Emerging Mechanisms of Resistance in Gram (-) Bacteria: Plasmid-Mediated MCR-1 and Fosfomycin Resistance

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Colistin resistance
The pre-\textit{mcr}-1 paradigm

- Colistin resistance had been most investigated for \textit{Salmonella} and \textit{Pseudomonas}
- Common pathway
  \[\text{Modification of lipid A by:}\]
  - Addition of phosphoethanolamine
  - Addition of 4-amino-4-arabinose
- Dependent on non-synonymous chromosomal mutations of two-component regulators (\textit{PhoPQ}, \textit{PmrAB})
Discovery of *mcr-1*

- The research group at China Agricultural University and South China Agricultural University noticed high rates of colistin resistance in *E. coli* collected from pigs in early 2014.
- The resistance could be transferred by conjugation, which led to the identification of *mcr-1* (mobile colistin resistance).
First report

- \( mcr-1 = 1,626 \text{ bp} \)
- Phosphoethanolamine transferase
- First found on a self-transmissible 57-kb IncI2 plasmid
First report

• Moderate homology with known phosphoethanolamine transferases
First report

- MCR-1 catalyzes transfer of phosphoethanolamine from phosphatidylethanolamine to lipid A
First report

- MCR-1 causes 8 to 16-fold increase in colistin MICs in *E. coli*
First report

- Prevalent in pigs and chickens (~28%), much less common in humans (patients or Healthy persons)
Colistin is not approved for human use in China.

12,000 tons of colistin is used in agriculture in China.

China is... one of the world’s highest users of colistin in agriculture. Driven largely by China, the global demand for colistin in agriculture is expected to reach 11,942 tonnes per annum by the end of 2015 (with associated revenues of $229.5 million), rising to 16,500 tonnes by the year 2021, at an average annual growth rate of 4.75%. Of the top ten largest producers of colistin for veterinary use, one is Indian, one is Danish, and eight are Chinese. Asia (including China) makes up 73.1% of colistin production with 28.7% for export including to Europe.
Apocalypse Pig: The Last Antibiotic Begins to Fail

POSTED SAT, 11/21/2015

Antibiotic resistance: World on cusp of 'post-antibiotic era'

By James Gallagher
Health editor, BBC News website

POSTED SAT, 11/21/2015

What is a superbug?
Opening the floodgate...

Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015

<table>
<thead>
<tr>
<th>Isolate origin</th>
<th>No. of isolates analysed by WGS</th>
<th>No. of sequences positive for mcr-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL- and AmpC-producing <em>E. coli</em> isolates from Danish chicken meat (2012-2014)</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td>ESBL- and AmpC-producing <em>E. coli</em> isolates from imported chicken meat (2012-2014)</td>
<td>255</td>
<td>5</td>
</tr>
<tr>
<td>ESBL- and AmpC-producing <em>E. coli</em> isolates from human bloodstream infections (January 2014 - beginning of November 2015)</td>
<td>417</td>
<td>1</td>
</tr>
<tr>
<td>Carbapenemase-producing isolates from humans (January 2014 - beginning of November 2015)</td>
<td>117</td>
<td>0</td>
</tr>
</tbody>
</table>

1 human, 5 chicken *E. coli* isolates with *mcr-1* 2012-2015, Denmark

November 26 (first paper)
↓
December 4 (submitted)
↓
December 10 (published)
The flood keeps going

• Among diseased swine in Japan, 309 (45%) of the 684 E. coli strains were colistin-resistant
• mcr-1 was detected in 90 (13%) strains
• Proportion of mcr-1-positive Strains have increased since 2009

MCR-1 in food animals

- Carriage rates are high among food animals in some countries
  - ~30% of chickens in China
  - ~2% of chickens in France and the Netherlands
  - ~11% of turkeys in Germany
  - ~20% of calves in France
MCR-1 in companion animals

- A patient with UTI presented to a hospital in southern China
- Urine grew *mcr-1*-positive *E. coli*
- The patient works at a pet store
- 6/53 fecal samples from the pets at the store grew *mcr-1*-positive *E. coli*
- 4 of the pet isolates had identical PFGE pattern with the patient isolate
- Possible transmission among the pets and to the workers
MCR-1 in humans

- On the other hand, rates in strains causing human infections are extremely low
  - Global surveillance between 2014-2015
  - 19/13,525 (0.1%) of *E. coli*
  - 0/7,480 of *K. pneumoniae*
MCR-1 in humans

• Direct clinical impact of \textit{mcr-1} is unclear
• 2066 \textit{E. coli} and \textit{K. pneumoniae} bloodstream isolates collected in Chinese hospitals
  – Collected between 2013 and 2014
  – Only 20/1495 \textit{E. coli} and 1/571 \textit{K. pneumoniae} isolates had \textit{mcr-1}
  – Evenly split between community-onset and hospital-acquired bacteremia
  – 18/21 \textit{mcr-1}-positive isolates produced ESBL
  – 30-day mortality = zero

Quan J, et al. Lancet Infect Dis 2017; in press
Distribution of MCR-1
MCR-1 in USA

- First human case reported in May 2016
  - A female without recent travel history in Pennsylvania
  - Presented with UTI due to \textit{mcr-1}-positive, ESBL-producing \textit{E. coli}

'Nightmare Bacteria' Superbug Found for First Time in U.S

by MAGGIE FOX

Behind the scenes of a U.S. superbug discovery that made headlines around the world

By Lena H. Sun

Drug-resistant 'superbug' could make antibiotics useless

Here's how scientists discovered the antibiotic-resistant superbug
MCR-1 in USA

- **2nd case**
  - A patient in New York City, ESBL-producing *E. coli*
  - Details unknown

- **3rd case**
  - A male patient in New Jersey, NDM-producing *E. coli*
  - Originally from India, presenting with complicated UTI
  - Treated with piperacillin-tazobactam and trimethoprim-sulfamethoxazole, and discharged home

- **4th case**
  - A child who had just returned from the Caribbean
  - Diarrhea from Shiga-toxin-negative *E. coli* O157

Mediavilla JR, et al. mBio 2016;7:e01191
Vasquez AM, et al. MMWR 2016;65:979
MCR-1 in USA

- *mcr-1* is extremely rare in animals in the U.S.
  - 1/949 livestock isolates with *mcr-1*
  - **No 44,000 Salmonella** and 9,000 *E. coli/Shigella* isolates from retail meat
  - 2/2,003 chicken, turkey, cattle and pig isolates with *mcr-1*
MCR-1 in Latin America

• Reports from Latin America
• 9/87 colistin-resistant Enterobacteriaceae clinical isolates in Argentina had *mcr-1*
• 16/4,620 Enterobacteriaceae isolates from human, animal, food, environmental samples in Brazil had *mcr-1*
• *mcr-1* in *E. coli* from chicken meat in Brazil
  – Most of them with CTX-M ESBL
• *mcr-1* in *E. coli* from footpads of Magellanic penguins in Brazil
  – With CTX-M ESBL

More topics on mcr-1

• Discovery of mcr-2
  – *E. coli* from pigs and cows in Belgium
  – 1,617-bp phosphoethanolamine transferase gene with 77% nucleotide identity with mcr-1
  – Confers colistin MICs of 4-8 μg/ml
  – Found in 11% of colistin-resistant *E. coli* from livestock
  – Screening should include both mcr-1/mcr-2 moving forward
More topics on *mcr-1*

- Crystal structure of catalytic domain of MCR-1
  - Predicted phosphorylation of Thr285 as the catalytic nucleophile
  - No obvious phosphatidylethanolamine binding site
  - T285A reverts colistin MIC close to the control

*Stojanoski V, et al. BMC Biol 2016;14:81*
More topics on mcr-1

- Could mcr-1 impact lactose-non-fermenters?
  - Introduction and expression of mcr-1 in A. baumannii and P. aeruginosa
  - MIC increase significant for A. baumannii but not P. aeruginosa

<table>
<thead>
<tr>
<th>Strain</th>
<th>PCR for mcr-1</th>
<th>MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>E. coli ATCC 25922 (pMQ124-mcr-1)</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>E. coli YD626</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>E. coli YD626 (pMQ124-mcr-1)</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>A. baumannii ATCC 17978</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>A. baumannii ATCC 17978 (pMQ124WH1266-mcr-1)</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>A. baumannii SM1536</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>A. baumannii SM1536 (pMQ124WH1266-mcr-1)</td>
<td>+</td>
<td>32</td>
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<tr>
<td>A. baumannii D773</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>A. baumannii D773 (pMQ124WH1266-mcr-1)</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 47085</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 47085 (pMQ124-mcr-1)</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>P. aeruginosa TRPA087</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa TRPA087 (pMQ124-mcr-1)</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>P. aeruginosa TRPA179</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa TRPA179 (pMQ124-mcr-1)</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>P. aeruginosa B542455</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>P. aeruginosa B542455 (pMQ124-mcr-1)</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883 (pMQ124-mcr-1)</td>
<td>+</td>
<td>64</td>
</tr>
<tr>
<td>K. pneumoniae 4081916</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>K. pneumoniae 4081916 (pMQ124-mcr-1)</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>K. pneumoniae 2110291</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>K. pneumoniae 2110291 (pMQ124-mcr-1)</td>
<td>+</td>
<td>32</td>
</tr>
<tr>
<td>K. pneumoniae 2352892</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>K. pneumoniae 2352892 (pMQ124-mcr-1)</td>
<td>+</td>
<td>64</td>
</tr>
<tr>
<td>K. pneumoniae 4225188</td>
<td>-</td>
<td>&gt;128</td>
</tr>
<tr>
<td>K. pneumoniae 4225188 (pMQ124-mcr-1)</td>
<td>+</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>
More topics on *mcr-1*

- Phosphoethanolamine modification occurs in both species
- *A. baumannii* may be the next species to acquire *mcr-1* once it gets to hospitals
More topics on *mcr-1*

- Report of a hybrid plasmid co-harboring $bla_{NDM-5}$ and *mcr-1*
  - Long expected but first confirmation

Take home messages - MCR-1

- *mcr*-1 is already common in *E. coli* in livestock in some countries
- *mcr*-1 is very uncommon in humans at this time
- Next threats include association with potent beta-lactamases (e.g. NDM), transfer of *mcr*-1 to more resistant species (e.g. *A. baumannii*), and into healthcare settings
- Agricultural use of colistin needs to be addressed, which is the very likely culprit of this phenomenon
Fosfomycin

• Fosfomycin is a cell-wall synthesis inhibitor initially reported in 1969
• Fosfomycin inhibits the cytosolic N-acetylglucosamine enolpyruvyl transferase (MurA), which catalyzes formation of N-acetylmuramic acid
• It uses glycerol-3-phosphate (GlpT) and glucose-6-phosphate (UhpT) transport systems for cell entry
Fosfomycin

- In the U.S., an oral formulation (tromethamine) is approved for the treatment of uncomplicated UTI.
- Intravenous formulation (disodium) is available for invasive infections in some European countries.
- Intravenous formulation is being developed in the U.S. for the treatment of various infections caused by resistant bacterial pathogens.

http://www.zavante.com/pipeline.html
Fosfomycin

- The 2011 IDSA/ESCMID guidelines on treatment of uncomplicated UTIs now include as first-line recommendations:
  - Nitrofurantion
  - Trimethoprim-sulfamethoxazole
  - Fosfomycin
  - Pivmecillinam
Fosfomycin

- Use of fosfomycin at the University of Pittsburgh Medical Center
  - From 0 order in 2009 to >500 orders per year in 2013

![Graph showing fosfomycin usage from 2009 to 2013 with a peak in 2013 after the publication of the IDSA guideline.](image-url)
Activity of fosfomycin

- Susceptibility breakpoint for *E. coli* is MIC of 64 μg/ml
- After a 3-g oral dose, the maximum serum concentration is 22-32 μg/ml
- A high urine concentration (1,000 to 4,000 μg/ml) is achieved and remains over 100 μg/ml for 30 to 48 h
Resistance to fosfomycin

1. Reduced permeability – *most common*
   - Glycerol-3-phosphate transporter (GlpT)
   - Glucose-6-phosphate transporter (UhpT)
   - Resistant mutants tend to be less fit

2. MurA modification/overexpression
   - Cys115 as an acid-base catalyst
   - Cys115Asp is observed in fosfomycin-resistant species

3. Inactivation of fosfomycin – *most concerning*
   - FosA/FosB/FosX

Resistance to fosfomycin
Activity of fosfomycin

- *E. coli*
  - ~99% of 120 ESBL-producing *E. coli* strains are susceptible to fosfomycin (Minnesota)
  - 100% of 105 ESBL-producing *E. coli* strains are susceptible to fosfomycin (Norway)
  - Of ~670 ESBL/AmpC-producing *E. coli* strains at UPMC (2011-2016), 14 were resistant to fosfomycin
    - 2 produced plasmid-mediated FosA (glutathione-S-transferase)
    - 4 were missing UhpT (glucose-6-phosphate transporter)

Zykov IN, et al. Infect Dis (Lond) 2016;48:99
Activity of fosfomycin

- *Klebsiella pneumoniae*
  - MICs of *K. pneumoniae* are higher than *E. coli*
  - Fosfomycin is usually not considered for *K. pneumoniae* infections
Fos

- Metalloenzyme superfamily
- FosA
  - Mn$^{2+}$/K$^+$-dependent glutathione-S-transferase
  - Homodimer
  - Gram-negatives
- FosB
  - Mn$^{2+}$-dependent thiol-S-transferase
  - Gram-positives
- FosX
  - Mn$^{2+}$-dependent epoxide hydrolase
  - Rare in pathogens

FosA

- Transferable fosfomycin resistance in *Serratia marcescens* strains in Spain
  - *fosA* Located on 12.5-kb Tn2921
  - Tn2921 is derived from *Enterobacter cancerogenus*

Baquero F, et al. Chemotherapy 1977;S1:133
Garcia-Lobo JM, Ortiz JM. J Bacteriol 1982;151:477
The most common plasmid-mediated \textit{fosA} is \textit{fosA3}

- \textit{fosA3} was initially discovered in 2/192 ESBL-producing \textit{E. coli} isolates in Japan (2006 & 2008) with transferable fosfomycin resistance
- 70\% identity to \textit{fosA}^{\text{Tn2921}}
- Bracketed by insertion sequence IS26

\textbf{FosA}

\begin{center}
\begin{figure}
\includegraphics[width=\textwidth]{figure.png}
\end{figure}
\end{center}
FosA

- Plasmid-mediated **fosA** (*fosA3*) is commonly detected among *E. coli* of animal origin in China
  - 58/661 *E. coli* from chickens and 21/323 *E. coli* from dogs and cats harbored *fosA3* in southern China
  - Fosfomycin MICs are high
    - >256 mg/L

Plasmid-mediated *fosA* of *K. pneumoniae* origin in *E. coli*

- A fosfomycin-resistant *E. coli* clinical strain found in Pittsburgh possessed *fosA* of *K. pneumoniae* chromosomal origin (*fosA6*) on a plasmid
- A similar phenomenon has also been reported in China and Hong Kong
FosA

- Plasmid-mediated $fosA$ of *K. pneumoniae* origin in *E. coli*
  - The level of resistance conferred by $fosA^{KP}$ in *E. coli* is consistent with the level of natural fosfomycin resistance of *K. pneumoniae*

<table>
<thead>
<tr>
<th>Table 1. MICs for <em>E. coli</em> clones carrying various $fosA$ genes and <em>K. pneumoniae</em> clinical isolates with chromosomal $fosA$ genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (pBCSK−)</td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (pFosA$^{PMK1}$)</td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (pFosA6)</td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (pFosA$^{ST37}$)</td>
</tr>
<tr>
<td><em>E. coli</em> TOP10 (pFosA$^{ST258}$)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> NDM01 (FosA$^{PMK1}$)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> CRKpE6 (FosA$^{ST37}$)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> CRKpC1 (FosA$^{ST258}$)</td>
</tr>
<tr>
<td><em>E. coli</em> 5588 (fossA negative)</td>
</tr>
</tbody>
</table>
FosA

- FosA3 appears to like NDM-1!
  - NDM-1/FosA3-producing Enterobacteriaceae isolates from humans in duck in China (genes on separate plasmids or the same plasmid)
  - *Salmonella* Corvallis with two genes on the same plasmid from a wild bird in Germany

FosA

• FosA3 in Latin America
  – 213 UTI isolates from a hospital in Bolivia
  – 89% were *E. coli* or *K. pneumoniae*
  – 95-98% were susceptible to fosfomycin
  – 3/24 ESBL-producing *E. coli* and 1 *K. pneumoniae* were *fosA3*-positive
Take home messages - Fosfomycin

- Fosfomycin resistance in *E. coli* is still rare
- Plasmid-mediated resistance due to FosA3 is spreading in China, mostly in animals
- Potential threats are spread in humans and association with multidrug resistance, especially NDM-1
Acknowledgments