In vitro pharmacodynamics of fosfomycin against clinical isolates of carbapenem-resistant *Klebsiella pneumoniae*

**Jocelyn Teo**¹, Audrey Goh², Tze-Peng Lim¹, Si-Xuan Tan¹, Yiying Cai¹, Winnie Lee¹, Tse-Hsien Koh¹, Thuan-Tong Tan¹, Andrea Kwa¹,²,³

¹ Singapore General Hospital  
² National University of Singapore  
³ Duke-NUS Medical School
Background


Fosfomycin

Retains activity against several MDR pathogens

- Unique chemical structure and mechanism of action
- Unlikely for development of cross-resistance

Therapeutic potential of IV formulation against systemic CRE infections

- Only oral formulation approved by FDA for acute uncomplicated UTI caused by E. coli and E. faecalis

Knowledge gap in PK and PD

- Discovered more than 40 years ago

To investigate the *in vitro* pharmacodynamics of fosfomycin disodium against clinical isolates of carbapenem-resistant *Klebsiella pneumoniae*

- Minimum Inhibitory Concentrations (MICs)
- Population Analysis Profiles (PAPs)
- Time-Kill Studies (TKS)
Selection Criteria

8 clinical isolates of *Klebsiella pneumoniae*

- **Non-stool** origin
- **Carbapenem resistant** phenotype
- **Diverse** resistance mechanisms
- Varied **fosfomycin** susceptibility

ATCC 13883 *K. pneumoniae* reference strain
Susceptibility testing

Agar Dilution

**fosA gene detection**

- Presence of *fosA* gene was detected via PCR according to a published protocol\(^1\)

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Population Analysis Profiles

1. Log phase culture

![Image of a log phase culture with 10^8 CFU/mL]

2. 10-fold serial dilutions

![Image of serial dilutions with concentrations 10^8 to 10^3]

3. Spread 50µL

![Image showing spread of 50µL with concentration less than MIC and equal to or more than MIC]

4. Incubate

![Image showing incubation at 35°C for 24h]

Concentration less than MIC:
- 10^6
- 10^7
- 10^8

Concentrations equal to or more than MIC:
- 10^5
- 10^4
- 10^3

Time-kill Studies

1. Shaking water bath at 35 °C for 24h
2. Log phase culture of 10^5 CFU/mL
3. Dilute, plate, incubate

Fosfomycin (0.5x MIC – 2048mg/L)

1mL + 15mL

5. Plot time-kill profiles
6. Inhibitory sigmoid Emax model fitted to the mean bacterial killing effect at 24h with ADAPT II software

# Susceptibility

Based on **EUCAST** clinical breakpoints for **Enterobacteriaceae**

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Isolate No.</th>
<th>Site of Culture</th>
<th>MIC (mg/L)</th>
<th>Susceptible to Fosfomycin</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>KP</td>
<td>1963</td>
<td>Blood</td>
<td>≥64</td>
<td>No</td>
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<tr>
<td>2</td>
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<td>Urine</td>
<td>≥64</td>
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<td>KP</td>
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<td>Urine</td>
<td>≥64</td>
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<td>Urine</td>
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</tr>
</tbody>
</table>

- **8 CR-KP**
- **Four fosfomycin resistant**
- **Four fosfomycin susceptible**
- **Four KP isolates (4 – 16mg/L)**

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Population Analysis Profiles

- **Heteroresistance detected in all isolates**
- **Regardless whether S/R and fosA+/−**
- **Concentration ↑, frequency ↓**
- **At 2048mg/L, resistant subpopulations existed in the majority of the isolates**
Heteroresistance detected in all isolates

Regardless whether S/R and fosA+/-

Concentration ↑, frequency ↓

At 2048mg/L, resistant subpopulations existed in the majority of the isolates
Time-kill Studies

“Low” MICs
- MIC 4mg/L
- MIC 8mg/L
- MIC 8mg/L

“Medium” MICs
- MIC 16mg/L
- MIC 64mg/L
- MIC 64mg/L

- Killing occurred up till 2h–4h, followed by regrowth.
- Maximum killing was >3Log_{10} CFU/mL.
- Bactericidal activity (≥3Log_{10} CFU/mL ↓ in 24h bacterial counts) was not observed in any isolates.
- Regardless whether S/R and fosA+/-
“High” MICs

- Killing occurred up till 2h – 4h, followed by regrowth
- Maximum killing was >3Log_{10} CFU/mL ↓
- Bactericidal activity (≥3Log_{10} CFU/mL ↓ in 24h bacterial counts) was not observed in any isolates
- Regardless whether S/R and fosA+/−
Inhibitory Sigmoid-Emax Effect Models

"Low" MICs
- MIC 4mg/L
- MIC 8mg/L

"Med" MICs
- MIC 16mg/L
- MIC 64mg/L
- MIC 64mg/L

"Low" MICs
- MIC 4mg/L
- MIC 8mg/L

"Med" MICs
- MIC 16mg/L
- MIC 64mg/L
- MIC 64mg/L
Beyond 256 – 512mg/L, no additional killing was observed.

Bacterial counts remained around 5Log_{10} CFU/mL regardless whether S/R and fosA+/−.

**Inhibitory Sigmoid-Emax Effect Models**
Conclusions

• Fosfomycin monotherapy should not be used in systemic infections caused by carbapenem-resistant *K. pneumoniae*

• Despite low fosfomycin MICs, heteroresistance is high in these organisms

• Fosfomycin MICs do not predict the extent of bacterial killing
Questions to ponder..

• What is the maximum systemic fosfomycin exposure achievable?
• Fosfomycin-based combination therapy may be explored to overcome CRE infections?
• What about other carbapenem-resistant organisms; *Enterobacter cloacae, E. coli.*?
Acknowledgments

Funding Agencies

• National Medical Research Council
• SingHealth Foundation
• Singapore General Hospital Research Grants

Special thanks to Ms Audrey Goh for in part contribution to the slides