



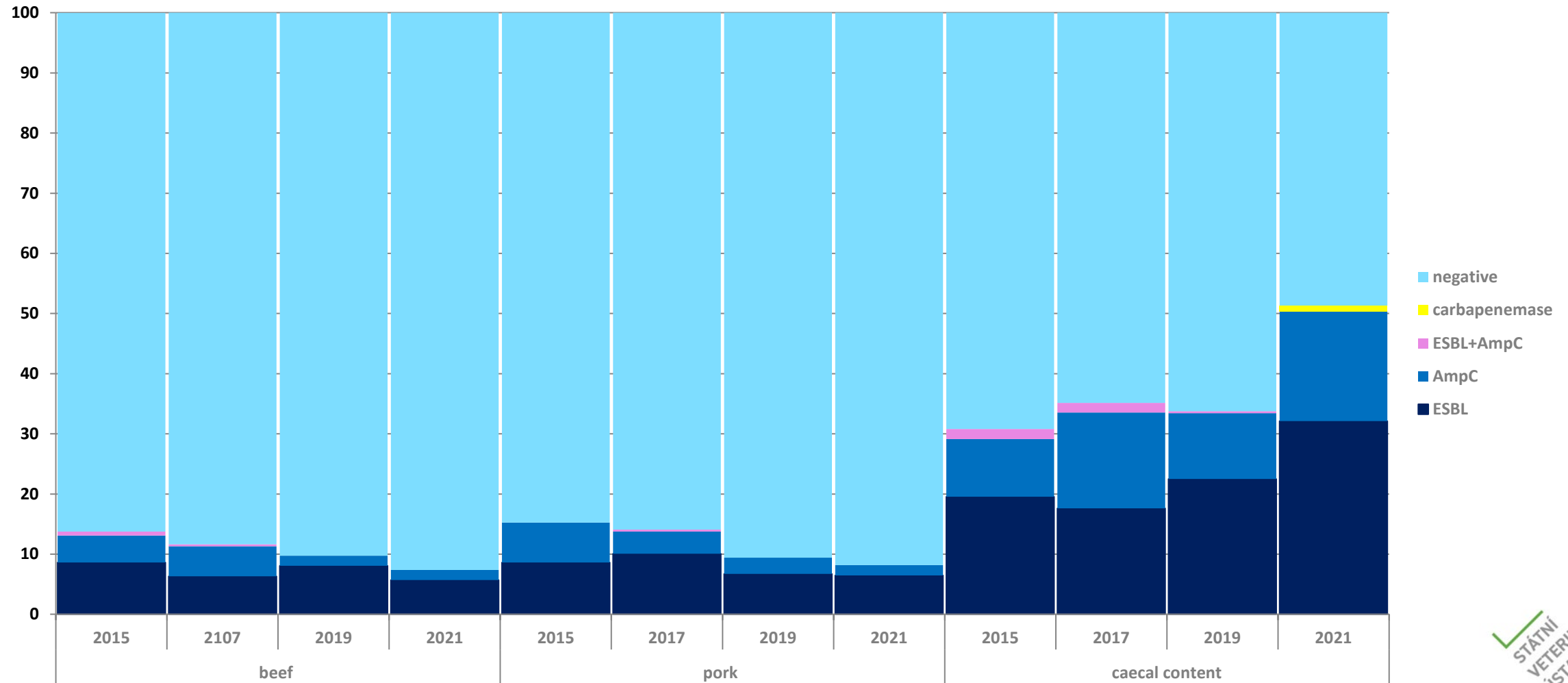
**Detection of NDM-5 positive *Escherichia coli*
from monitoring 2021 in Czechia**

Tomáš Černý

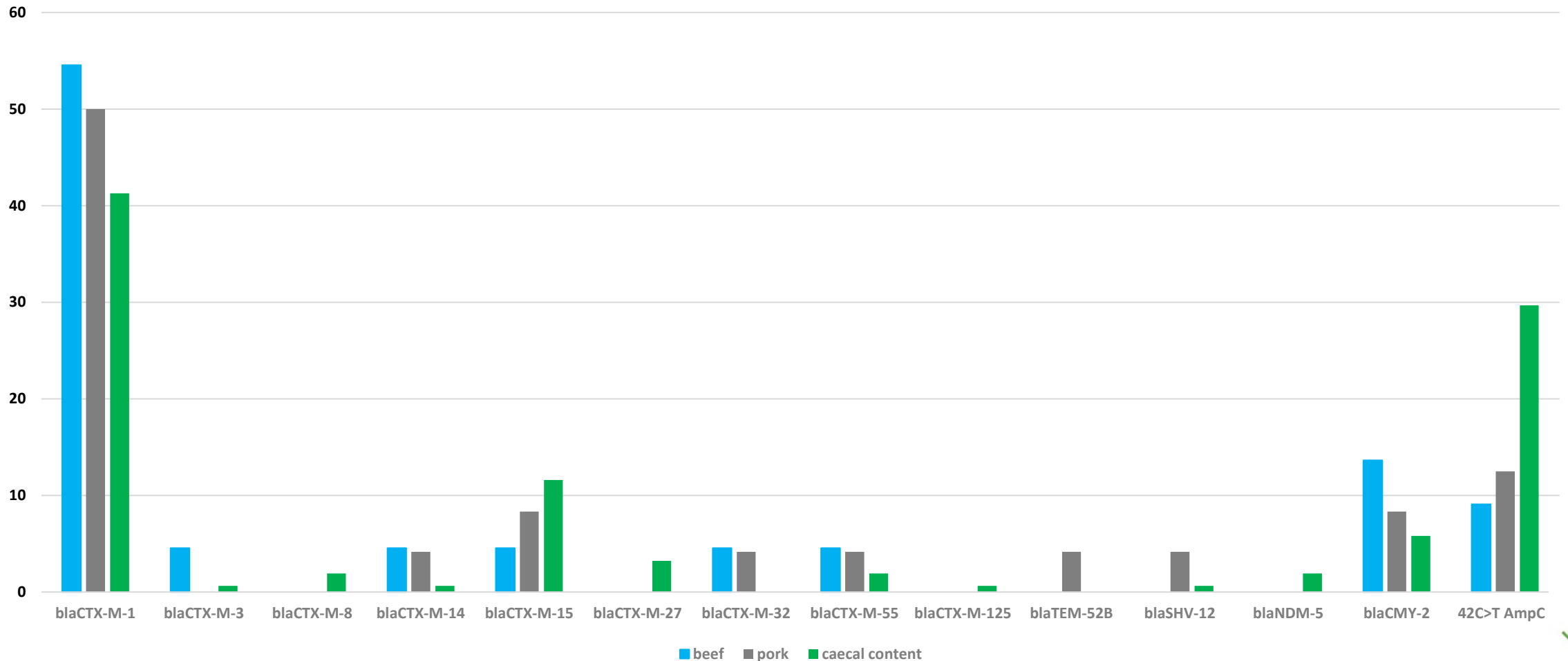
SVI Prague

NRL for AR

Comparison of the relative frequencies of ESBL/AmpC/carbapenemase phenotypes in *Escherichia coli* isolates detected under Decision 2020/1729 EU in 2015, 2017, 2019 and 2021 in Czechia



Comparison of relative frequencies of genotypes in ESBL/AmpC/carbapenemase producers detected according to Decision 2020/1729 EU in 2021 in Czechia



Timeline of detection *Escherichia coli* harboring blaNDM-5 gene (anonymized metadata)



Sample origin: CZ 91011121

Sampling place: CZ 123

Matrix: pig caecal content

09.07.



2021

2022

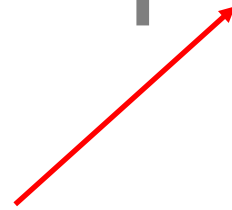


15.01.

Matrix: pig caecal content

Sampling place: CZ 123

Sample origin: CZ 12345678

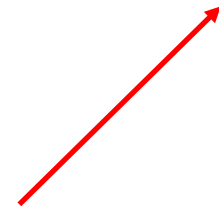


04.10.

Matrix: pig caecal content

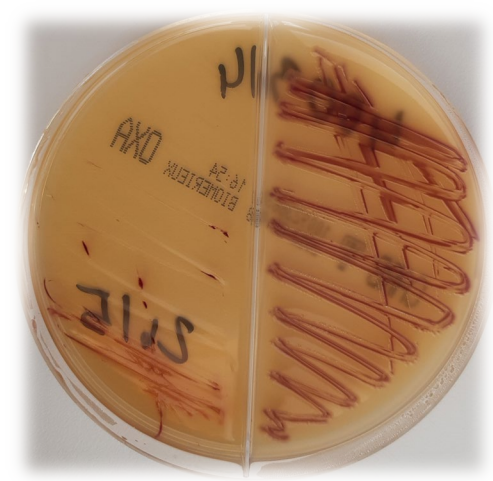
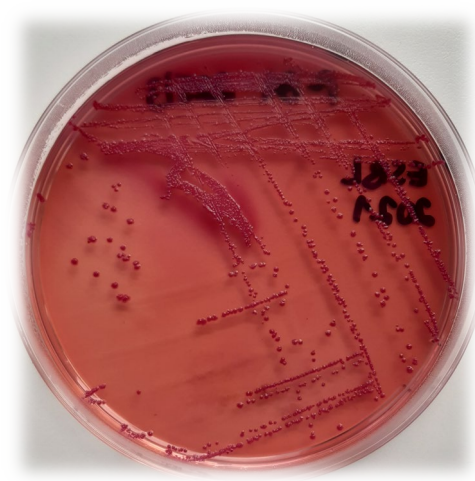
Sampling place: CZ 123

Sample origin: CZ 31415161



Parallel tests that are performed from the same sample of pig intestine contents

- *Escherichia coli* - commensal >> non-selective cultivation TBX >> MALDI-TOF >> MIC >> EUVSEC III +(II)
- *Escherichia coli* - ESBL, AmpC producers >> selective cultivation McC+CTX >> MALDI-TOF >> NGS
- *Escherichia coli* - carbapenemase producers >> selective cultivation CS >> MALDI-TOF >> NGS



Summary results of phenotypic tests of *Escherichia coli* isolated from focused caecal samples

Sample No.	Programme	Phenotype	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	FOX	ETP	FOT	TRM	IMI	MERO	TAZ	FEP	F/C	T/C
21-P-BA-225-3-K	CARB/OXA*	Karba+T	>32	8	≤4	1	≤0,25	>8	>4	≤1	≤4	>32	≤0,25	8	≤8	8	≤0,015	>64	>2	>64	32	16	8	>128	16	>64/4	>128/4
21-P-BA-225-1	COMENSAL	AmpC	>32	4	≤4	≤0,5	≤0,25	8	4	≤1	≤4	≤2	≤0,25	≤8	≤8	≤0,03	≤0,015	64	0,06	8	4	0,25	≤0,03	8	0,25	8/4	8/4
21-P-BA-4003-4-K	CARB/OXA	Karba	>32	4	≤4	1	≤0,25	>8	>4	≤1	≤4	≤2	≤0,25	≤8	≤8	8	≤0,015	>64	>2	>64	32	4	8	>128	16	>64/4	>128/4
21-P-BA-4003-2	COMENSAL	CSuT+W+Nal+Cip	4	≤2	≤4	≤0,5	≤0,25	≤0,25	≤0,25	≤1	16	16	>16	>512	64	≤0,03	1	4	≤0,015	≤0,25	4	≤0,12	≤0,03	≤0,25	≤0,06	≤0,06/4	≤0,12/4
21-P-BA-4003-4	ESBL/AmpC	AmpC+Nal+Cip	>32	4	≤4	≤0,5	≤0,25	8	4	≤1	>64	≤2	≤0,25	≤8	≤8	≤0,03	0,25	32	0,03	4	8	≤0,12	≤0,03	8	0,25	4/4	8/4
21-P-BA-5466-2-K	CARB/OXA	Karba+T	>32	2	≤4	1	≤0,25	>8	>4	≤1	≤4	>32	≤0,25	16	≤8	8	≤0,015	>64	>2	>64	32	8	8	>128	16	>64/4	>128/4
21-P-BA-5466-1	COMENSAL	ASuT+W	>32	4	≤4	≤0,5	≤0,25	≤0,25	≤0,25	≤1	≤4	>32	>16	>512	≤8	≤0,03	≤0,015	4	≤0,015	≤0,25	4	≤0,12	≤0,03	≤0,25	≤0,06	≤0,06/4	≤0,12/4
21-P-BA-5466-2	ESBL/AmpC	ESBL+SuT	>32	4	≤4	≤0,5	≤0,25	1	>4	≤1	≤4	≤2	>16	>512	≤8	≤0,03	≤0,015	4	≤0,015	32	8	≤0,12	≤0,03	1	4	0,12/4	0,25/4

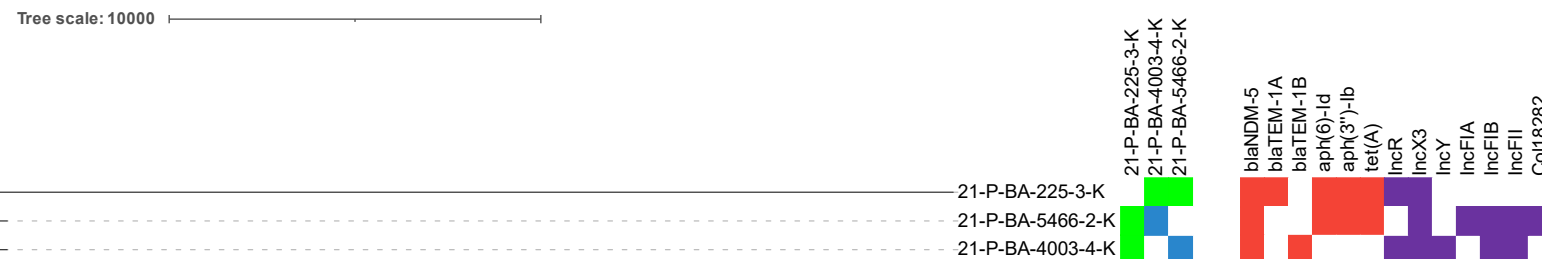
*- On the CS plate was detected identical isolate as on the McC+CTX plate

Epidemiology of *Escherichia coli* harboring blaNDM-5 strain

Sampling date	Programme	Sampling place	Origin	Matrix	No. of sample	Phenotype	Genes	ST	cgST	O-antigen	H-antigen
15.01.2021	CARB/OXA	CZ 123	CZ 12345678	pig caecal content	21-P-BA-225-3-K	Carba + T	blaNDM-5	10	34258	O101	H9
15.01.2021	COMENSAL	CZ 123	CZ 12345678	pig caecal content	21-P-BA-225-1	AmpC	??	??	??	??	??
15.01.2021	ESBL/AmpC*	CZ 123	CZ 12345678	pig caecal content	21-P-BA-225-3-K	Carba + T	blaNDM-5	10	34258	O101	H9
09.07.2021	CARB/OXA	CZ 123	CZ 91011121	pig caecal content	21-P-BA-4003-4-K	Carba	blaNDM-5	898	33479		H48
09.07.2021	COMENSAL	CZ 123	CZ 91011121	pig caecal content	21-P-BA-4003-2	CSuT+W+Cip	-	??	??	??	??
09.07.2021	ESBL/AmpC	CZ 123	CZ 91011121	pig caecal content	21-P-BA-4003-4	Na+Cip+AmpC	blaCMY-2	75	114622	O112ab	H8
04.10.2021	CARB/OXA	CZ 123	CZ 31415161	pig caecal content	21-P-BA-5466-2-K	Carba + T	blaNDM-5	898	4945	O88	H48
04.10.2021	COMENSAL	CZ 123	CZ 31415161	pig caecal content	21-P-BA-5466-1	ASuT+W	-	??	??	??	??
04.10.2021	ESBL/AmpC	CZ 123	CZ 31415161	pig caecal content	21-P-BA-5466-2	ESBL+SuT	blaCTX-M-1	23	130956	O8	H9

*- On the McC+CTX plate was detected identical isolate as on the CS plate

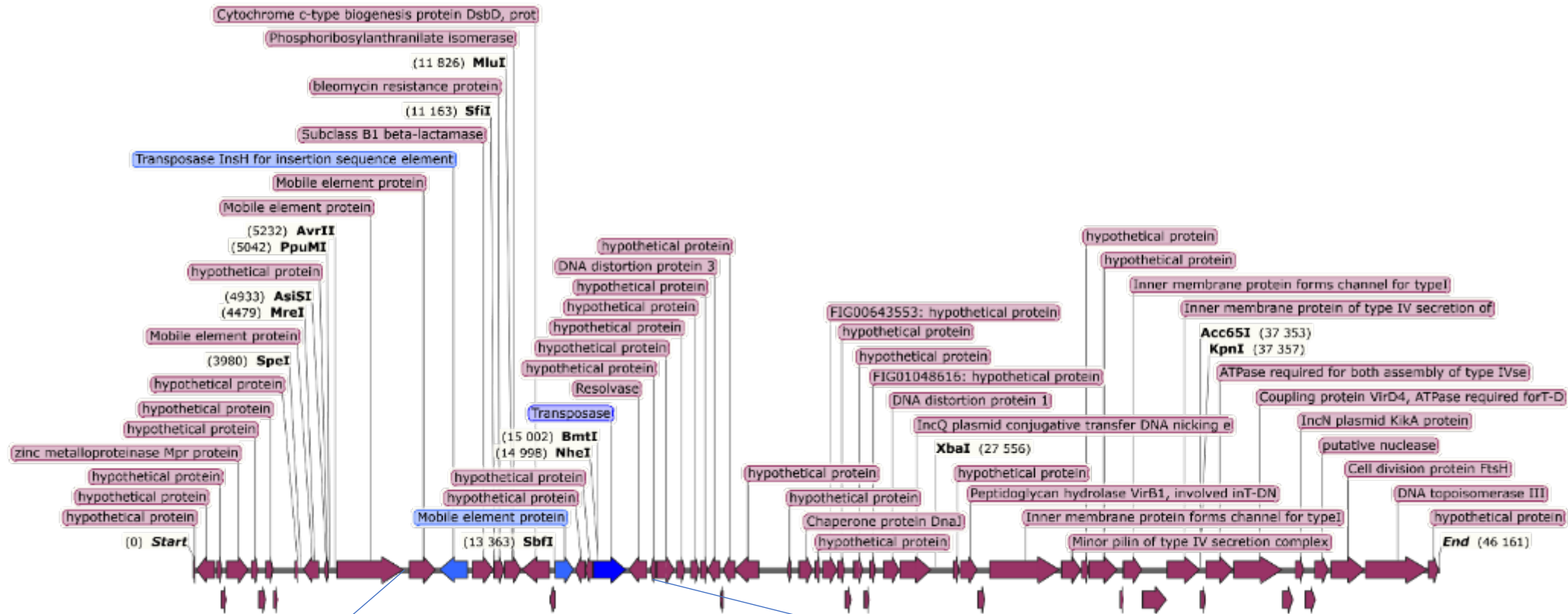
Sample No. / SNPs distance	21-P-BA-225-3-K	21-P-BA-4003-4-K	21-P-BA-5466-2-K
21-P-BA-225-3-K	0	26178	26180
21-P-BA-4003-4-K	26178	0	727
21-P-BA-5466-2-K	26180	727	0



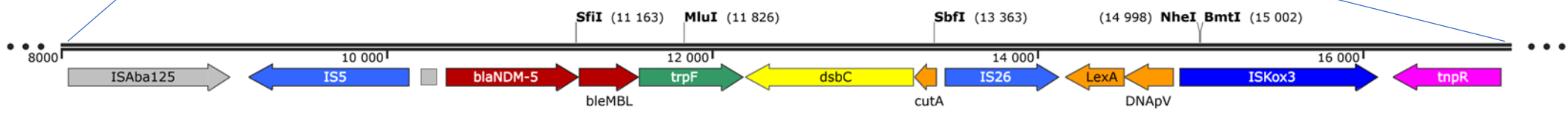
Carbapenemase – blaNDM-5 (overview)

- First capture of *Escherichia coli* harboring blaNDM-5 in GB (Hornsey, M., et. al., 2011)
- Captures of blaNDM-5 in the Czech Republic since 2011 (Hrabák, J., et. al., 2012; Pasková, V. , et. al., 2018; Chudějová, K., et. al., 2021)
- The gene was carried on different plasmids IncF, IncX3, IncL/M, IncH, IncA/C2 (Zhu, Y.,Q. et. al., 2016; Giufrè. M. at. al., 2018)
- IncX3 identified as carrier of blaNDM-x genes in Europe, Asia, America (Wu, W., et.al, 2019)
- IncX3 is the most important carrier of the blaNDM-5 gene in enterobacteria (Shen, Z., 2018; Xi, L., 2018; Krishnaraju, M., 2015)
- *Escherichia coli* IncX3/blaNDM-5 was detected:
 - in poultry (Yang, L., et al., 2016, Zhang, F., et al., 2019)
 - from cow's milk (Yaici, L., et al., 2016)
 - in pigs (Pak-Leung, H., et. al., 2018; Xiaofeng, H., 2021)
- Sequence ISAb125-IS5-blaNDM-5-IS26 detected on IncX3 (~46 Kb) (Pak-Leung H., et. al. 2018)
- High conjugation activity of IncX3/blaNDM-5 plasmid was demonstrated (Wang, Y., et al., 2018, Xiaofeng, H., 2021)
- Presence of IncX3/blaNDM-5 plasmid also demonstrated in environmental samples (Ito, Y., et. al., 2022)

Plasmid IncX3 as a carrier of the blaNDM-5 gene

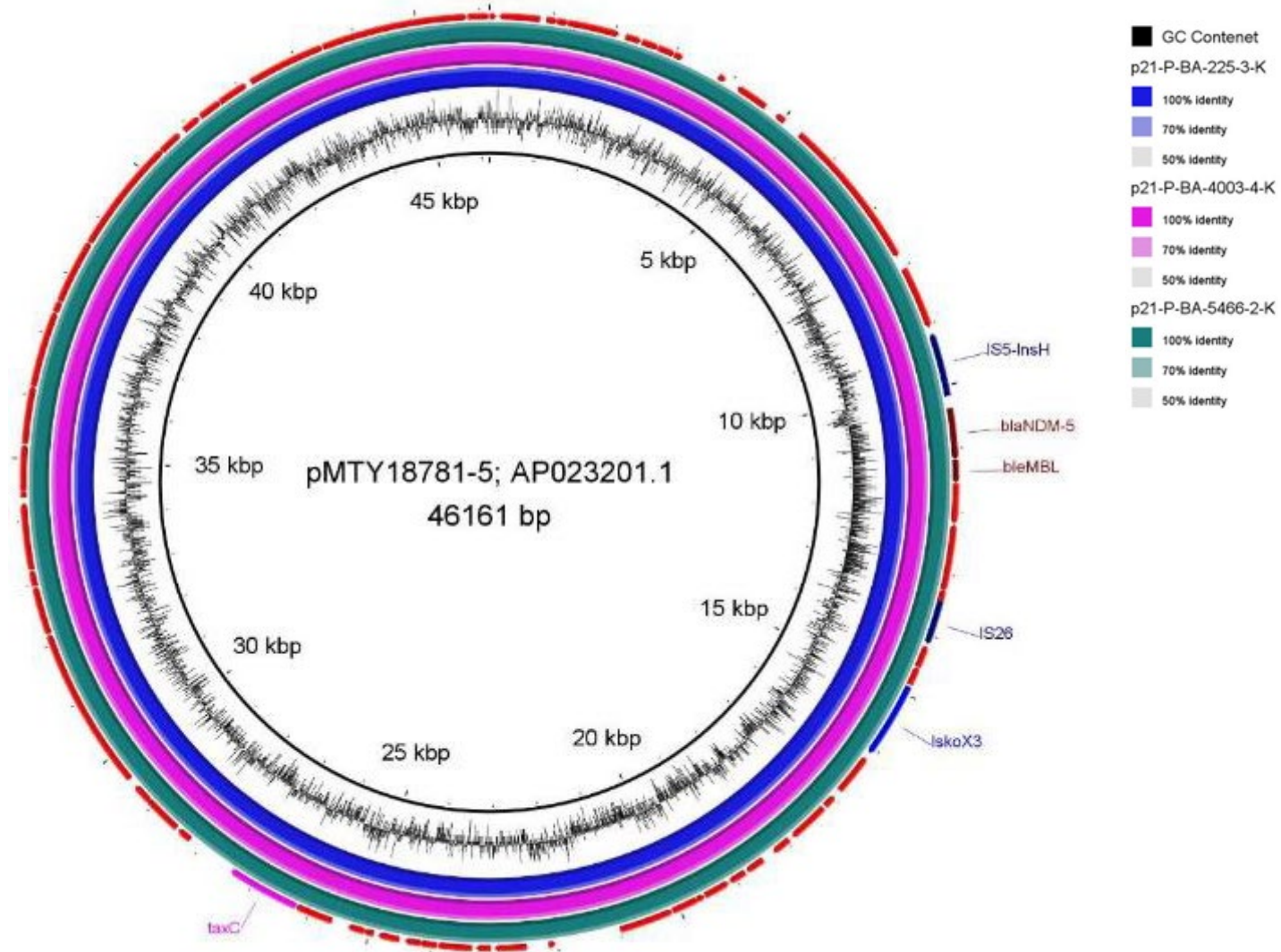


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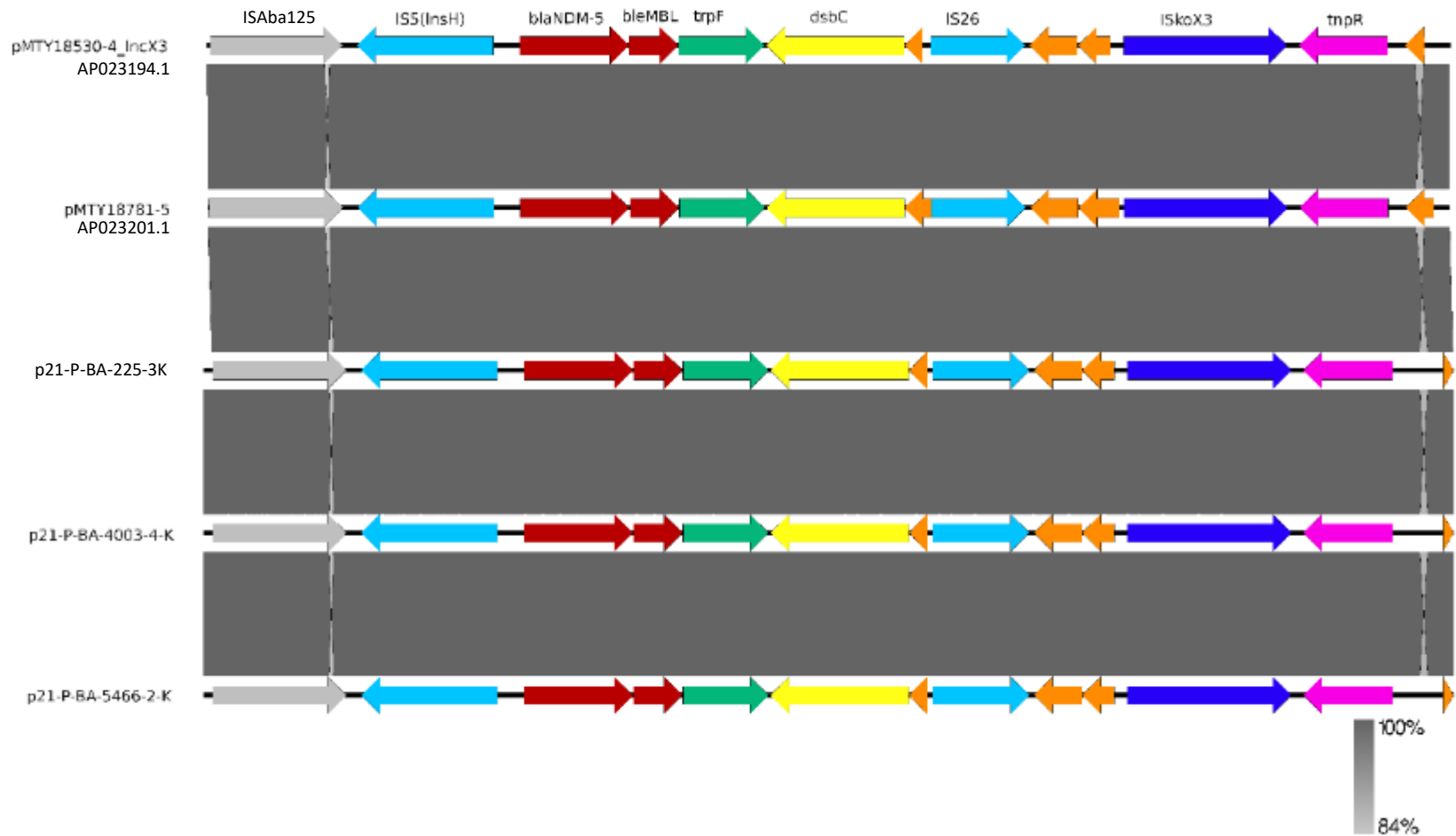


pMTY18781-5
46 161 bp

Comparison of detected IncX3/blaNDM-5 plasmids

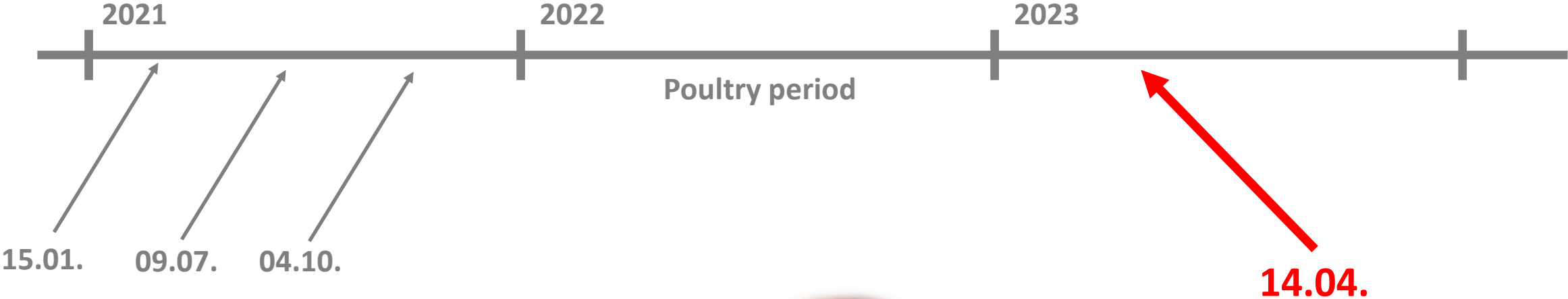


Comparison of the ISAb125-IS5-blaNDM-5-ISkoX3 segments topography

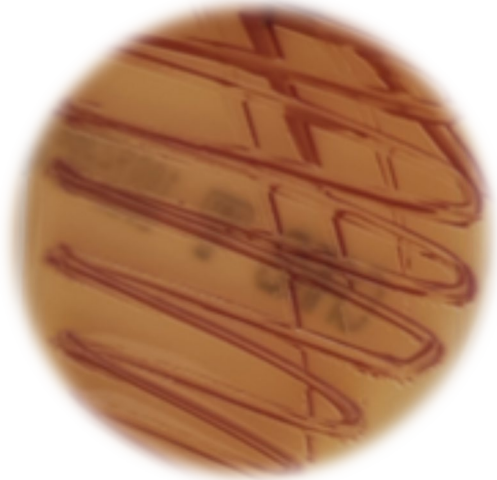


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Does the story continue???



Different *Escherichia coli* strain
Same blaNDM-5 harbored in IncX3 plasmid
Same matrix: pig caecal content
Same sampling place
Different sample origin

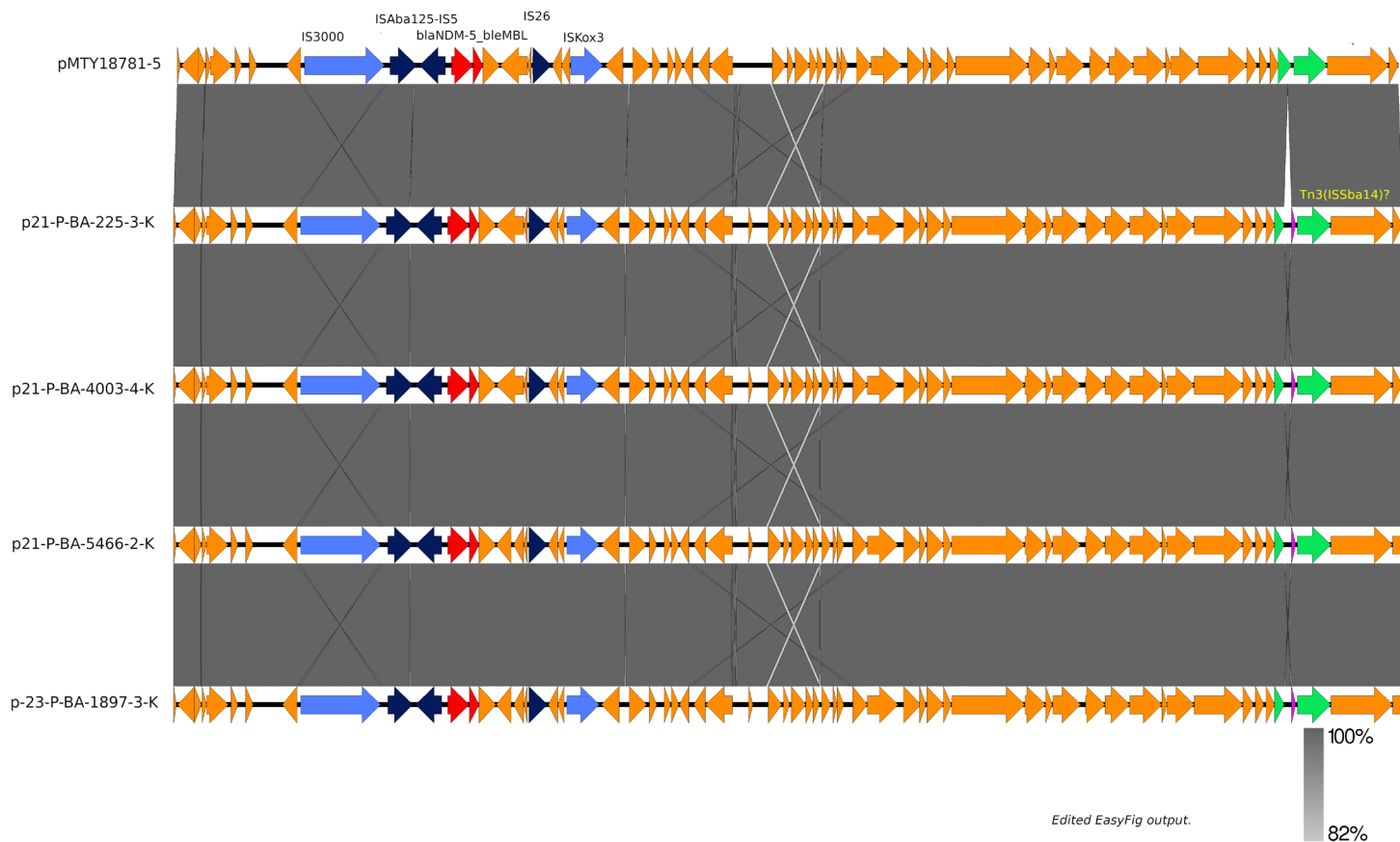


Different *Escherichia coli* strain
CSuT+carbapenemase phenotype
Same matrix: pig caecal content
Same sampling place
Different sample origin

Preliminary results of the sequencing *Escherichia coli* 23-P-BA-1897-3-K

Escherichia coli O27:H12; GS: 5576083 pb

- ST 10; cgST 28330
- IncX3, IncR, IncI1-I plasmids were detected
- IncX3 (~46 Kb), blaNDM-5, bleMBL
- IncR (~23 Kb), blaTEM-1A
- IncI1-I (~113 Kb), blaCTX-M-1, floR, sul2, tet(A)
- UPEC genotype: cnf1, papA_F12, shiB



The plasmid was detected on another MDR *Escherichia coli* isolate two years after initial capture

The IncX3 plasmid of this strain was identical to the plasmids detected in 2021 (46452 pb)

Plasmids detected by us show high homology with plasmids found in the BLAST database

Conclusion - risks analysis

- Presence of complex multifactorial system
- Horizontal transmission
- Vertical transmission
- Presence of different subpopulations in the samples
- Persistence in the environment
- Cross contamination
- The role of selection pressure
- Critical points of the food chain
- Identification of the intensity and frequency of occurrence of risk factors



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