GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

BfR
Bundesinstitut für Risikobewertung

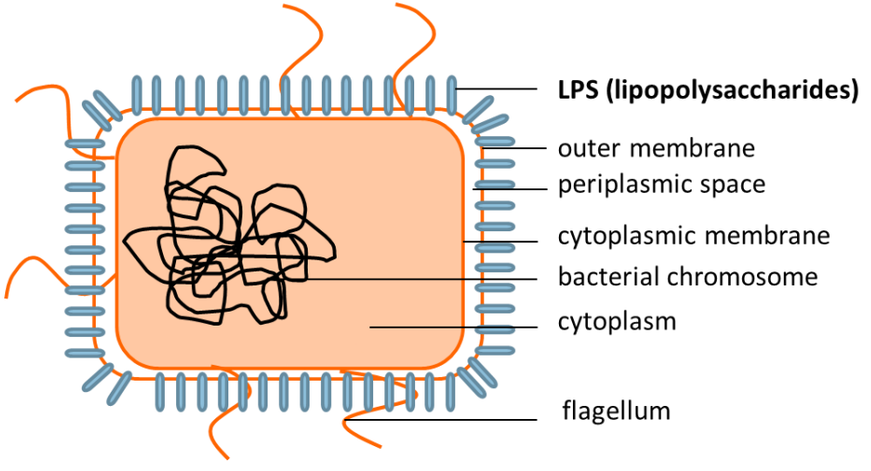
Plasmid-mediated colistin resistance in German *Salmonella enterica* strains isolated from livestock, food and the environment

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
- last resort antibiotic



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

Colistin and *mcr* mediated colistin resistance

mcr-1: the first described mobile colistin resistance gene

- discovered in *E. coli* and *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

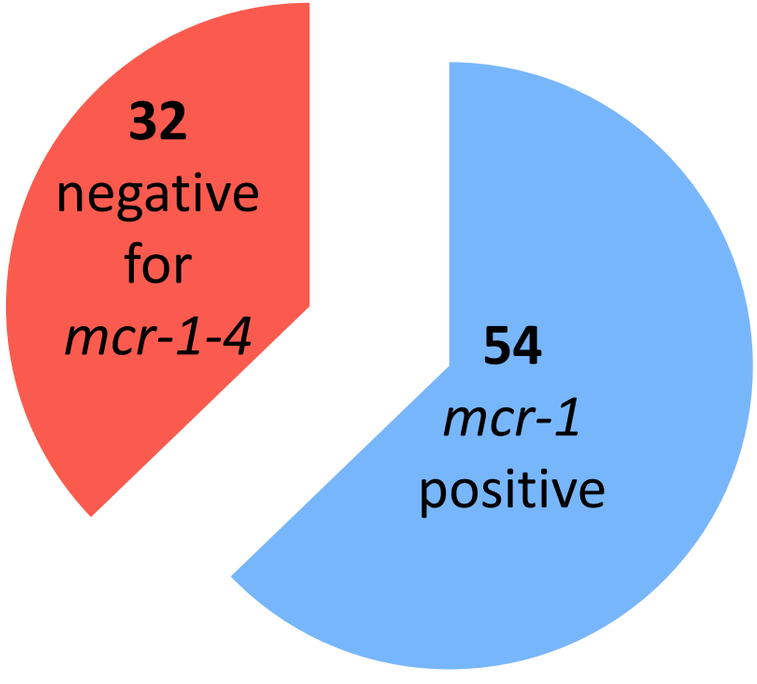
Colistin and *mcr* mediated colistin resistance

First description of the respective *mcr* variants:

***mcr* variant**	**reference**	**date of publication**	**country**	**organism**	**source**
mcr-1.1	Liu *et al.*	26.11.2015	China	*E. coli*	pigs, retail meat (chicken and pork), patients
				K. pneumoniae	patients
mcr-2.1	Xavier *et al.*	07.07.2016	Belgium	*E. coli*	calves and piglets
mcr-3.1	Yin *et al.*	27.07.2017	China	*E. coli*	pigs
mcr-4.1	Carattoli *et al.*	03.08.2017	Italy	*S.* Typhimurium	pigs
			Spain & Belgium	*E. coli*	piglets
mcr-5.1	Borowiak *et al.*	18.09.2018	Germany	*S.* Paratyphi B *d*Ta+	poultry and chicken meat
mcr-6.1	AbuOun *et al.*	11.08.2017	UK	*M. pluranimalium*	pigs
mcr-7.1	Yang *et al.*	01.07.2018	China	*K. pneumoniae*	chicken
mcr-8.1	Wang *et al.*	04.07.2018	China	*K. pneumoniae*	pigs and chicken

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

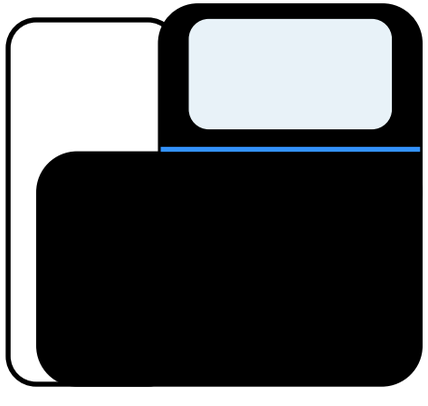
Study on 86 colistin resistant German *Salmonella* Paratyphi B *d*Ta+ isolates (2011-2016)



- 12/32 *mcr-1-4* negative isolates showed a unique resistance profile:

AMP, CIP, COL, NAL, SMX, STR, TET, TMP

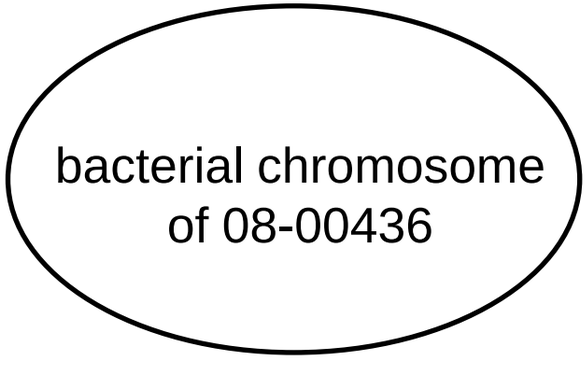
one isolate (13-SA01718) was selected for sequencing



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

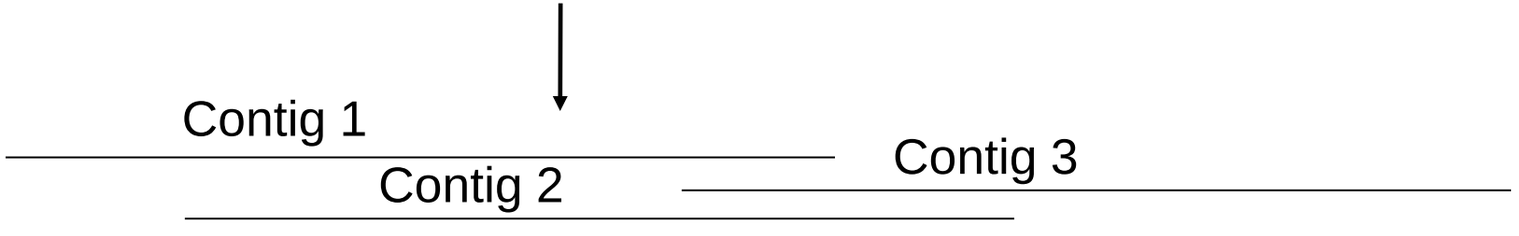
13-SA01718
sequencing raw reads

mapping against a bacterial chromosome



collecting unmapped reads

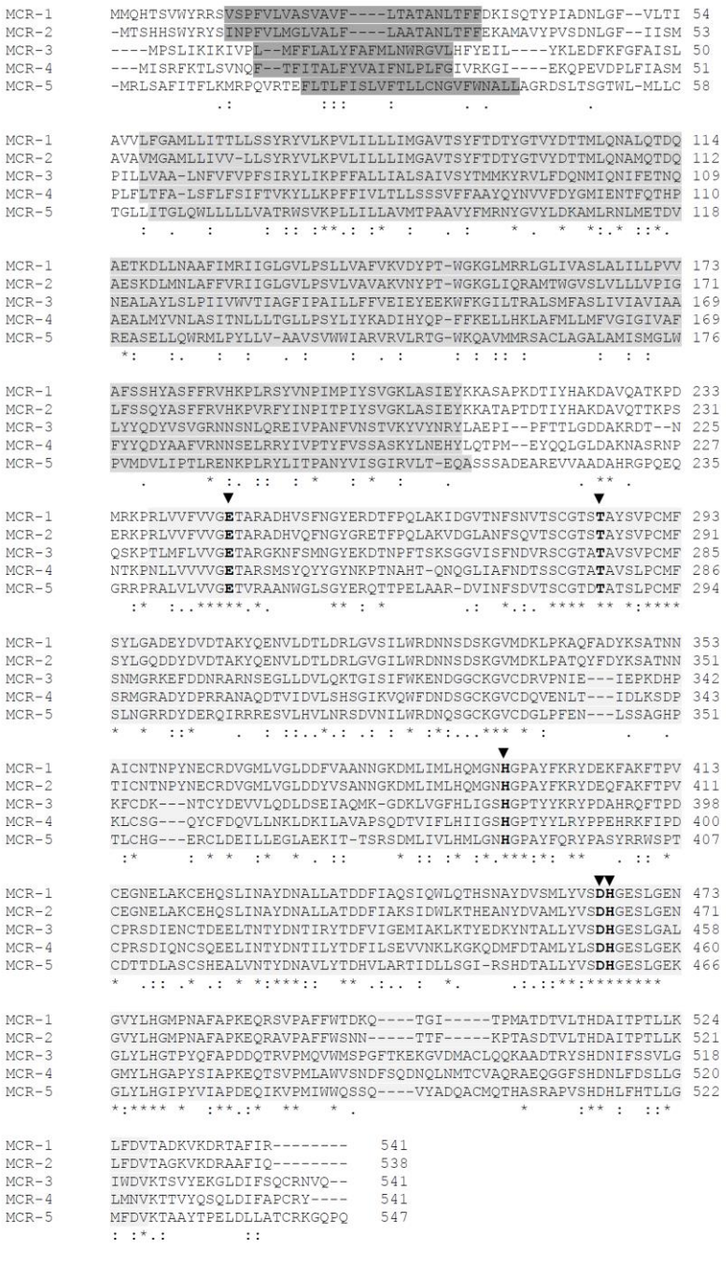
assembly



RastTK annotation

predicted phosphoethanolamine transferase = ***mcr-5***

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

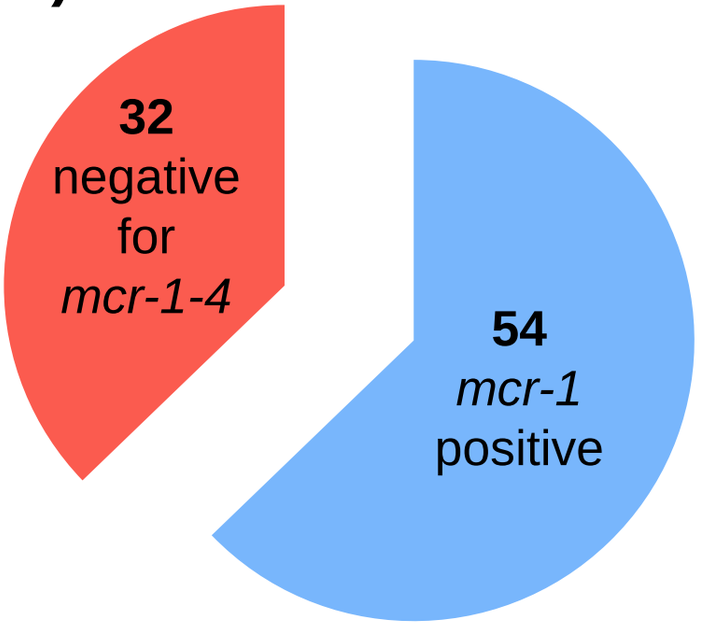


MCR-5 characteristics:

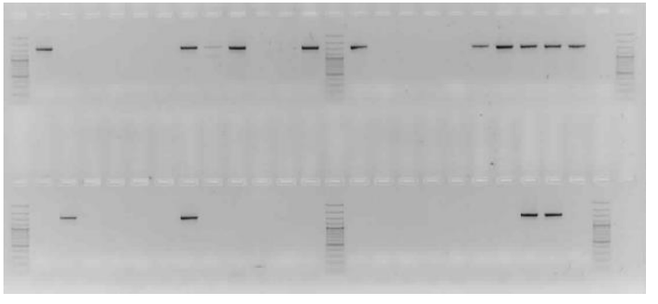
- 1,644 bp; 547 amino acids
- amino acids identity:
  MCR-1: 36.11%
  MCR-2: 35.29%
  MCR-3: 34.72%
  MCR-4: 33.71%
- SMART protein domain structure:
  - transmembrane domain
  - domain of unknown function
  - sulfatase domain
- conserved residues for colistin resistance:
  E248, T286, H389, D458 and H459

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

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



***mcr-5* PCR screening**

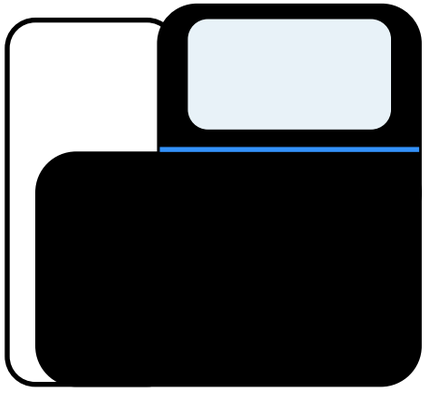


14 isolates *mcr-5* positive isolates

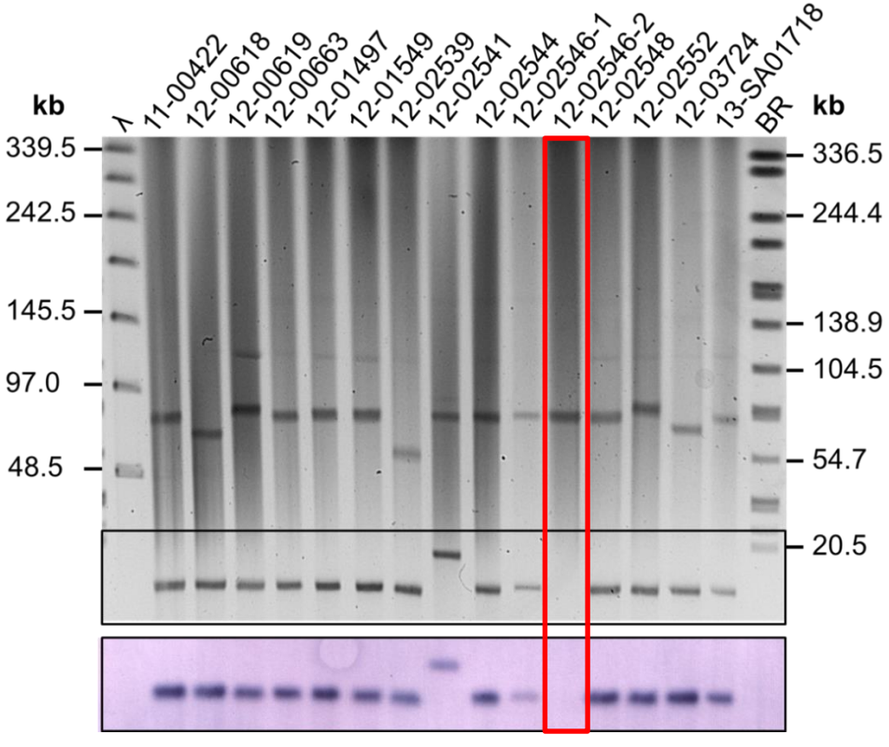
poultry

environment

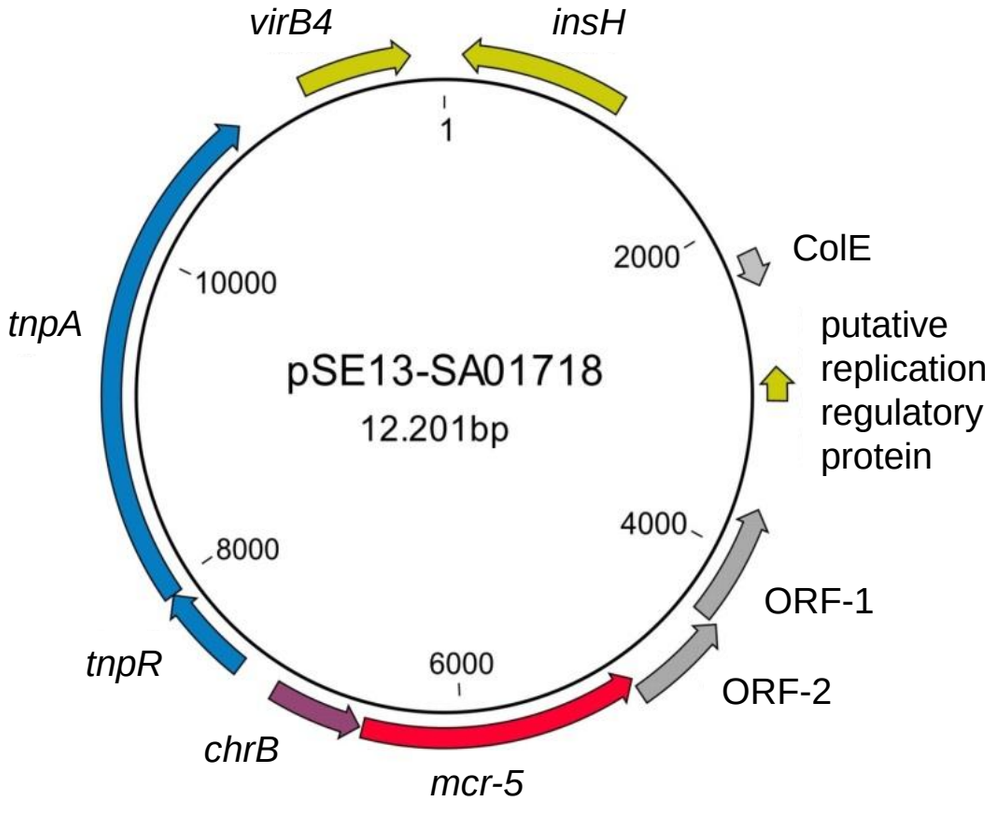
chicken meat



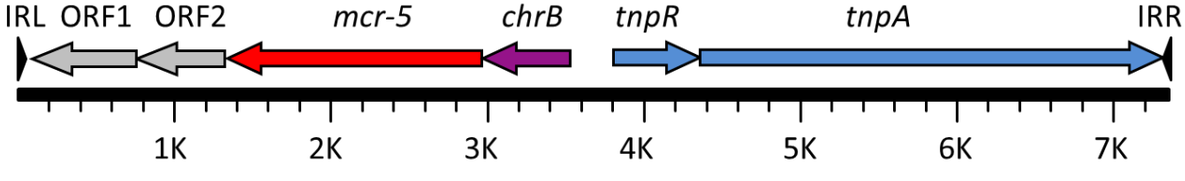
Discovery of *mcr-5* in *Salmonella* using WGS data



S1-PFGE, Southern Blot & Hybridization



- in *Salmonella* Paratyphi B *d*Ta+ *mcr-5* is harbored by ColE-like plasmids and associated with a Tn*3* family transposon (Tn*6452*)
- Tn*6452* integration in the bacterial chromosome was observed



Transposon Tn*6452*

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

J Antimicrob Chemother 2017; **72**: 3317–3324
doi:10.1093/jac/dkx327 Advance Access publication 18 September 2017

Journal of Antimicrobial Chemotherapy

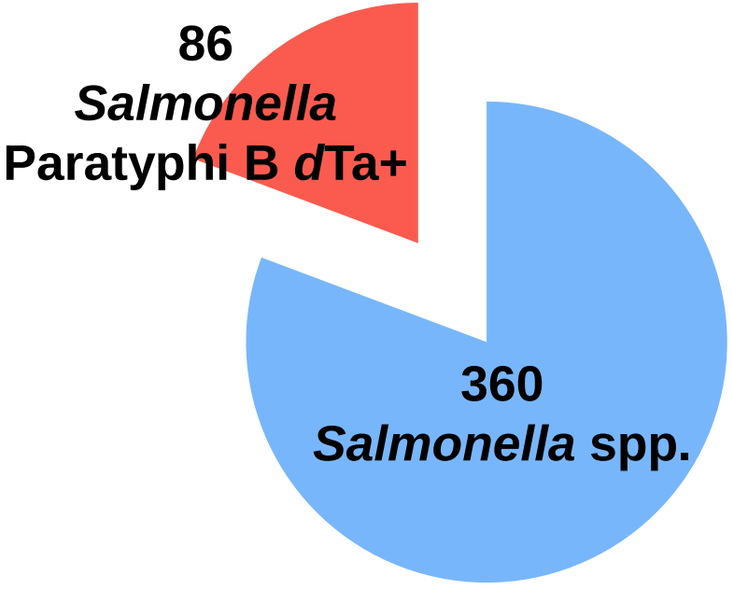
Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in *d*-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B

Maria Borowiak[1], Jennie Fischer[1], Jens A. Hammerl[1], Rene S. Hendriksen[2], Istvan Szabo[1] and Burkhard Malorny[1]*

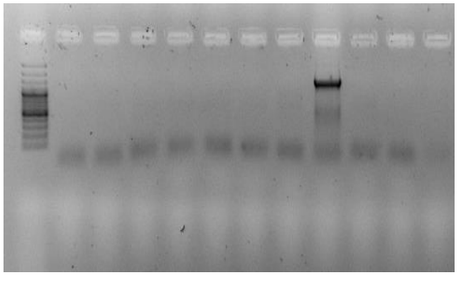
[1]German Federal Institute for Risk Assessment, BfR, Department for Biological Safety, Berlin, Germany; [2]National Food Institute, Technical University of Denmark, WHO Collaborating Center for Antimicrobial Resistance in Foodborne Pathogens and European Union Reference Laboratory for Antimicrobial Resistance, Kgs Lyngby, Denmark

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

Screening on 360 additional colistin resistant *Salmonella* isolates (2011-2018)



***mcr-5* PCR screening**

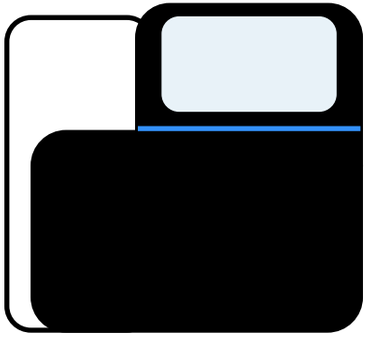


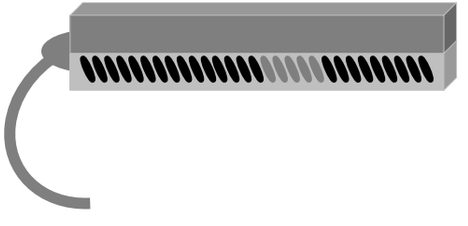
9 additional *mcr-5* positive isolates:

2x *Salmonella* sp.
4x *Salmonella* Typhimurium
3x *Salmonella* Typhimurium monophasic

pig

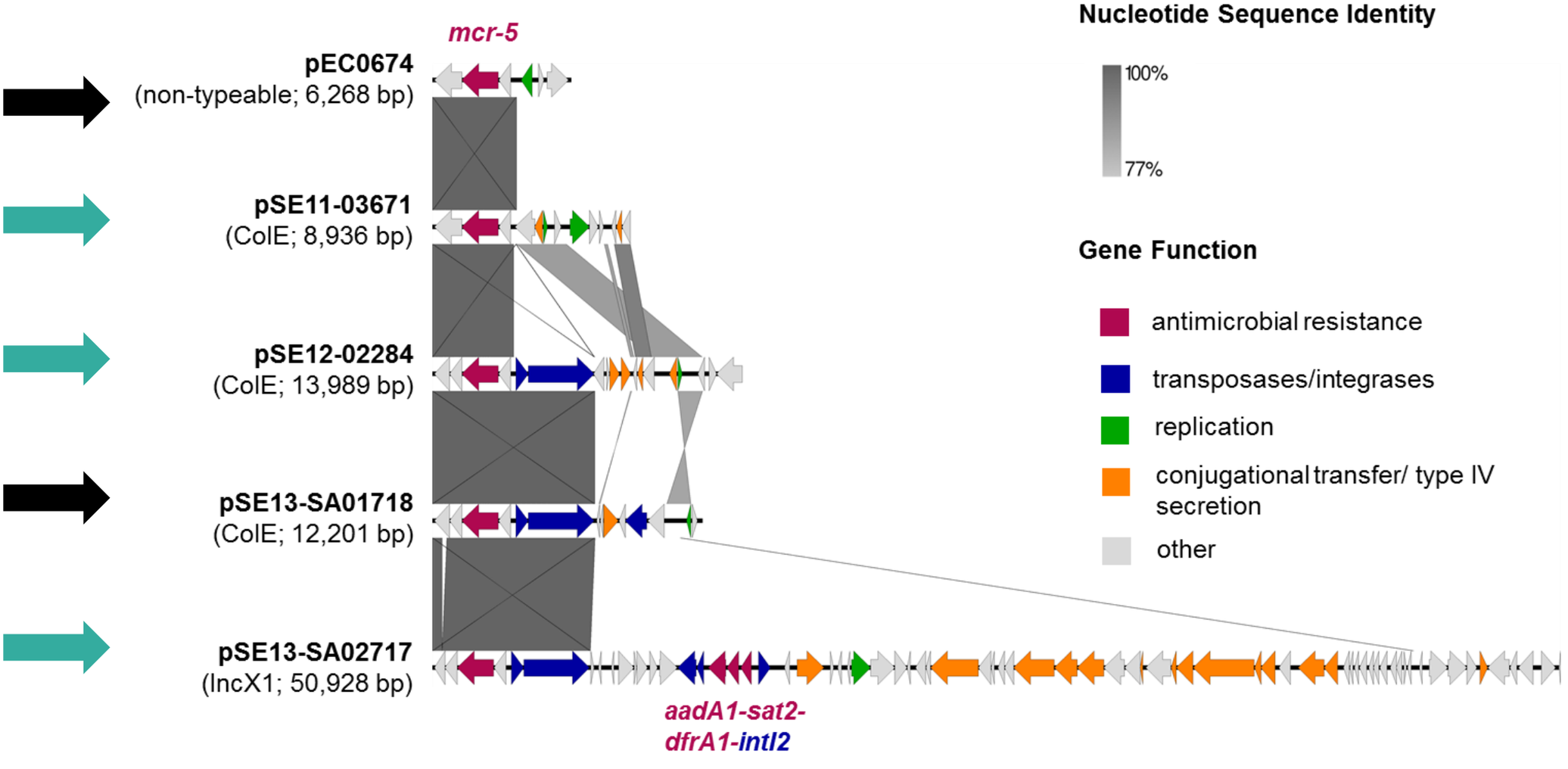
pork





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

mcr-5 plasmid diversity in *Salmonella* Typhimurium



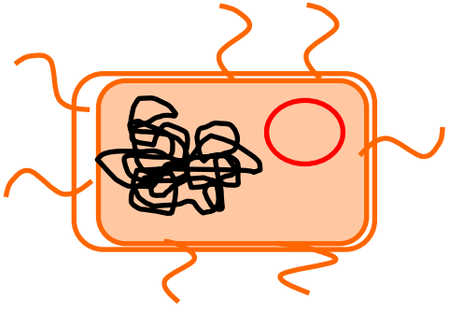
Characterization of further *mcr-5* harboring *Salmonella*

mcr-5 mobility associated with conjugative plasmids

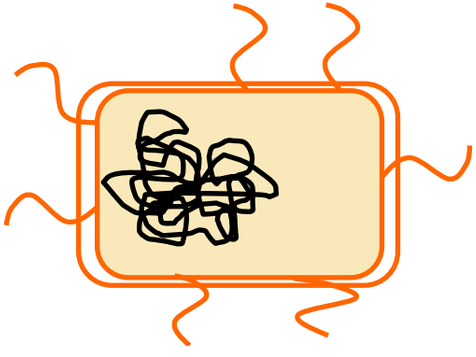
pSE13-SA02717

50,928 bp
IncX1

Salmonella



E. coli K12 J53



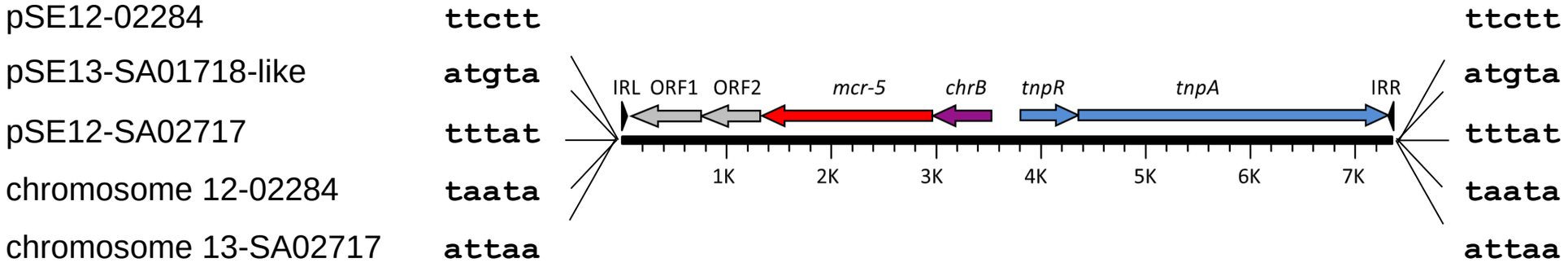
pSE13-SA01718-like

12,201 bp
ColE

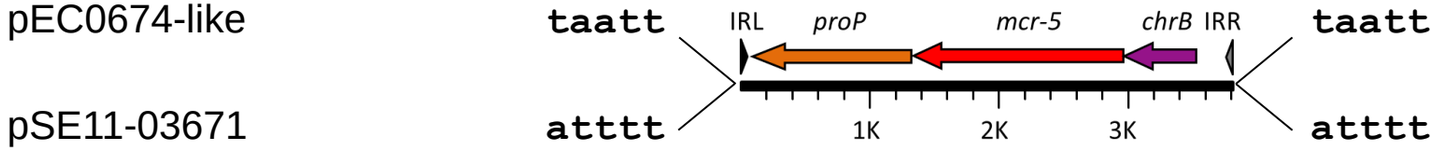
Characterization of further *mcr-5* harboring *Salmonella*

mcr-5 mobility associated with Tn*6452* and putative mobile insertion cassettes

mcr-5 located on Tn*6452*



mcr-5 located on a mobile insertion cassette



Global distribution of *mcr-5*

Organisms harboring *mcr-5*:

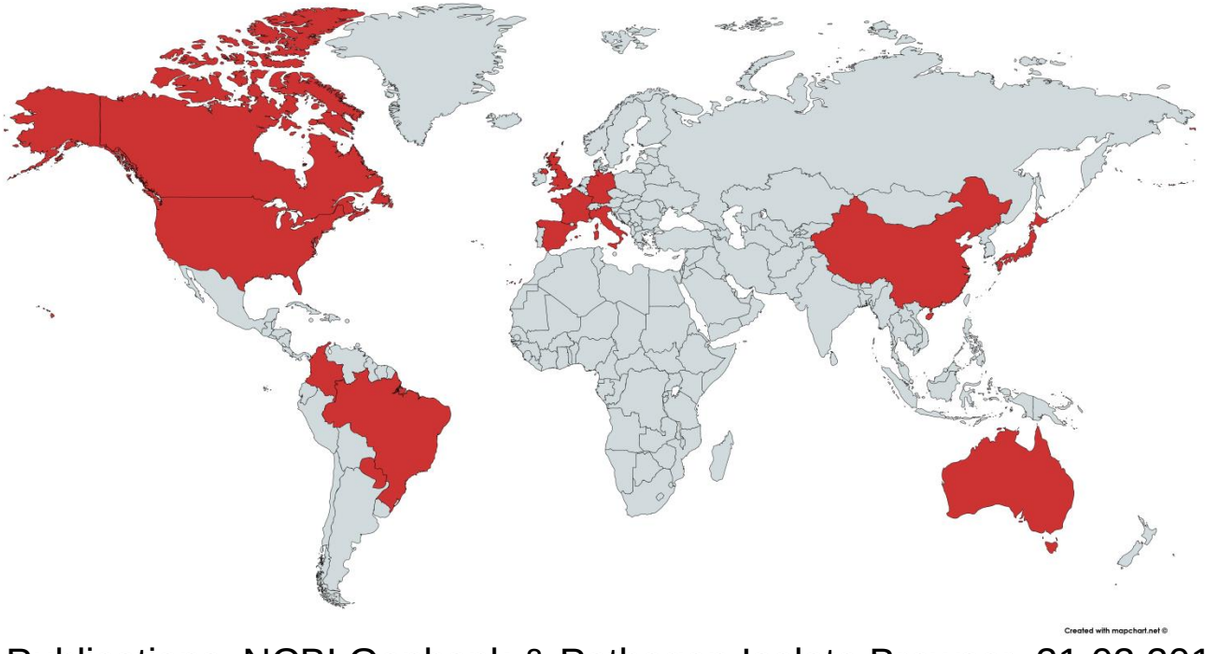
Publications:

Salmonella enterica
Escherichia coli
Pseudomonas aeruginosa
Aeromonas hydrophila

Blast:

Pigmentiphaga sp.
Cupriavidus gilardii

Countries reporting *mcr-5*

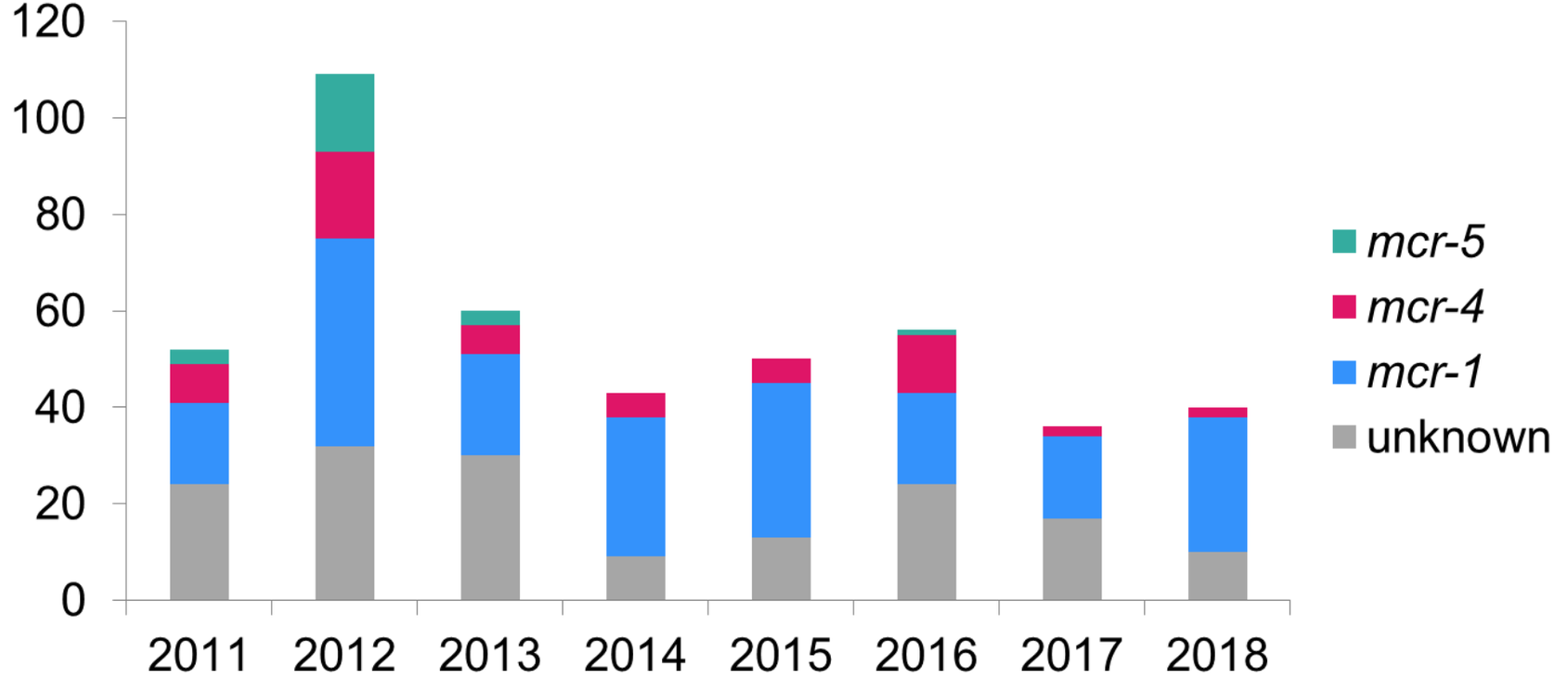


based on Publications, NCBI Genbank & Pathogen Isolate Browser, 21.03.2019

Prevalence of *mcr* genes in German *Salmonella* isolates

Multiplex PCR screening on 446 colistin resistant *Salmonella* isolates

- *mcr-1* to *mcr-5* multiplex PCR by Rebelo *et al.* 2018
- *mcr-6* to *mcr-8* multiplex PCR by Borowiak *et al.* (unpublished)



Distribution of *mcr* genes in *Salmonella* isolates over the years

Prevalence of *mcr* genes in German *Salmonella* isolates

Multiplex PCR screening on 446 colistin resistant *Salmonella* isolates

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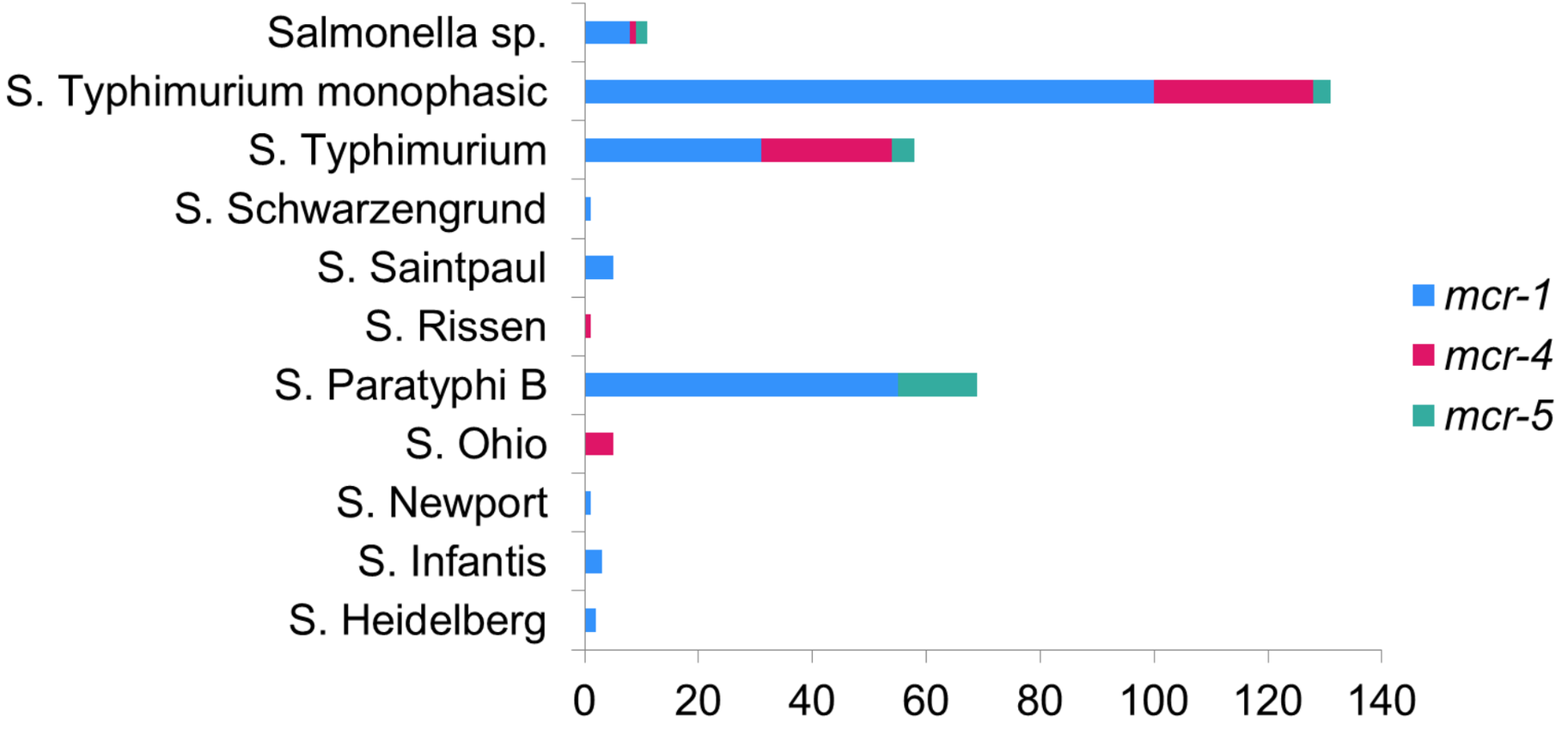

Distribution of *mcr* genes in different *Salmonella* serovars

Prevalence of *mcr* genes in German *Salmonella* isolates

Multiplex PCR screening on 446 colistin resistant *Salmonella* isolates

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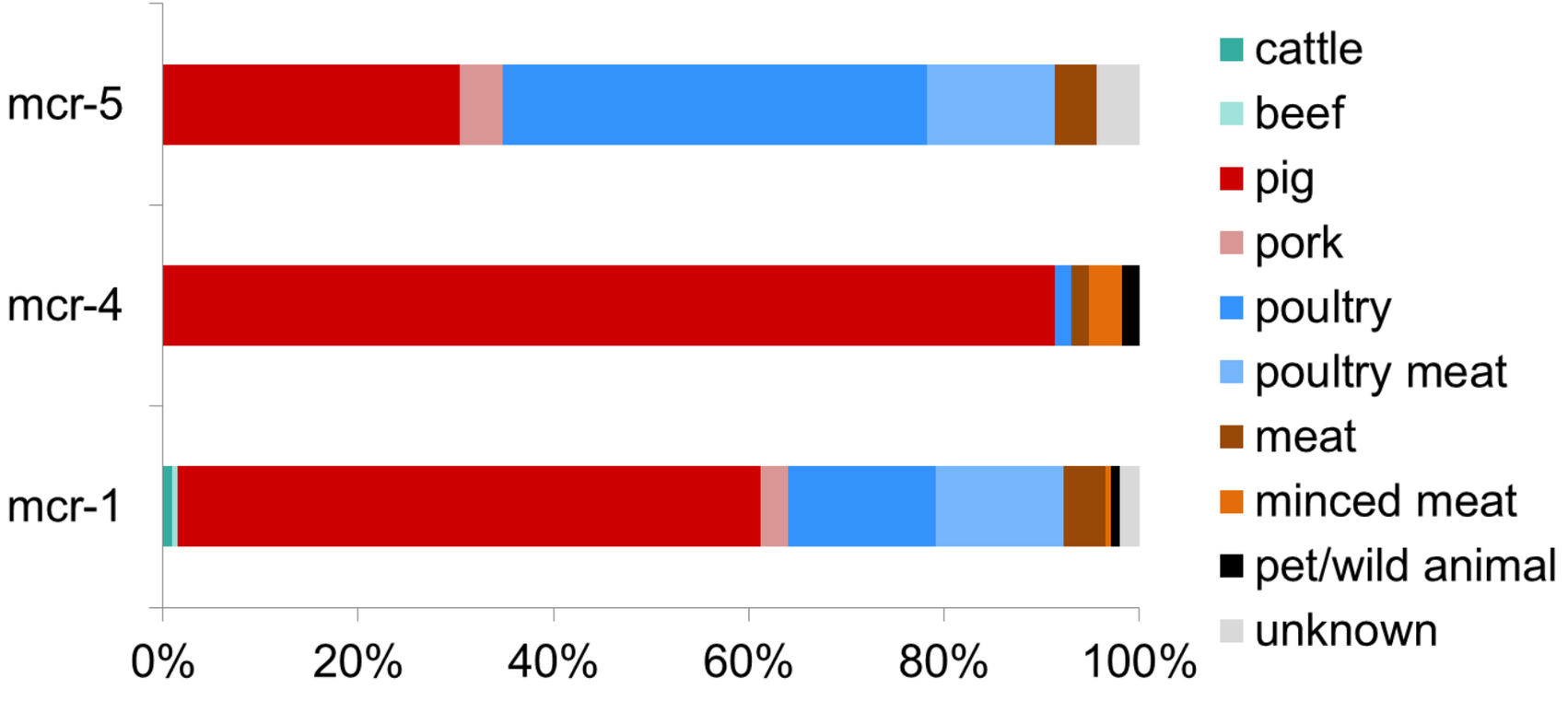

Isolation sources of *mcr* harboring *Salmonella* isolates

Summary

- colistin resistant ***Salmonella*** *i*solates from animals, livestock and food in Germany harbor ***mcr-1*** **(46.2 %)**, ***mcr-4*** **(13.0 %)** or ***mcr-5*** **(5.2 %)** genes

- ***mcr*** **positive** ***Salmonella*** *enterica* were mainly isolated from **pig** and **poultry** production, but can be also found in **cattle** as well as **pet animals**

- ***mcr*** genes can be found in more than **9 different** *Salmonella enterica* **serovars** including *S.* Typhimurium and *S.* Paratyphi B *d*Ta+

- sequencing of selected *mcr* positive isolates is planned

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

  - **5 different** *mcr-5* harboring **plasmids** were described
  - one plasmid was **conjugative** and another plasmid could be co-mobilized in conjugation studies
  - *mcr-5* was either located on a **transposon (Tn*6452*)** or a **putative mobile insertion cassette**
  - in three isolates **integration of Tn*6452* in the bacterial chromosome** was observed

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Dr. Istvan Szabo

Unit 43
Dr. Jens A. Hammerl

***mcr-5* in *Salmonella* Paratyphi B *d*Ta+:**
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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.

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Thank you for your attention

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