In vivo development of tigecycline resistance in *Klebsiella pneumoniae*

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Klebsiella pneumoniae

- A member of Enterobacteriaceae
- Common opportunistic pathogen responsible for hospital-acquired infections
- Cause urinary tract infections, pneumonia, bacteremia and soft tissue infections
Antibiotic resistance among *K. pneumoniae* is increasing

Carbapenemase-resistance trends among *K. pneumoniae* clinical isolates in China

Data was collected from CHINET surveillance network.
Tigecycline

Vaida Šeputienė, Medicina (Kaunas) 2010; 46(4):240-8
Regulation of AcrAB

RamR mutations in clinical isolates of *K. pneumoniae* with reduced susceptibility to tigecycline

Schematic diagram of the genomic region comprising *ramR* and *ramA* of *K. pneumoniae* MGH 78578

M. Hentschke et al., AAC, 2010
Study on development of tigecycline resistant *K. pneumoniae* during tigecycline therapy

Patient history

• A 50-year-old man was admitted to Huashan Hospital in Shanghai on September 10, 2015, with pain in the left side of scrotum.

• On admission, he had leukocytosis and computed tomography showed abscess in his left scrotum.

• Culture from urine was positive for *K. pneumoniae*, which was resistant to almost all antibiotics tested, except for tigecycline and polymyxin.
Tigecycline resistance in *K. pneumoniae* that was evolved during tigecycline therapy

The timeline of antibiotic usage and phylogenetic distance analysis of 4 consecutively isolated *K. pneumoniae*
The tigecycline resistant *K. pneumoniae* isolates exhibit high expression level of *acrB*

qRT-PCR assessment of transcriptional level of *ramA* and *acrB*.
Regulation of *acrAB*

![Diagram of regulation of acrAB]

AcrR

RamA

RamR

**mutation**
The tigecycline resistant *K. pneumoniae* isolates have a 12-bp deletion in the RBS region upstream of *ramR*

**Aligned sequences of the *ramR* regions among 4 *K. pneumoniae* clinical isolates**
Gene transcription and translation

TSS rbs ramR

transcription

mRNA

translation

protein

RamR

Tttgtttaaacctgcgtgagaaaaaagtagtggttgctcggtccaaaggagtgaagataaaag......

Kp-1S

tttgcttcacatgcttagtccatgeoagatgagctgtatagcttagtccatgeoagatgagctgtatagc...

Kp-3R

Tttgcttcacatgcttagtccatgeoagatgagctgtatagc......
The tigecycline resistant *K. pneumoniae* isolates are not defective in *ramR* transcription, but have impaired RamR protein production.

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**A**

**Q-RT-PCR**

**B**

**Coomassie gel**

**C**

**Immunoblot**
XylE reporter analysis

XylE:

- Codes for Catechol (2,3) dioxygenase (C23O)
- C23O catalyzes the conversion of colorless catechol into yellow 2-hydroxymuconic semialdehyde

Catechol \[ \rightarrow \text{C23O} \rightarrow 2\text{-hydroxymuconic semialdehyde} \]
Complementation of a functional $ramR$ restores suppression of the expression of $acrB$ in KP-3R and KP-4R.
Complementation of a functional *ramR* partially restores susceptibility to tigecycline.

<table>
<thead>
<tr>
<th>Strains</th>
<th>KP-1S</th>
<th>KP-2S</th>
<th>KP-3S</th>
<th>KP-4S</th>
<th>KP-3R/pMY43-Ara</th>
<th>KP-4R/pMY43-Ara</th>
<th>KP-3R/pMY43+Ara</th>
<th>KP-4R/pMY43+Ara</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (µg/ml)</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Summary

• Tigecycline resistant isolates can emerge after 41 days of tigecycline treatment.

• Deletion of RBS of \textit{ramR} is the mechanism contributing to rapid emergence of tigecycline resistance \textit{in K. pneumoniae} during tigecycline therapy.
Thank you