Challenging drugs for AST: e.g. fosfomycin, polymyxins

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Why do we do susceptibility testing?
To try to predict the probability of successful treatment

How do we do susceptibility testing?
Using a reference method or a validated alternative

What is the major prerequisite?
A relationship between labresult (reference method or alternative) and outcome
Essentials of PKPD

ACTIVITY
in vitro (MIC)

CONCENTRATIONS
in vivo (PK)

DOSING
regimen

ANTIMICROBIAL EFFICACY
(Microbiological Cure)

Other factors

CLINICAL EFFICACY
(Clinical Cure)
The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach

Preclinical PK/PD studies

Correlation exposure–effect

Clinical PK/PD studies

Correlation exposure–effect

Qualitative relationship (PK/PD index)

Quantitative relationship (value PK/PD index)

PD target

Clinical dosing regimen

Monte Carlo simulations

Initial PK/PD breakpoint

MCS robustness target population dose adjustments

PK/PD breakpoint

MIC distributions

FIG. 7. Summary of the process of setting pharmacokinetic/pharmacodynamic (PK/PD) breakpoints by EUCAST.
The Case of Fosfomycin

- As an old drug developed more than 40 years ago has not undergone the rigorous development and regulatory scrutiny to which are new agents subject
- The “label” was not updated as new information became available
- As with all old antibiotics without redevelopment:
  - Susceptibility breakpoints may not be correct
  - Doses were never optimized using present pkpd approaches
  - Indications may not all be valid
Why do we do susceptibility testing?

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Susceptibility testing fosfomycin

- Gold standard: agar dilution (ISO 20776-1)
Susceptibility testing fosfomycin

- Gold standard method: agar dilution
- Other available methods:
  - Disc diffusion
  - Gradient tests
  - Automated systems:
    - Vitek
    - Phoenix

How do the methods perform in comparison to the gold standard method?
### Background information (EUCAST)

#### Enterobacteriaceae (new taxonomy: Enterobacterales*)

<table>
<thead>
<tr>
<th>Miscellaneous agents</th>
<th>MIC breakpoint (mg/L)</th>
<th>Disk content (µg)</th>
<th>Zone diameter breakpoint (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S ≤</td>
<td>R &gt;</td>
<td></td>
</tr>
<tr>
<td>Fosfomycin iv</td>
<td>32²</td>
<td>32²</td>
<td>200^B</td>
</tr>
<tr>
<td>Fosfomycin oral (uncomplicated UTI only)</td>
<td>32²</td>
<td>32²</td>
<td>200^B</td>
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</table>

2. Agar dilution is the reference method for fosfomycin. MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturers’ instructions for commercial systems.

C. Zone diameter breakpoints apply to *E. coli* only. For other Enterobacteriaceae, use an MIC method.

D. Ignore isolated colonies within the inhibition zone (see pictures below).

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Examples of inhibition zones for *Escherichia coli* with fosfomycin.

a-c) Ignore all colonies and read the outer zone edge.

d) Record as no inhibition zone.
Fosfomycin and *E. coli*

![Graph showing MIC values for Fosfomycin against *E. coli*.](image)

**MIC**
- Epidemiological cut-off (ECOFF): 8 mg/L
- Wildtype (WT) organisms: ≤ 8 mg/L

5117 observations (7 data sources)
• 10 laboratories in the Netherlands
• In total:
  • 771 *E. coli*
  • 201 *K. pneumoniae*
• Inclusion criteria: ESBL-positive isolates (from urine, blood or respiratory samples in 2016 or 2017)
• Methods:
  • Agar dilution (gold standard, central laboratory)
  • Disc diffusion (oxoid)
  • Gradient tests (Etest and MTS)
  • Automated systems (Vitek and Phoenix)
• Interpretation: EUCAST criteria
Distribution- agardilution

Graph A: Fosfomycin / Escherichia coli
- MIC range: 0.002 - >128 mg/L
- No. of Microorganisms

Graph B: Fosfomycin / Klebsiella pneumoniae
- MIC range: 0.002 - >128 mg/L
- No. of Microorganisms

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Reading challenges
Results – Categorical agreement – overall numbers

![Bar chart showing the number of categorical errors for different tests: Etest, Mic Test Strip, Vitek2, and Phoenix. The chart indicates the number of categorical agreements and categorical errors, with very major errors highlighted in red.]

ESCMID eLibrary © by author
Error rates - overall results - percentages -

**Error rates**

- **Etest**
- **Mic Test Strip**
- **Vitek2**
- **Phoenix**

**Major error rate**

\[
\text{Major error rate} = \frac{\# \text{ of major errors}}{\text{total } \# \text{ of susceptible isolates}} \times 100
\]

**Very major error rate**

\[
\text{Very major error rate} = \frac{\# \text{ of very major errors}}{\text{total } \# \text{ of resistant isolates}} \times 100
\]

JWM Amsterdam 13-04-2019
### Error rates per micro-organism

**Table 1.** Categorical agreement and error rates for five antimicrobial susceptibility testing methods compared with agar dilution MIC results

<table>
<thead>
<tr>
<th>Method</th>
<th>isolates tested</th>
<th>categorical agreement</th>
<th>ME rate</th>
<th>VME rate</th>
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<tbody>
<tr>
<td>Etest</td>
<td>773</td>
<td>99.0% (765/773)</td>
<td>0.1% (1/743)</td>
<td>23.3% (7/30)</td>
<td>198</td>
<td>84.8% (168/198)</td>
<td>14.2% (25/176)</td>
<td>22.7% (5/22)</td>
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<tr>
<td>MTS</td>
<td>769</td>
<td>99.2% (763/759)</td>
<td>0.1% (1/742)</td>
<td>18.5% (5/27)</td>
<td>196</td>
<td>87.2% (171/196)</td>
<td>11.5% (20/174)</td>
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<tr>
<td>Vitek2</td>
<td>774</td>
<td>99.0% (766/774)</td>
<td>0.3% (2/742)</td>
<td>18.8% (6/32)</td>
<td>201</td>
<td>94.5% (190/201)</td>
<td>4.0% (7/176)</td>
<td>16.0% (4/25)</td>
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<td>Phoenix</td>
<td>208</td>
<td>99.5% (207/208)</td>
<td>0.0% (0/200)</td>
<td>12.5% (1/8)</td>
<td>201</td>
<td>93.0% (187/201)</td>
<td>6.3% (11/176)</td>
<td>12.0% (3/25)</td>
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<td>774</td>
<td>98.4% (762/774)</td>
<td>1.1% (8/751)</td>
<td>12.9% (4/31)</td>
<td>—</td>
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- Categorical agreement: high, especially for *E coli*
- Major errors (reference method S, and test-method R):
  - *E coli*: 1-1.1%
  - *K pneumoniae*: 4-14.2%
Very major error rates

**Table 1.** Categorical agreement and error rates for five antimicrobial susceptibility testing methods compared with agar dilution MIC results

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- **Very Major errors** (reference method R, and test-method S):
  - *E coli*: 12.5 – 23.3 %
  - *K pneumoniae*: 12-22.7%
- All methods used in routine laboratories have problems detecting resistant strains
Disc diffusion: difference with new ECOFF?

Suggested breakpoint $S \geq 16\text{mm}$

**E. coli**

- ECOFF from this study
- Current EUCAST breakpoint

**K. pneumoniae**

- Current EUCAST breakpoint
- ECOFF from this study
Disk Diffusion

Fosfomycin 200µg vs MIC E.coli

Number of isolates

Zone Diameter (mm)

- >128
- 128
- 64
- 32
- 16
- 8
- 4
- 2
- 1
- 0.5
- 0.25
Disk Diffusion

Fosfomycin 200µg vs MIC K. pneumoniae

Zone Diameter (mm)

Number of isolates

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Conclusion

• Total of 972 isolates
  • 57 gold standard method isolates: R
• ESBL positive, from clinical cultures
• *E. coli* and *K. pneumoniae*
• All methods commonly used in routine laboratories have problems with detecting resistance
Solutions?

• It has been suggested to use a different inoculum only for fosfomycin
Susceptibility testing with Etest vs reference

-3 -2 -1 0 1 2 3

Mean MIC

Red line: perfect test

Difference between MICagar - MICetest
Susceptibility testing with Etest vs agar

Díez-Aguilar AAC. 2015 p1158.
Standard inoculum  

Low inoculum  

It looks better, but it is not convenient in the routine laboratory……  

Díez-Aguilar AAC. 2015 p1158.
Overall conclusion

- Main resistance mechanism are mutations resulting in changes in the influx in the bacterial cell
- Up to now there is not a reliable method that can easily used in a clinical routine laboratory
- Problem is that methods fail to detect resistant strains
Why do we do susceptibility testing?
To try to predict the probability of successful treatment

How do we do susceptibility testing?
Using a reference method or a validated alternative

What is the major prerequisite?
A relationship between labresult (reference method or alternative) and outcome
PD of fosfomycin – oral dosing
• Simulates normal human urodynamics on a 1:16 scale
• Multiple bladder compartments ($n = 16$) run in parallel
In vitro pharmacokinetic simulation

Dynamic changes in fosfomycin concentrations:

$C_{\text{max}} \approx 2000 \text{ mg/L at 7.5 h}$

Elimination $t_{1/2} = 6.9$-hours

Conc. $>128$ mg/L for 40-hours

Abbott et al, JAC 2018
Bladder model 3 days
Fosfomycin oral

(a) E. coli

#4807 (MIC 32 mg/L)

#1231 (MIC 16 mg/L)

#12620 (MIC 2 mg/L)

Time (h)

Log$_{10}$ cfu/mL

L.O.D.

(b) E. cloacae

#10 (MIC 64 mg/L)

#9 (MIC 32 mg/L)

#94 (MIC 1 mg/L)

Time (h)

Log$_{10}$ cfu/mL

L.O.D.

(c) K. pneumoniae

#50 (MIC 8 mg/L)

#17 (MIC 4 mg/L)

#31865 (MIC 2 mg/L)

Time (h)

Log$_{10}$ cfu/mL

L.O.D.


Resistant subpopulations
Pharmacodynamics

- Quantitative growth on MHA
- Concordant 72h PD outcomes
- Reduced time to re-growth seen in urine

16 isolates

8 killed  \( \rightarrow \) 8 re-grew

Urine

2 / 8 *E. coli*
2 / 4 *E. cloacae*
4 / 4 *K. pneumoniae*

72h PK/PD Exposure-response results indicate that after a single 3g dose:

- 72h PK/PD Exposure-response results indicate that after a single 3g dose:
  - *E. coli* and *E. cloacae* isolates with an MIC > 4 mg/L would not be reliably killed
  - **Questionable utility** against *K. pneumoniae* isolates, regardless of baseline MIC
  - Baseline HLR subpopulation is an important predictor for re-growth

- EUCAST fosfomycin MIC susceptible breakpoint is ≤ 32 mg/L

- MIC cut-off found in exposure-response analysis in the bladder infection *in vitro* model is ≤ 4 mg/L
Relevance of the results

- EUCAST fosfomycin MIC susceptible breakpoint is ≤ 32 mg/L
- MIC cut-off found in exposure-response analysis in the bladder infection in vitro model is ≤ 4 mg/L
- This value agrees with the suggested E. coli ECOFF of 4 mg/L\(^1\)
  - Note: K. pneumoniae isolates have higher MICs (ECOFF 32 mg/L)
  - Further questions the utility against these isolates

\(^1\)Van Den Bijl

[Graphs showing bacterial distribution]
Repeated dosing against *P. aeruginosa*

- 16 isolates, dosed once daily and either once or 7 days exposure
- Agar dilution MICs 1 - >1024 mg/L
- Dose once: all isolates regrew
- Dose 7 times all isolates regrew
Species and baseline resistance are more predictive than fosfomycin MIC for therapeutic success in urinary tract infections

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2. Erasmus Medical Centre, Rotterdam, The Netherlands

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J. Dekker\textsuperscript{2}
RA. Wijma\textsuperscript{2}
BCM. de Winter\textsuperscript{2}
AY. Peleg\textsuperscript{1}
JW. Mouton\textsuperscript{2}
• 44 clinical isolates
  • 24 *E. coli*; 20 *K. pneumoniae*
  • 42 (95%) ESBL-producing pathogens
  • 38 (86%) from a urinary source

• Selected with a range of fosfomycin MIC values (by agar dilution)
Efficacy of fosfomycin

- *E. coli*
  - all isolates in WT killed
  - MICs>2 mg/L: often efficacy but regrowth did occur

- *K. pneumoniae*
  - Only 3/20 were killed

Description method for *E. coli*
Not very effective against *K. pneumoniae*
Hollow fiber model 4 days
Fosfomycin IV

Dosing regimen: 4g Q24
Daily dose: 4g
$C_{\text{max}}$: 200 mg/L
AUC: 7443 mg.h/L

Dosing regimen: 4g Q8
Daily dose: 12g
$C_{\text{max}}$: 200 mg/L
AUC: 15757 mg.h/L
PD analysis HFIM

(a) *C. freundii*, *E. cloacae* and *E. coli* isolates

24-hours

- ELₐ₀ = 75.2
- R² = 0.6936

- ELₐ₀ = 7.0
- R² = 0.6084

- ELₐ₀ = 33.2
- R² = 0.8692

96-hours

- ELₐ₀ = 212.0
- R² = 0.7554

- ELₐ₀ = 27.2
- R² = 0.4965

- ELₐ₀ = 55.1
- R² = 0.2563

(b) *K. pneumoniae* isolates

24-hours

- ELₐ₀ = 87.3
- R² = 0.8556

- ELₐ₀ = 9.0
- R² = 0.8189

- ELₐ₀ = 42.1
- R² = 0.8331

96-hours

- ELₐ₀ = 5026.0
- R² = 0.8545

- ELₐ₀ = 377.8
- R² = 0.8526

- ELₐ₀
- R²

Legend:
- *C. freundii*
- *E. cloacae*
- *E. coli*
- *K. pneumoniae*
Fosfomycin – current assessment

- Breakpoints are (far) too high
  - Covers only *E. coli* Wild Type,
  - BOTH ORAL and IV

- Klebsiella is NOT a good target, primarily due to resistance mechanisms and high MICs (e.g. FosA)
Why do we do susceptibility testing?

To try to predict the probability of successful treatment  NO

How do we do susceptibility testing?

Using a reference method or a validated alternative  NO

What is the major prerequisite?

A relationship between lab result (reference method or alternative) and outcome SOMETIMES
Conclusions

• There is at present no reliable susceptibility method for fosfomycin
• New alternatives are being explored
• Clinical breakpoints of *E. coli* should be modified
• Studies with *K. pneumoniae* indicate that this species is not a very good target
• A similar conclusion for *P. aeruginosa*
• The effectiveness in combination therapy is another issue