Genetic characterization of lipopolysaccharide-modifying genes involved in polymyxin resistance in *E. coli* and *K. pneumoniae* carrying MCR-1 by sequential time-kill experiments approach

Hariyanto IH

INSERM U1070 – Pharmacology of Antimicrobial Agents

POITIERS

15e congrès national de la SFM, 30 septembre - 2 octobre 2019, Paris
Occurrence of carbapenemase-producing Enterobacteriaceae (K. pneumoniae and E. coli) in 38 European countries

European Centre for Disease Prevention and Control, Stockholm, 2016

European Antimicrobial Resistance Surveillance Network (EARS-Net), 2017
Antibiotic development and antimicrobial resistance

Solution?

Combine 2 or 3 antibiotics

or

Re-introduce ‘old’ antibiotics

COLISTIN
Colistin (polymyxin E) & polymyxin B

- Polymyxins class; Cationic Antimicrobials Peptides (CAMPs)
- In 1970s, it was replaced by newer antibiotic because of its side effect (nephrotoxicity >20%)*
- Early 1990s, It is increasingly being used as a “Last resort drug” to overcome infections caused by multidrug-resistant GNB (MDR(-))
- In particular *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* & *E. coli*
- Infections caused by GNB are the most difficult infections to treat because of their ability to develop into the intrinsic drug resistance


Polymyxin Resistance Reports

Introduction

Mechanism of Resistance

Methods

Results

Conclusion

Spread of colistin resistant non-mucoid Pseudomonas aeruginosa among chronically infected Danish cystic fibrosis patients

Helle Krogh Johansen a,⁎, Samuel M. Moskowitz b, Oana Ciofă b, Tiejana Pressler a, Niels Hnibly a,⁎

⁎ Department of Clinical Microbiology, Dept. 89/1 and Danish Cystic Fibrosis Centre, Dept. 50/6, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

a Division of Pulmonary Medicine, Children’s Hospital and Regional Medical Center and University of Washington School of Medicine, Seattle, Washington 98195, USA

Received 30 September 2003, received in revised form 27 January 2004; accepted 4 February 2004

Available online 20 March 2004

Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies

Yun Cai, Dong Chei, Rui Wang⁎, Beilei Liang and Non Bai

Department of Clinical Pharmacology, the PLA General Hospital, Beijing 100853, People’s Republic of China

EMJ Infect Dis 2017;21(1):98-101

Brief communication

Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years

Flávia Rossi a,⁎, Raquel Girardello a, Ana Paula Cury a,⁎, Thais Sabato Romano Di Gioia a,⁎, Joã o Nóbrega de Almeida Jr a,⁎, Alberto José da Silva Duarte a

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Decreased Susceptibility to Polymyxin B during Treatment for Carbapenem-Resistant Klebsiella pneumoniae Infection

All Reports from clinical isolates
LPS Modification

Mechanism of Resistance

L-Ara4N

PEtN
Molecular Pathway of LPS Modification

Two-Component regulatory systems (TCS)

Olaitan et al. 2014. Frontiers in Microbiology | Antimicrobials, Resistance and Chemotherapy
Plasmid-Mediated Resistance

MCR: Mobilizable Colistin Resistance
→ Phosphoethanolamine transferase (addition of PEtN to lipid A)
Objective

Role of MCR-1 in the development of additional adaptive resistance to polymyxins by an original approach of sequential time-kill study
**Introduction**

**Mechanism of Resistance**

**Methods**

**Results**

**Conclusion**

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*All strains were kindly provided by P. Nordmann*

**Population Analysis Profils (PAPs)**

**MICs Determination (After TKC)**

**DNA Sequencing**
- To determine the order of nucleotides in specific DNA
- Genome mutations

**RT – qPCR Analysis**
- RNA Extraction → cDNA
- Quantitatively measure gene expression based on RNA transcript levels

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**Susceptible**

- *E.coli J53*
- *K. pneumoniae R2292*

**Resistant**

- *E.coli J53_MCR-1*
- *K.pneumoniae R2292_MCR-1*

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1st Time-Kill Curve

2nd Time-Kill Curve

3rd Time-Kill Curve

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Biomolecular analysis

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To determine the order of nucleotides in specific DNA

Genome mutations
### MICs Result (mg/L)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E. coli J53</th>
<th>K. pneumoniae R2292</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>+ MCR-1</td>
</tr>
<tr>
<td>Colistin (CST)</td>
<td>0.25</td>
<td>2-4</td>
</tr>
<tr>
<td>Polymixin B (PMB)</td>
<td>0.25</td>
<td>2</td>
</tr>
</tbody>
</table>

**WT** : Wild-type (non-carrying-MCR-1)

+ MCR-1 : inserted by plasmid MCR-1

Susceptible : MICs < 2 µg/mL

Resistant : MICs ≥ 2 µg/mL

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**TIME-KILL CURVE ANALYSIS**
Colistin (CST) & Polymyxin B (PMB) shown rapid and concentration-dependent bacterial killing during Time-Kill Curve (TKC).

The highest concentration of antibiotic where bacteria can regrow over $10^6$ CFU/mL after 30 hours considered as MAXIMUM REGROWTH CONCENTRATION.

For all WT Strains (not-carrying-MCR-1), the regrowth was stable and observed at 0.5x MIC (0.125 mg/L) in both of 1st and 2nd TKC.

No Adaptation was found.

RT-qPCR Analysis.
Genes expression level after sequential TKC for WT strains

➢ No different gene expression was shown between 1st and 2nd TKC for both species

➢ Down-expression of *phoP* and over-expression of *lpxM* for *E. coli* in CST & PMB

➢ Presumably were triggered by polymyxins pressure
Sequential Time-Kill Curve

**Colistin vs MCR-1 transconjugants**

- **COLISTIN vs E. coli_MCR-1**
  - Maximum regrowth concentrations are: 2x MIC (4 mg/L) for 2\(^{nd}\) TKC and 4x MIC (8 mg/L) for 3\(^{rd}\) TKC

- **COLISTIN vs K. pneumoniae_MCR-1**
  - Maximum regrowth concentration increased gradually up to 4x MIC (8 mg/L) for 2\(^{nd}\) TKC and 32x MIC (64 mg/L) for 3\(^{rd}\) TKC
Both MCR-1 transconjugants strains adapted rather slowly in PMB than in CST

- Maximum regrowth concentrations are: 1x MIC (2 mg/L) for 2nd TKC and 2x MIC (4 mg/L) for 3rd TKC

- Maximum regrowth concentrations are: 4x MIC (8 mg/L) for 2nd TKC and 8x MIC (16 mg/L) for 3rd TKC

NO ANTIBIOTIC DEGRADATION was found AFTER 30 HOURS

Both MCR-1 transconjugants strains adapted rather slowly in PMB than in CST

- Maximum regrowth concentrations are: 1x MIC (2 mg/L) for 2nd TKC and 2x MIC (4 mg/L) for 3rd TKC

- Maximum regrowth concentrations are: 4x MIC (8 mg/L) for 2nd TKC and 8x MIC (16 mg/L) for 3rd TKC
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Population analysis profiles (PAPs)

Bacterial population

S

R

Spiral plating in MH agar with various concentrations of ATB (0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 10 mg/L)

Both carrying-MCR-1 strains had NO ABLE TO GROW in the presence of antibiotics at concentrations higher than 2 mg/L (cutoff at 10^6 CFU/mL)

REGROWTH in SEQUENTIAL TKC WERE NOT CAUSED by RESISTANT SUBPOPULATION
<table>
<thead>
<tr>
<th>Strain</th>
<th>Colistin</th>
<th>Polymyxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>0,25</td>
<td>0,125</td>
</tr>
<tr>
<td>EC_1st TKC</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>EC_2nd TKC</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>EC_MCR-1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>EC_MCR-1_1st TKC</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>EC_MCR-1_2nd TKC</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>EC_MCR-1_3rd TKC</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>KP</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>KP_1st KC</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>KP_2nd KC</td>
<td>0,25</td>
<td>0,25</td>
</tr>
<tr>
<td>KP_MCR-1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>KP_MCR-1_1st TKC</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>KP_MCR-1_2nd TKC</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>KP_MCR-1_3rd TKC</td>
<td>512</td>
<td>128</td>
</tr>
</tbody>
</table>

**MICs (mg/L) after Sequential Time-Kill Curve**

- **EC**: E. Coli J53
- **KP**: K. Pneumoniae R2292
- **EC_MCR-1**: E. Coli carrying-MCR-1
- **KP_MCR-1**: K. Pneumoniae carrying-MCR-1

The presence of MCR-1 may be able to develop High-level polymyxin resistance (HLPR).

K. Pneumoniae_MCR-1 highly adapted in CST & PMB high concentration.

**Introduction**

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**Biomolecular Studies**

LOW-Level Resistance

HIGH-Level Resistance
DNA Sequencing

- 7 genes were determined
- Analysis was performed for all strains before and after sequential TKC
- NO mutations were found
Gene expression profiles by RT-qPCR
Before Sequential TKC
(no contact with antibiotic)

➢ All genes had over-expressed in \textit{E.coli} since MCR-1 plasmid was firstly inserted

➢ NO overexpression in \textit{K.pneumoniae_MCR-1}

\textbf{Fig.} Relative expression of genes for \textit{E.coli} J53 and \textit{K.pneumoniae} R2292 carrying-MCR-1 before Sequential Time-Kill Curves was performed (n=3)

*(P < 0.05)
For *E. coli* _MCR-1_, all genes were **down-expressed** in CST & PMB high concentration

**arnT** extremely **over-expressed** up to 29-fold for *K. pneumoniae_ _MCR-1_ in CST, but only 4.5-fold in PMB

**Results**

\[ \text{arnT} \rightarrow 4\text{-amino-4-deoxy-L-Arabinose (L-Ara4N)} \text{ addition to LPS} \]

**KP_MCR-1** highly adapted in CST than in PMB
Plasmid-Mediated Resistance

**Mechanism of Resistance**

**MCR**: Mobilizable Colistin Resistance

$\rightarrow$ Phosphoethanolamine transferase (addition of PETN to lipid A)

K. pneumoniae_MCR-1 well adapted better than EC_MCR-1 in both polymyxins antibiotics
CONCLUSION

- The presence of MCR-1 facilitated the step-by-step resistance

- Polymyxin B less induce the resistance than in colistin
PERSPECTIVE

➢ Reversibility study (up to 2-6 months)
➢ Whole genome sequencing
➢ Structural changes of lipid A
Acknowledgements

Special Thanks

Pr William COUET

Dr Julien BUYCK
Déclaration de conflit d’intérêt

Pour cette présentation, je déclare n’avoir aucun conflit d’intérêt.
MERCI !

Is it because I'm gram negative?