

„Insights in CPE detection and characterization from German food production chain”

09.10.2024, EURL-AR workshop

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Department for Biological Safety

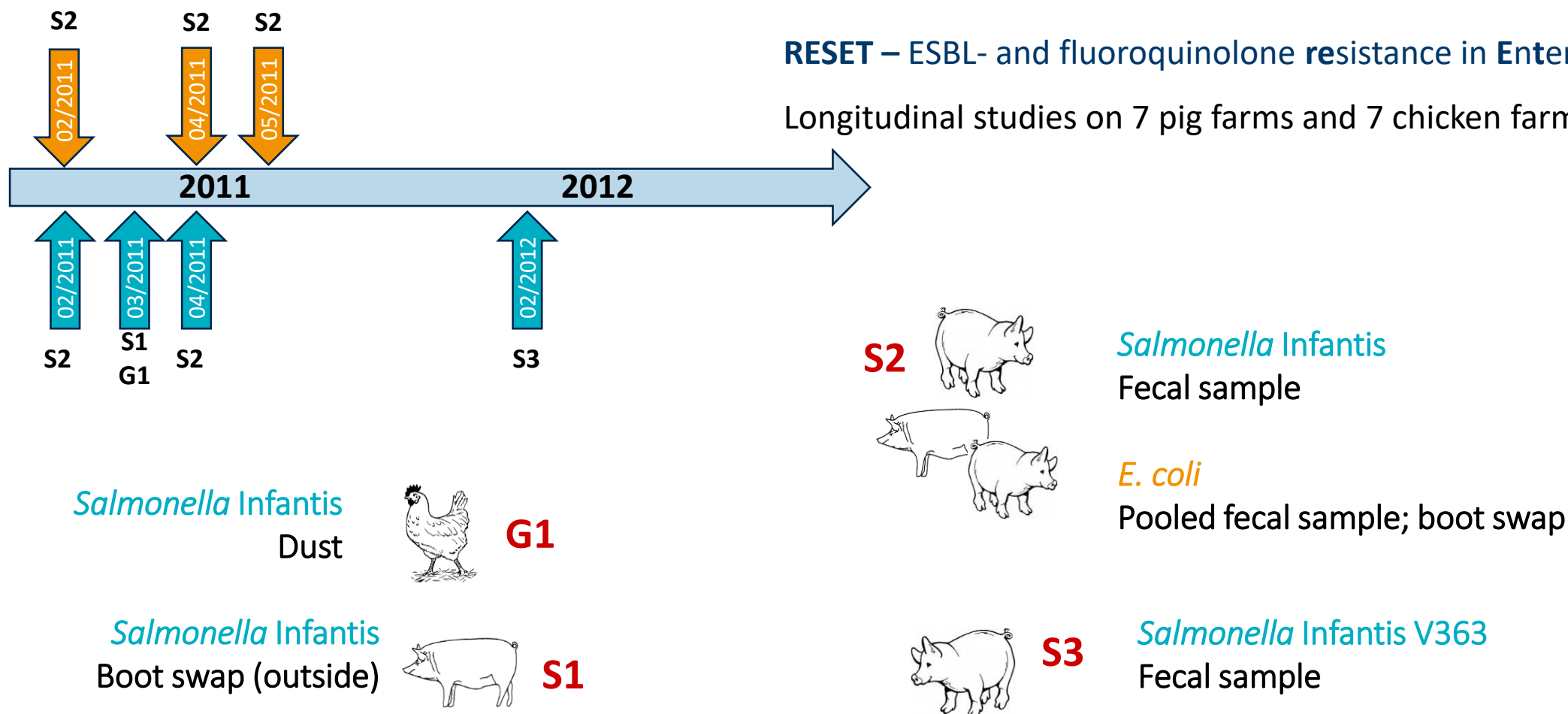
Recap

Beginning of the CPE story

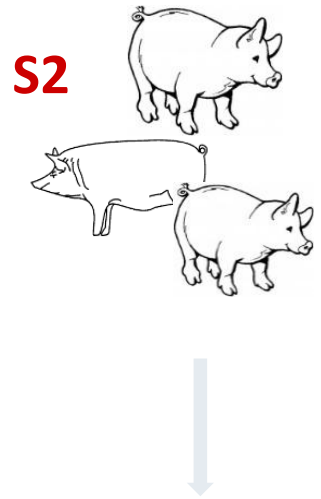
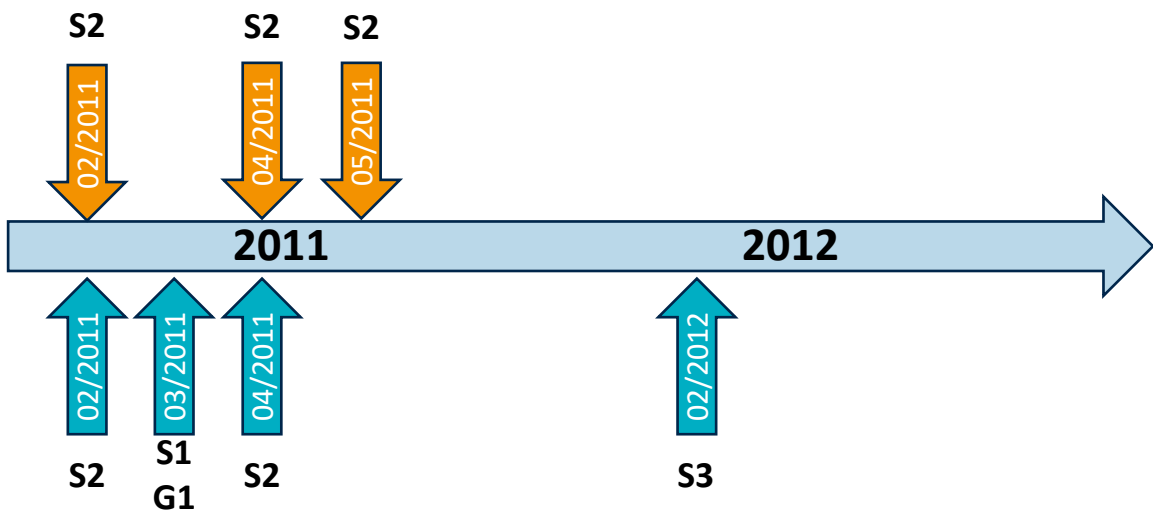
2011 – first detection of VIM-1 producing

RESET – ESBL- and fluoroquinolone resistance in Enterobacteriaceae

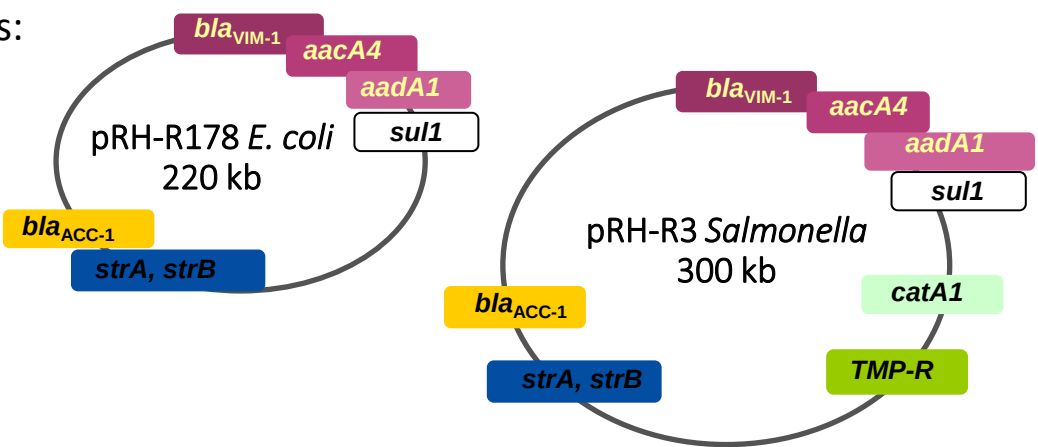
Longitudinal studies on 7 pig farms and 7 chicken farms



2011 – first detection of VIM-1 producing



Inc-HI2 plasmids:





Contents lists available at [ScienceDirect](#)

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Comparative genome analysis of IncHI2 VIM-1 carbapenemase-encoding plasmids of *Escherichia coli* and *Salmonella enterica* isolated from a livestock farm in Germany

Linda Falgenhauer^{a,b}, Hiren Ghosh^{a,b}, Beatriz Guerra^{c,1}, Yancheng Yao^{a,b}, Moritz Fritzenwanker^{a,b}, Jennie Fischer^c, Reiner Helmuth^c, Can Imirzalioglu^{a,b}, Trinad Chakraborty^{a,b,*}

Importance of the results and consequences

- first detection of CPE in animal production worldwide
 - development of isolation method for CP *E. coli* from meat and caecal samples
 - in 2014: start of voluntary monitoring of CP *E. coli* in EU
- in Germany monitoring voluntary in 2016 and mandatory since 2017 (in EU since 2021)

DTU Food
National Food Institute



LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing
E. coli from fresh meat

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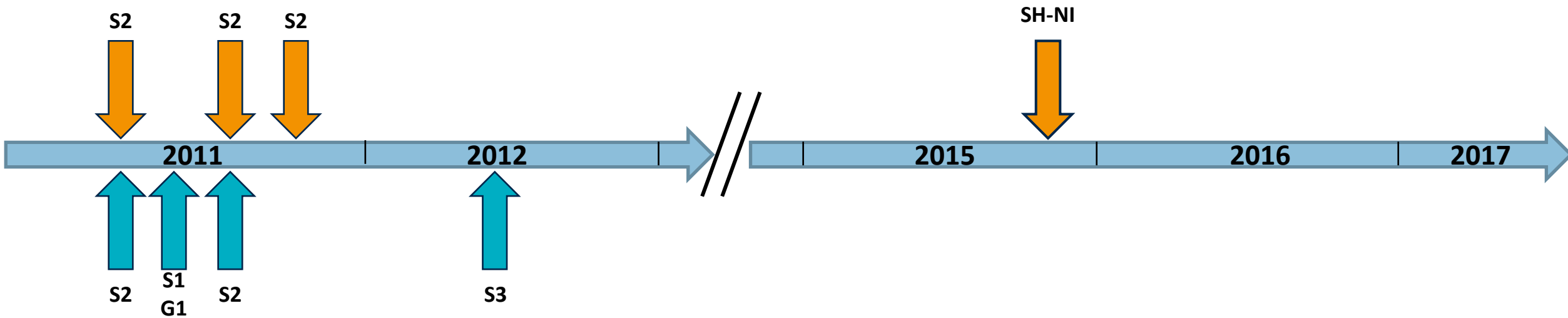
LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing
E. coli from caecal samples

<https://www.eurl-ar.eu/protocols.aspx>

CPE in food production chain

CPE in food production chain



12/2015: VIM-1 *E. coli*

Fattening pig at slaughter

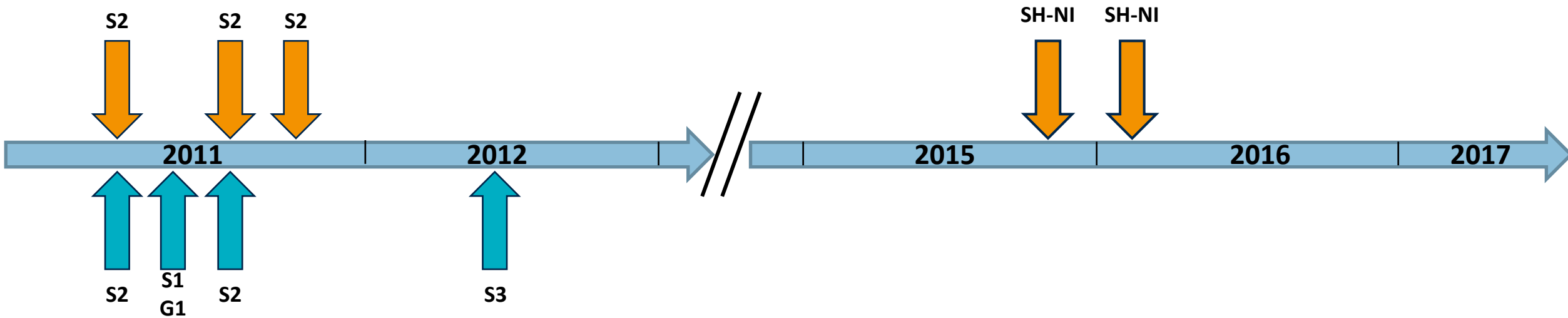
→ ESBL Monitoring

MIC: MERO 0.5 to 0.25 mg/L

ETP 0.12 mg/L

IMI 2 mg/L

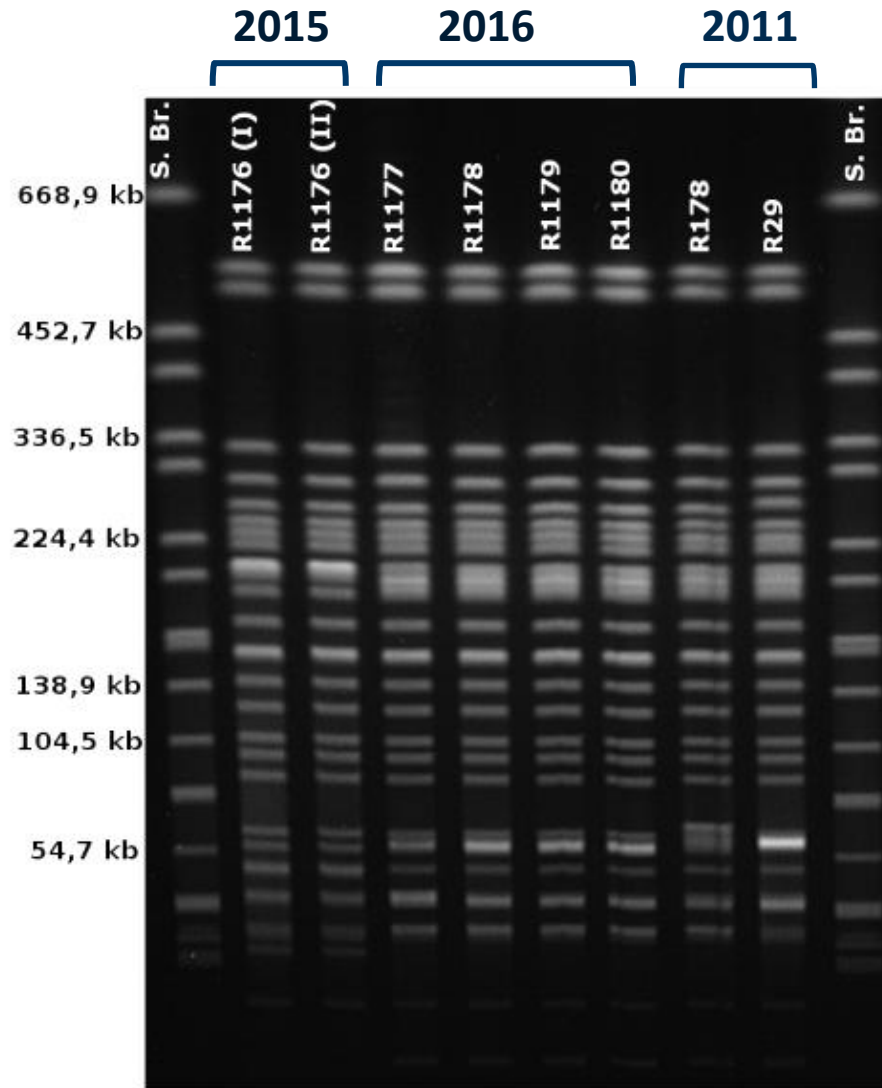
CPE in food production chain



12/2015: VIM-1 *E. coli*; fattening pig at slaughter

03/2016: next slaughter batch; 1/6 samples positive; 4 isolates

CPE in food production chain

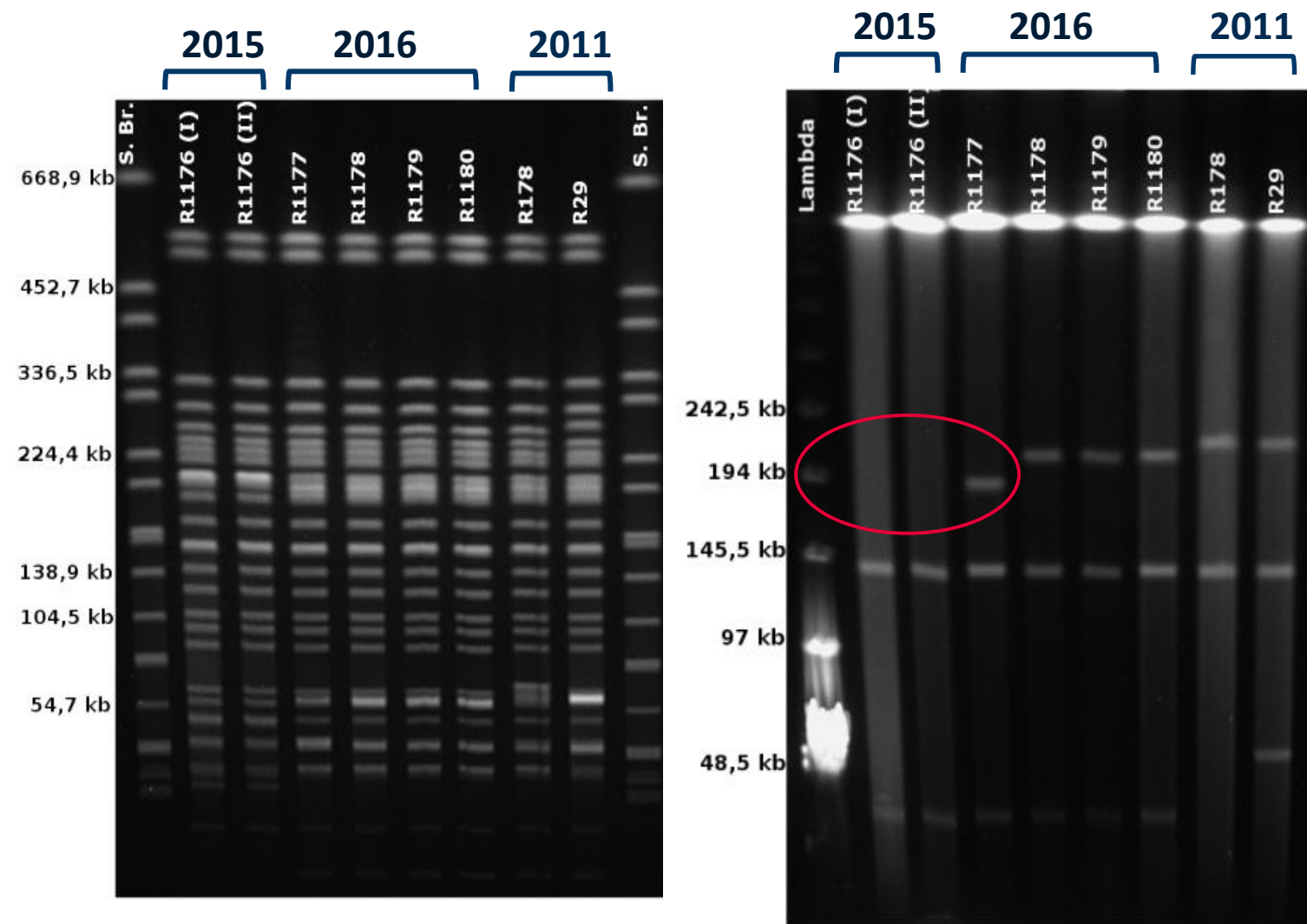


Strains closely related



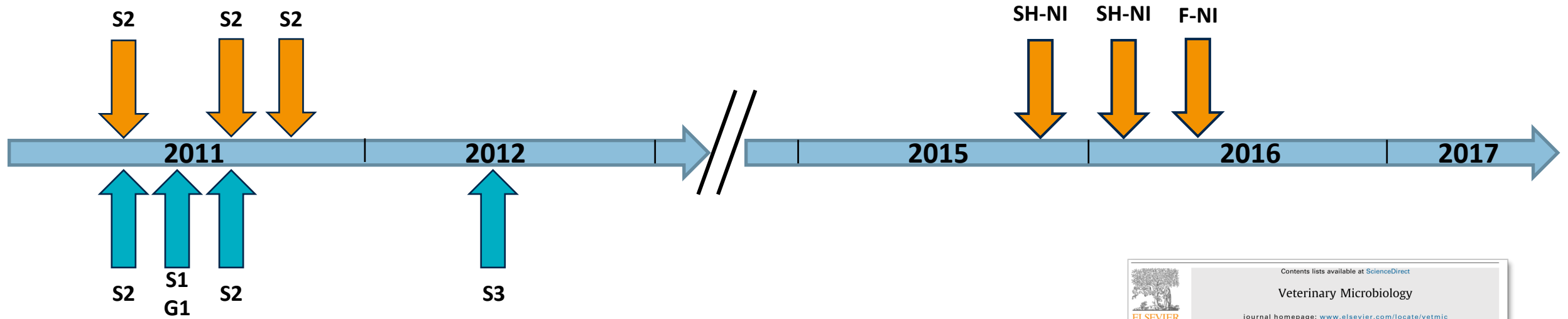
Farms not directly related

CPE in food production chain



	R1176	R1177- R1180	R29/ R178
Date of isolation	12/2015	03/2016	05/2011
bla-genes	VIM-1 TEM-1	VIM-1 ACC-1 TEM-1	VIM-1 ACC-1
MLST	ST88	ST88	ST88
Integron	Class 1/ bla _{VIM-1} , aacA4, aadA1	Class 1/ bla _{VIM-1} , aacA4, aadA1	Class 1/ bla _{VIM-1} , aacA4, aadA1
Plasmid	-	180-200 kb / IncHI2	220 kb/ IncHI2

CPE in food production chain

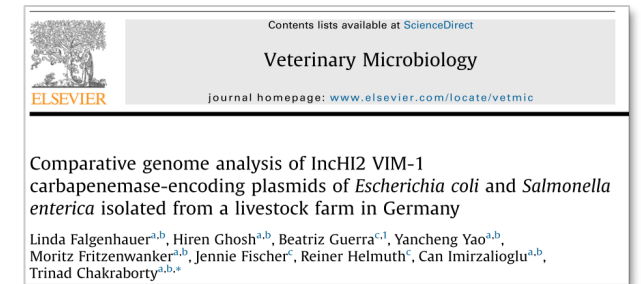


Trace-back study: 6 barns → 4 positive samples [32 isolates] from 3 barns

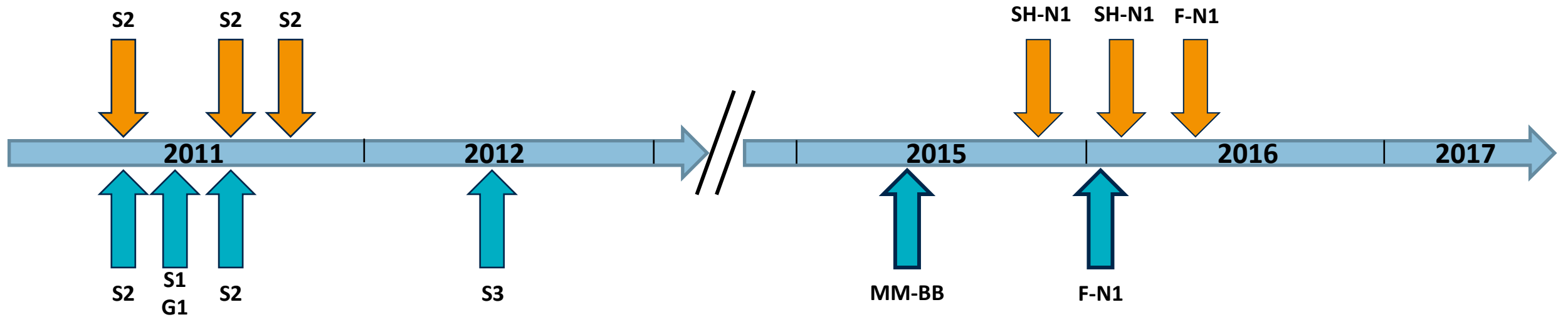
→ 2 samples: chromosomal *bla*_{VIM-1}

→ 2 samples: plasmids of variable sizes

→ Detection with 3 different isolation procedures



CPE in food production chain



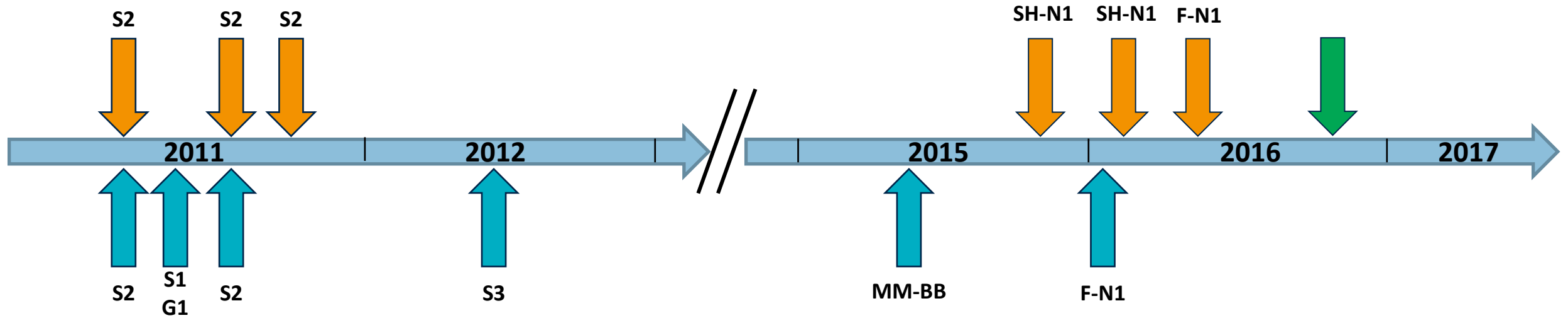
→ what is the link
between the 5 different
pig farms?

NRL-Salmonella:

2015: VIM-1 producing *Salmonella* from minced meat (retrospective detection)

2016: diagnostic sample from infected piglet → same farm (N1) as *E. coli*

CPE in food production chain



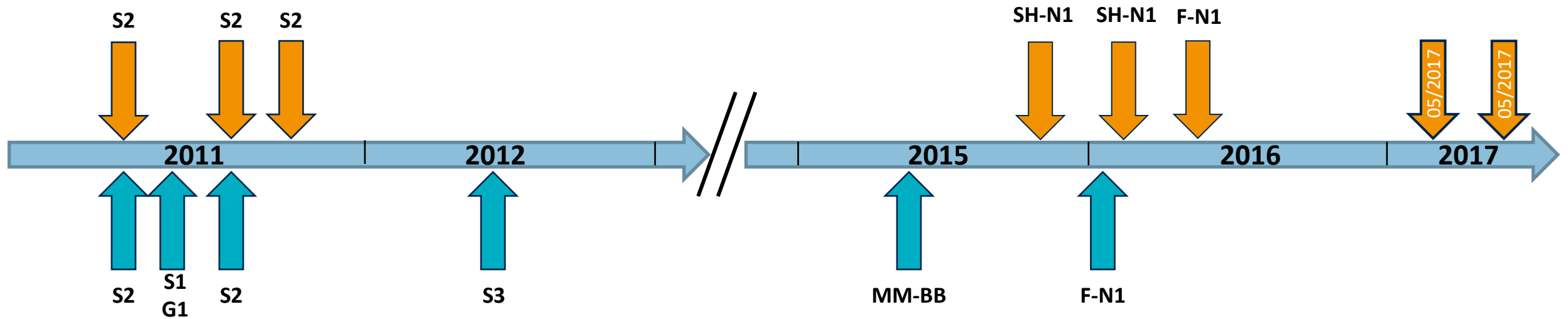
- Link might be the piglet producer
- Sampling by Freie Universität 04/2017 →
- Breeding farm closed for other reasons
- No further clones detected

Detection of VIM-1-Producing *Enterobacter cloacae* and *Salmonella enterica* Serovars Infantis and Goldcoast at a Breeding Pig Farm in Germany in 2017 and Their Molecular Relationship to Former VIM-1-Producing *S. Infantis* Isolates in German Livestock Production

Nicole Roschanski,^{a,*} Sead Hadziabdic,^b Maria Borowiak,^b Burkhard Malorny,^b Bernd-Alois Tenhagen,^b  Michaela Projahn,^{a,*} Annemarie Kaesbohrer,^{b,c} Sebastian Guenther,^{a,*} Istvan Szabo,^b Uwe Roesler,^a Jennie Fischer^b

Problem solved?

CPE in food production chain



05/2017: - first isolate within the CPE monitoring

- fecal sample of a fattening pig

12/2017: - CPE detected from fattening pig at slaughter within **ESBL** monitoring (negative in CPE monitoring)

→ Both bla_{VIM-1} positive

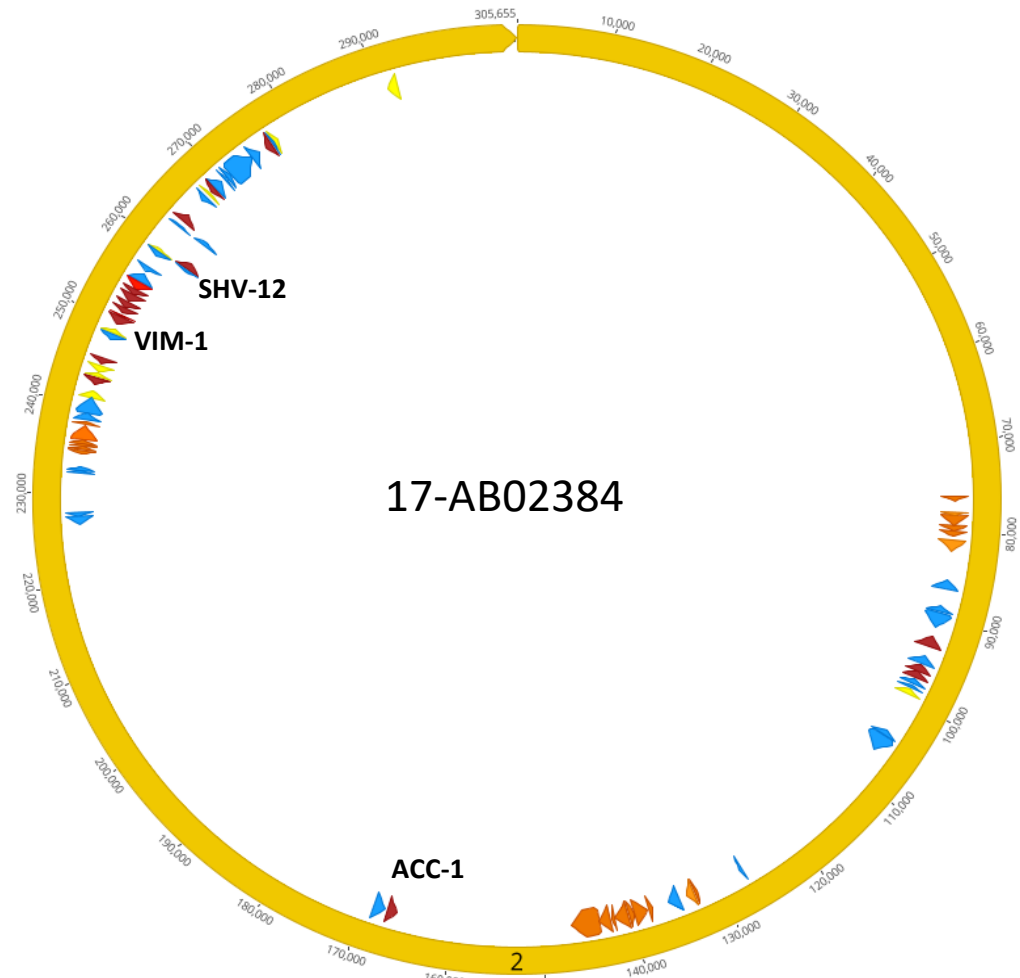
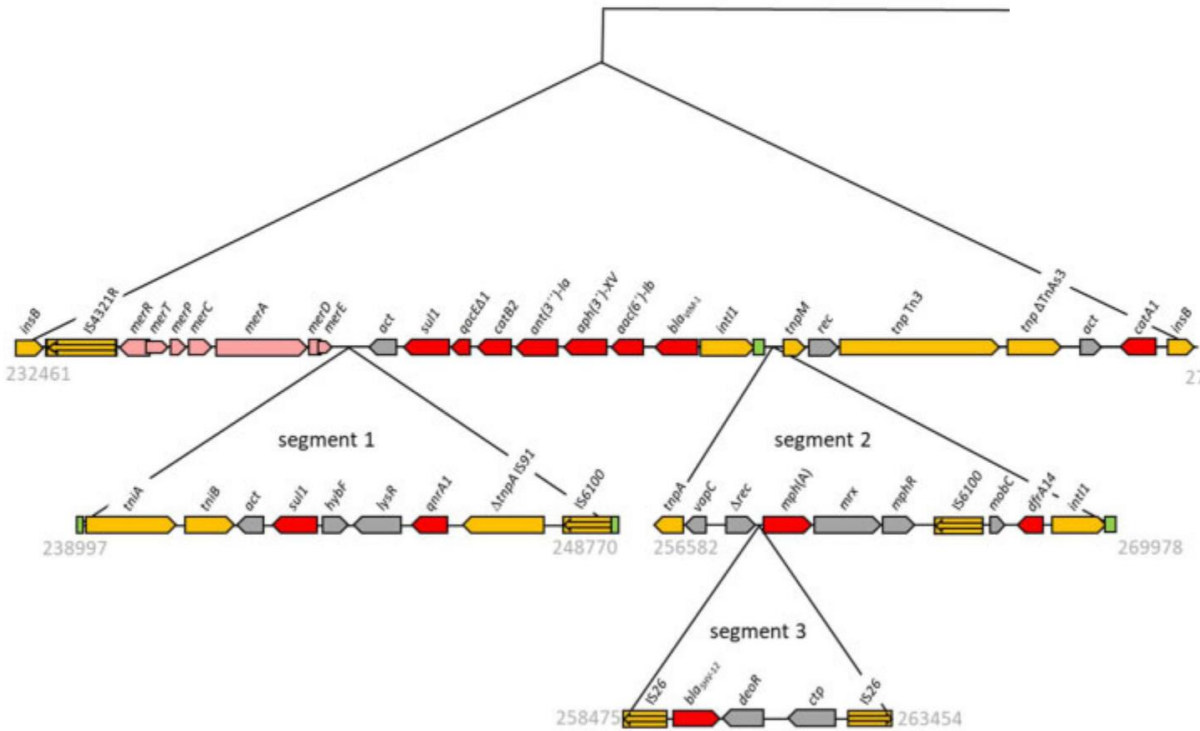
CP *E. coli* in food production chain 2017

	17-AB01027	17-AB02384	(2011-)2016
Origin	Fattening pig Primary production	Fattening pig At slaughter	Fattening pigs At slaughter /PP
Monitoring	CPE	ESBL	ESBL
MSLT	ST48	ST7593	ST88
Plasmid	+	+	-/+
-type	IncHI2 (fusion with IncF?)	IncHI2	(IncHI2)
-size	> 400 kb	300 kb	180-220 kb
Resistance genes a) beta-lactamases b) other	a) <i>bla</i> _{ACC-1} ; <i>bla</i> _{VIM-1} ; b) <i>aadA1</i> *, <i>aac(6')-Ib4</i> *, <i>aph(6)-Id</i> , <i>aph(3'')-Ib</i> , <i>catA1</i> , <i>dfrA5</i> , <i>floR</i> , <i>sul1</i> , <i>sul2</i> , <i>tet(A)</i>	a) <i>bla</i> _{ACC-1} ; <i>bla</i> _{SHV-12} <i>bla</i> _{VIM-1} *; b) <i>aadA1</i> *; <i>aac(6')Ib4</i> *, <i>aph(3')-XV</i> *; <i>aph(3'')-Ib</i> ; <i>aph(6)-Id</i> , <i>catA1</i> ; <i>catB2</i> *; <i>mph(A)</i> , <i>qnrA1</i> , <i>floR</i> , <i>dfrA14</i> , <i>sul1</i> ,	a) (<i>bla</i> _{ACC-1} , <i>bla</i> _{TEM-1}); <i>bla</i> _{VIM-1} ; b) <i>aac(6')Ib4</i> , <i>aadA1</i> *, <i>aadA2</i> , <i>aph(3'')-Ib</i> ; <i>aph(6)-Id</i> , <i>dfrA12</i> , <i>cmlA1</i> , <i>sul1</i> , <i>sul2</i> ,
Integron /Transposon	<i>Class1</i> *; Tn21	<i>Class1</i> *; Tn21	<i>Class1</i> *; Tn21

CP *E. coli* in food production chain 2017

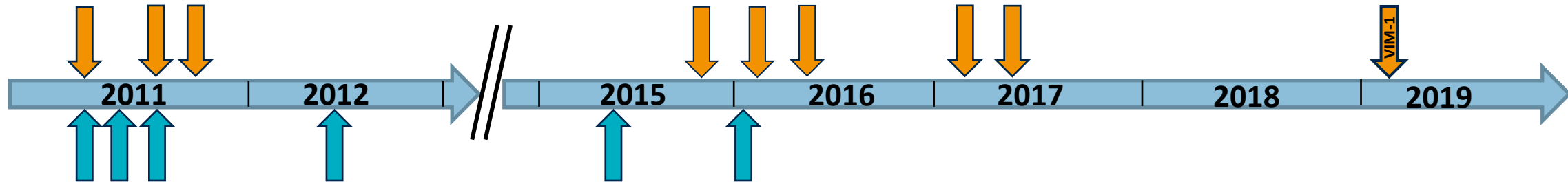
Co-occurrence of the *bla*_{VIM-1} and *bla*_{SHV-12} genes on an IncHI2 plasmid of an *Escherichia coli* isolate recovered from German livestock

N. Pauly^{1*}, J. A. Hammerl¹, S. Schwarz², M. Grobbel¹,
D. Meemken³, B. Malorny¹, B.-A. Tenhagen¹,
A. Käsbohrer^{1,4} and A. Irrgang¹



CP *E. coli* in German food production chain 2019 - increasing diversity

CP *E. coli* in food production chain 2019



19-AB01133: VIM-1 producing *E.coli*
CPE monitoring

Isolate	AMP	AZI	CHL	CIP	COL	ERP	FEP	FOT	FOX	GEN	IMI	MERO	NAL	SMX	TAZ	TET	TGC	TMP
19-AB01133	>64	16	16	>8	≤1	>2	>32	>64	>64	32	8	8	>128	>1024	>8	4	≤0.25	>32
TK_19-AB01133	>64	8	8	≤0.015	≤1	>2	>32	>64	>64	4	8	4	≤4	>1024	>8	4	≤0.25	>32
K12 J53	4	8	≤8	≤0.015	≤1	≤0.015	≤0.06	≤0.25	4	2	0.25	≤0.03	≤4	≤8	≤0.5	4	0.5	≤0.25

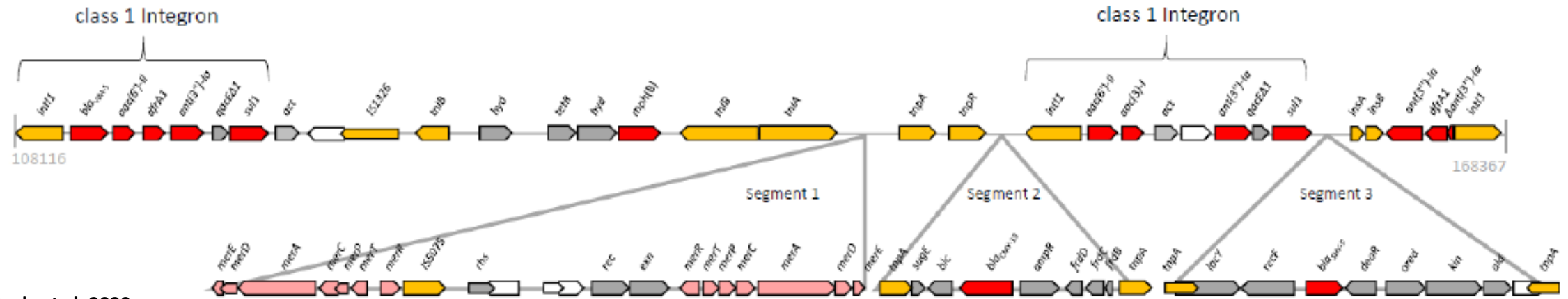
ne ST

Garcia-Grealls et al. 2020

Plasmid: 190 kb IncA/C₂

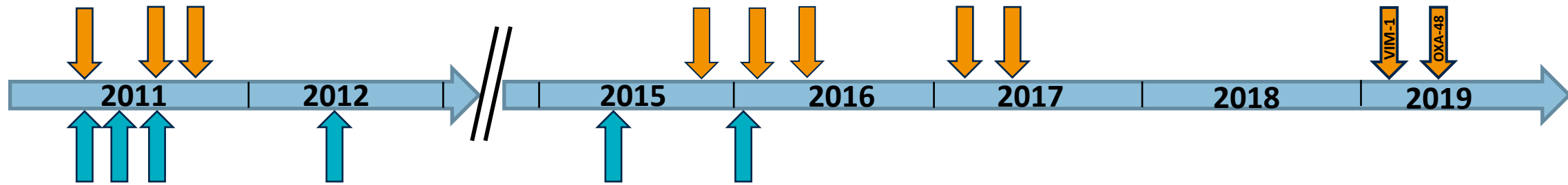
almost identical to plasmid described by Garcia-Grealls et al 2020 + same ST

from *Klebsiella* or *Enterobacter*; backbone from clinical *E. coli* isolates



Pauly et al. 2020

CP *E. coli* in food production chain 2019



Isolate	Origin	Phenotype	AMR Genes	Phylogenetic Group/MLST	Plasmid Size	Inc- Group
19-AB01443	Pig, fecal sample	AMP, ETP, FEP, FOT, IMP, MERO, TEMOCI	<i>bla</i> _{OXA-48}	B1 / ST295	63 kb	IncL/M

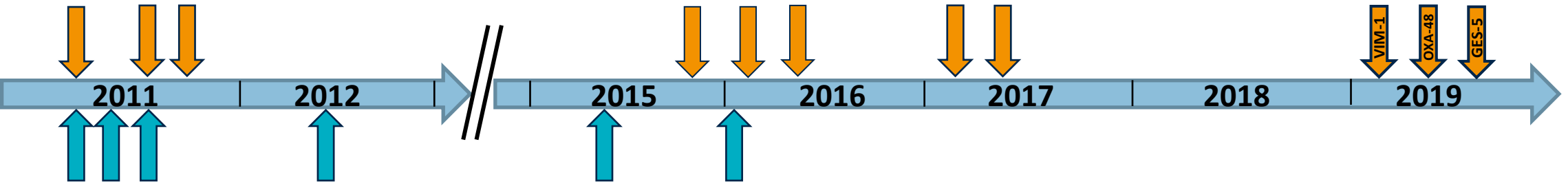
First detection in German food production chain (CPE monitoring)

Plasmid identical to widely spread plasmid detected from different Enterobacterales from human worldwide

Highly transferable between different species

No further functional resistance genes encoded

CP *E. coli* in food production chain 2019

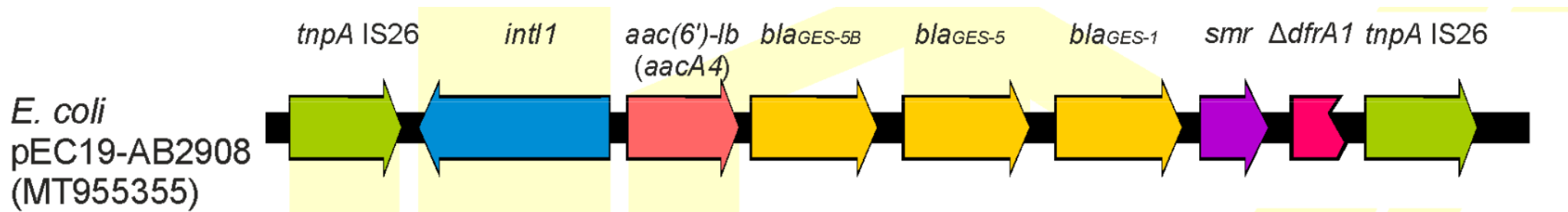


Phylogeny	Phenotypic Resistance	Resistance Genes	Plasmids	Virulence Associated Genes
-ST1084 -phylogenetic group B1	AMP, ETP, FEP, FOT, FOX, MERO, SMX, TAZ, TET, TMP	<i>aac(6')-Ib3, ant(3'')-Ia, aph(3'')-Ib, aph(6)-Id, bla_{GES-1}, bla_{GES-5}, bla_{GES-5B}, bla_{TEM-1B}, dfrA1, mph(B), sul-1, sul-2, tet(A)</i>	12 kb (pEC19-AB02908) 227 kb	<i>afaA, afaD, cma, cvaC, hlyF, hra, iroN, iss, lpfA, ompT, sitA, terC, traT</i>

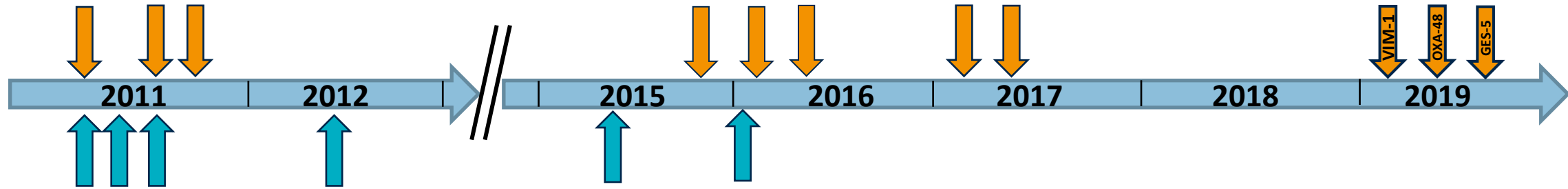
First detection in German food production chain (CPE monitoring)

GES-5 producing *E. coli* 19-AB02908

- Phenotypically resistant to MERO (MIC ≥ 0.25 mg/L) and ETP (MIC ≥ 0.25 mg/L) not to IMI (MIC ≥ 0.5 mg/L)
- 12 kb plasmid
- three *bla*_{GES} genes sequentially located in an incomplete class-1 integron
- Additional 227 kb IncF plasmid harboring resistance genes against several antimicrobials and heavy metals, as well as virulence associated genes typical for uropathogenic *E. coli*



CP *E. coli* in food production chain 2019



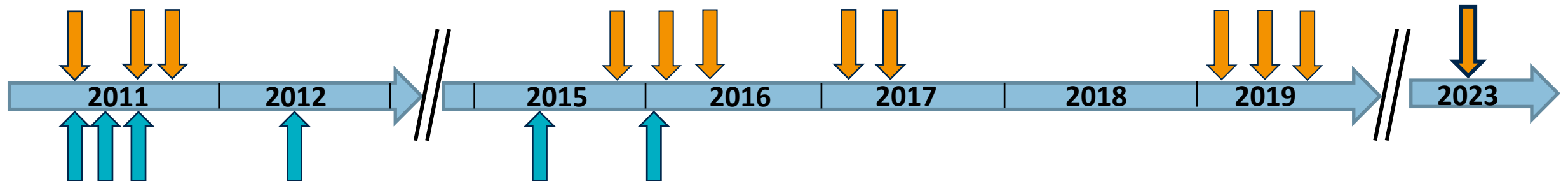
→ Increasing diversity in 2019

→ All three CP *E. coli* detected on selective CPE agar

→ Investigations on all three farms conducted → no further spread and persistence within the farms

**CP *E. coli* in German food
production chain 2023
- old problem back-**

CP *E. coli* in food production chain 2023

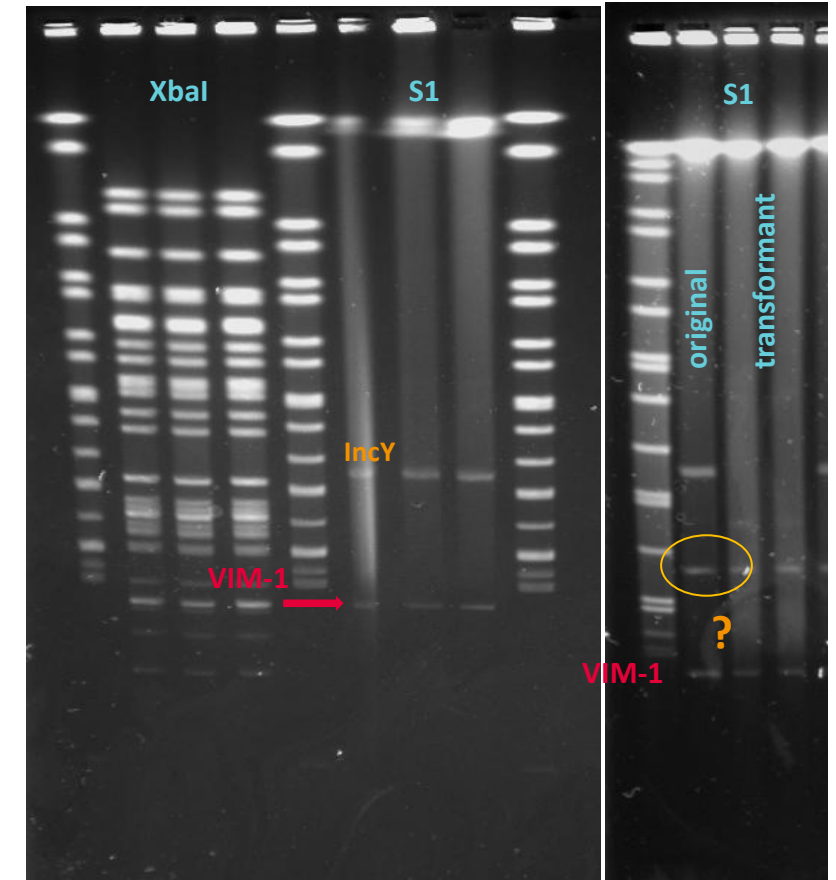


- **23-AB02114:** VIM-1 producing
- First CPE from fattening calf in Germany
- Only calf which was sampled at slaughter in Brandenburg in 2023
- Detected in ESBL monitoring, **negative** by CPE selective cultivation
- No growth on ChomID Carba even after several cultivation steps on CTX

MICs: **MERO** 0.06 to 0.12 mg/L; **IMI** 1 mg/L; **ETP** 0.03 mg /L

VIM-1 producing *E. coli* 23-AB02114

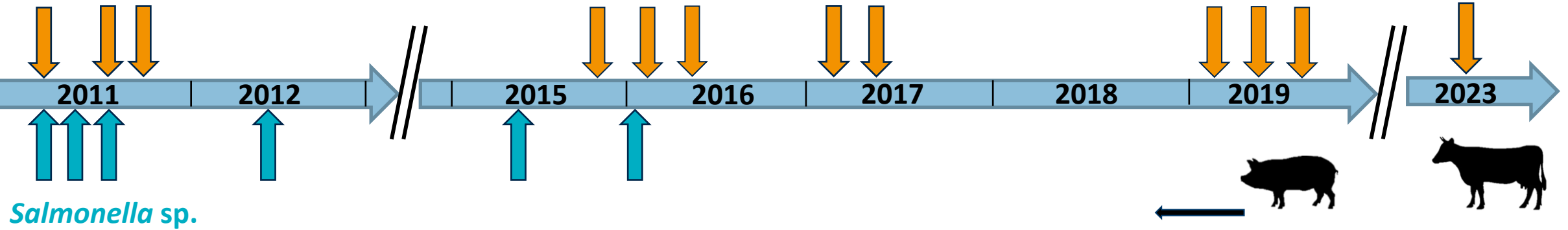
- short-and long read sequencing → closed genome
- 100 kb **IncY**
- 16,776 bp **circular element** → typical class1 integron harboring *bla*_{VIM-1} *aac*(6')-Ib/*aac*(6')-II; *aadA*1
- Homologs to *Vibrio tubiashii*
- Transferable by transformation
- Additional third circular element by PFGE
- no trace-back investigations of the farm (closed)



Summary

CPE in German food production chain

E. coli



- Since 2011: Detections of the carbapenemase VIM-1 in *E. coli* and *S. Infantis* in German livestock (pigs)
- Up to 2019: High similarity of the *bla*_{VIM-1}-carrying plasmids, also across species
- In 2019: Increased variance (VIM-1, OXA-48, and GES-producing *E. coli*); all detected *E. coli* from fattening pigs
- In 2023: first VIM-1 producing *E. coli* from calf

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Isolation CP E. coli from fecal samples (NI)

