Characterization of *mcr*-5-harboring plasmids and mobile genetic elements in *Salmonella* using short-read and long-read sequencing technologies

12th International Meeting on Microbial Epidemiological Markers (IMMEM XII)

Maria Borowiak
Colistin and *mcr* mediated colistin resistance

**colistin:**
- cationic antimicrobial peptide
- interacts with lipid A in LPS → membrane disruption
- frequently used in animal production
- considered as critically important antibiotic in human medicine

**mcr- mobile colistin resistance**
- mediated by plasmid-encoded phosphoethanolamine transferases → addition of phosphoethanolamine to lipid A in the LPS layer results in reduced binding of colistin

Gao *et al.*, 2016 [https://doi.org/10.1371/journal.ppat.1005957](https://doi.org/10.1371/journal.ppat.1005957)
Colistin and \textit{mcr} mediated colistin resistance

\textit{mcr-1}: the first described mobile colistin resistance gene

- discovered in \textit{E. coli} and \textit{K. pneumoniae} isolates from livestock, meat and patients in China
- transferable by horizontal gene transfer
- detected in more than ten Enterobacteriacea species
- globally distributed
- classified as major public health threat

Sun \textit{et al.}, 2018 https://doi.org/10.1016/j.tim.2018.02.006
# Colistin and *mcr* mediated colistin resistance

First description of the respective *mcr* variants:

<table>
<thead>
<tr>
<th><em>mcr</em> variant</th>
<th>reference</th>
<th>date of publication</th>
<th>country</th>
<th>organism</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mcr</em>-1.1</td>
<td>Liu <em>et al.</em></td>
<td>26.11.2015</td>
<td>China</td>
<td><em>E. coli</em></td>
<td>pigs, retail meat (chicken and pork), patients</td>
</tr>
<tr>
<td><em>mcr</em>-2.1</td>
<td>Xavier <em>et al.</em></td>
<td>07.07.2016</td>
<td>Belgium</td>
<td><em>K. pneumoniae</em></td>
<td>calves and piglets</td>
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<tr>
<td><em>mcr</em>-3.1</td>
<td>Yin <em>et al.</em></td>
<td>27.07.2017</td>
<td>China</td>
<td><em>E. coli</em></td>
<td>pigs</td>
</tr>
<tr>
<td><em>mcr</em>-4.1</td>
<td>Carattoli <em>et al.</em></td>
<td>03.08.2017</td>
<td>Italy</td>
<td><em>S. Typhimurium</em></td>
<td>pigs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spain &amp; Belgium</td>
<td><em>E. coli</em></td>
<td>piglets</td>
</tr>
<tr>
<td><em>mcr</em>-5.1</td>
<td>Borowiak <em>et al.</em></td>
<td>18.09.2017</td>
<td>Germany</td>
<td><em>S. Paratyphi B dTa+</em></td>
<td>poultry and chicken meat</td>
</tr>
<tr>
<td><em>mcr</em>-6.1</td>
<td>AbuOun <em>et al.</em></td>
<td>11.08.2017</td>
<td>UK</td>
<td><em>M. pluranimalium</em></td>
<td>pigs</td>
</tr>
<tr>
<td><em>mcr</em>-7.1</td>
<td>Yang <em>et al.</em></td>
<td>01.07.2018</td>
<td>China</td>
<td><em>K. pneumoniae</em></td>
<td>chicken</td>
</tr>
<tr>
<td><em>mcr</em>-8.1</td>
<td>Wang <em>et al.</em></td>
<td>04.07.2018</td>
<td>China</td>
<td><em>K. pneumoniae</em></td>
<td>pigs and chicken</td>
</tr>
<tr>
<td><em>mcr</em>-9.1</td>
<td>Carroll <em>et al.</em></td>
<td>07.05.2019</td>
<td>USA</td>
<td><em>S. Typhimurium</em></td>
<td>patient</td>
</tr>
</tbody>
</table>
Discovery of mcr-5 in *Salmonella* using WGS data

Study on 86 colistin resistant German *Salmonella* Paratyphi B dTa+ isolates (2011-2016)

- 12/32 mcr-1 negative isolates showed a unique resistance profile:
  - AMP, CIP, COL, NAL, SMX, STR, TET, TMP
  - one isolate (13-SA01718) was selected for sequencing

poultry  
chicken meat
Discovery of *mcr-5* in *Salmonella* using WGS data

13-SA01718
sequencing raw reads

mapping against a
bacterial chromosome

collecting unmapped
reads

assembly

bacterial chromosome
of 08-00436

Contig 1

Contig 2

Contig 3

RastTK annotation

predicted phosphoethanolamine transferase = *mcr-5*
Discovery of *mcr-5* in *Salmonella* using WGS data

Study on 86 colistin resistant German *Salmonella* Paratyphi B *dTα*+ isolates (2011-2016)

- **32** negative for *mcr-1*
- **54** *mcr-1* positive

*poultry*

*environment*

*chicken meat*

*mcr-5* PCR screening

14 isolates *mcr-5* positive isolates

MiSeq sequencing
Discovery of *mcr-5* in *Salmonella* using WGS data

- In *Salmonella* Paratyphi B *dTa+ mcr-5* is harbored by ColE-like plasmids and associated with a Tn3 family transposon (Tn6452)
- Tn6452 integration in the bacterial chromosome was observed
Characterization of further \textit{mcr-5} harboring \textit{Salmonella} \\

Screening on 360 additional colistin resistant \textit{Salmonella} isolates (2011-2018) \\

- 86 \textit{Salmonella} Paratyphi B \textit{dTa}+ \\
- 360 \textit{Salmonella} spp. \\

\textit{mcr-5} PCR screening \\

9 additional \textit{mcr-5} positive isolates: \\
5x \textit{Salmonella} Typhimurium \\
4x \textit{Salmonella} Typhimurium monophasic \\

\text{pig} \\
\text{pork} \\

\text{MiSeq & Minlon sequencing}
Characterization of further \textit{mcr-5} harboring \textit{Salmonella}

\textit{mcr-5} plasmid diversity in \textit{Salmonella} Typhimurium

Characterization of further $mcr$-5 harboring *Salmonella*

$mcr$-5 mobility associated with conjugative plasmids

- **pSE13-SA02717**
  - *Salmonella*
  - 50,928 bp IncX1

- **pSE13-SA01718-like**
  - *E. coli K12 J53*
  - 12,201 bp ColE

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Characterization of further *mcr-5* harboring *Salmonella*

*mcr-5* mobility associated with Tn6452 and putative mobile insertion cassettes

### mcr-5 located on Tn6452

- pSE12-02284
- pSE13-SA01718-like
- pSE12-SA02717
- chromosome 12-02284
- chromosome 13-SA02717

### mcr-5 located on a mobile insertion cassette*

- pEC0674-like
- pSE11-03671


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Summary

• **23 mcr-5** positive *Salmonella* isolates were analyzed using WGS:

  - **5 different** *mcr-5* harboring **plasmids** were observed (1 conjugative and 1 comobilizable)

  - *mcr-5* was either located on a **transposon (Tn6452)** or a mobile insertion cassette

  - in three isolates **integration of Tn6452 in the bacterial chromosome** was observed
Global distribution of *mcr-5*

Organisms harboring *mcr-5*:

- *Salmonella enterica*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Aeromonas hydrophila*
- *Pigmentiphaga sp.*
- *Cupriavidus gilardii*

Countries reporting *mcr-5*:
Global distribution of *mcr*-5

Organisms harboring *mcr*-5:

- *Salmonella enterica*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Aeromonas hydrophila*
- *Pigmentiphaga sp.*
- *Cupriavidus gilardii*

Sources of isolates and samples tested positive for *mcr*-5:

- Pig
- Pork
- Poultry meat
- Ready-to-eat food
- Patients
- Hospital environment
- Hospital sewage
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mcr-5 in *Salmonella Paratyphi B* dTa+:
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Disclaimer: The conclusions, findings and opinions expressed in this presentation reflect only the view of the authors and not the official position of the European Food Safety Authority.
Thank you for your attention

German Federal Institute for Risk Assessment
Max-Dohrn-Str. 8-10 • 10589 Berlin, GERMANY
Phone +49 30 - 184 12 - 0 • Fax +49 30 - 184 12 – 99 0 99
bfr@bfr.bund.de • www.bfr.bund.de/en