

Phylogeny of *Salmonella* Paratyphi B variant Java harbouring *mcr-1*

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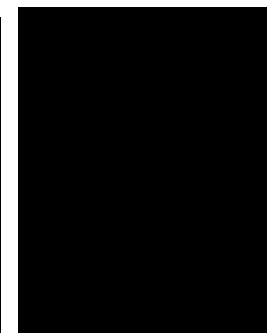
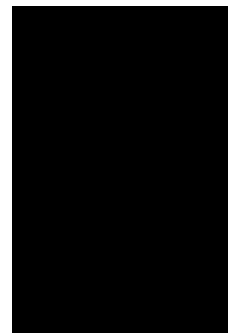
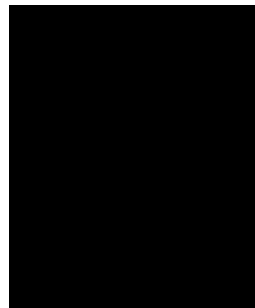
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Salmonella enterica subsp. *enterica* Paratyphi B (dT+) variant Java

Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Salmonella*
Species: *S. enterica*
Subspecies: *S. enterica* subsp. *enterica*
Serovar: Paratyphi B dT+ (4,[5],12:b:1,2)
Variant: Java

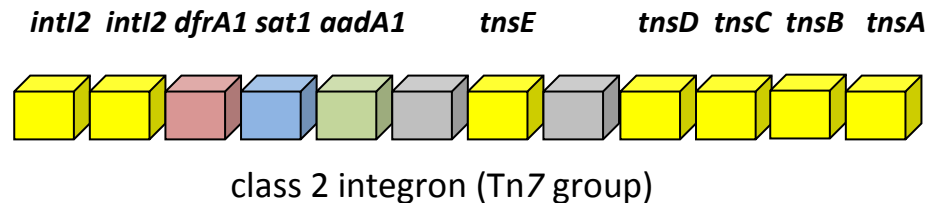


- d-tartrate-fermenting (dT+)
- zoonotic pathogen
- can lead to gastroenteritis
- frequently found in poultry



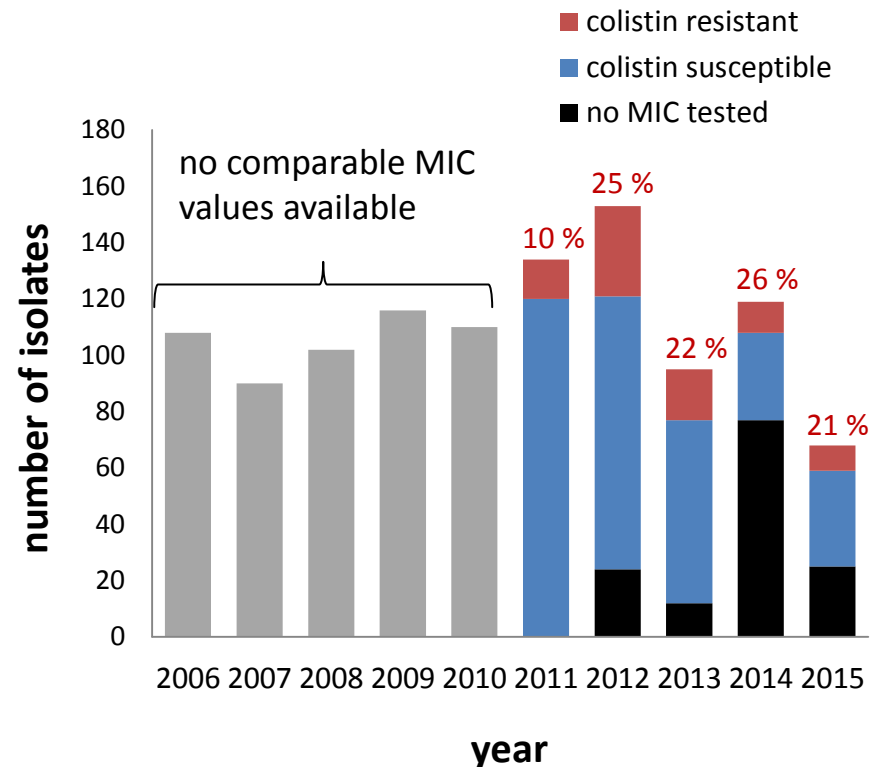
Salmonella enterica subsp. *enterica* Paratyphi B (dT+) variant Java

- persistent multi-drug resistant lineage in Germany
 - chromosomally located class 2 integron [*dfrA1-sat1-aadA1*]
 - chromosomal point mutation in gyrase gene (quinolone resistance)
 - acquisition of diverse resistance plasmids (ESBL-genes, *mcr-1*,...)



Phenotypic colistin resistance *in S. Paratyphi B (dT+)* variant Java

- NRL for Analysis and Testing of Zoonoses in Germany received ca. 90 *S. Paratyphi B (dT+)* isolates from food and animals per year
- comparable MIC values for colistin are available since 2011
- 10 to 26 % of the tested isolates have a microbial resistance (MIC ≥ 4) for colistin

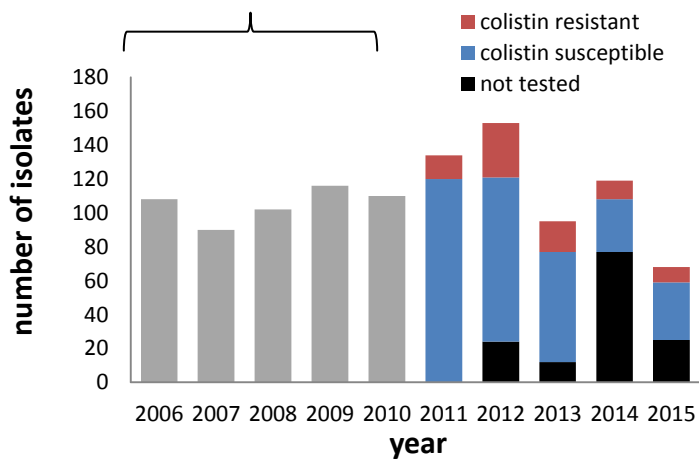


Strain selection, PCR screening & sequencing

strain selection

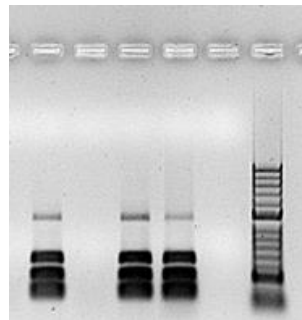
every second strain & strains with similar resistance profile to *mcr-1* + strains

all strains with a MIC ≥ 4



394 isolates

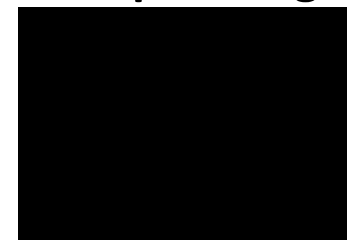
multiplex PCR screening



3 PCR product combinations (5', center, 3')

mcr-1
positive isolates

Whole genome sequencing

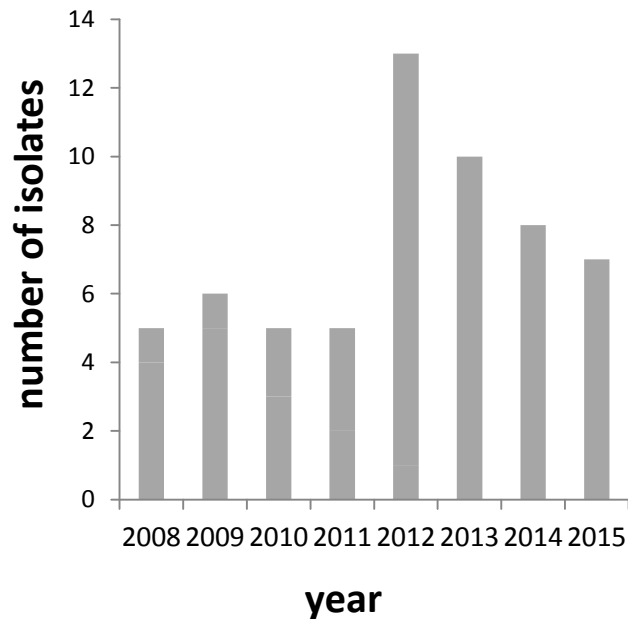


MiSeq (all isolates)

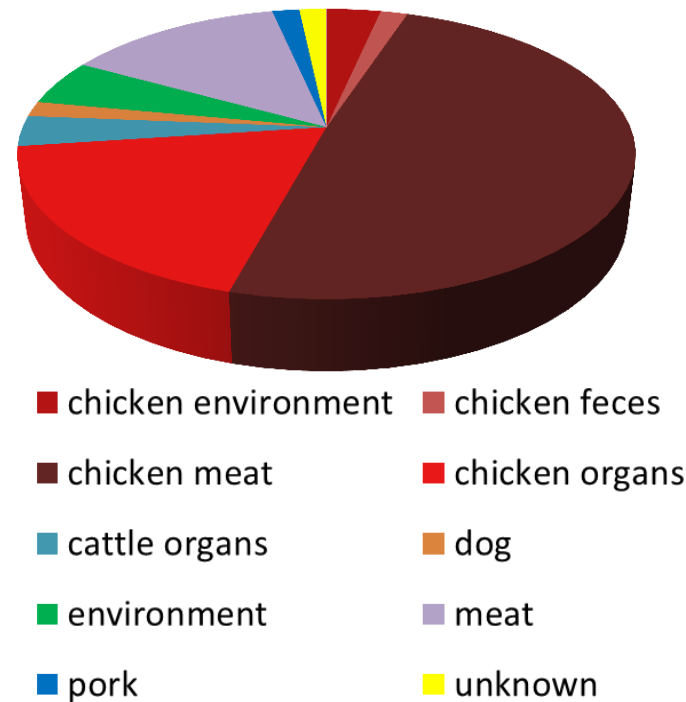


PacBio (earliest isolate)

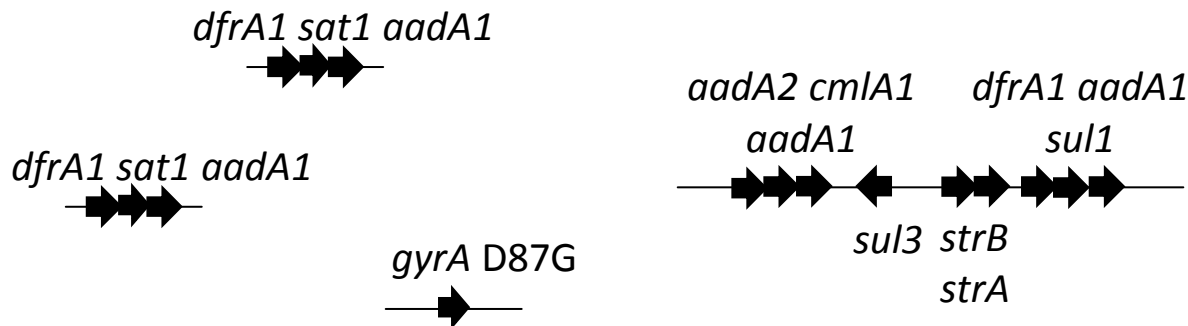
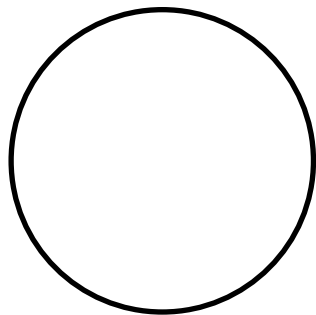
mcr-1 positive *S. Paratyphi* B (dT+) variant Java



- 59 epidemiological unlinked *mcr-1* positive isolates were identified
- most isolates were recovered from chicken
- 58 isolates belonged to ST-28 and one isolate to ST-3663



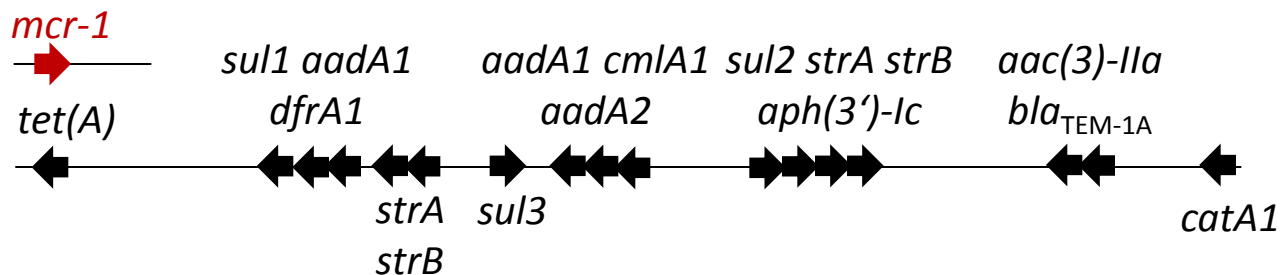
The earliest identified isolate: 08-00436 (PacBio sequence) from chicken skin



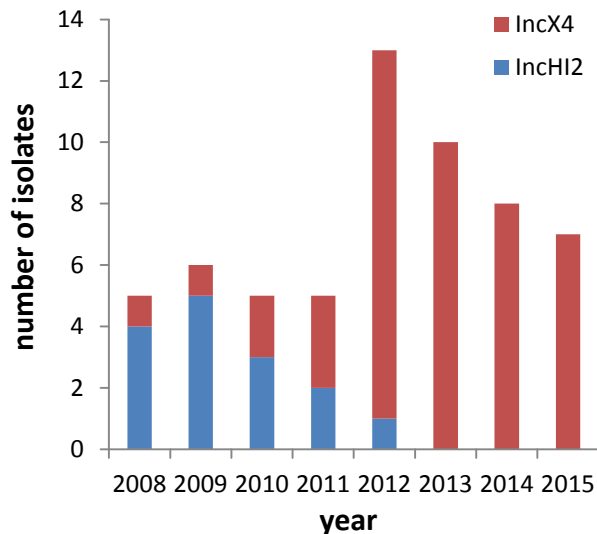
bacterial chromosome: 4,751,926 bp

○
Plasmid 1: 264,914 bp
IncHI2, IncHI2A, IncQ1

●
Plasmid 2: 54,067 bp
IncI1

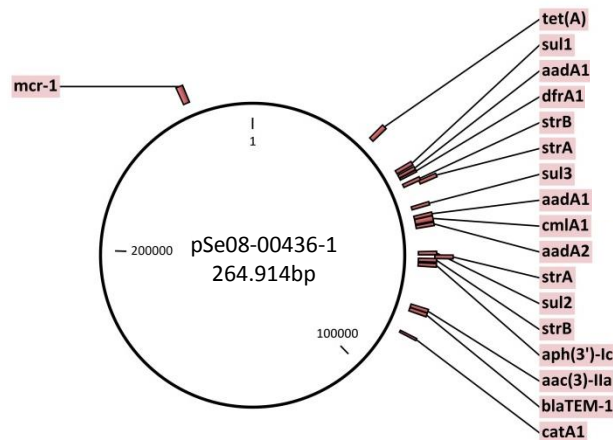


mcr-1 plasmid localization



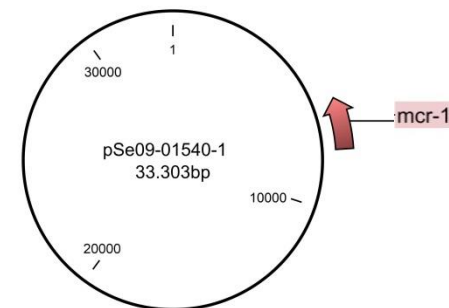
- *mcr-1* is located on IncHI2 or IncX4 type plasmids

IncHI2 type plasmid from 08-00436



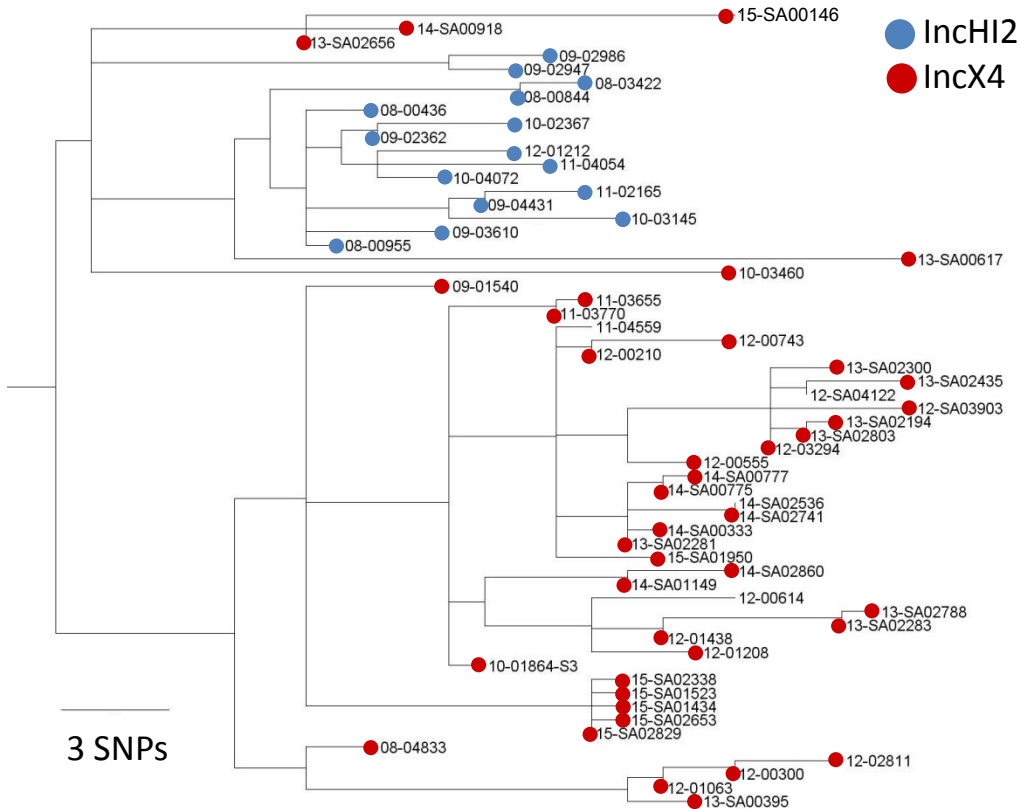
- large plasmid with lots of resistance genes

IncX4 type plasmid from 09-01540



- smaller plasmid with *mcr-1* as the only resistance gene

Phylogeny of *S. Paratyphi* B variant Java harbouring *mcr-1*



- Neighbor-joining phylogenetic tree based on whole genome sequence data of 59 *mcr-1* positive isolates
- Reference: bacterial chromosome from the earliest isolate identified (08-00436 PacBio sequence)

- ***S. Paratyphi B (dT+)* variant Java has evolved to colistin resistance through acquisition of *mcr-1* in at least 2 independent events**
→ multi-resistant IncHI2 plasmids and IncX4 plasmids
- ***mcr-1* positive isolates are still circulating in the German chicken production**
→ 5 colistin resistant isolates harboring *mcr-1* in 2016
(10 % of all *S. Paratyphi B* subjected to MIC testing)
- **these findings underline the importance of further monitoring and surveillance**

Acknowledgment

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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