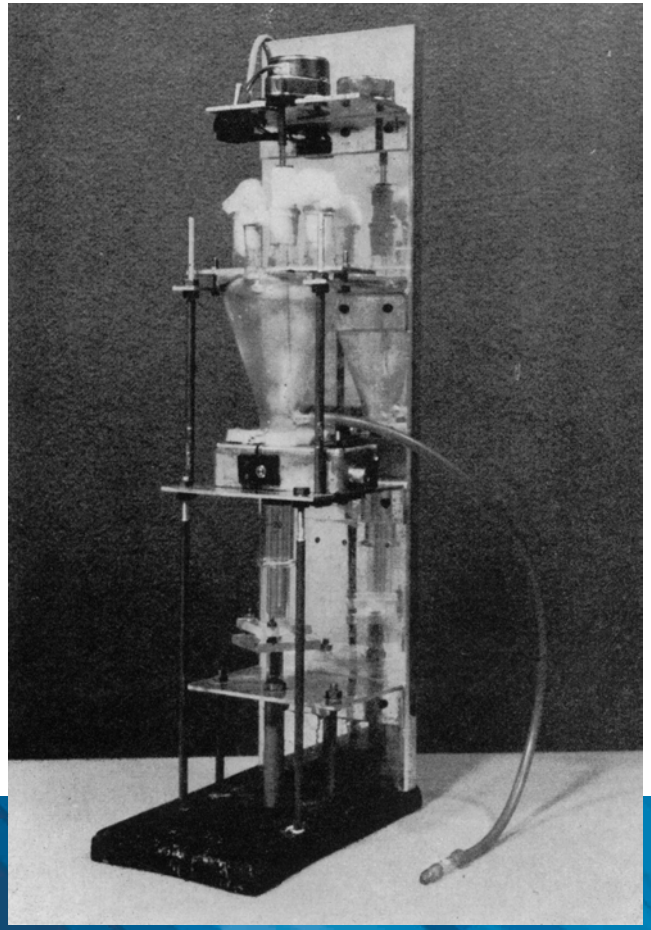
- Original UTI *in vitro* model (1966)
  - Fresh broth added at 1 mL/min
  - Intermittent simulated voiding
  - Reduces volume to 30 mL

- Normal urodynamics alone can reduce bacterial density, without any antimicrobials added to the *in vitro* system



Site specific breakpoints

Dynamic PK/PD bladder infection *in vitro* modelling



O'Grady F & Pennington JH. **Br J Exp Pathol** (1966)

Abbott IJ *et al.* **J Antimicrob Chemother** (2018)

Dynamic bladder infection model

Publications to date

- Fosfomycin efficacy and emergence of resistance among *Enterobacteriaceae* in an *in vitro* dynamic bladder infection model. **JAC** (2018).
- Impact of bacterial species and baseline resistance on fosfomycin efficacy in urinary tract infections. **JAC** (2019).
- Oral fosfomycin efficacy with variable urinary exposures following single and multiple doses against Enterobacterales: the importance of heteroresistance on growth outcome. **AAC** (2020).
- Evaluation of pooled human urine and synthetic alternatives in a dynamic bladder infection *in vitro* model simulating oral fosfomycin therapy. **JMM** (2020).
- Efficacy of single and multiple oral doses of fosfomycin against *Pseudomonas aeruginosa* urinary tract infections in a dynamic *in vitro* bladder infection model. **JAC** (2020 – in press).
- Oral fosfomycin treatment for Enterococcal urinary tract infections in a dynamic *in vitro m*odel **AAC** (2020 - submitted).

Considerations

NB: **Personal opinion only** (not reflective of AusNAC/EUCAST)

Changes to oral fosfomycin breakpoints

If agar dilution (+ 25 mg/L G6P) remains the reference MIC method

- If the S category is reduced to ≤ 2 mg/L (Enterobacterales), this would
  - Accurately classify *E. coli* isolates that do not have a resistant subpopulation as susceptible
  - Classify the majority of wild-type *K. pneumoniae* as non-susceptible
- While *E. coli* isolates with MICs 4 – 32 mg/L may, or may not, have a resistant subpopulation
  - In these isolates, an additional heteroresistance screen could identify isolates that would still respond to therapy
- In the absence of agar dilution MIC, disk diffusion appears to perform better than gradient strip MICs
  - Although difficulties existing with reading results (whether or not to ignore colonies)

If the reference MIC method changes to BMD (without G6P):

- Potentially may reflect a more relevant MIC value for a urine-specific breakpoint
- May not need to greatly alter existing MIC breakpoint values

Conclusions

Fosfomycin frustrations… forever or finished?

- Target pathogens
  - Single dose oral fosfomycin remains an attractive and **efficacious option** for *E. coli* uUTIs
  - Also has good **bacteriostatic activity** against *Enterococcus* spp.
  - **Less certain** activity against other Enterobacterales and *Pseudomonas aeruginosa*
  - **Multi-dose regimens** promoted emergence of resistance (when heteroresistance present at baseline)
- Fosfomycin AST
  - Agar dilution MIC (with 25 mg/L G6P) appears to be a **poor gold standard** AST MIC method
    - Does not identify isolates with a resistant subpopulation important in treatment failure
- Clinical breakpoints
  - **EUCAST**: Plan to do Monte-Carlo simulations to account for PK variability and extrapolate to UTIs
  - **CLSI**: No change to current advice; will r/v all data about G6P; await PK-PD/animal data for non-*E. coli* species
- Await the outcome data from clinical trials
  - FORECAST: cUTI, iv to oral switch, ciprofloxacin vs. fosfomycin, daily to complete 10 days
  - FOREST: iv fosfomycin vs. meropenem bacteraemic UTI caused by ESBL-*E. coli*

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iOASIS
In-vitro **O**ptimisation of **A**ntimicrobials
for **S**uperbug **I**nfection**S**

Poster Number: P47
Session: Poster Session 2
Date: Friday 28 February, 2020
Time: 1530-1615

MONASH University

Susceptibility discrepancies

Some of other people who have published their “fosfomycin frustrations”

- Fuchs PC. Susceptibility testing quality control studies with fosfomycin tromethamine. *Eur J Clin Microbiol Infect Dis* 1997; **16**: 538-40.
- de Cueto M. In vitro activity of fosfomycin against extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: comparison of susceptibility testing procedures. *Antimicrob Agents Chemother* 2006; **50**: 368-70.
- Lopez-Cerero L. Evaluation of the Etest method for fosfomycin susceptibility of ESBL-producing Klebsiella pneumoniae. *J Antimicrob Chemother* 2007; **59**: 810-2.
- Diez-Aguilar M. In vitro activity of fosfomycin against a collection of clinical Pseudomonas aeruginosa isolates from 16 Spanish hospitals: establishing the validity of standard broth microdilution as susceptibility testing method. *Antimicrob Agents Chemother* 2013; **57**: 5701-3.
- Perdigao-Neto LV. Susceptibility of multiresistant gram-negative bacteria to fosfomycin and performance of different susceptibility testing methods. *Antimicrob Agents Chemother* 2014; **58**: 1763-7.
- Hirsch EB. Activity of fosfomycin and comparison of several susceptibility testing methods against contemporary urine isolates. *Int J Antimicrob Agents* 2015; **46**: 642-7.