RESET

Resistenzen
bei Tier und Mensch -
gemeinsame Forschung in Deutschland

15 YEARS
Science in the Service of Humanity

BfR
Bundesinstitut für Risikobewertung

RESET II- IP1 (MolBioRes)

Detection of VIM-1 carbapenemase encoding *E. coli* in German pig production

Alexandra Irrgang, Jennie Fischer, Silvia Schmoger, Bernd-Alois Tenhagen, Mirjam Grobbel, Jens Hammerl, Annemarie Käsbohrer

Background

RESET 1 IP3 (FU Berlin):

longitudinal studies of 7 pig farms (S1-S7) and 7 chicken farms (G1-G7)

IP1 (BfR)

molecular characterization of isolates

Detection of VIM-1 carbapenemase producing *E. coli* and *Salmonella* in samples of four different farms

Salmonella Infantis R3
pooled dust

G1

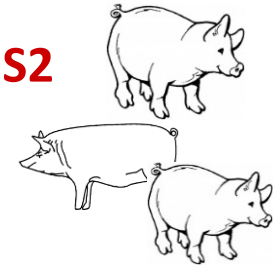
Salmonella Infantis R25
boot swaps (outside)

S1

Salmonella Infantis V363
feacal sample

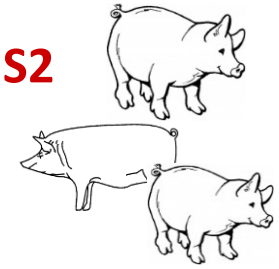
S3

S2



Salmonella Infantis R27
feacal sample
E. coli R29, R178
pooled feacal sample, boot swap

Background



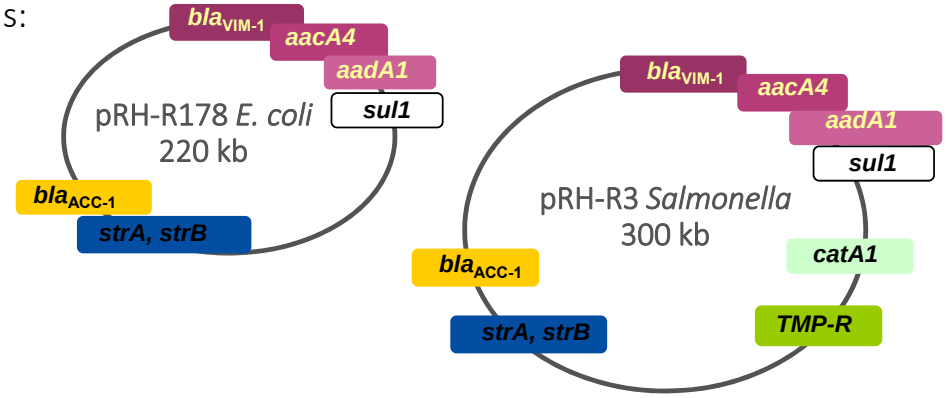
Salmonella Infantis R27 feacal sample

E. coli R29, R178

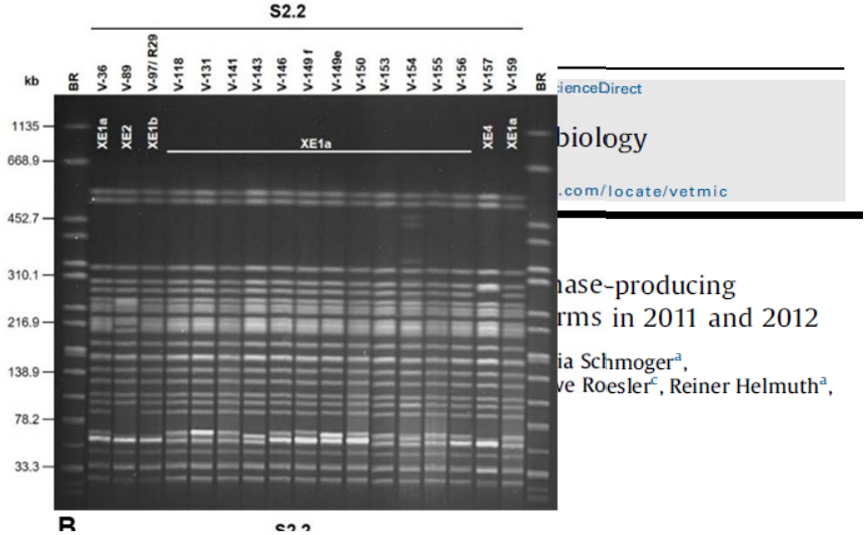
pooled feacal sample, boot swap

retrospective study

Inc-HI2 plasmids:



further 35 *E. coli isolates* obtained from dust, faeces, boot swaps, flies, liquid manure



ienceDirect

biology

.com/locate/vetmic

ase-producing
rms in 2011 and 2012

ia Schmoger[a],
ve Roesler[c], Reiner Helmuth[a],

Contents lists available at ScienceDirect

Veterinary Microbiology

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

ELSEVIER

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,*]

RESET2 IP1 →Task 6: Screening for new carbapenemases producing Enterobactericeae in samples from animal, food and environment

IP1 ⟹ National Reference Laboratory for Antimicrobial Resistance (NRL-AR)

Monitoring on:
i) commensal *E. coli*
ii) ESBL producing *E. coli*
iii) carbapenemases producing *E. coli*

⟹ **Screening for carbapenem resistant isolates**

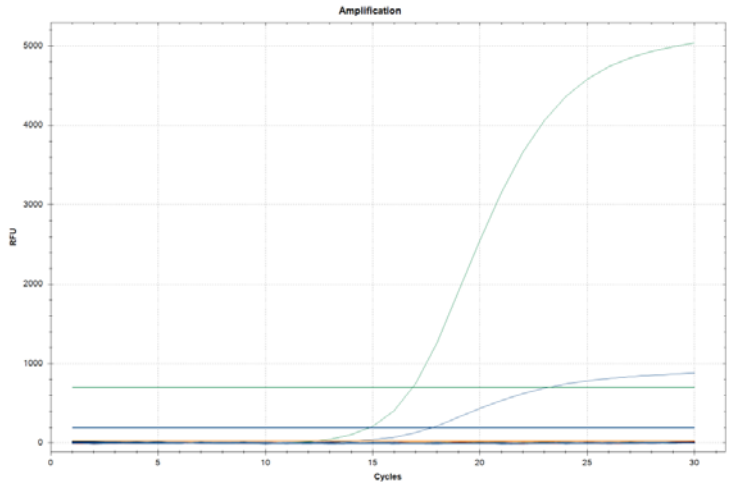
⇓ MIC

MERO ≥ 0.12 mg/L
ETP ≥ 0.12 mg/L
IMI ≥ 1 mg/L

⟸ **24** isolates in 2015

real-time PCR

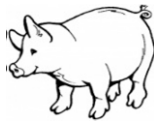
Results of the Screening for Carbapenemases March 2016



1/24 isolates positive for *bla*$_{VIM}$

PCR/sequencing: **VIM-1**

R1176:



obtained from swine colon content

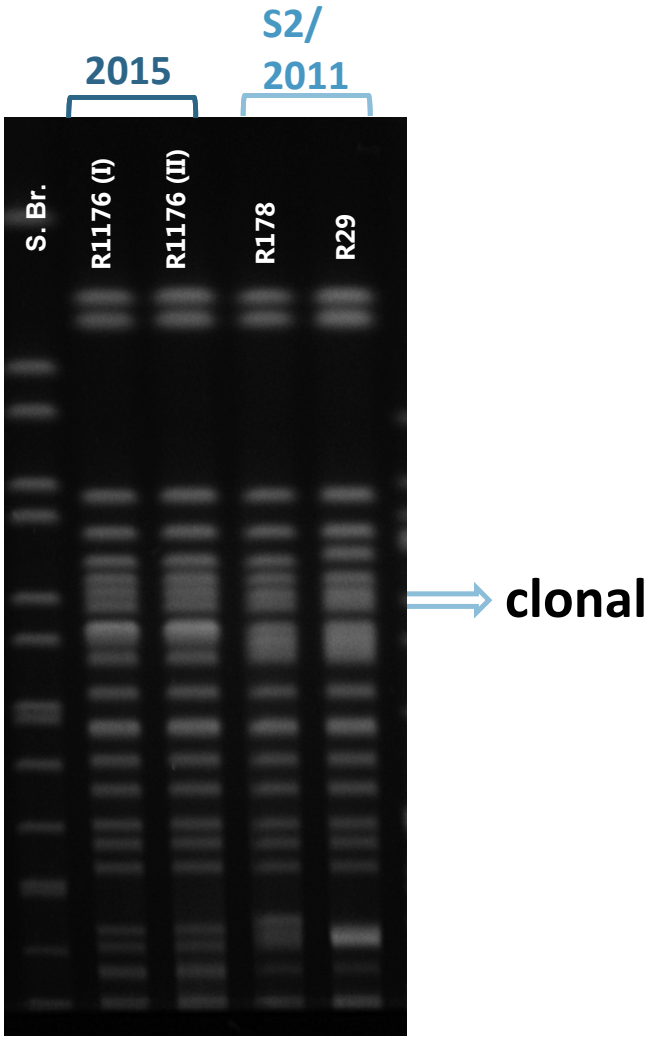
December 2015 (ESBL monitoring)

MIC: MERO 0.5 / 0.25 mg/L

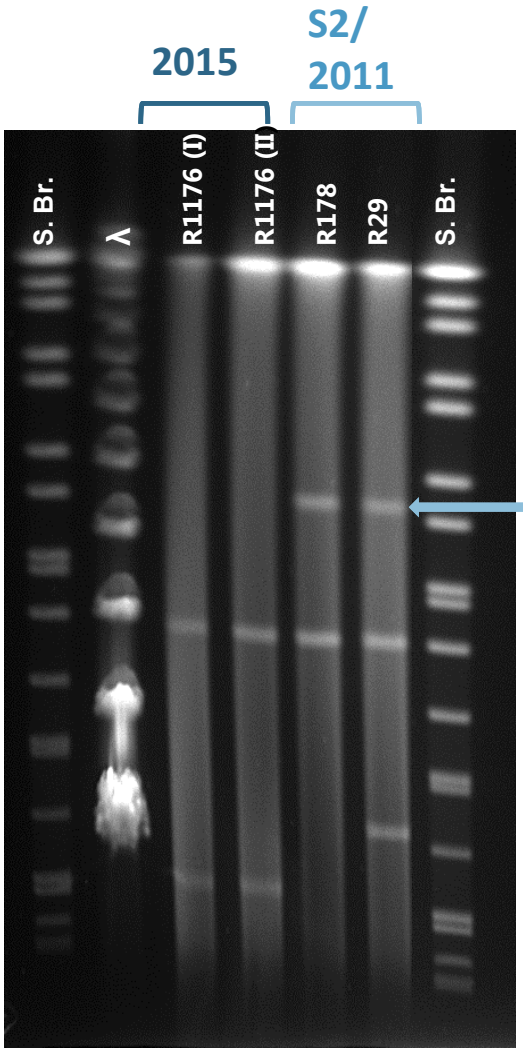
ETP 0.12 mg/L

IMI 2 mg/L

Molecular Characteristics of the *E. coli* Isolate R1176



clonal



	R1176	R29/ R178
bla genes	VIM-1 TEM-1	VIM-1 ACC-1
MLST	ST88	ST88

known VIM-plasmid missing

→ negative by southern blot hybridization

→ chromosomally located

XbaI **S1 nuclease**

Unique finding?

slaughter house

additional colon content samples from the same producer

6 samples send to BfR

enrichment selective/non-selective

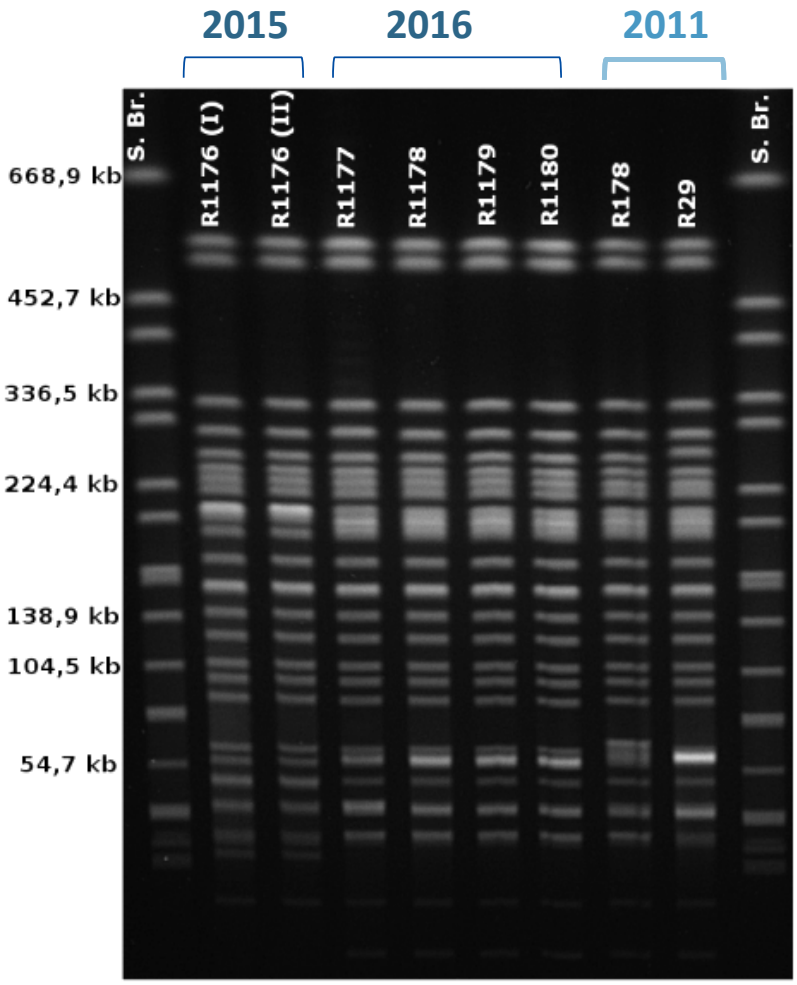
plating on selective culture media
-CTX 1mg/L
-MEM 0.125 mg/L
- MEM 0.06 mg/L

direct plating
-CTX 1mg/L
-MEM 0.125 mg/L
-MEM 0.06 mg/L

single colonies picked → PCR

Characteristics of the slaughter house Isolate

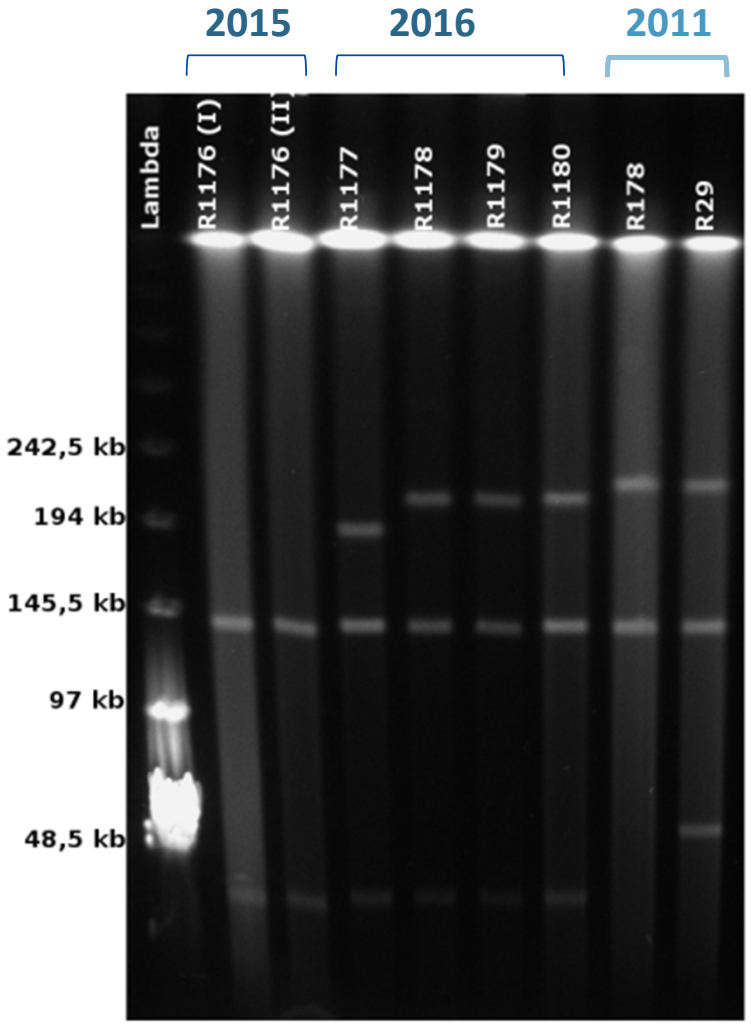
→1 sample (4 isolates) positive for *bla*$_{VIM}$



	R1176	R1177-R1180	R29/ R178
Date of isolation	12/2015	04/2016	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	-	?	220 kb/ IncHI2

Characteristics of the slaughter house Isolate

S1 PFGE



	R1176	R1177-R1180	R29/ R178
Date of isolation	12/2015	04/2016	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

Characteristics of the slaughter house Isolate

J Antimicrob Chemother
doi:10.1093/jac/dkw479

Recurrent detection of VIM-1-producing *Escherichia coli* clone in German pig production

Alexandra Irrgang†, Jennie Fischer†, Mirjam Grobbel, Silvia Schmoger, Tanja Skladnikiewicz-Ziemer, Katharina Thomas, Andreas Hensel, Bernd-Alois Tenhagen and Annemarie Käsbohrer*

	R1176	R1177-R1180	R29/ R178
Date of isolation	12/2015	04/2016	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

Persistence at farm vs. contamination at slaughter??

Sampling:

1) pooled faeces of piglets arriving at the farm

6 samples send to BfR April 2016

2) pooled faeces from fattening pigs and their environment

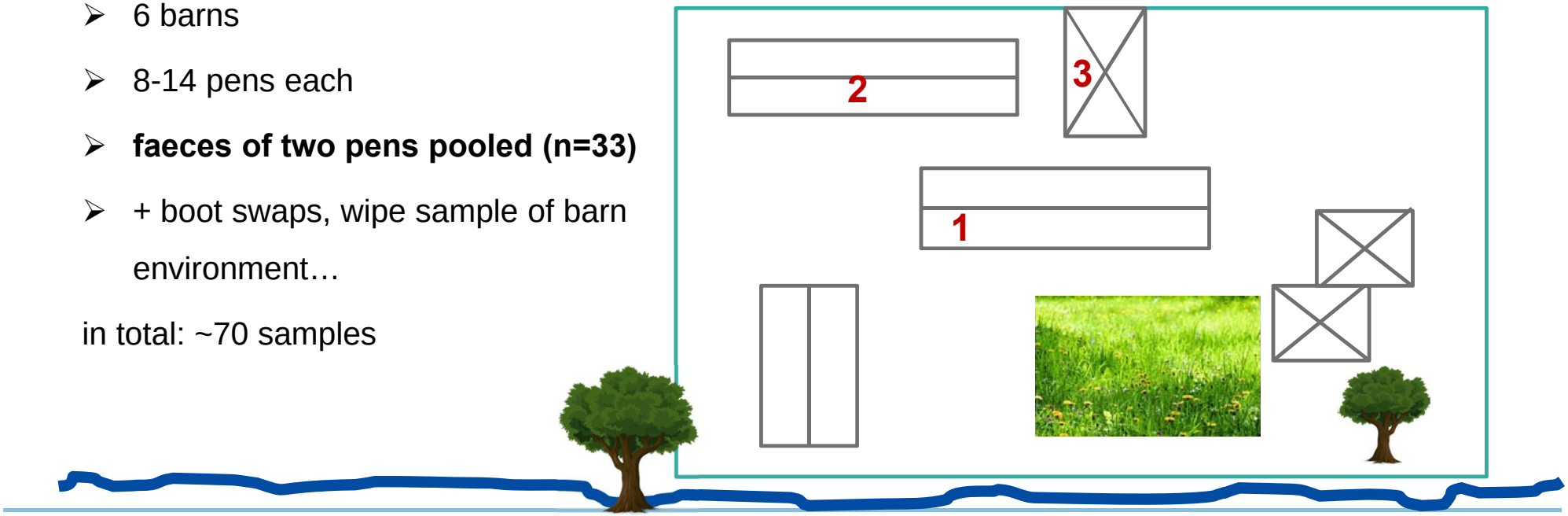
sampling in May 2016

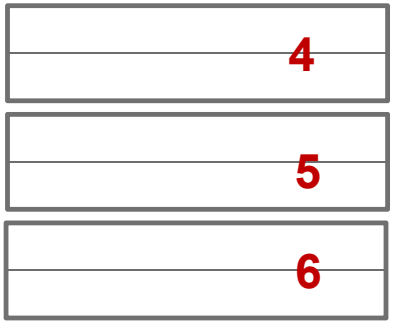
2. Sampling on the pig farm

fattening farm:

- 6 barns
- 8-14 pens each
- **faeces of two pens pooled (n=33)**
- + boot swaps, wipe sample of barn environment…

in total: ~70 samples





2. Isolation method

Recommended protocol by EFSA (meat and caeca):

Unselective pre-enrichment in Buffered Peptone water (1:10)

↓ 37° C / o.n.

selective agar plates →

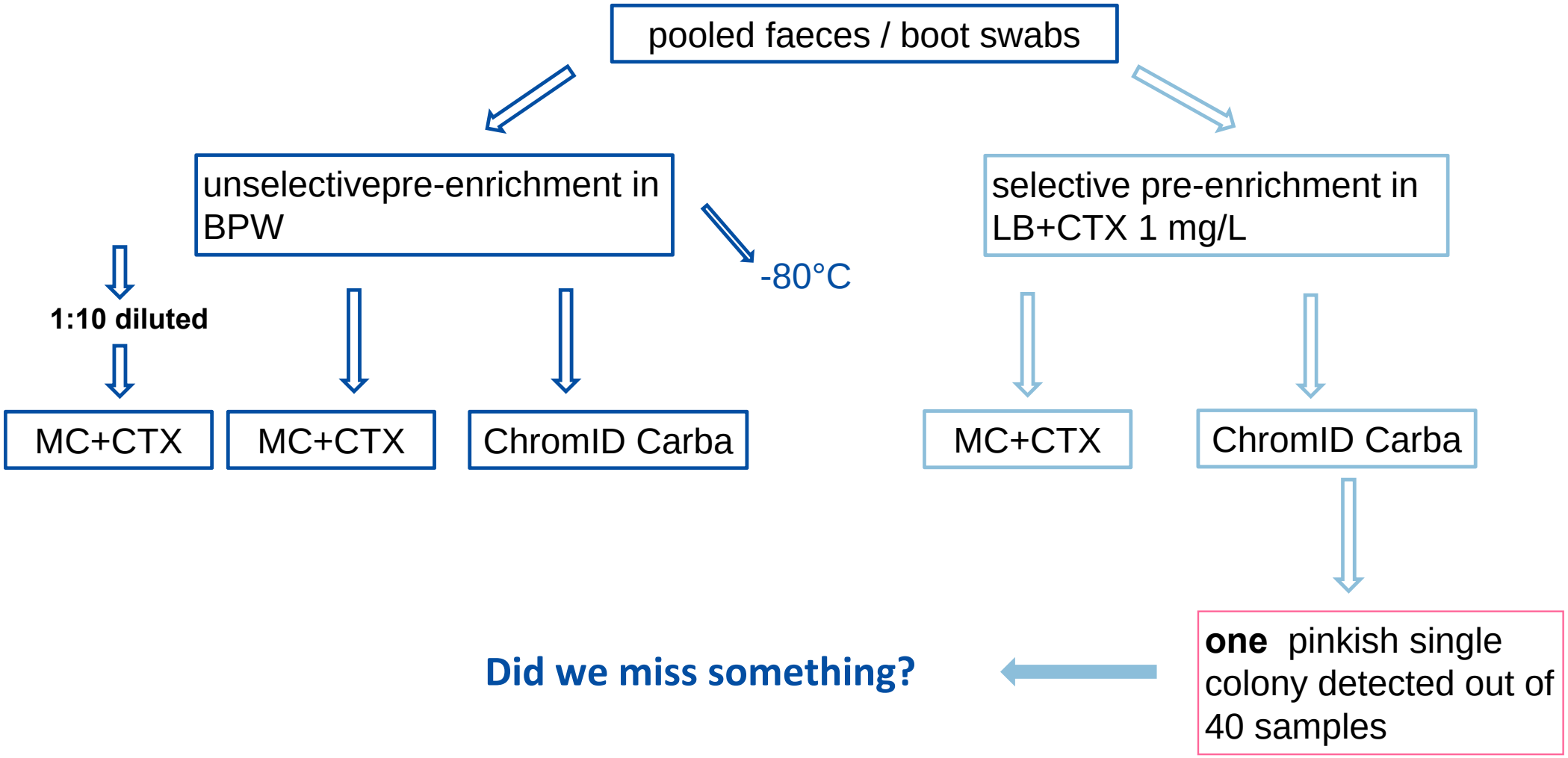
↓ 37° C / o.n.

subcultivation of presumptive colonies

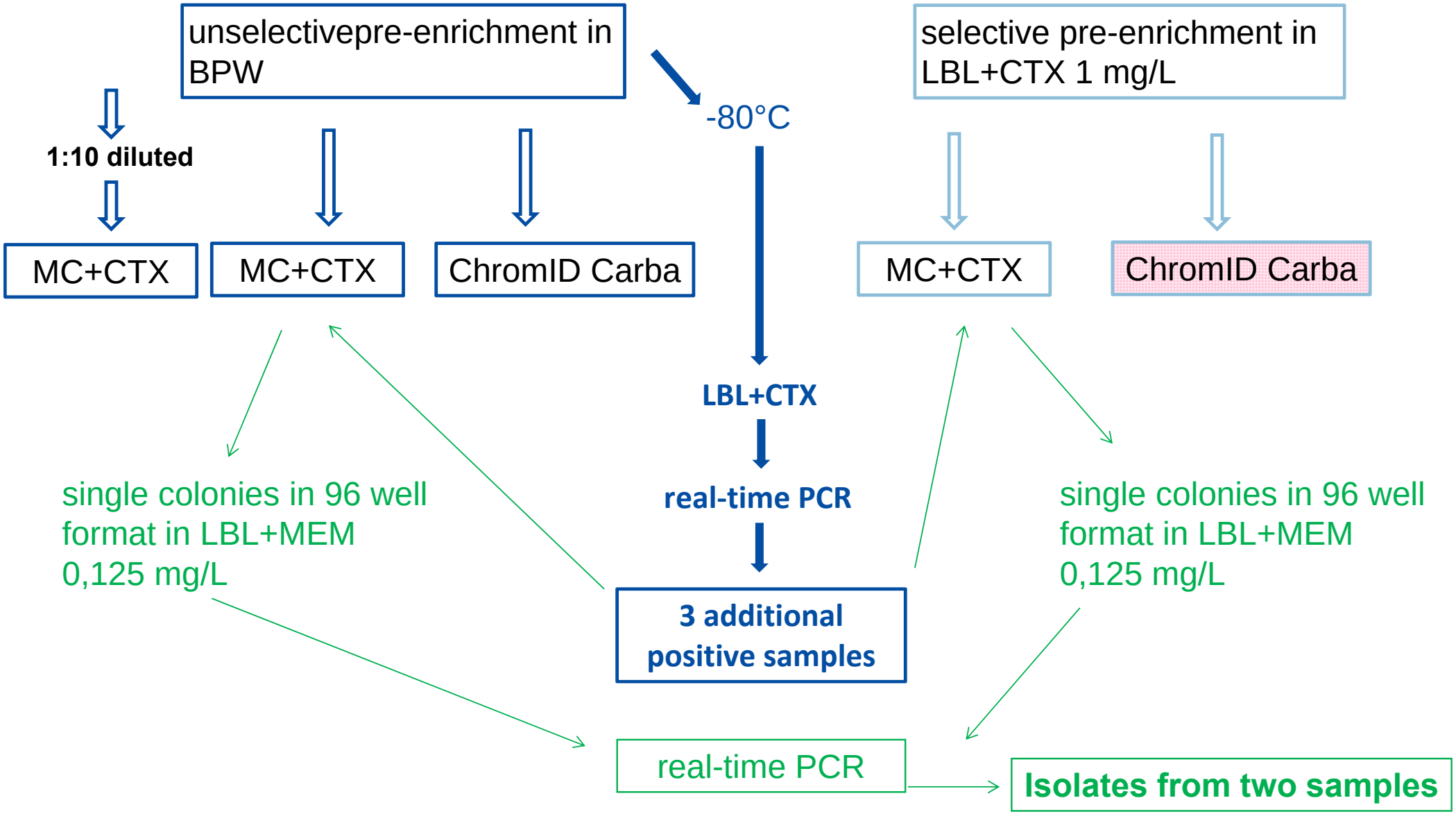
faecal samples

over-growing by
Pseudomonas aeroginosa

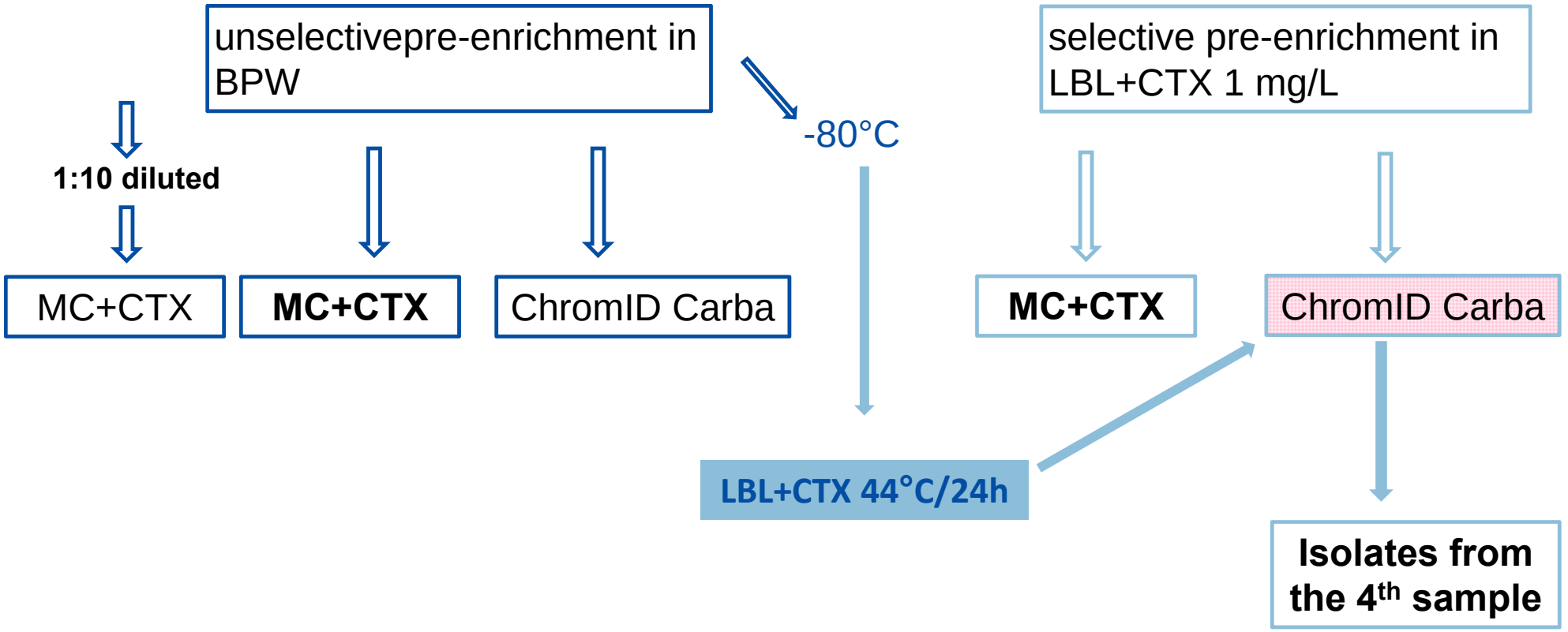
2. Isolation procedure



2. Isolation procedure – PCR screening



2. Isolation procedure

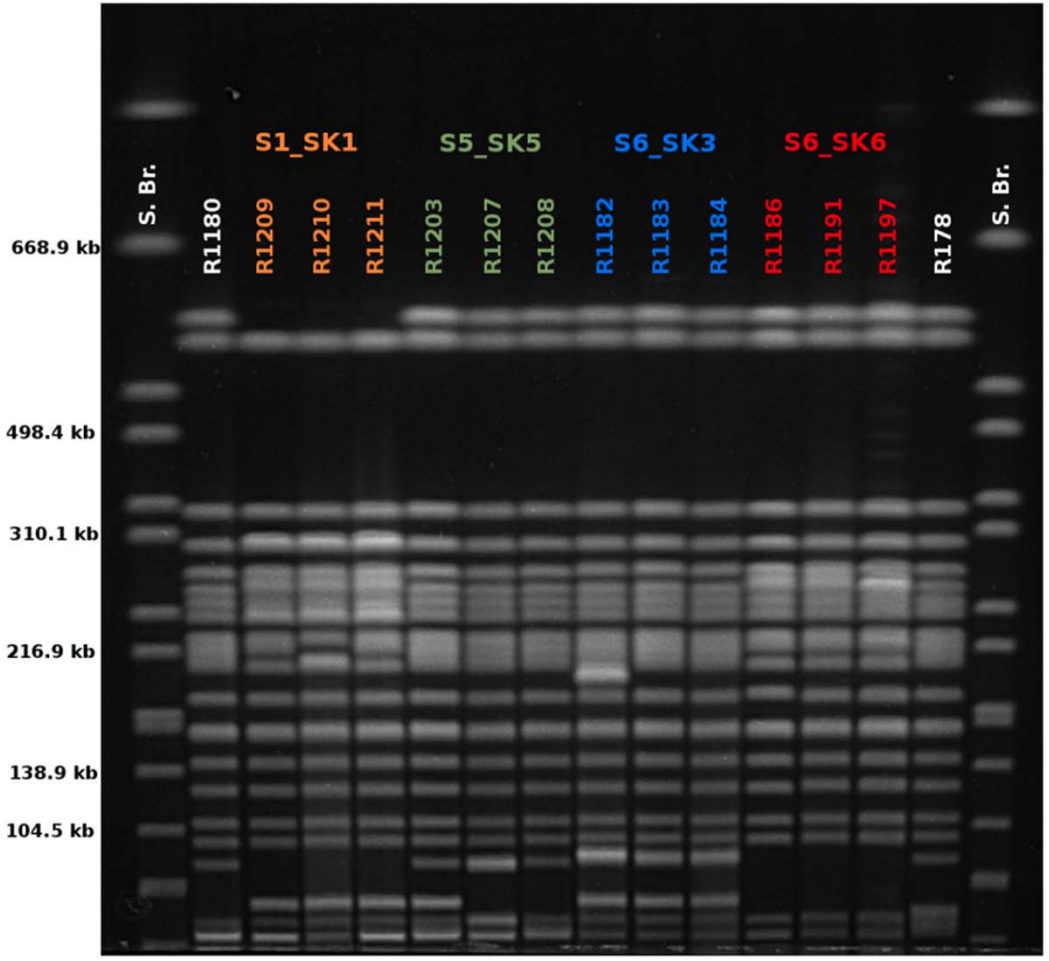


Investigation fattening farm - Isolates

→ 4 positive feacal samples:

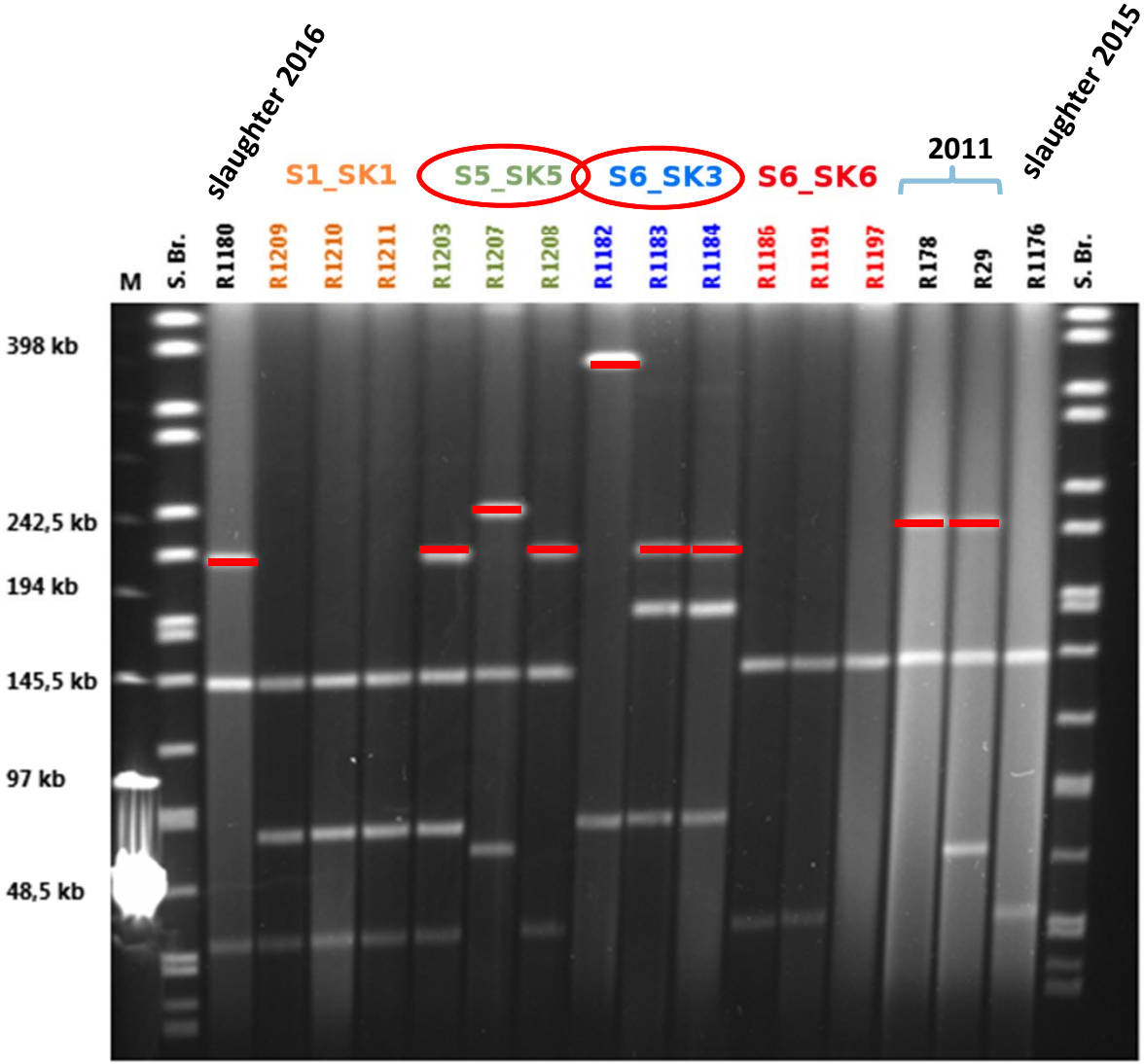
	Barn	No. of positive samples	No. of isolates
→	1	1	5
	5	1	6
	6	2	3 / 18
Σ	3	4	32

Investigation fattening farm - characterization of the isolates



→ high similarities

Investigation fattening farm - characterization of the isolates



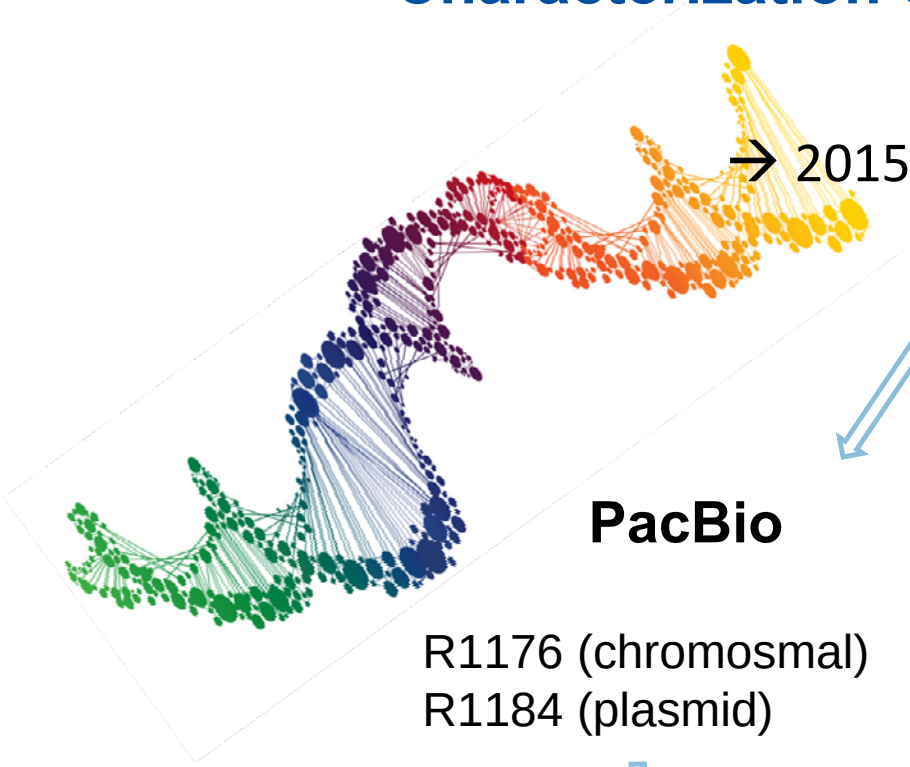
→ variability of the plasmids

→ two samples → *bla*$_{\text{VIM-1}}$ chromosomally located

Overview of VIM-1 producing isolates in German pig production

	R29/ R178	R1176	R1177-R1180	Barn 1 R1209-R1213	Barn 5 R1203-R1208	Barn 6 Sample 3 R1182-R1184	Barn 6 Sample 6 R1185-R1202
Date of isolation	05/2011	12/2015	04/2016	05/2016	05/2016	05/2016	05/2016
Source	Faeces / boot swab	Colon content	Colon content	Faeces	Faeces	Faeces	Faeces
bla-genes	VIM-1 ACC-1	VIM-1 TEM-1	VIM-1 ACC-1 TEM-1	VIM-1 TEM-1 ±TEM206	VIM-1 ACC-1 (TEM-1 ± TEM206)	VIM-1 ACC-1 TEM-206	VIM-1 ±TEM-1
MLST	ST88	ST88	ST88	ST88	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*	Class 1/ bla_{VIM-1}, *aacA4*, *aadA1*	Class 1/ bla_{VIM-1}, *aacA4*, *aadA1*	Class 1/ bla_{VIM-1}, *aacA4*, *aadA1*	Class 1/ bla_{VIM-1}, *aacA4*, *aadA1*
plasmid	220kb/ IncHI2	-	180-200 kb / IncHI2	-	>200kb IncHI2	>200kb IncHI2	-

Characterization of the isolates - sequencing



→ 2015/2016: Σ 37 Isolates

PacBio

R1176 (chromosmal)
R1184 (plasmid)

„reference genomes“

Miseq (Illumina)

R1176 (chromosmal; at slaughter)
R1180 (plasmid, slaughter)
R1182 (plasmid, farm, barn 6, sample 3)
R1184 (plasmid, farm, barn 6, sample 3)
R1191 (chromosomal, farm, barn 6, sample 6)
R1203 (plasmid, farm, barn 5)
R1207 (plasmid, farm, barn 5)
R1208 (plasmid, farm, barn 5)
R1209 (chromosomal, farm, barn1)

Figure: https://research.medicine.umich.edu