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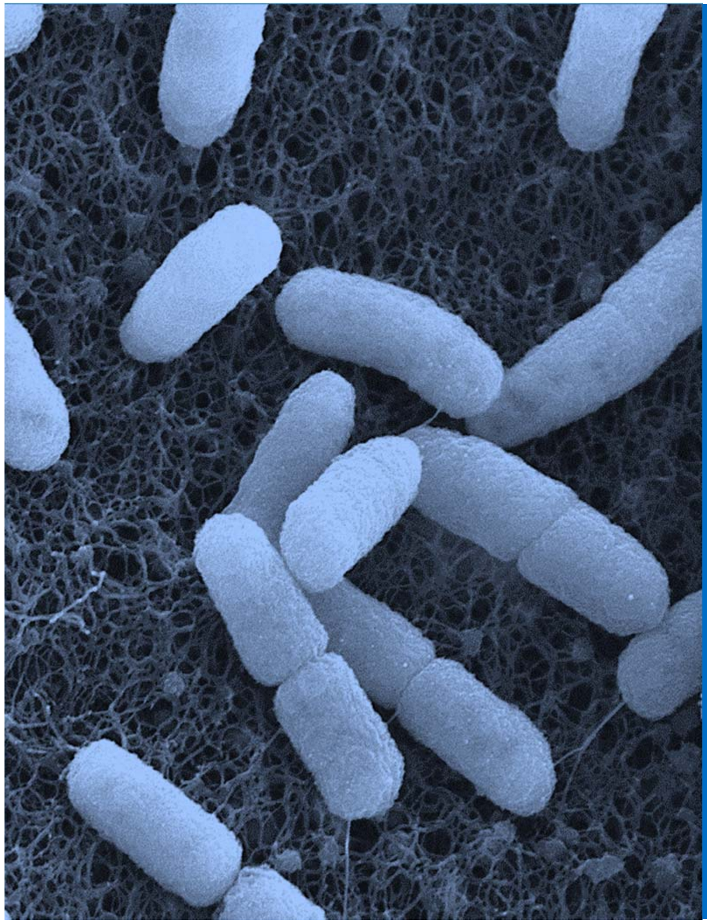


NGS-based analysis of AmpC-beta-lactamase CMY-2-producing *Escherichia coli* from humans, livestock and food in Germany

Michael Pietsch
Berlin, 27.04.2017

RESET & MedVetStaph – Wissenschaftliches Abschlusssymposium, 26.-28. April 2017, Berlin

AmpC β-lactamases

Bacterial enzymes that hydrolyze penicillins, cephalosporins and cephamycins → resistance

- Chromosomal-encoded AmpC β-lactamases
  - inducable or constitutive high-level expression of the *ampC* gene, e.g. in *Enterobacter cloacae* and *Citrobacter freundii*
  - constitutive low-level expressed ampC genes (*E. coli*)

  modifications in regulatory mechanisms result in *ampC* overexpression

mobilization event

- Plasmid-encoded AmpC β-lactamases (pAmpC)

Fig. Mo S. *et al.* 2016

pAmpC β-lactamases

- Mobilized formerly chromosomal *ampC* genes
- Acquisition by various species by plasmid or transposon integration, e.g. *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumoniae*, *Proteus mirabilis*
- *ampC* overexpression due to a promotor upstream of *ampC*

AmpC β-lactamase:	Origin
CMY	*C. freundii*
ACC	*H. alvei*
ACT	*E. cloacae*
FOX	
MOX	*A. hydrophila*
DHA	*M. morgannii*

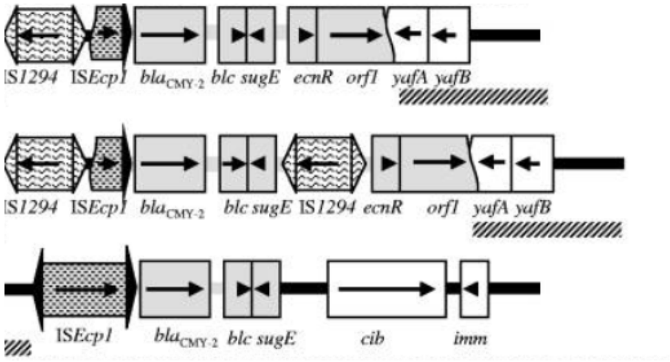


Fig. Verdet C. *et al.* 2009

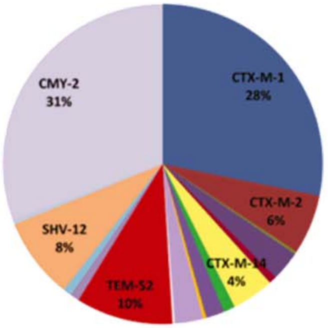
- Most prevalent pAmpC in Enterobacteriaceae: DHA, CMY, esp. CMY-2

pAmpC CMY-2: prevalence in Germany/Europe

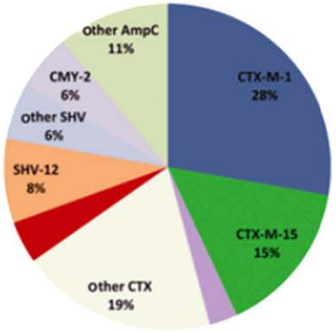
RESET studies on livestock animal and food:

- **Ca. 10 %** of all *E. coli* isolates from livestock/food with resistance to cephalosporins produced pAmpC, mainly **CMY-2**

Poultry

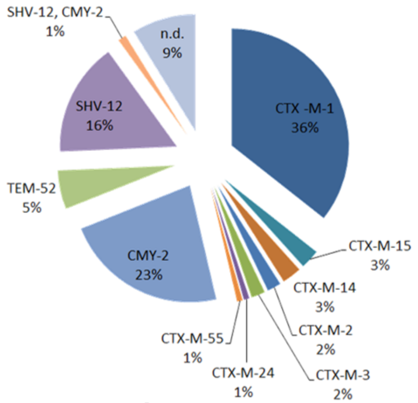


Companion animals

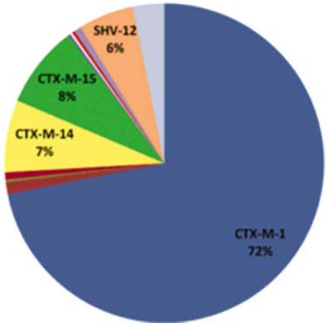


Food products poultry (n=264)

Jennie Fischer; ECCMID 2016



Cattle/Pig



Cephalosporin-resistance and β-lactamases in *E. coli*

- Ewers *et al.* Clin Microbiol Infect 2012:
- Ca. **6%** of *E. coli* from cattle/pig and companion animals harboured **CMY-2**
- Ca. **30%** of *E. coli* from poultry harboured **CMY-2**

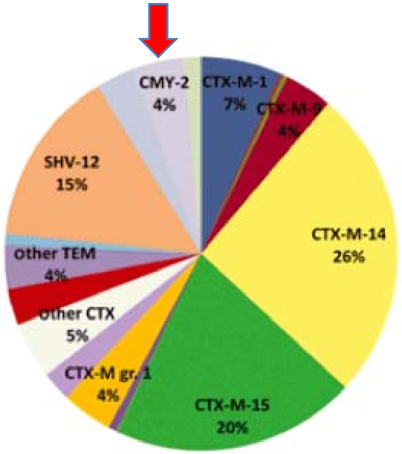
pAmpC CMY-2: prevalence in Germany/Europe

Prevalence in *E. coli* isolates from humans

- Proportion of CMY-2- *E. coli* as commensal: 1 % (Valenza G.*et al.* 2014)
- Proportion in human infections in Denmark: <1% (Jørgensen RL, *et al.* 2010)
- Proportion of CMY-2-*E. coli* in ambulance/hospital: ca. 1 %

  (France: 1% Pascual V.*et al.* 2015)

Humans

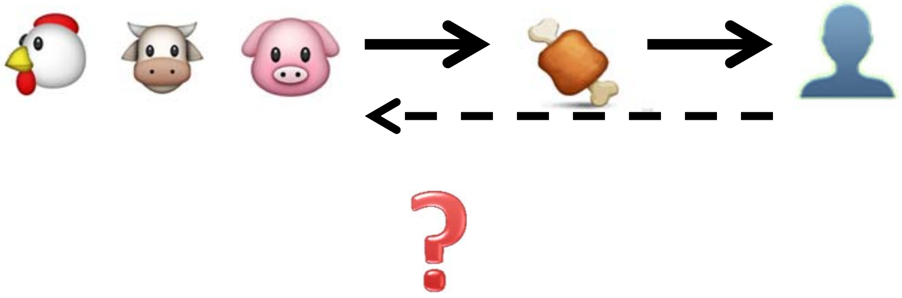


Ewers C., *et al.* 2012

Study aims

Molecular characterization of CMY-2-*E. coli* isolates from humans, livestock animals and food:

- Characterize the phylogeny of CMY-2 producing *E. coli*
- Investigate the genetic environment and plasmid location of $\mathit{bla}_{\text{CMY-2}}$
- Evaluate possible resistance transfer ways between the different reservoirs



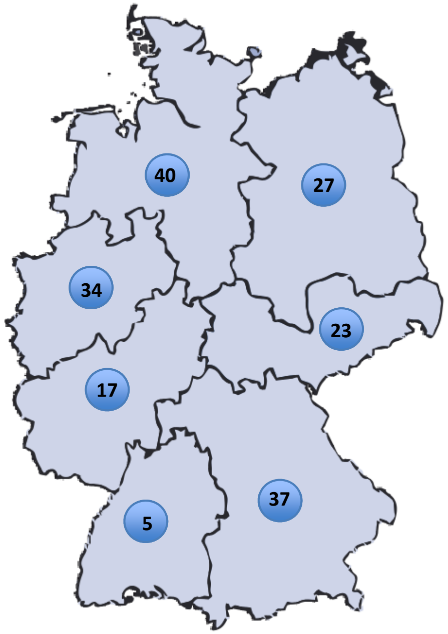
Study design - Isolates

- Selection of CMY-2 positive *E. coli* from livestock animals, food and humans (RESET database)
- Isolates were collected in different studies performed the scope of the RESET research consortium in Germany 2009-2013:

RESET

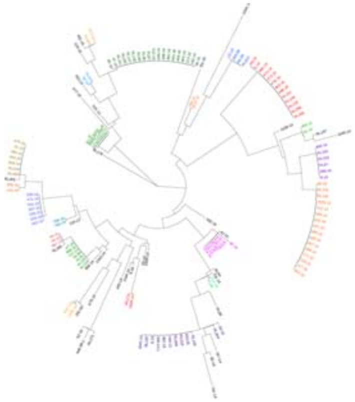
Source	Number of *E. coli* isolates
human	51
animal*	56
food	63

*chicken n=41, turkey n=4, pig n=9, cattle n=2



Study design – Whole genome analysis

- Sequencing of 170 selected isolates via Illumina MiSeq
- *De novo* assemblies and mapping pipelines
  - A5 and BWA-SW
- Phylogenetic analysis by Multilocus SequenceTyping (MLST) and core-genomeMLST (cgMLST)
  - SeqSphere+, Geneious
- Analysis of plasmid replicon and backbone structures
  - pMLST and Inc types
- Analysis of the direct genetic environment of *bla*$_{CMY-2}$



Results

Resistance and resistance genes

- 50% of all **CMY-2-*E*.** *coli* carried additional β-lactamase genes:
  - *bla*$_{\text{TEM}}$ (n=72), *bla*$_{\text{CTX-M}}$ (n=8), *bla*$_{\text{OXA}}$ (n=6), *bla*$_{\text{SHV}}$ (n=5)
- 12 **CMY-2**-*E. coli* were *mcr-1* positive (plasmid-mediated colistin resistance)
- 7 **CMY-2**-*E. coli* were *aac(6*')-*Ib-cr* positive
- 6 **CMY-2**-*E. coli* presented *qnr* genes (*qnrS* n=4 and *qnrB* n=2)

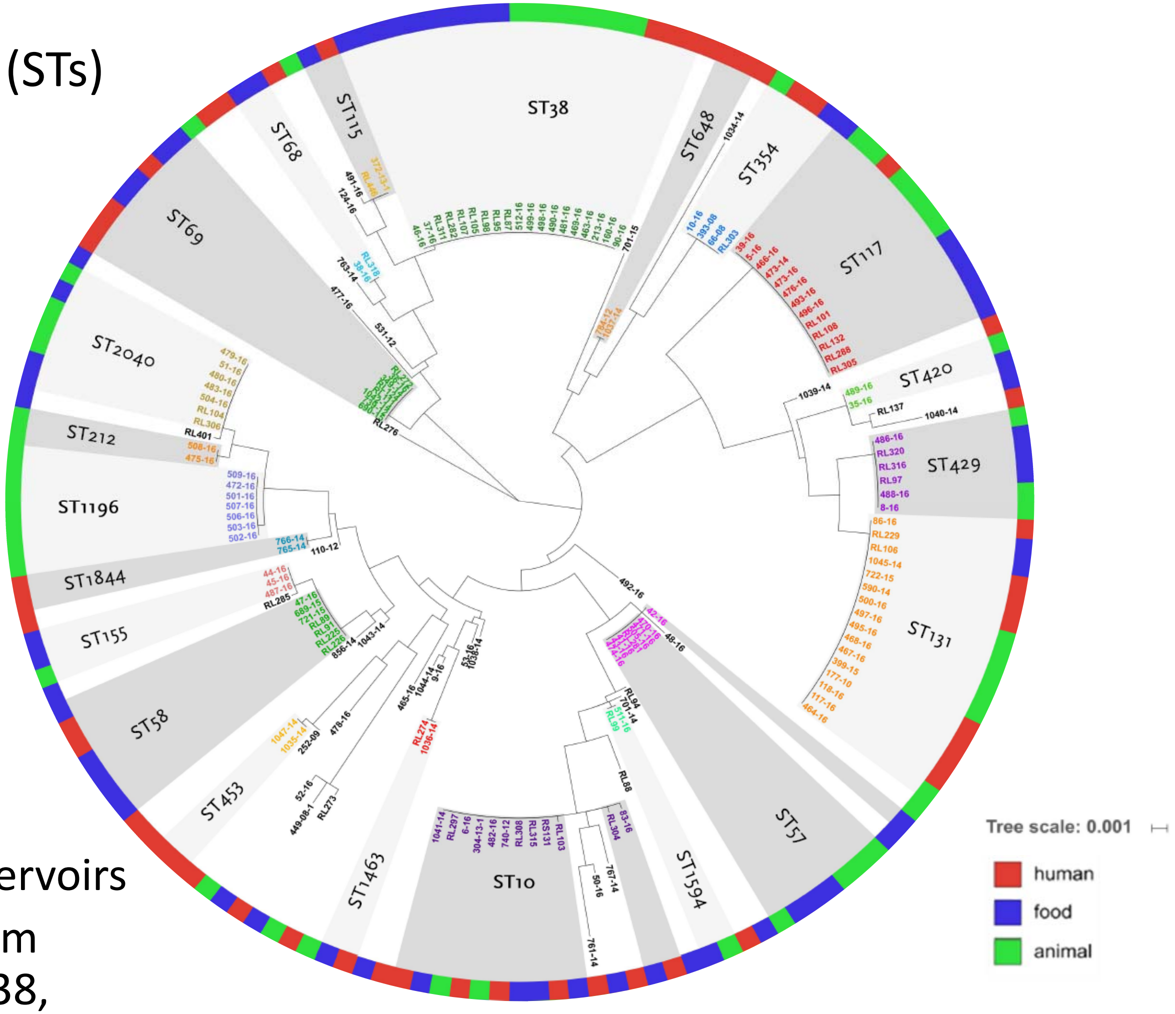
Plasmid content

- High diversity of plasmid replicons were observed:
  - IncFIB (n=138), IncFII (n=90), IncI1 (n=87), IncK (n=80)
  - 16 further Inc types

Bacterial strain typing: MLST Analysis

59 different sequence types (STs)

ST	Number
ST-38	19
ST-131	16
ST-117	13
ST-10	9
ST-2040	8
ST-69	8
ST-1196	7
ST-57	7
ST-58	7
ST-429	6
others	70

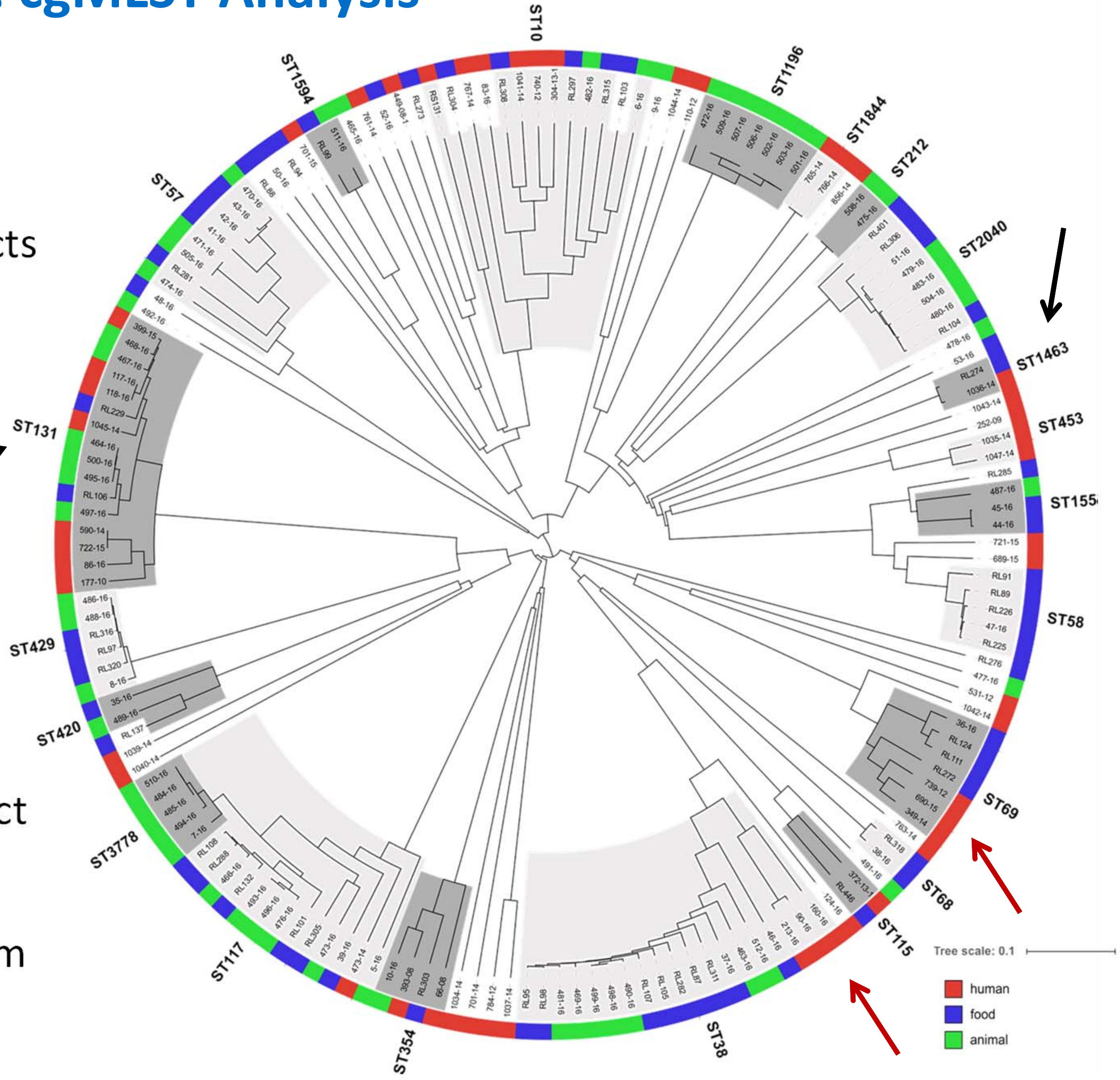


The large variety:

- STs are distributed in all reservoirs
- Overlap of CMY-2-*E. coli* from animal and food: ST131, ST38, ST57, ST117, 2040, ST429

Bacterial strain typing: cgMLST Analysis

- *Ad hoc* cgMLST based on 2547 targets
- Higher discriminatory effects



- Human *E.coli* cluster distinct from poultry or food *E.coli*
- Closely related isolates from human and animal (ST131, ST1463)

E. coli-ST131

Successful pandemic clone *E. coli* O25b:H4-ST131

- associated with the global dissemination of CTX-M-15 type ESBL
- associated with resistance to fluoroquinolones
- associated with the human host and infections in humans, particularly UTIs

RESET ESBL studies on *E. coli* O25b:H4-ST131 from human livestock animal and food:

ESBL:	human	ambulant: 40% (Pietsch M. *et al.* 2015)
		nosocomial: 32.3% (Pietsch M. *et al.* 2015)
		colonization: 12.3% (Valenza G. *et al.* 2014)
		companion animal: 5.6% (Ewers C. *et al.* 2010)
		livestock: 0 – 1.8% (Nicolas-Chanoine MH. *et al.* 2014)

Current AmpC study on *E. coli* O25b:H4-ST131

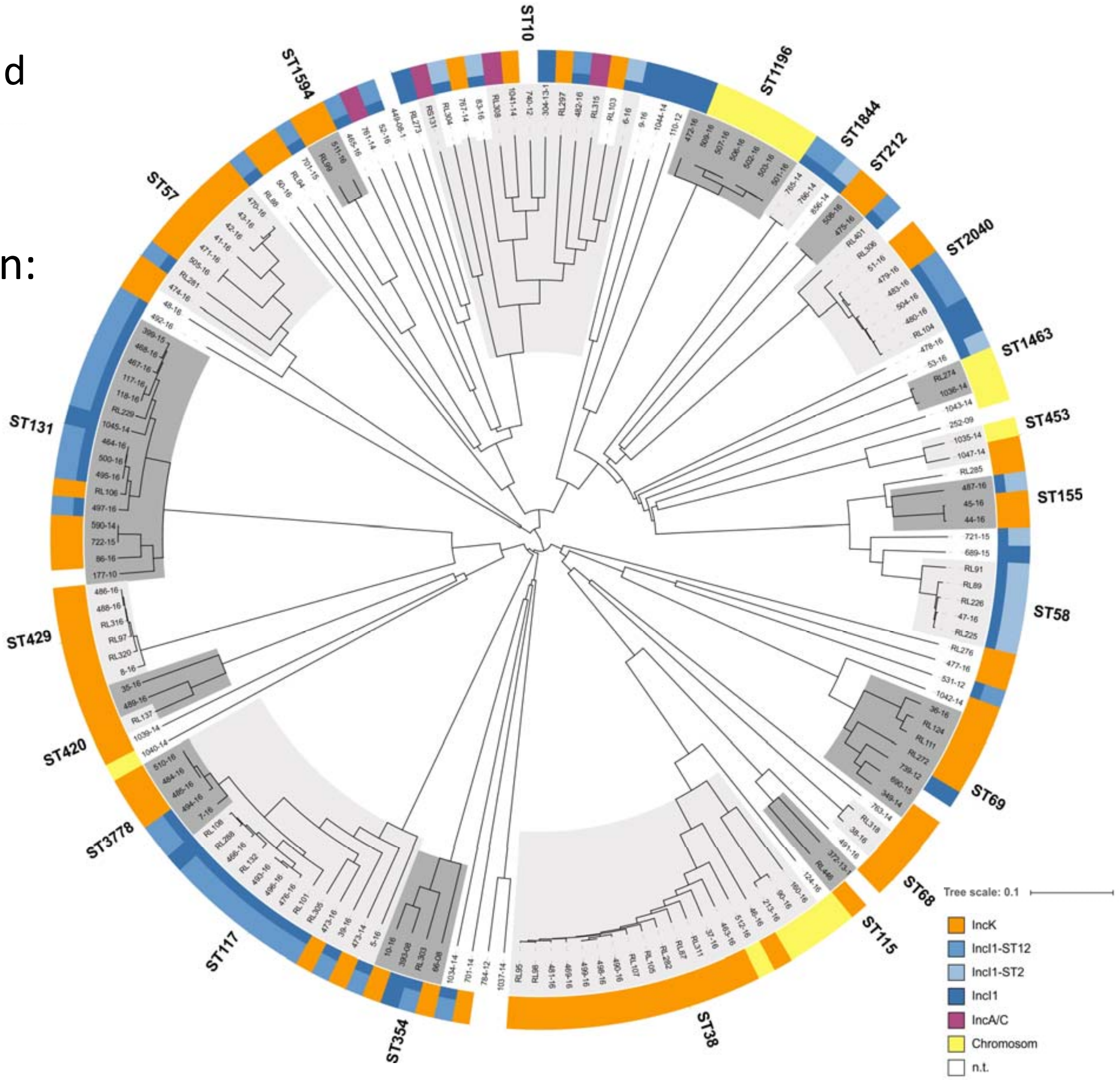
CMY-2:	human	14.3% (isolates from ambulant and nosocomial settings)
		6.7% (isolates from animals and food products - poultry)
		11% (clinical isolates from Norway) (Naseer U. *et al.* 2009)

Plasmid Analyses

1. reference based mapping and *de novo* assembly

bla$_{CMY-2}$ and plasmid replicon:

bla$_{CMY-2}$ localization	Amount
IncK2	76
IncI1	65
IncA/C	4
Chromosome	15
untypeable	7



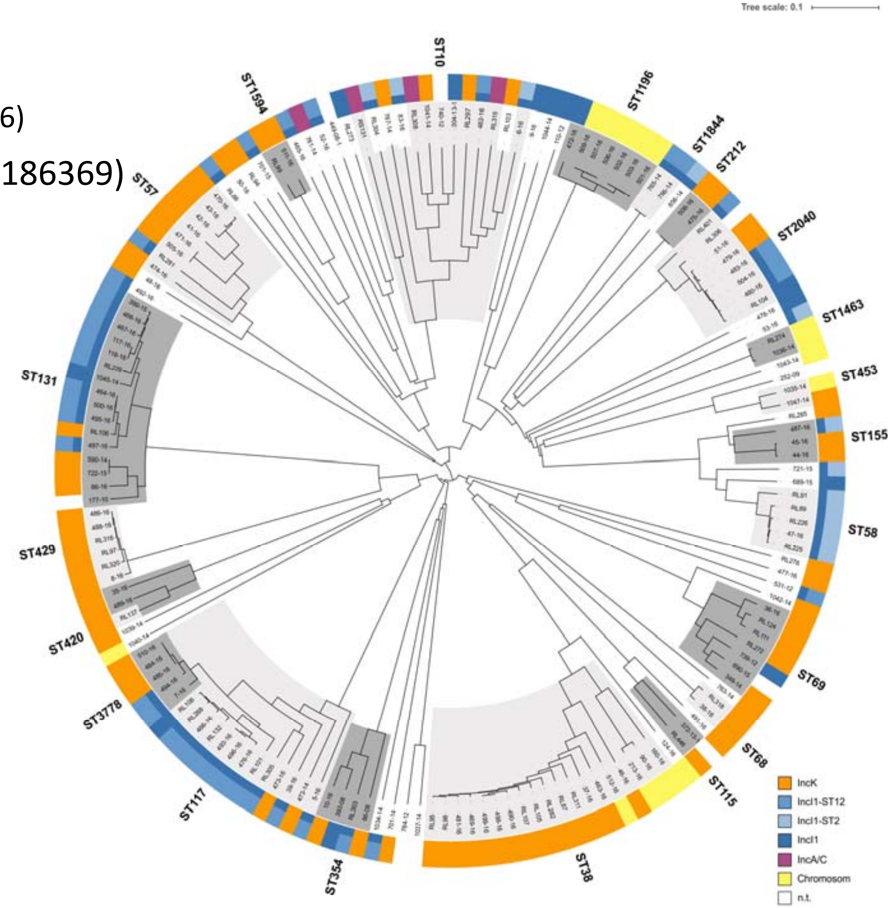
Plasmid Analysis

Seven highly conserved plasmid structures were identified

IncK2: KR905386 (79kb) (Seiffert *et al.* 2017)
KR905384 (86kb) (Vogt *et al.* 2014)
p486-16 (115kb) (alteration of KR905384)
IncI1: KT186369 (90kb) (**pST12**) (Hansen *et al.* 2016)
p1045-14 (99kb) (**pST12**) (alteration of KT186369)
CP012929 (96kb) (**pST2**) (Labbe *et al.* 2016)
IncA/C: CP014658 (160kb) (Nguyen *et al.* 2016)

Chromosomal localization:

- Two mayor variants (C. Verdet *et al.* 2009)

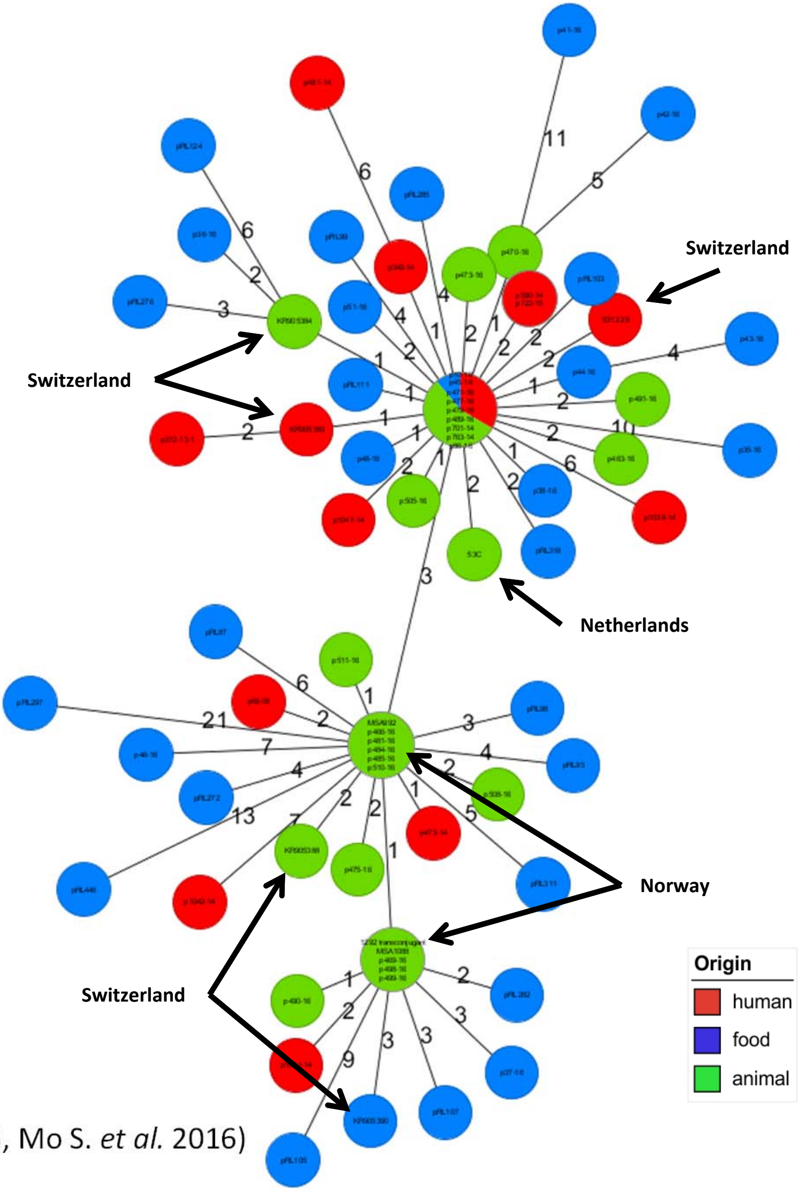


Plasmid Analysis – IncK plasmids

- **cgMLST IncK plasmid (based on 78 targets)**
- all IncK consists of new described IncK2
  (Seiffert N. *et al.* 2017)
- *bla*$_{\text{CMY-2}}$-IncK2 plasmids found in various *E. coli*-STs from different reservoirs

ST	Number
ST-38	15
ST-57	8
ST-429	6
ST-69	6
ST-131	3
ST-3778	3
ST-10	2
ST-115	2
ST-117	2
ST-155	2
ST-1594	2
ST-2040	2
ST-212	2
ST-354	2
ST-420	2
ST-68	2
single variants	15

- International comparison: highly homogenious IncK cluster distributed in divers *E. coli* clones from different timeframes
- Similar findings in Netherlands, Sweden, Norway, Switzerland
  (Seiffert N. *et al.* 2017, Berg E.S. *et al.* 2017, Voets G. M. *et al.* 2013, Mo S. *et al.* 2016)



Summary

- **High diversity** in *bla*$_{CMY-2}$-positive *E.coli* STs from different reservoirs
  - cgMLST more discrimintory -> higher diversity of STs and reservoirs
  - Higher resolution/discriminatory power of cgMLST/SNP-based methods needed for population based studies
  - Prevalent types:

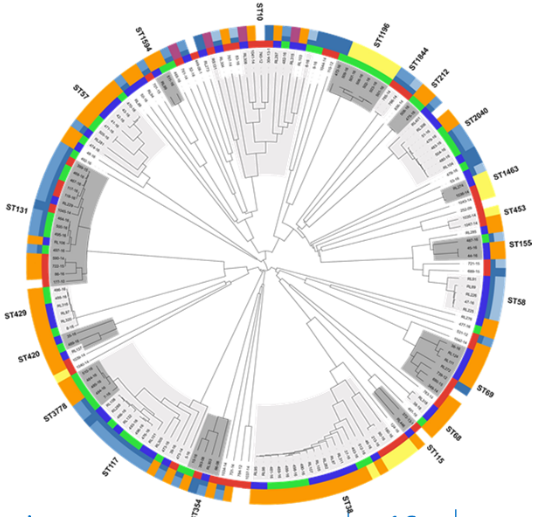
    IncK-ST38 in *E.coli* isolated from poultry

    ST131 *E.coli* isolated from **all reservoirs** and with ***bla*$_{CMY-2}$** on different plasmids

    - noticeable different distribution than ESBL-ST131 *E.coli*

- Location of *bla*$_{CMY-2}$ in *E. coli* mainly on **IncK** and **IncI1 plasmids**

- No correlation of *bla*$_{CMY-2}$-positive *E. coli* strain types with distinct plasmid types

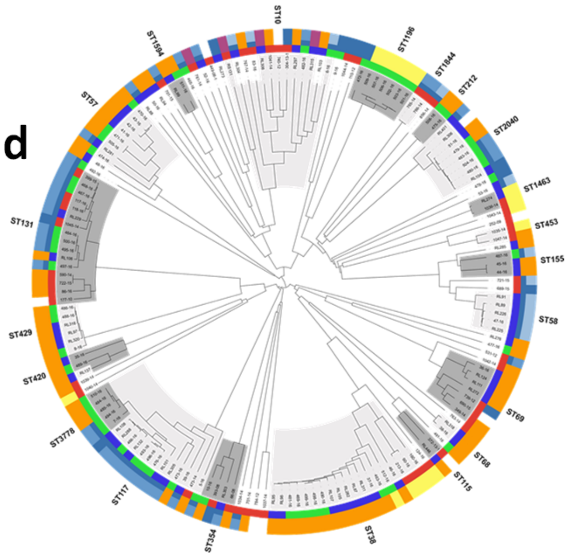


Summary

- *bla*$_{\text{CMY-2}}$ plasmids of type **IncK**
  - very homogeneous
  - potential for dissemination between reservoirs
  - highly stable
  - **ST38-IncK CMY-2-*E. coli*** seems to circulate in the **European broiler production**

- *bla*$_{\text{CMY-2}}$ plasmids of type **IncI1**
  - more diverse than *bla*$_{\text{CMY-2}}$-IncK2 plasmids, but also stable in distribution of types and plasmid backbone

- **Horizontal transfer of *bla*$_{\text{CMY-2}}$ rather than clonal spread seems as the most likely way of transmission across animal and human reservoirs**



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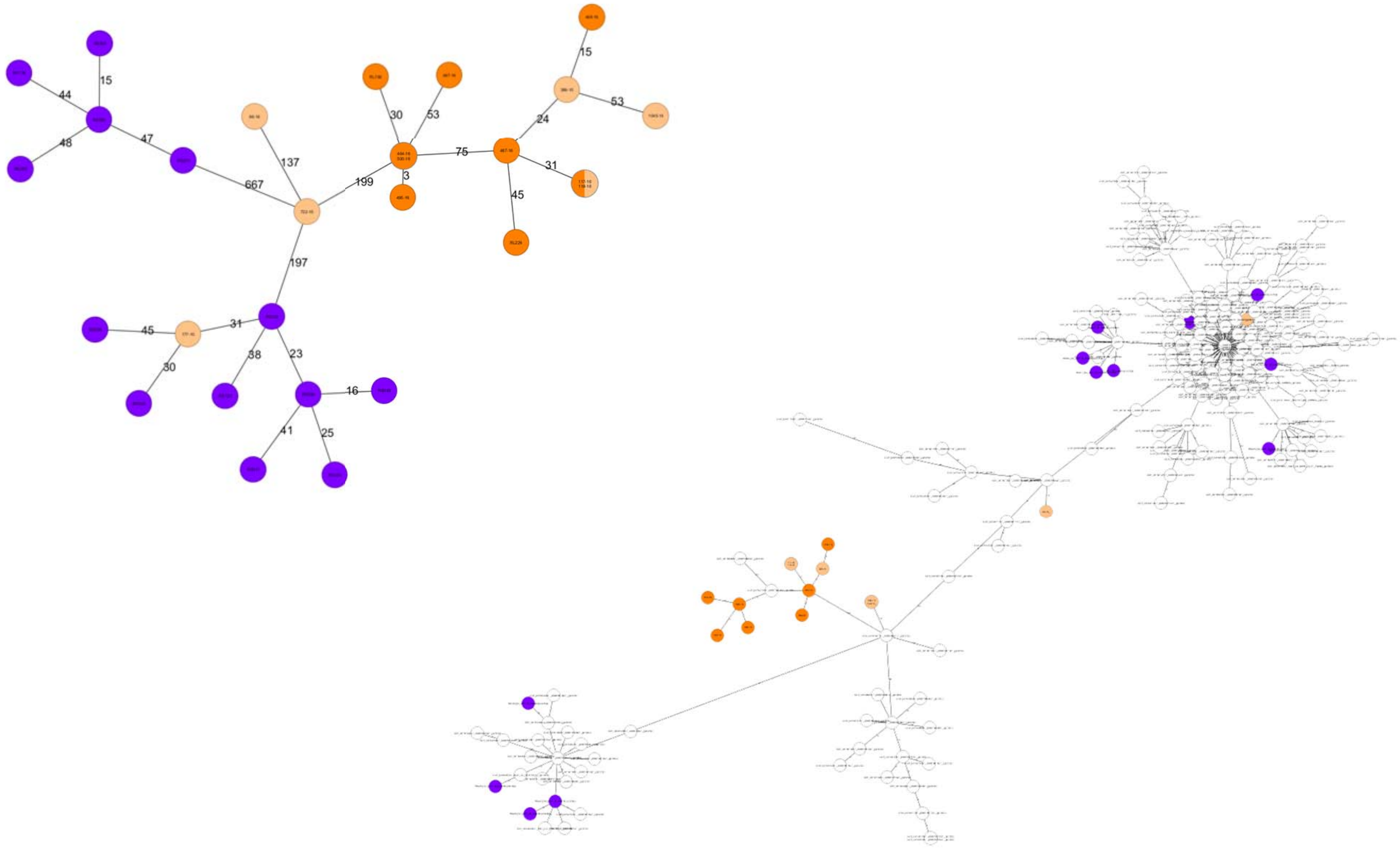
E. coli-ST131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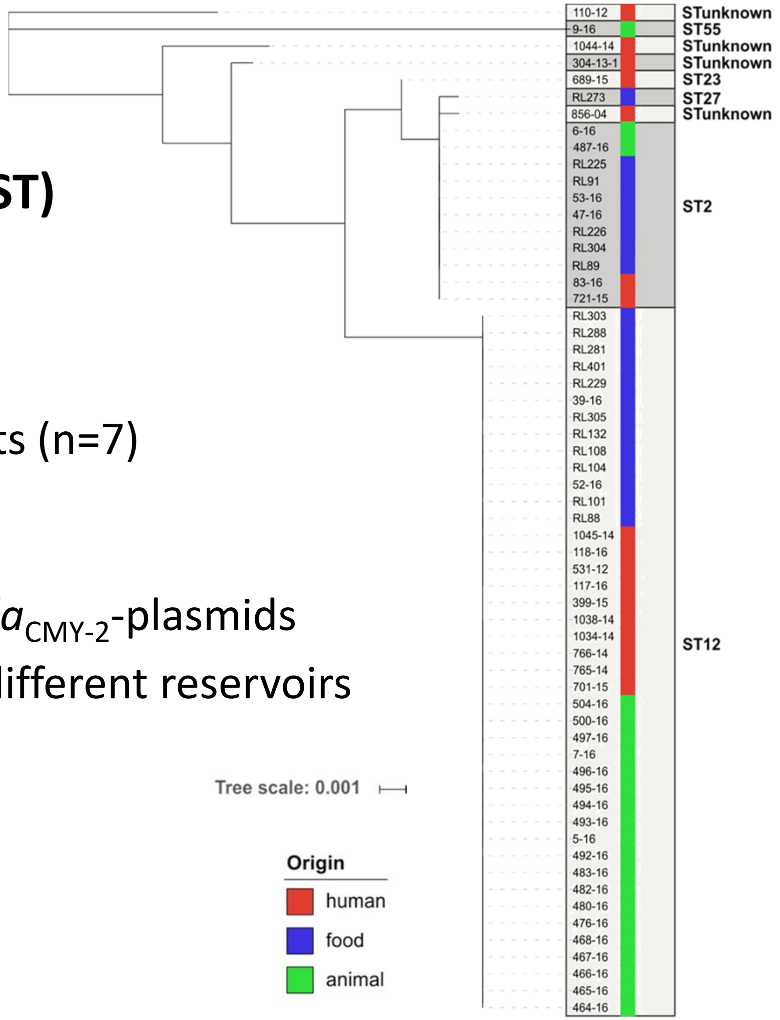
38
23
16
41
25

Plasmid Analysis – IncI1 plasmids

- **Plasmid subtyping (pMLST)**

- pST12 (n=40)
- pST2 (n=12)
- further (single locus) variants (n=7)
- non typeable (n=6)

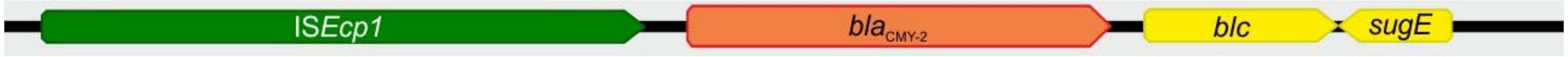
- high similarity among the *bla*$_{CMY-2}$-plasmids of one pST in isolates from different reservoirs



ST	Number
ST-117	11
ST-131	11
ST-58	7
ST-2040	5
ST-10	3
ST-1844	2
ST-354	2
ST-3778	2
ST-1196	1
ST-1286	1
ST-1421	1
ST-155	1
ST-162	1
ST-1818	1
ST-2077	1
ST-224	1
ST-23	1
ST-2485	1
ST-348	1
ST-393	1
ST-457	1
ST-540	1
ST-57	1
ST-6008	1
ST-69	1
ST-744	1
ST-75	1
ST-86	1
ST-88	1
ST-93	1

Mobilization of the *bla*$_{\text{CMY-2}}$ gene

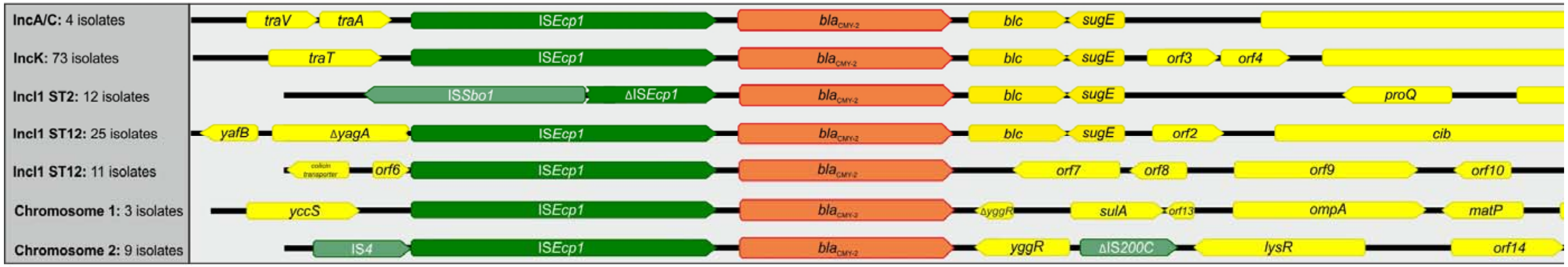
- IS*Ecp1* element is associated with *bla*$_{\text{CMY-2}}$ genes



  - Mobility of a transposition unit and resistance determinant - also described for ESBL genes, e.g. *bla*$_{\text{CTX-M}}$)
  - Promotor for high level expression of neighbouring genes
  - Is reported to mobilize and transport genes onto plasmids easy and efficient

  - Mobilization of *bla*$_{\text{CMY}}$ genes from *Citrobacter freundii* (Verdet C. *et al.* 2009)

Results: genetic environment of *bla*$_{\text{CMY-2}}$



- different genetic environments within different plasmids suggests different acquisition events:
    - possibility that IncK and IncA/C or IncI1 ST12 exchanged *bla*$_{\text{CMY-2}}$ transposon structure?

- Structures were identical to previously described plasmids in Europe (Voets G. *et al.* 2013, Mo S. *et al.* 2016, Hansen K. *et al.* 2016)

- No linkage of plasmid and genetic environment to a specific *E. coli*-ST