Emergence and spread of resistances against last resort antibiotics under the One Health perspective

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5. Better tackling AMR from animal sector
AMR had been listed as a serious threat of global public health by WHO

Antimicrobial resistance (AMR) – a crisis in waiting

No more evident is the need for a collaborative global health response than with AMR, which threatens the foundation of modern health systems and can undermine efforts to achieve the SDGs.

As bacterial infections grow more resistant to antibiotics, companies are pulling out of antibiotics research and fewer new antibiotics are being approved.

AMR estimated to kill more than 700,000 people globally per year today.

If AMR is not addressed, 10 million people are expected to die annually because of drug resistance by 2050.

In the last 25 years virtually no new antibiotics have been developed.

The world can expect to lose about 100 trillion USD worth of economic output by 2050 if antimicrobial drug resistance is not tackled.

A continued rise in antimicrobial resistance would lead to a global reduction of 2% - 3.5% in Gross Domestic Product (GDP) by 2050.

The usage of antimicrobials in farm animals

Antimicrobials play an important and irreplaceable role in animal production

- Over 50% of antimicrobials used in food animal
- Started to be used as feed additives in 1950s and in late 1970s in China

Antimicrobial consumption

A: Comparison of antimicrobials
B: Comparison of animal species

Science 357, 1350 (2017)
China is one of the biggest consumer of veterinary drugs

At least 50,000 tons of antibacterial drugs were used in animals per year, and more than 50% of the antibacterial drugs were used as feed additive (growth promotion)

Category statistic of new veterinary drugs approved in 1987-2015

Data source: China veterinary drug information network

Proportion of various veterinary chemicals used in China’s breeding industry in 2016

Data source: China animal health product association
The usage of antibiotics in farm animals in China ranks the top of the world.

Antibiotic usages in per kilogram of animal product.

- European: 60 mg/PCU; American: 90 mg/PCU
- China: $\sim 300$ mg/PCU.

Consumption levels varied considerably between countries (2013)
Large difference of antimicrobial usage in farm animals among EU countries

Comparisons of the average use of antimicrobials in 30 EU countries in 2016

ESVAC report 2016
The wide- and over-use of antimicrobial brings two issues:
- Antimicrobial residues
- Antimicrobial resistance
A one-health approach to antimicrobial resistance
Summary of categorization and prioritization of antimicrobials categorized as Critically Important, Highly Important and Important

<table>
<thead>
<tr>
<th>Antimicrobial class</th>
<th>CRITICALLY IMPORTANT ANTIMICROBIALS</th>
<th>C1</th>
<th>C2</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIGHEST PRIORITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporins (3rd, 4th and 5th generation)</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Macrolides and ketolides</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Polymyxins</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td><strong>HIGH PRIORITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Ansamycins</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Carbenepens and other penems</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Glycyclones</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<td>Lipopeptides</td>
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<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Monobactams</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

**C1 Criterion 1**

The antimicrobial class is the sole, or one of limited available therapies, to treat serious bacterial infections in people.

**C2 Criterion 2**

The antimicrobial class is used to treat infections in people caused by either: (1) bacteria that may be transmitted to humans from nonhuman sources, or (2) bacteria that may acquire resistance genes from nonhuman sources.
2. Polymyxins and mobile colistin resistance gene *mcr*

- Polymyxin B and polymyxin E (colistin) are cationic polypeptides, with broad-spectrum activity against Gram-negative bacteria

**Presence of mcr-1 and colistin resistance in *E. coli* of chicken origin during 1970–2014**

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The risk factor for mcr-1-carrying *E. coli* isolates (infection & colonisation)

- The colonization and infection of *mcr-1* in patients were associated with antibiotic usage
- The inpatient *mcr-1* colonization is probably associated with the animal farms and animal-derived food

Prevalence of \textit{mcr-1} in Chinese populations

\textbf{Study design}

\textbf{Exclusion criteria}
- Antibiotics < 3 months
- Gastroenteritis
- Neonates
- Pregnancy
- Known chronic diseases
- Vegetarians (n=30)

\textbf{Admission}
(One health center chosen from each of 30 Provinces)

\textbf{Fecal Samples from Healthy Subjects}
1\textsuperscript{st} Jun 2016 to 30\textsuperscript{th} Sep 2016
n=5,159

- MCR-1 positive samples
  n=774

- MCR-1 positive \textit{E. coli}

\textbf{Sequencing criteria}
- Sequence all MCRPEC if 10 or less, or sequence 10 isolates from each province if number of MRCPEC is > 10

\textbf{Univariable Analyses and Multivariable Logistic Regression Analysis}

\textbf{Epidemiology}

\textbf{Inclusion criteria}
- Subject consent
- Enrollment over 5 days

\textbf{Inclusion criteria}

\textbf{Microbiology}

\textbf{MCR-1 negative}
n=4,385

\textbf{Whole Genome Sequencing}
(n=287)

\textbf{Core-genome, Heat Map and MLST Analysis}
(all sequencing isolates)
- Fecal Samples (n=5,159) were collected from healthy individuals from 30 provinces/municipalities during Jun to Sep of 2016, 774 were positive for *mcr-1* (average of 15.0%)
- The prevalence of MCRPEC in health human is ranging from 3.7% to 32.7% across China
### Resistance profiles of 774 *mcr-1*-positive *E. coli*

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Number of resistant isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>140 (18.1%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>9 (1.2%)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>38 (4.9%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>107 (13.8%)</td>
</tr>
<tr>
<td>Ticarcillin/Clavulanic acid</td>
<td>448 (57.9%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>301 (38.9%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10 (1.3%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>550 (71.1%) (28.9% 1-2 mg/L)</td>
</tr>
<tr>
<td>Cefoperazone/Sulbactam</td>
<td>18 (2.3%)</td>
</tr>
</tbody>
</table>
Bayesian analysis of the population structure (BAPS) revealed four distinct lineages among the 287 MCRPEC isolates.
Minimum spanning trees revealed the genetic diversity of MCRPEC

- **287 isolates** belong to **135 STs**
- **ST10** (46, 16.0%) is the most prevalent clone in human carriages
The association of floR, tetA, oqxA/B and blaTEM with mcr-1 was higher than other antimicrobial resistance genes.
### Associated factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographical zoning of aquaculture conditions</td>
<td>low*</td>
<td>0.5 (0.3-0.7)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>medium*</td>
<td>0.7 (0.5-0.9)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>high*</td>
<td>1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Daily aquatic product intake (g/day) *</td>
<td>≤100*</td>
<td>0.6 (0.5-0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;100*</td>
<td>1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total population *</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Annual animal production (10 thousand tons)</td>
<td>sheep*</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annual animal-derived food consumption (kg/person)</td>
<td>meat*</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>pork*</td>
<td></td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>mutton*</td>
<td></td>
<td>0.003</td>
</tr>
</tbody>
</table>

*significant variable in multivariable logistic regression analysis (p<0.05)
Possible transfer route of mcr-1 from animal to human

Prevalence of MCR-1 in aquaculture production chain

- MCRPEC were identified in different sectors of the production chain. Limited SNPs in several sub-clusters of strains from aquaculture production chain and human gut in Shenzhen area.

Shen Y, et al. Environ Int, 2019 130, 104708
3. Carbapenems and \( \text{bla}_{\text{NDM}} \)

NDM variants are the most widely-spread carbapenemases

- \( \text{bla}_{\text{NDM}} \) was discovered in more than 55 countries or areas
- NDM-producing bacteria are increasing identified and more than 40 bacterial species are related

Global distribution of NDM by country and region (2018)


N. Deborah Friedman, 2015; M Berrazeg, 2014; Khan AU, 2017
NDM has been spread worldwide in animals

- China has become an endemic area of NDM in animals
- NDM-producing bacteria from food animals has been reported from Shandong, Guangdong, Sichuan, and Jiangsu provinces.
Increasing trend of NDM-positive Gram-negative bacteria in Chinese farm animals

- *Escherichia coli* from pigs (2015, Zhang RM, et al.)
- NDM is predominant carbapenemase in animal including NDM-17 and NDM-20, two novel NDM variants (Liu Z et al 2017, and Liu Z et al; 2018)
The detection rate of carbapenem-resistant Enterobacteriaceae from farm animals in China

<table>
<thead>
<tr>
<th>Date</th>
<th>Provinces</th>
<th>Animals</th>
<th>Samples</th>
<th>Number of Samples</th>
<th>Detection rate of CRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-12</td>
<td>Shandong, Sichuan, Guangdong</td>
<td>Broiler Pig</td>
<td>Cloacal swab, Anal swab</td>
<td>396</td>
<td>0</td>
</tr>
<tr>
<td>2013.10</td>
<td>Guangdong</td>
<td>Broiler</td>
<td>Cloacal swab</td>
<td>245</td>
<td>20.8%</td>
</tr>
<tr>
<td></td>
<td>Shandong</td>
<td>Broiler</td>
<td>Cloacal swab</td>
<td>355</td>
<td>21.1%</td>
</tr>
<tr>
<td>2014.11</td>
<td>Shandong</td>
<td>Broiler</td>
<td>Cloacal swab</td>
<td>90</td>
<td>55.6%</td>
</tr>
<tr>
<td>2015.01</td>
<td>Shandong</td>
<td>Broiler</td>
<td>Cloacal swab</td>
<td>90</td>
<td>64.4%</td>
</tr>
<tr>
<td>2015.04</td>
<td>Shandong</td>
<td>Broiler</td>
<td>Cloacal swab</td>
<td>90</td>
<td>60.0%</td>
</tr>
</tbody>
</table>

Data from: National Veterinary Drug Safety Evaluation Center
Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production

<table>
<thead>
<tr>
<th>Sample sites</th>
<th>Date of sampling</th>
<th>Source</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery farm 1</td>
<td>2015.05</td>
<td>Chicken cloacae</td>
<td>60</td>
</tr>
<tr>
<td>Hatchery farm 2</td>
<td>2015.05</td>
<td>Chicken cloacae</td>
<td>60</td>
</tr>
<tr>
<td>Commercial chicken farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2014.11, 2015.02, 2015.08</td>
<td>Chicken cloacae, flies</td>
<td>150</td>
</tr>
<tr>
<td>B</td>
<td>2014.11, 2015.02, 2015.05, 2015.08</td>
<td>Chicken cloacae, birds, farmers, dogs and flies</td>
<td>278</td>
</tr>
<tr>
<td>C</td>
<td>2014.11</td>
<td>Chicken cloacae</td>
<td>30</td>
</tr>
<tr>
<td>D</td>
<td>2014.11, 2015.05</td>
<td>Chicken cloacae</td>
<td>60</td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>2014.11</td>
<td>Chicken caeca, sewage</td>
<td>53</td>
</tr>
<tr>
<td>Supermarkets</td>
<td>2015.08</td>
<td>Chicken meat</td>
<td>48</td>
</tr>
<tr>
<td>In total</td>
<td></td>
<td></td>
<td>739</td>
</tr>
</tbody>
</table>
The presence of NDM and MCR-1 in chicken production chain

- *mcr-1* was found in each section, NDM was observed in all section except for the hatchery farms
Presence of NDM and MCR-1 in different hosts in and around farm B

- NDM and mcr-1 presented in chickens, birds, files, dogs and farmers; NDM-carrying *E. coli* in files collected from chicken house (I) and dog house (II) are higher than that from outside of the farm B.
161 *E. coli* contains 3 types of NDM-MGE; transfer elements including *ISCR1*, *intl1*, *IS3000*, *ISAba125* and *IS5* were observed in the flanking region of NDM gene.
Three types of $\text{bla}_{\text{NDM}}$ MGEs are disparately disseminated among the 161 $E. \text{coli}$ strains.
Genomic analysis of NDM and/or mcr-1 carrying E. coli of various origin

NO: Norway
US: United States
CN: China
AU: Australia
SG: Singapore
CO: Columbia

▲: mcr-1 positive strains

Chickens from four farms
Files of farm B
Dogs of farm B
Chickens from supermarkets
Chickens from slaughterhouse
Anal swabs of farmers from farm B
Swallow feces
blaNDM-negative E. coli from chickens
Human origin from other region of the world
The possible transmission routes of NDM and mcr-1 in poultry production chain

- mcr-1 can transfer from hatcheries to supermarket, NDM entered into the commercial farms through environmental media (bird, fly and human), then further contaminated the abattoir and supermarkets.

4. Glycyclines (tigecycline) and tet(X)

- **tet(X3) and tet(X4)** were identified from *A. baumannii* and *E. coli* in animals and mediated high-level resistance to tigecycline (MIC 32–64 mg l⁻¹), eravacycline and omadacycline.
- Located on conjugative plasmids and could be transferred by ISVsa3-mediated transposition.
- Both can be detected in animals, food of animal origin and humans.
- **48%** of the antimicrobials used in animals are tetracyclines.
5. Better tackling AMR from animal sector

- Reinforce the management of registration, production, distribution, and use of veterinary antibiotics.
- Improve the management of food animal production and reduce the antibiotic use.
- Promote the diagnosis of animal diseases and precision medicine.
- Develop new antimicrobial agents and antimicrobial substitutes.
- Monitor the antibiotic use, residues and antimicrobial resistance in bacteria.
- Initiate the risk assessment of both antibiotic residues and the emerging of novel antimicrobial resistance in bacteria.
- Educate the public about the awareness of the hazard of antibiotic overuse.
- Promote the international cooperation for prevention and control of antimicrobial resistance.
- “One Health” approach should be better implemented in national and international level.
A one-health group
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