

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

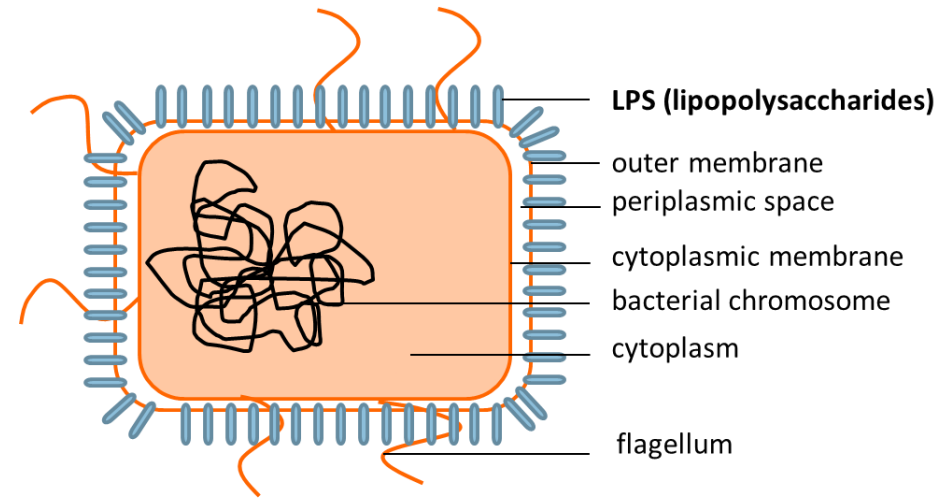
**Maria Borowiak**



# 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 Enterobacteriaceae species
- globally distributed
- classified as major public health threat

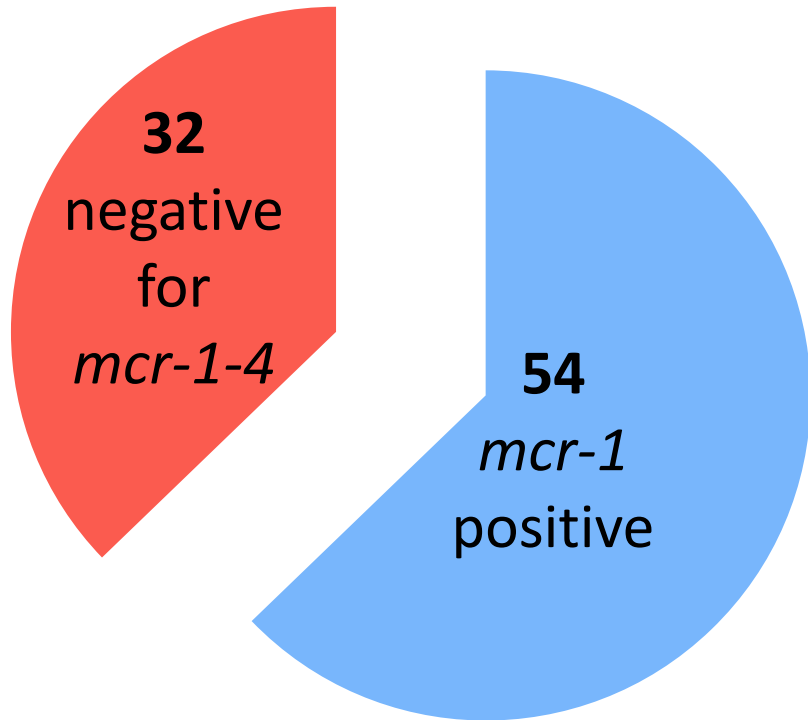
# Colistin and *mcr* mediated colistin resistance

## First description of the respective *mcr* variants:

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

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

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

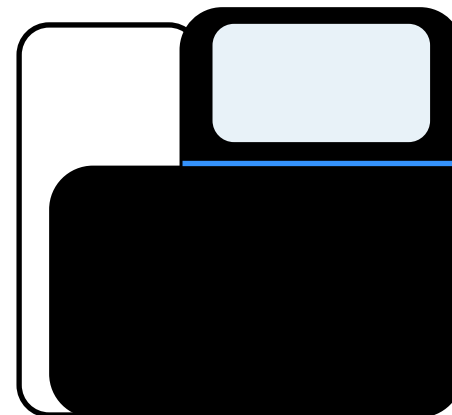


- 12/32 *mcr-1-4* negative isolates showed a unique resistance profile:

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



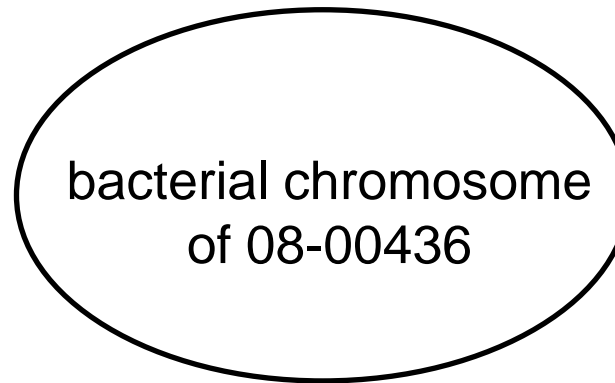
one isolate (13-SA01718)  
was selected for sequencing



# 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



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

## MCR-5 characteristics:

```

MCR-1 MMQHTSVWYRRS VSPFVLVASVAVF----LTATANLTFE DKISQTYPIADNLGF--VLTI 54
MCR-2 -MTSHHSWYRYS INPFVLMGLVALF----LAATANLTFE EKAMAVYVPSDNLGF--IISM 53
MCR-3 ----MPSLIKIKIVPL--MFFLALYFAFMLNWRGVLHFYBIL----YKLEDFKFGFAISL 50
MCR-4 --MISRFKTLVNOE--TFITALFYVAIFNLPLFGLVIRKGI----EKQPEVDPLFIASM 51
MCR-5 -MRLSAFITFLKMRPQVRTE FTLTLFISLVFTLLCNGVFWNALL AGRDSLTSGTWL-MLLC 58
      .:      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 AVVLFGAMLLITLTLSSYRYVLPKVLILLIMGAVTSYFTDITYGTVDYDTTMLQNALQTDQ 114
MCR-2 AVAVMGAMLLIIV-LLSYRYVLPKVLILLIMGAVTSYFTDITYGTVDYDTTMLQNAQTDQ 112
MCR-3 PILLVAA-LNFVFPVPSIRYLKIPFFALLIALSAIVSYTMMKYRVLFQDNMIQNIIFETNQ 109
MCR-4 PLFLTFA-LSFLFSIFTVKYLLKPPFIVLTLSSSVFFAAQYVNVVFDYGMIENTFQTHE 110
MCR-5 TGLLITGLQWLLLLLVATRWVSKPELLLLAVMTAAVYFMRNYGVYLDKAMLRNLMETDV 118
      .:      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 AETKDLLNAAFIMRI IGLGVLPSLLVAVKVDYPT-WGKGLMRRLLGLIVASLALILLPVV 173
MCR-2 AESKDLMLNAFVRI IGLGVLPSVLVAVAKVNYPT-WGKGLIQRAMTWGVSIVLLLVPIG 171
MCR-3 NEALAYLSLEPIVWVTIAGFIPAILLFFVEIEYEKWFKGLTRALSMFASLIVIAVIA 169
MCR-4 AEALMYNVLASITNLLLGLLPSYLYKADHYQP-FFKELLHKLAFMLLMFVIGIVAVF 169
MCR-5 REASELLQWRMLPYLLV-AAVSVWVIARVRLRTG-WKQAVMMRSACLALAMISGLW 176
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 AFSSHYASFFRVHKLPLRSYVNPIMPISVGLASIEYKASAPKDTIYHAKDAVQATKPD 233
MCR-2 LFSSQYASFFRVHKLPRFYINPITPIYSVGLASIEYKATAPTDTIYHAKDAVQTKPS 231
MCR-3 LYYQDYVSVGRNNSLQREIVPANFVNSTVKYVYNRVLAPEI--PFTLLGDDAKRDT--N 225
MCR-4 FYYQDYAAFRNNSLRRYIVPTVYVSSASKYLNEHYLQTPM--EYQQLGLDAKNASRNP 227
MCR-5 FVMMDVLIPTLRNKPLRYLITPANYVISGIRVLT-EQASSADEAREVVADARHPGPEQ 235
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 MRKPRLVVFFVVG ETARADHVSNFYERDTPQLAKIDGVNFSNVTSCTGTA TAVSVPCMF 293
MCR-2 ERKPRLVVFFVVG ETARADHVQFNQYGRETFPQLAKVVDGLANFSQVTSCTGTA TAVSVPCMF 291
MCR-3 QSKPTLMFLVVG ETARGKNFSMNGYEKDTNPFTSKSGGVISFNVDVRSCTGTA TAVSVPCMF 285
MCR-4 NTKPNLLVVVVG ETARMSYQYGYKNKPTNAHT-QNQGIAFNDSCTGTA TAVSLPCMF 286
MCR-5 GRRPRALVLVVG ETVRAANWGLSGYERQTTPELAAR-DVINFSVTSCTGTA TATSLEPCMF 294
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 SYLGADYDVTAKYQENVLDTLDRLGVSILWRDNNSDSKGVMDKLPKAQFADYKSAATNN 353
MCR-2 SYLGQDDYDVTAKYQENVLDTLDRLGVSILWRDNNSDSKGVMDKLPATQYFDYKSAATNN 351
MCR-3 SNMGRKFDNDRARINSEGLLDVQLKTGISIFWKENDGGCKGVCDRVFNIE---IEPKDHP 342
MCR-4 SRMGRADYDFRRANQDQTVIIDLVSLSGKIKVQWFDNDSGCKGVCQVENLT---IDLKSDP 343
MCR-5 SLNGRRDYDERQIRRRSVLHVLRNSDVNILLWRDNQSGCKGVCGLPFEN---LSSAGHP 351
      * * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 AICNTNPNYNECRDVGMVLVGLDDFVAANNKMDMLIMLHQMGNGHPAYFKRYDEKFAKFTPV 413
MCR-2 TICNTNPNYNECRDVGMVLVGLDDYVSANNKMDMLIMLHQMGNGHPAYFKRYDEQFAKFTPV 411
MCR-3 KFCDK---NTCYDEVVLQDLSEIAQMK-GDKLVGFHLIGSHGPTYKRYPDADRQFTPD 398
MCR-4 KLCSG---QYCFDQVLLNKLKDLAVAPSQDQTVIFLHIIHSHGPTYKRYPPPEHRKFIPD 400
MCR-5 TLCHG---ERCLDEILLEGLAERIT-TSRSDMLIVLHMLGNHGPAYFQRYPASYSRRWSP 407
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 CEGNELAKCEHQSLINAYDNALLATDDFIAQSIQWLQTHSNAYDVSMLYVSDHGESLGEN 473
MCR-2 CEGNELAKCEHQSLINAYDNALLATDDFIAKSIDWLKTHEANYDVAMLYVSDHGESLGEN 471
MCR-3 CPRSDIENCTDEELTNTYDNTIRYDFVIGEMIAKLTIEDKYNTALLYVSDHGESLGEN 458
MCR-4 CPRSDIQNSQEELINTYDNTILYDFILSEVVNKLKGGQDMFDTAMLYLSDHGESLGEN 460
MCR-5 CDTTDLASCSEALVNTYDNAVLYTDHVLARTIDLDSGI-RSHDPTALLYVSDHGESLGEN 466
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

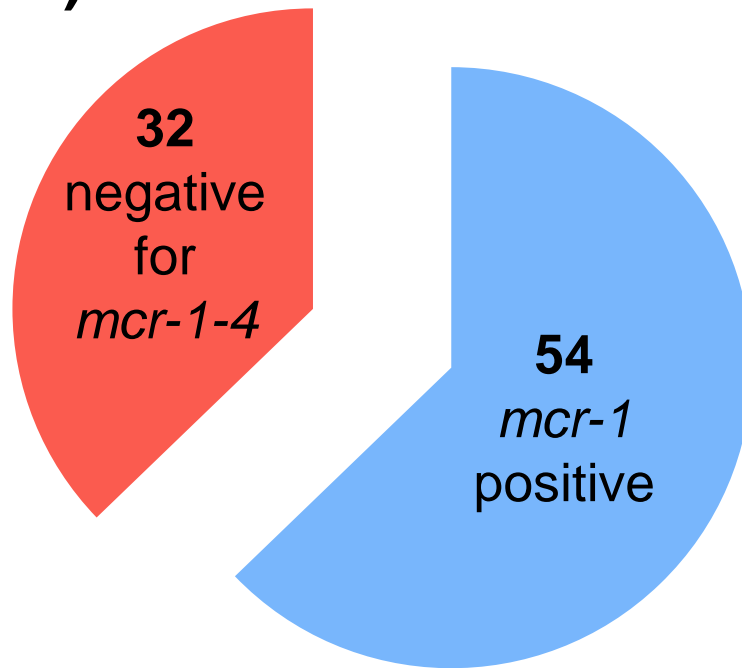
MCR-1 GVYHLGMPNAPAPKEQSRVPAFFWTDKQ----TGI-----TPMATDVLTHDAITPTLLK 524
MCR-2 GVYHLGMPNAPAPKEQRAVPAFFWSNN----TTF-----KPTASDVLTHDAITPTLLK 521
MCR-3 GLYLHGTPYQFAPDDQTRVPMQVWMSPEGFTKEKGVDMACQKAAADTRYSHDNIFSSVLG 518
MCR-4 GMYLHGAPYSIAPKEQTSVPMMLAVNSNDFSQDNQNLMTCAQRAEQGGFSDHNLFDSSLG 520
MCR-5 GLYLHGIPYVIAPDEQIKVPMIWWQSSQ----VYADQACMQTHASRAPVSHDHLFHTLLG 522
      * :      .:      .:      .:      .:      .:      .:      .:      .:      .:

MCR-1 LFDVTADKVKDRTA FIR----- 541
MCR-2 LFDVTAGKVKDRAA FIQ----- 538
MCR-3 IWDVKTSVYEKGLDIFSQCRNVQ-- 541
MCR-4 LMNVKTTVYQSQLDIFAPCRY---- 541
MCR-5 MFDVKTAAAYTPELDLLATCRKGQFQ 547
      .:      .:      .:      .:      .:
  
```

- 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  $\alpha$ Ta+ isolates (2011-2016)



poultry



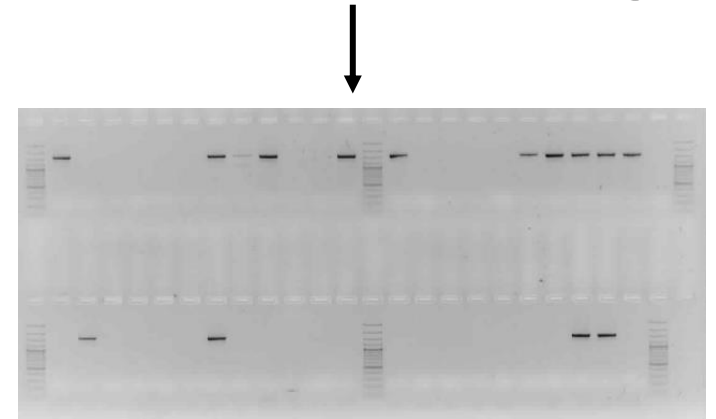
environment



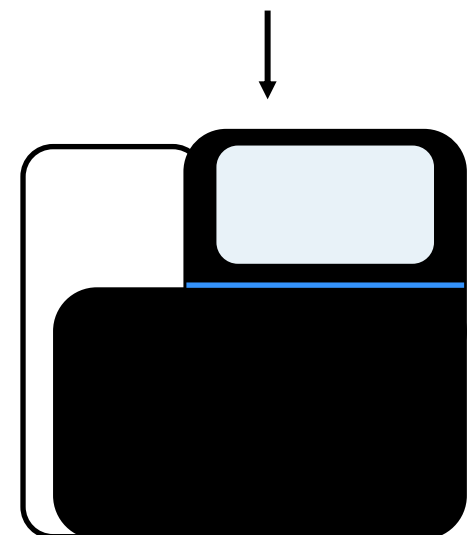
chicken meat



*mcr-5* PCR screening

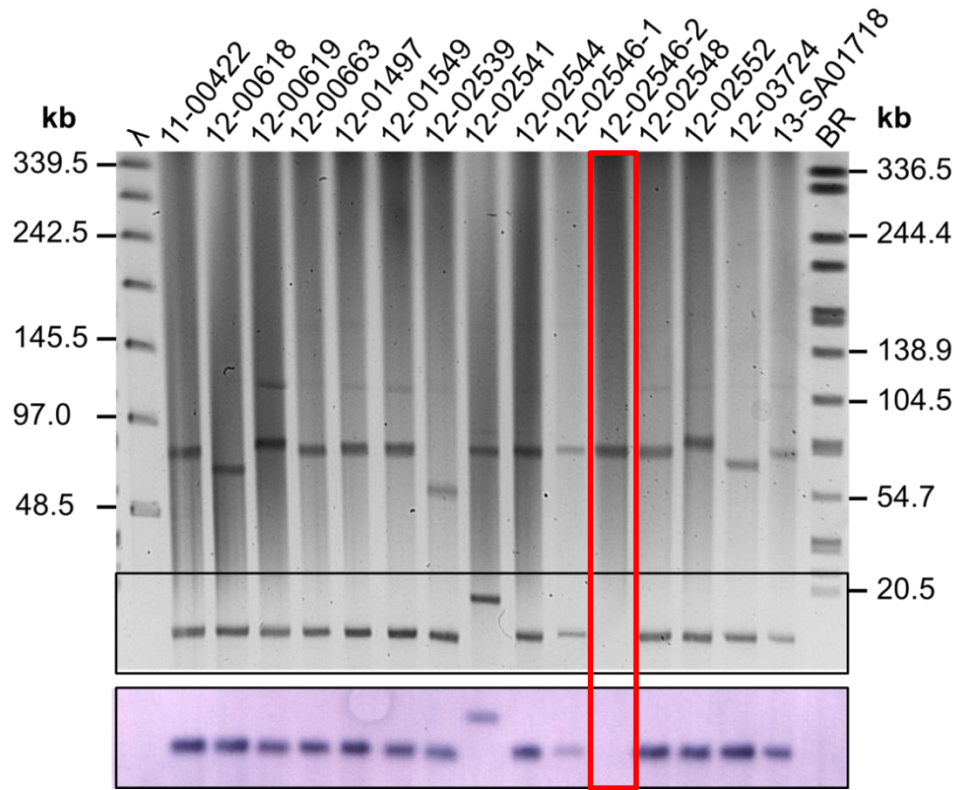


14 isolates *mcr-5* positive isolates

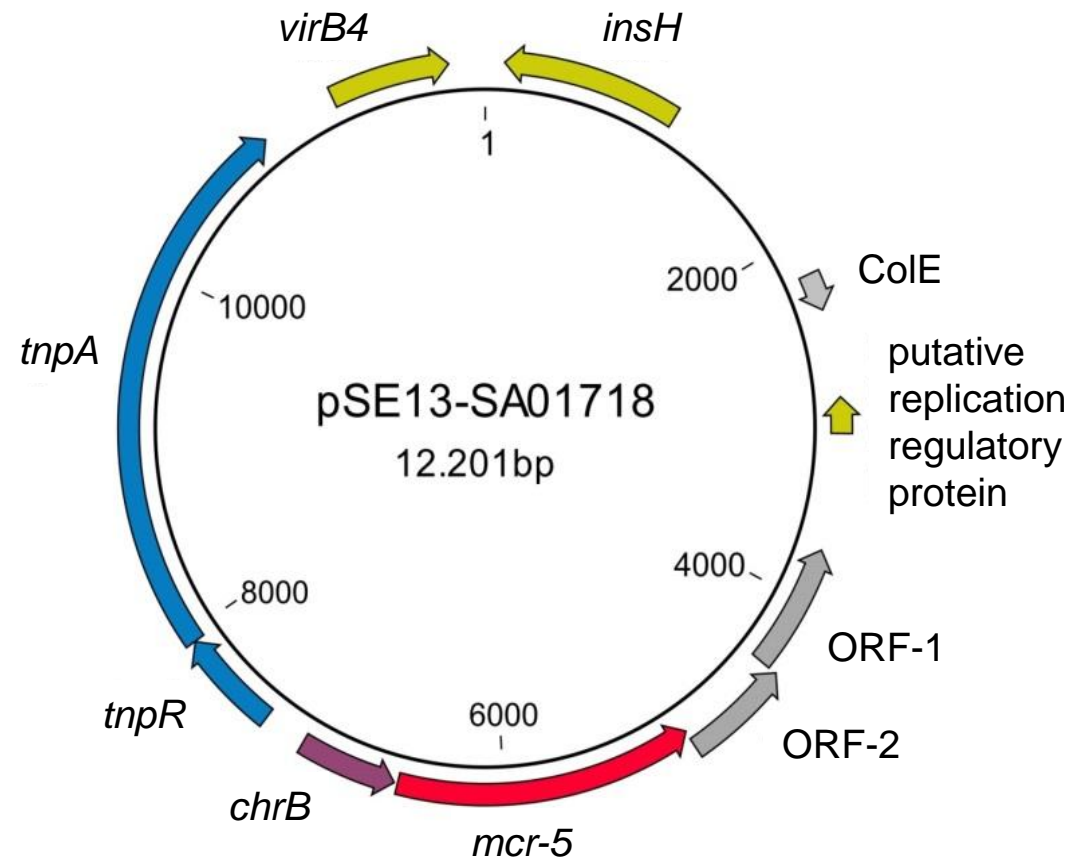




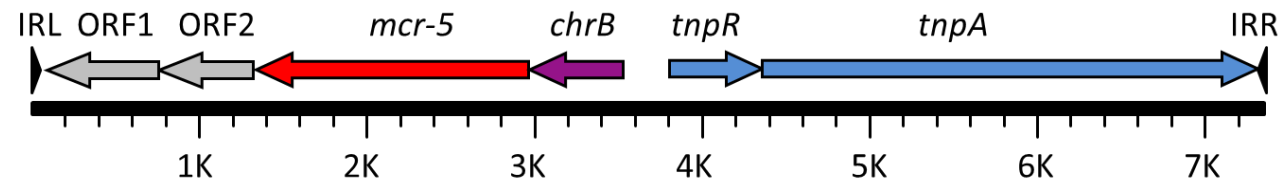
# Discovery of *mcr-5* in *Salmonella* using WGS data



S1-PFGE, Southern Blot & Hybridization



- in *Salmonella* Paratyphi B  $\Delta$ Ta+ *mcr-5* is harbored by ColE-like plasmids and associated with a Tn3 family transposon (Tn6452)
- Tn6452 integration in the bacterial chromosome was observed



Transposon Tn6452

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

Journal of  
Antimicrobial  
Chemotherapy

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

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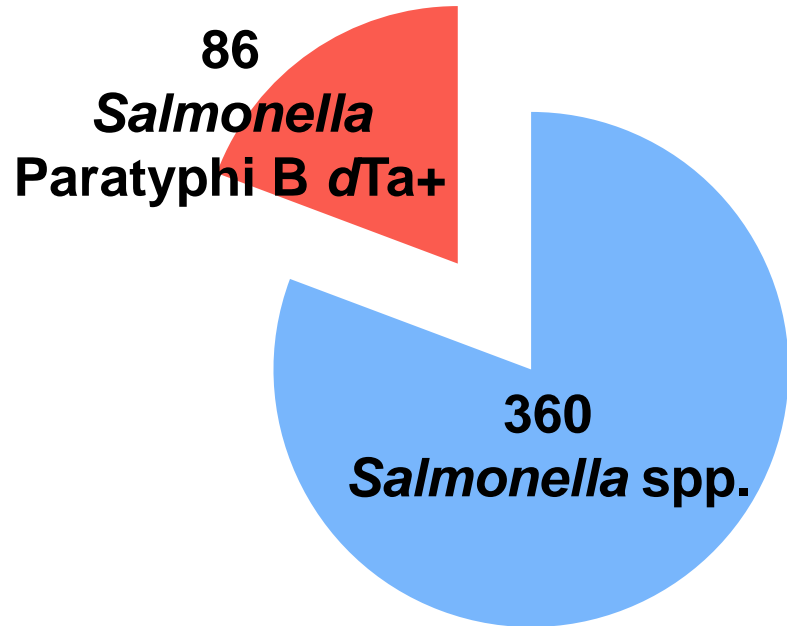
## Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in *d*-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B

Maria Borowiak<sup>1</sup>, Jennie Fischer<sup>1</sup>, Jens A. Hammerl<sup>1</sup>, Rene S. Hendriksen<sup>2</sup>, Istvan Szabo<sup>1</sup> and Burkhard Malorny<sup>1\*</sup>

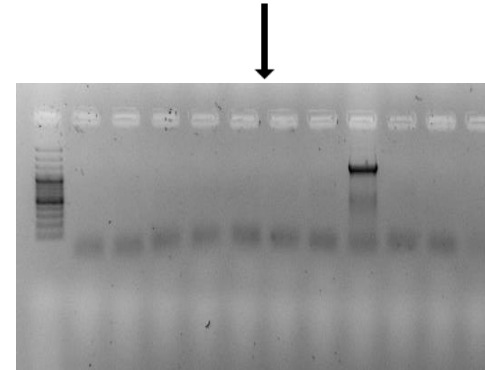
<sup>1</sup>German Federal Institute for Risk Assessment, BfR, Department for Biological Safety, Berlin, Germany; <sup>2</sup>National Food Institute, Technical University of Denmark, WHO Collaborating Center for Antimicrobial Resistance in Foodborne Pathogens and European Union Reference Laboratory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

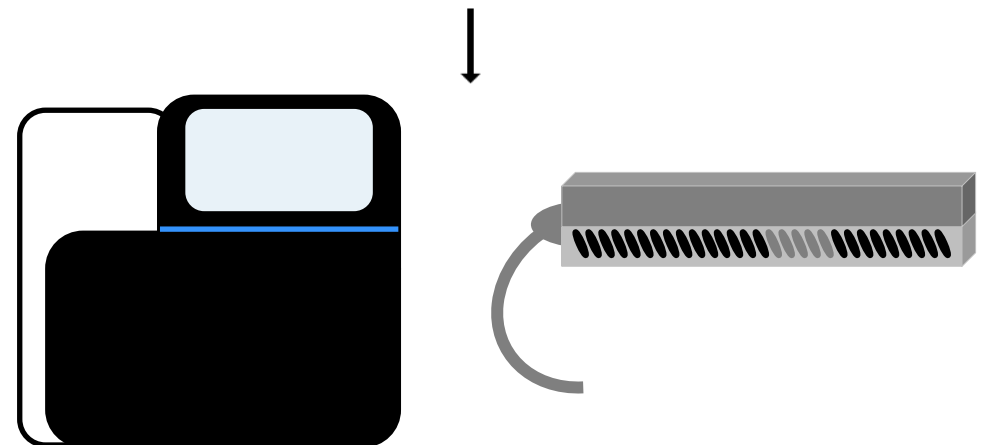


9 additional *mcr-5* positive isolates:  
2x *Salmonella* sp.  
4x *Salmonella* Typhimurium  
3x *Salmonella* Typhimurium monophasic

pig

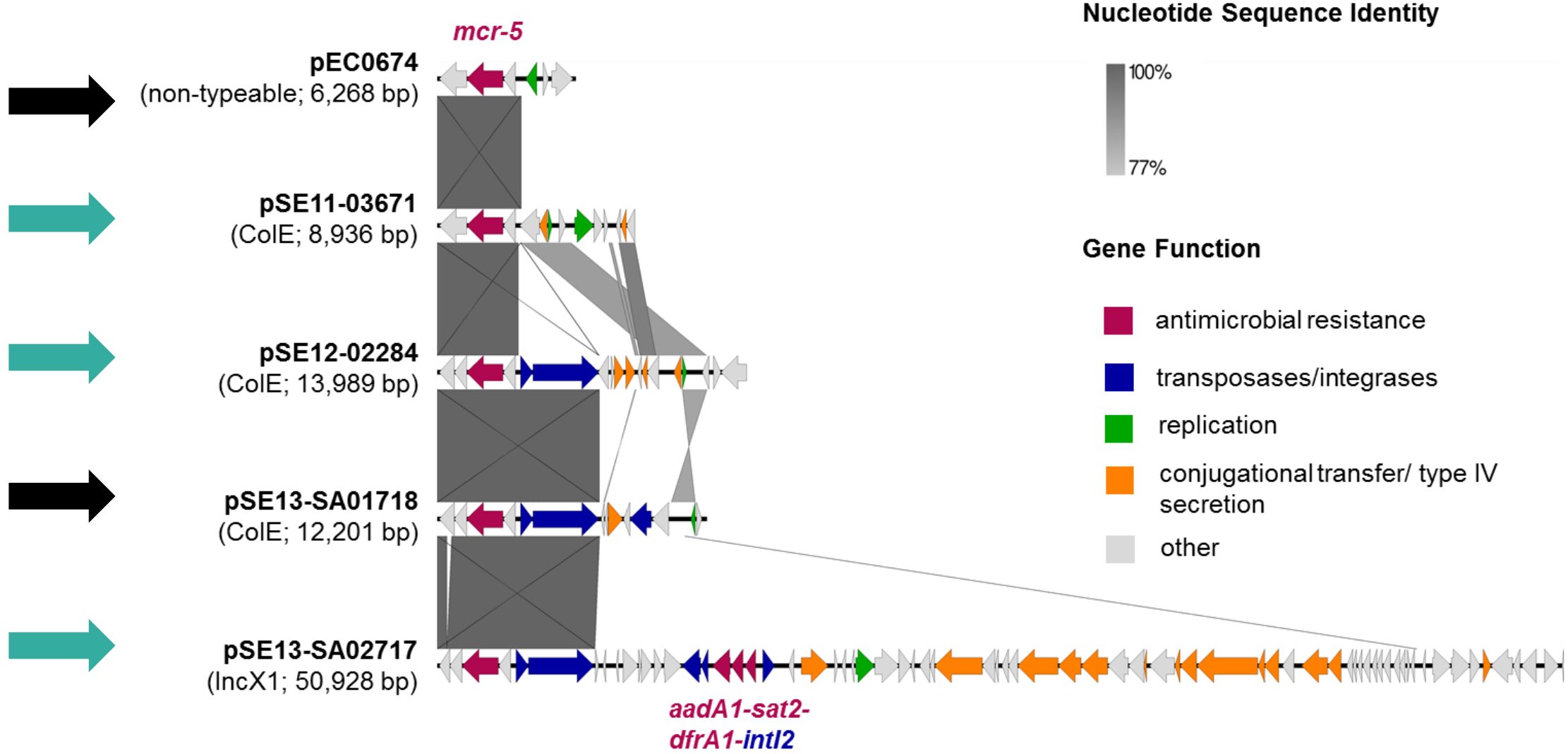


pork



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

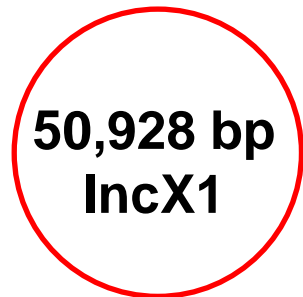
## *mcr-5* plasmid diversity in *Salmonella* Typhimurium



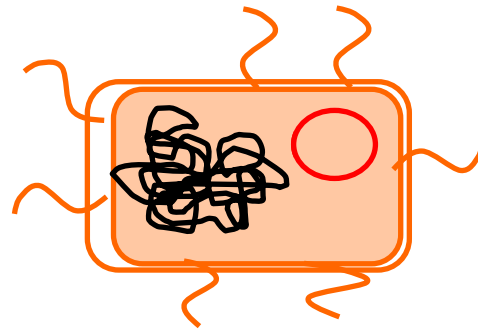
# Characterization of further *mcr-5* harboring *Salmonella*

## *mcr-5* mobility associated with conjugative plasmids

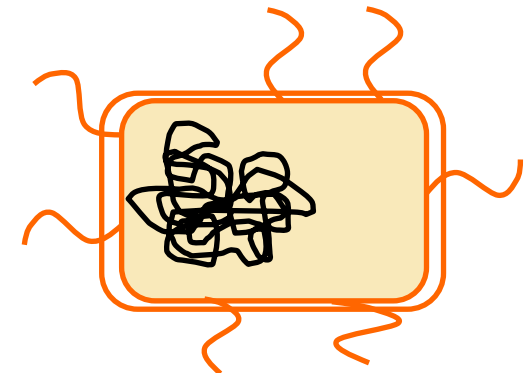
pSE13-SA02717



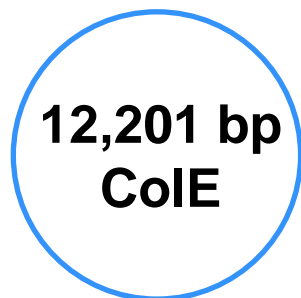
*Salmonella*



*E. coli* K12 J53



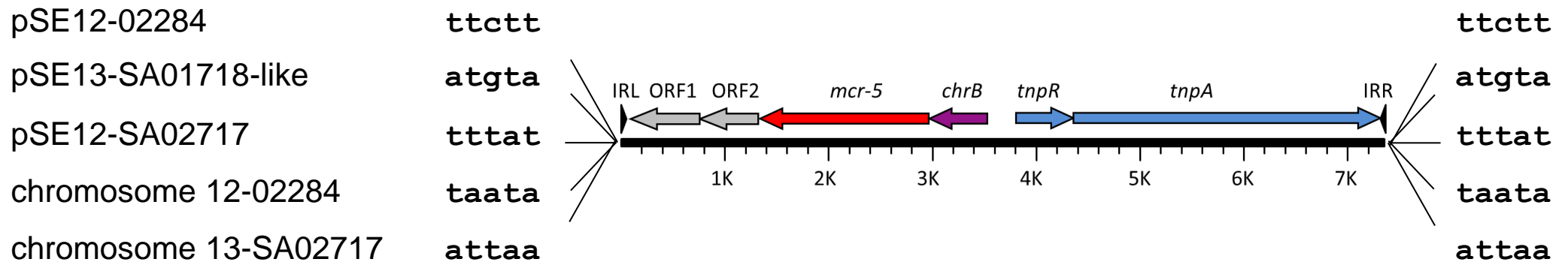
pSE13-SA01718-like



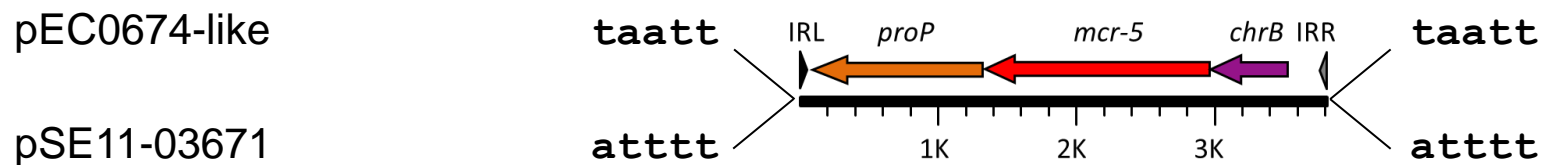
# Characterization of further *mcr-5* harboring *Salmonella*

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

### *mcr-5* located on Tn6452



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



# Global distribution of *mcr-5*

## Organisms harboring *mcr-5*:

### Publications:

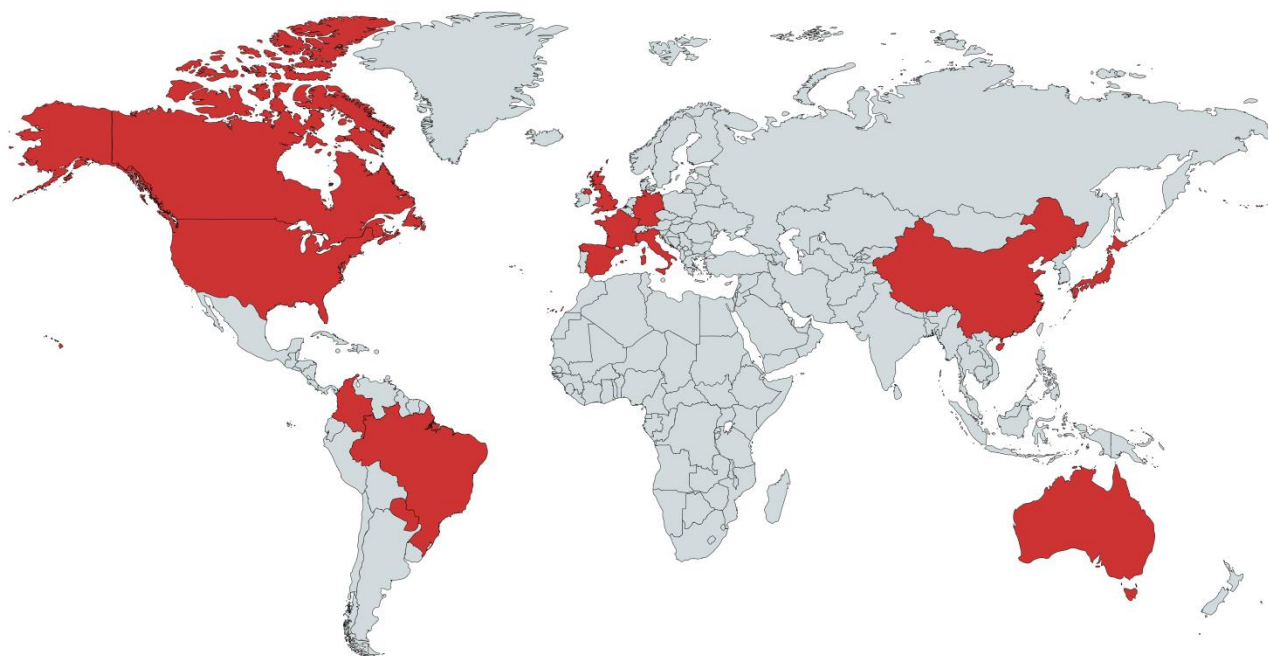
*Salmonella enterica*  
*Escherichia coli*

*Pseudomonas aeruginosa*  
*Aeromonas hydrophila*

### Blast:

*Pigmentiphaga* sp.  
*Cupriavidus gilardii*

## Countries reporting *mcr-5*

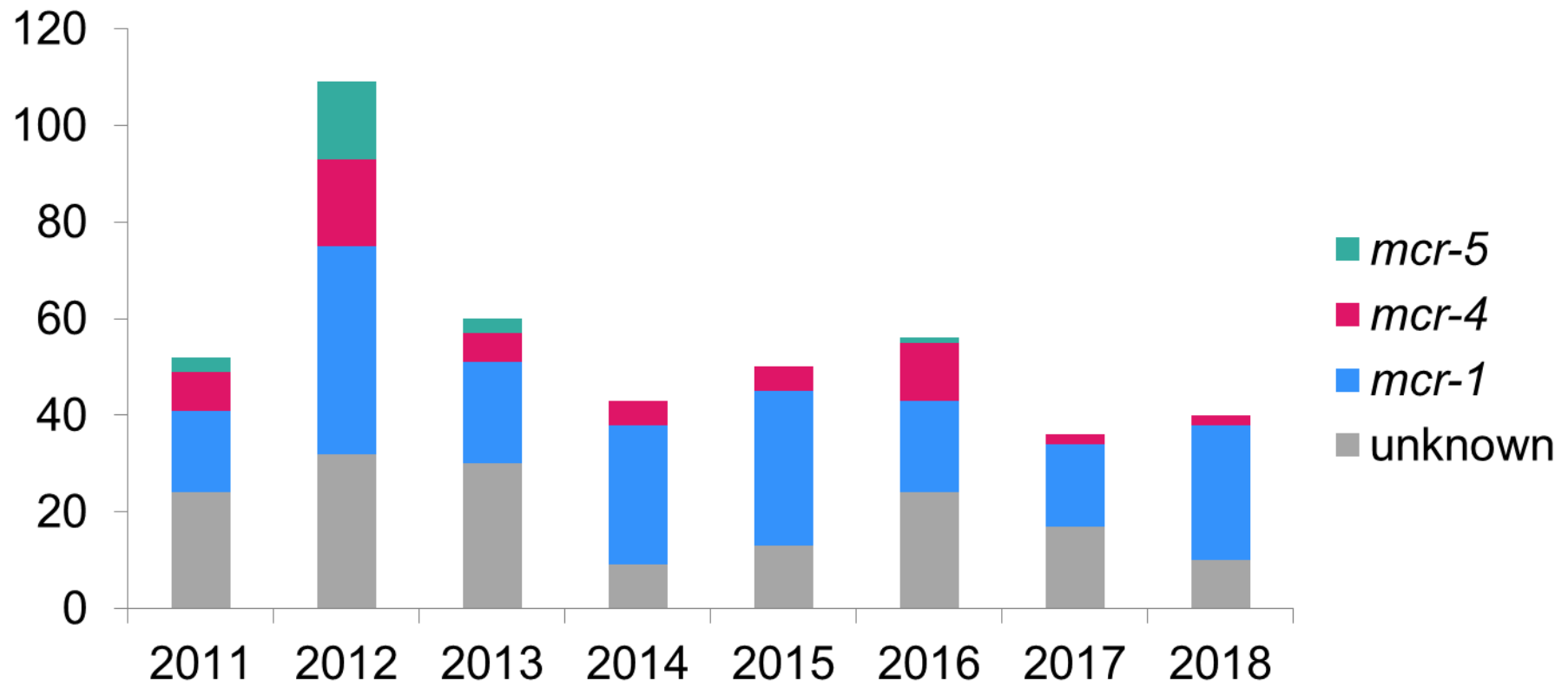


based on Publications, NCBI Genbank & Pathogen Isolate Browser, 21.03.2019

# 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)



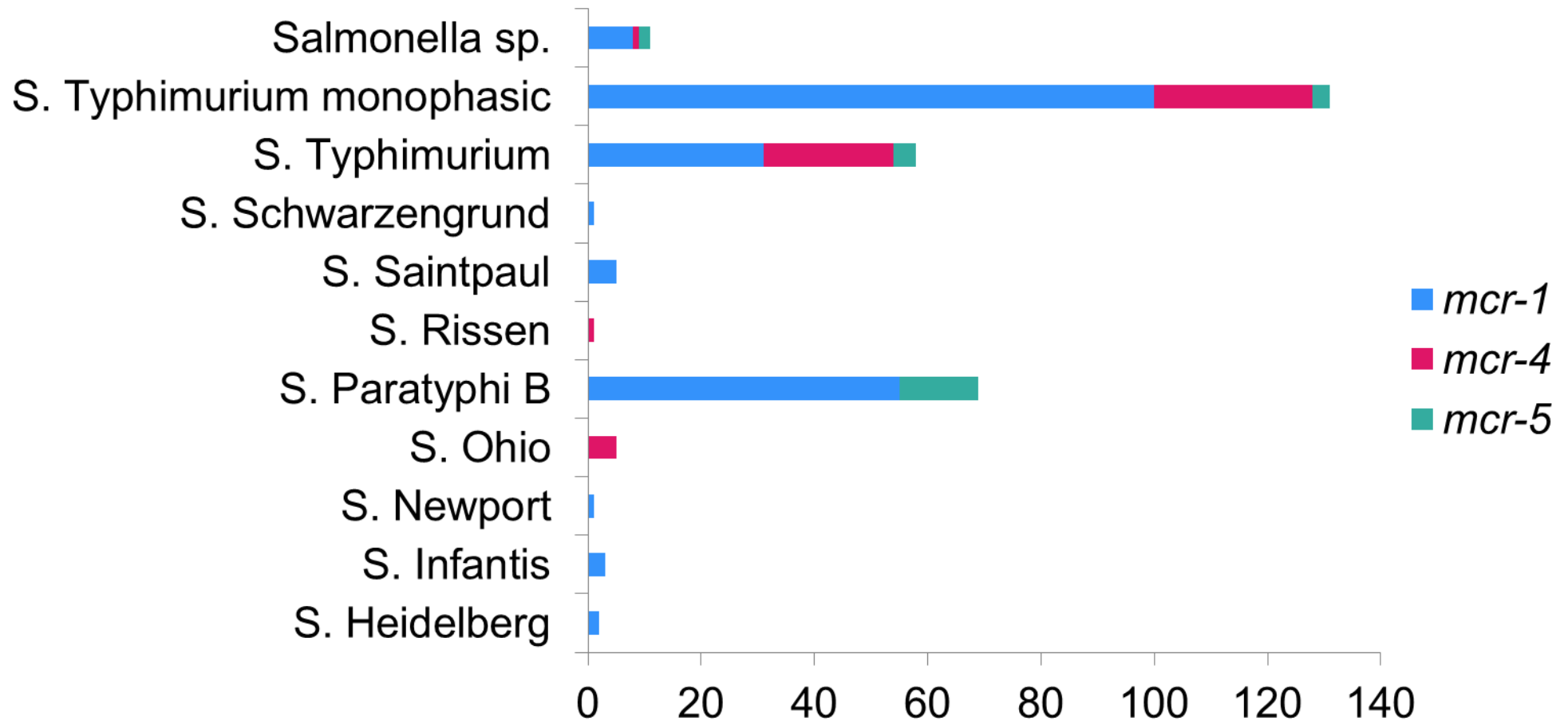
### Distribution of *mcr* genes in *Salmonella* isolates over the years



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

## Multiplex PCR screening on 446 colistin resistant *Salmonella* isolates

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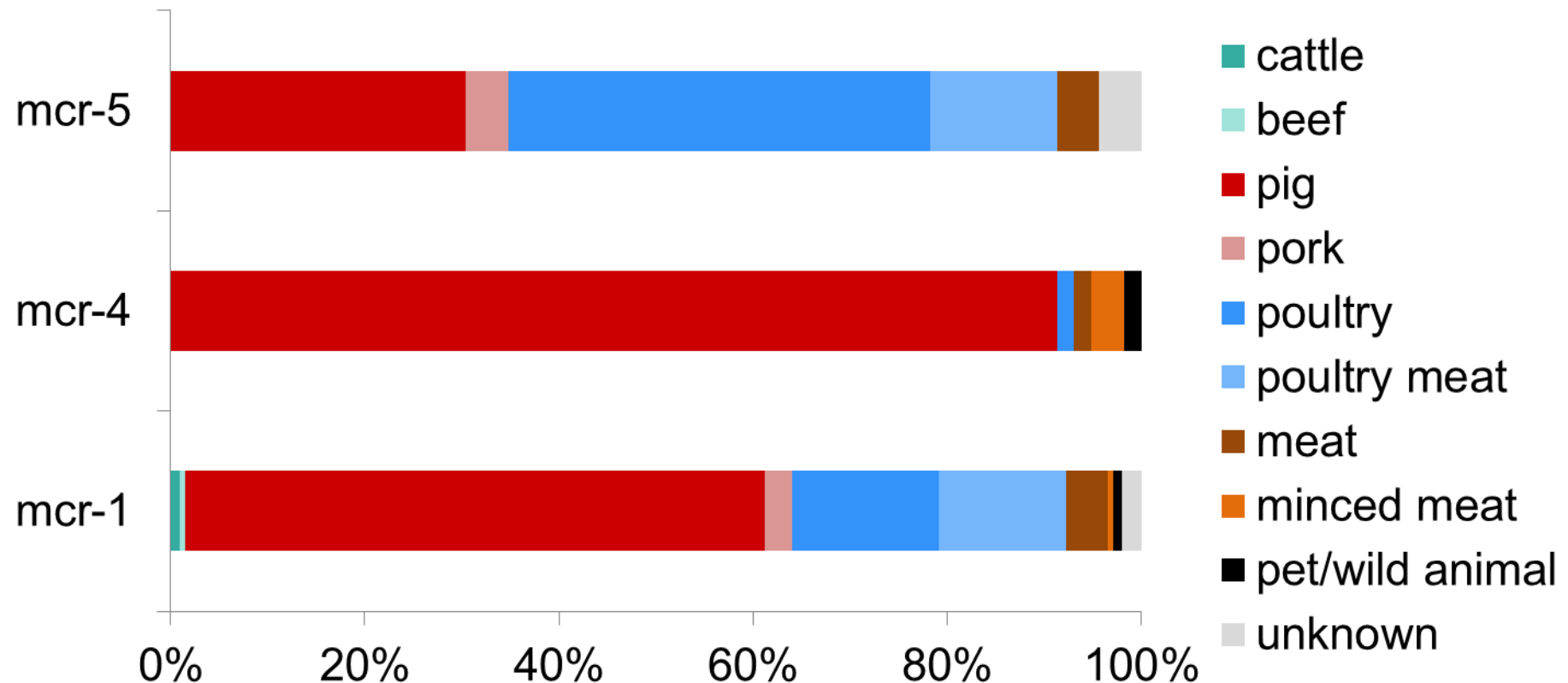


Distribution of *mcr* genes in different *Salmonella* serovars

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

## Multiplex PCR screening on 446 colistin resistant *Salmonella* isolates

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



### Isolation sources of *mcr* harboring *Salmonella* isolates

# Summary

- colistin resistant ***Salmonella*** isolates 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 dTa+*
- 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 (Tn6452)** or a **putative mobile insertion cassette**
  - in three isolates **integration of Tn6452 in the bacterial chromosome** was observed

# Acknowledgment

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Katharina Thomas

### Unit 42:

Dr. Jennie Fischer  
Dr. Istvan Szabo

### Unit 43

Dr. Jens A. Hammerl



Bundesinstitut für Risikobewertung



### *mcr-5* in *Salmonella Paratyphi B* $\Delta$ 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.

**Thank you for your attention**

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