Mécanismes d’adaptation et de Résistance de *Pseudomonas aeruginosa* à la colistine dans la mucoviscidose

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Polymyxins

- AntiMicrobial Peptides family
- Cyclic Polycationic lipopeptides
- Polymyxin E (colistin) and polymyxin B
- Colistin is produced by *Paenibacillus polymyxa colistinus*
- IV and nebulization
  - Colistin Methane-Sulfonate (CMS)
  - Acting as a prodrug
  - Without antimicrobial activity
  - Hydrolyzed in colistin
Membrane alterations

**OM**: Outer Membrane

**M**: Murein/PG

**PM**: Inner membrane

These molecules interact with the negative charges of the outer membrane.

Benincasa et al. *Antimicrob Agents Chemother* 2009, 53


No antibiotic

Polymyxin B 30µg/mL
Acquired Resistance to colistin

Report of SENTRY Antimicrobial Surveillance Program (2006-2009)
- 0.4% of *P. aeruginosa* and 0.9% of *Acinetobacter spp* are resistant to colistin
  
  *Gales AC et al. J Antimicrob Chemother. 2011, 9*

Emergence of strains resistant to colistin
- In **CF** patients
  - Low to high resistance levels (8 to 512 mg/L)

- In **non CF** patients
  - Low to moderate resistance levels (4 to 64 mg/L)
    *Wang CY et al. Clin Microbiol Infect. 2006, 12*
    *Falagas ME et al. J Antimicrob Chemother. 2007, 67*
    *Abraham N et al. FEMS Microbiol Lett. 2009, 298*
    *Schurek KN et al. Antimicrob Agents Chemother. 2009, 10*
    *Barrow K et al. Antimicrob Agents Chemother. 2009, 12*
    *Muller C et al. Antimicrob Agents Chemother. 2011, 3*
LPS modification

Modification of lipid A
Addition of 4-amino-4-deoxy-L-arabinose (Ara4N) to the 1 and 4’ phosphate groups

Lam JS, Front Microbiol. 2011; 2
Ernst RK et al. J. Infect. Dis. 2007, 196
**Resistance to colistin**

- Encoded by *arnBCADTEF-ugd* operon
- Responsible for synthesis and transport of Ara4N through the inner membrane
- Expression of *arn* operon is dependent on two-component systems

Classic two-component regulatory systems

64 Response regulators
63 Histidine kinases
Acquired resistance to polymyxins

- PhoP
- PmrB
- ParS
- CprS
- ColS

**PhoQ**

- **PhoP**
- **PmrA**
- **ParR**
- **CprR**
- **ColR**

**10 CF clinical strains isolated from chronically colistin treated CF patients**
- MIC to colistin >512 μg/mL


**11 CF clinical strains isolated from chronically colistin treated CF patients**
- MIC to colistin from **256 to >512** μg/mL


**CF clinical strain isolated from chronically colistin treated CF patients**
- MIC to colistin **4** μg/mL

*Muller C et al. Antimicrob Agents Chemother. 2011, 3*
Tolerance to colistin

PAO1 strain

Wild type population, MIC=2 µg/mL

Majority dies

Resistant adapted sub-population
Tolerance to colistin *ex-vivo*/*in vivo*

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Sexe</th>
<th>Infection by <em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9-22</td>
<td>H/F</td>
<td>No</td>
</tr>
<tr>
<td>II</td>
<td>23/63</td>
<td>H/F</td>
<td>No</td>
</tr>
<tr>
<td>III</td>
<td>19-51</td>
<td>H</td>
<td>Yes</td>
</tr>
<tr>
<td>IV</td>
<td>18-50</td>
<td>F</td>
<td>Yes</td>
</tr>
</tbody>
</table>

CF sputum

Mutant PAO1Δ*arn::luxCDABE*

Murine model of acute *P. aeruginosa* pneumonia
Colistin concentrations after nebulization

- 20 patients with ventilator-associated pneumonia (VAP)
- Gram negative bacteria only susceptible to polymyxins
- CMS administered at a dose of 80 mg (1 MUI) every 8 h for 7 days.

Conclusions

- Acquired resistance of clinical strains of *P. aeruginosa* to polymyxins is associated with mutations in proteins PmrB, PhoQ, ParS and/or ParR
- Resistance levels in CF strains (4 to 128 µg/mL)
- Additional *arn*-independent mechanisms likely contribute to elevated colistin resistance in CF strains
- Tolerance to polymyxins is a drug-induced, *arn*-independent, reversible adaptive process that helps *P. aeruginosa* to survive clinically relevant concentrations of drugs (up to 8 µg/mL)
- Pathogenicity of resistant strains in non CF and CF patients remains to be established.
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MDR, XDR and PDR

Wild type strain

Souche clinique XDR

Polymyxins remain the last hope

Magiorakos AP et al. Clin Microbiol Infect 2012; 18