Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

CRAB
Centro di Referenza Nazionale per l'Antibioticoresistenza

Update on Carbapenemase-producing *E.coli* from EU-harmonised AMR Monitoring in Italy, 2021-2023

18th EURL-AR Workshop 2024
9th October – Kgs. Lyngby, Denmark

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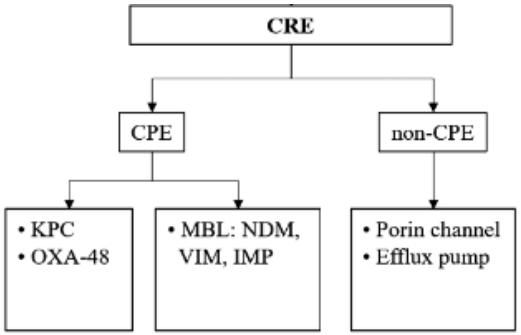
Istituto Zooprofilattico Sperimentale
del Lazio e della Toscana *M. Aleandri*

Carbapenemase-producing *Enterobacteriaceae*

Carbapenemase-producing *Enterobacteriaceae* (**CPE**) are gram-negative microorganisms resistant to **carbapenems**, considered "drug of last resort" or last choice antibiotic in human medicine (CIA)



Carbapenemases/β-lactamases

Serine-β-lactamases	Metallo-β-lactamases
Class A: **KPC, IMI, SME**, CTX-M, GES*, SHV, TEM	
	Class B: **NDM, VIM, IMP**
Class C: AmpC, ACT, CMY, DHA, FOX	
Class D: **OXA-48, OXA-181, OXA-23, OXA-40, OXA-58** OXA-1, OXA-2, OXA-9, OXA-10	

* Some variants may possess carbapenemase activity

Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria

Patrice Nordmann ✉, Laurent Poirel

Clinical Infectious Diseases, Volume 69, Issue Supplement_7, 1 December 2019, Pages S521–S528, https://doi.org/10.1093/cid/ciz824
Published: 13 November 2019

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Carbapenemase-producing *Enterobacteriaceae*

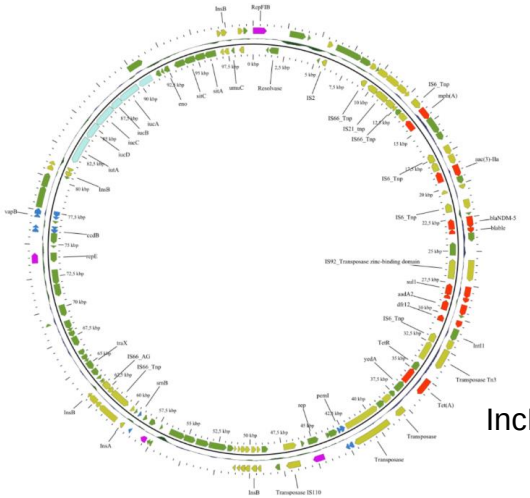
ELSEVIER

Veterinary Microbiology
Volume 256, May 2021, 109045

Short communication

Carbapenemase IncF-borne *bla*$_{NDM-5}$ gene in the *E. coli* ST167 high-risk clone from canine clinical infection, Italy

Patricia Alba [a], Roberta Taddei [b], Gessica Cordaro [a], Maria Cristina Fontana [b], Elena Toschi [b], Paolo Gaibani [c], Ilaria Marani [a], Angelo Giacomi [a], Elena L. Diaconu [a], Manuela Iurescia [a], Virginia Carfora [a], Alessia Franco [a]



IncFII, 2019, pig

Compared with international databases:
Very similar to:
- p51008369SK1_E (Switzerland) (Peterhans et al., 2018)
- pNDM-5-IT, from a clinical human isolate (Italy) (Giufrè et al., 2018)

The plasmid harbouring bla NDM-5 has been frequently detected in Europe
In Italy, it has been found in CPE of both human and animal origin

J Antimicrob Chemother 2020; **75**: 3475–3479
doi:10.1093/jac/dkaa374 Advance Access publication 24 August 2020

Journal of Antimicrobial Chemotherapy

Novel IncFII plasmid harbouring *bla*$_{NDM-4}$ in a carbapenem-resistant *Escherichia coli* of pig origin, Italy

Elena L. Diaconu, Virginia Carfora, Patricia Alba, Paola Di Matteo, Fiorentino Stravino, Carmela Buccella, Elena Dell'Aira, Roberta Onorati, Luigi Sorbara, Antonio Battisti* and Alessia Franco

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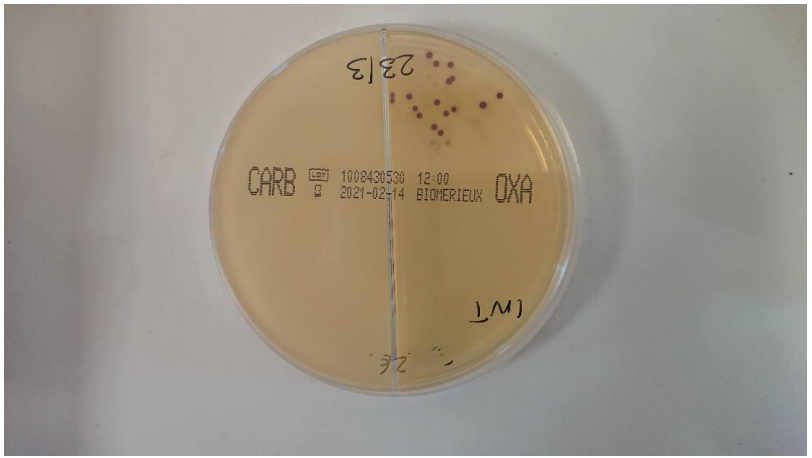
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana *M. Aleandri*

Commission Implementing Decision 2013/652/EC e Dec. (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria



Growth of *E.coli* OXA-48-like on the OXA half plate from ceacal content (monitoring)

APPENDIX 2

FLOW DIAGRAM
for detection ESBL/AmpC/carbapenemases
(including OXA-48 and OXA-48-like enzymes) in caecal samples

Non-selective pre-enrichment [item 1.4-1.5]

1 g of caecal sample in 9 mL of buffered peptone water (37°C ± 1°C, 18-22 h)

↓

Selective isolation

➔ of presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* [item 1.6]
Streak 10 µL of the incubated pre-enrichment culture in BPW onto **MacConkey agar plate supplemented with 1 mg/L of cefotaxime** (incubate at 44°C ± 0.5°C for 18-22 h)

➔ of presumptive carbapenemase (including OXA-48- and OXA-48-like)-producing *E. coli* [item 3.1 + 3.2]
Streak 10 µL of the incubated pre-enrichment culture in BPW onto **suitable selective agar plate(s).** (Commercially available chromogenic agar for isolation of carbapenemase-producing *E. coli* (including isolates producing only OXA-48 and/or OXA-48-like enzymes). (incubation according to manufacturer's instructions)

Method: Same lab procedure since 2014: Specific monitoring of CPE-producing E. coli
The EURL-AR protocol (by using commercial OXA-48-like + other Carbapenemases Biplate)

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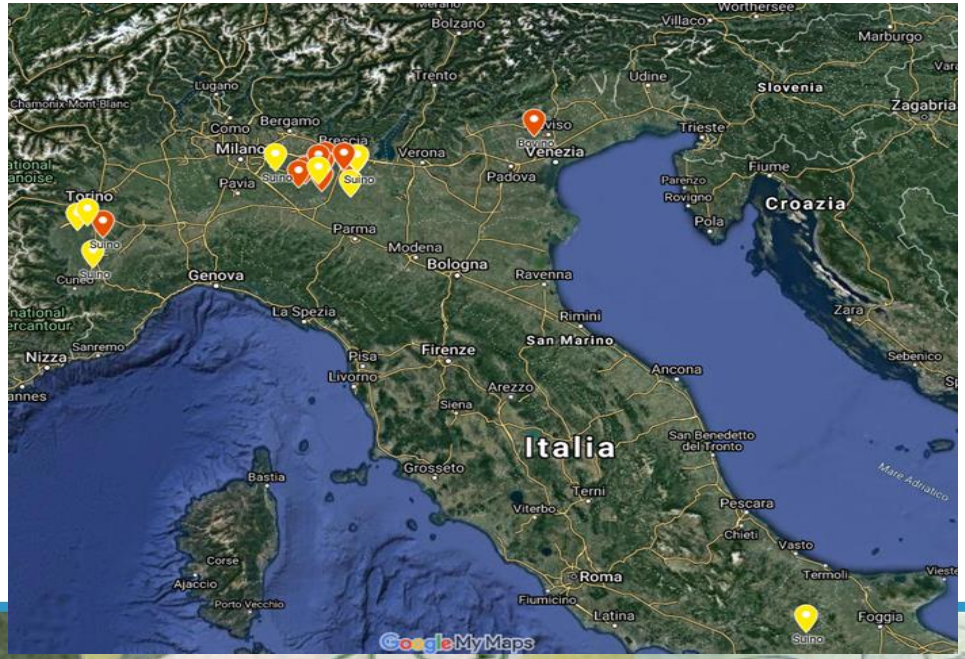
Update at December 2021 → **25 isolates** Oxa-48-like (24 OXA-181; 1 OXA-48) from EpiUnits sampled at slautherhouse (Dec (EU) 2020/1729) in 11 provinces (5 Regions)
n=21 from pigs (**6.98%**; 95% CI 4.37-10.47%; 21/301) **n=4** from bovines <12 months (**1.29%;** 95% CI 0.35–3.27%, 4/310)

PNAMR

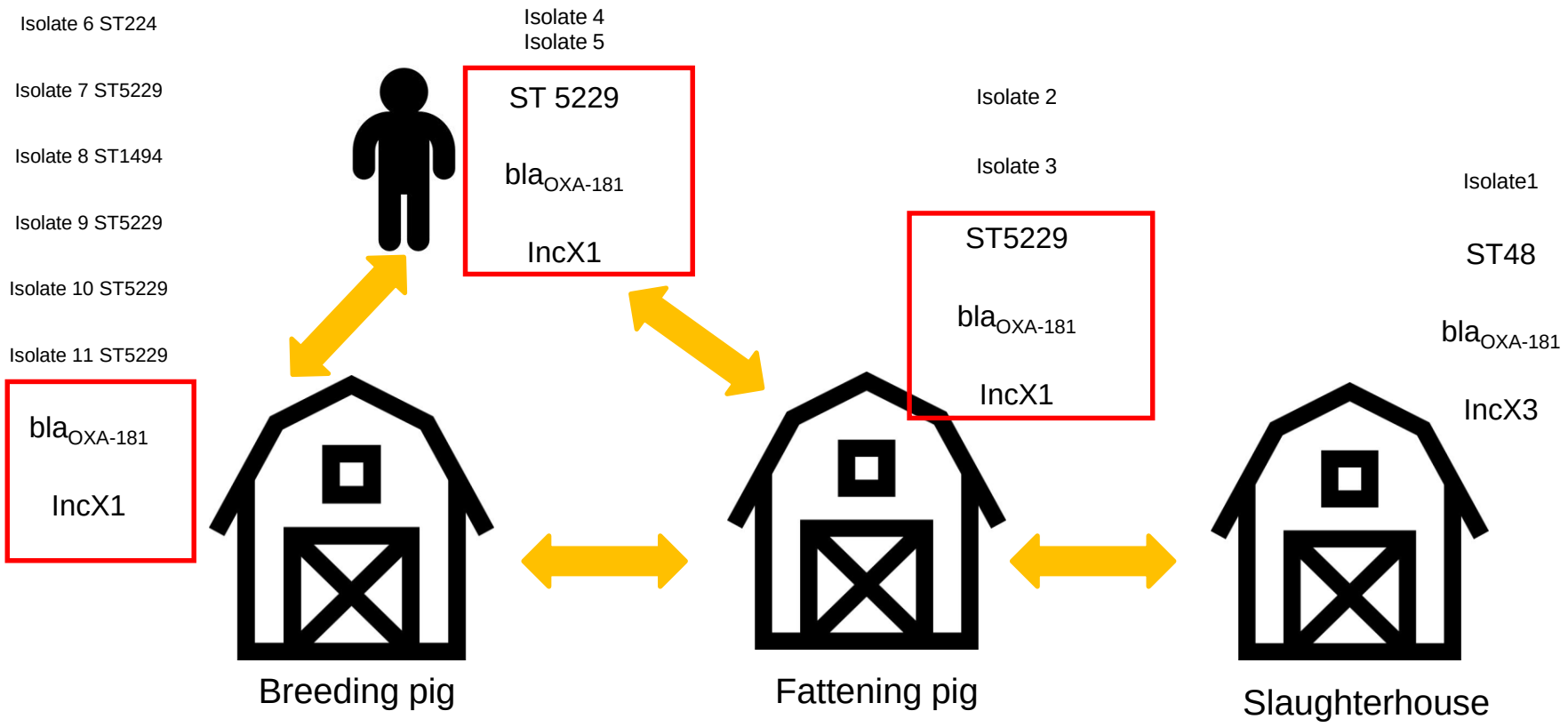
PNAMR/IE

IE



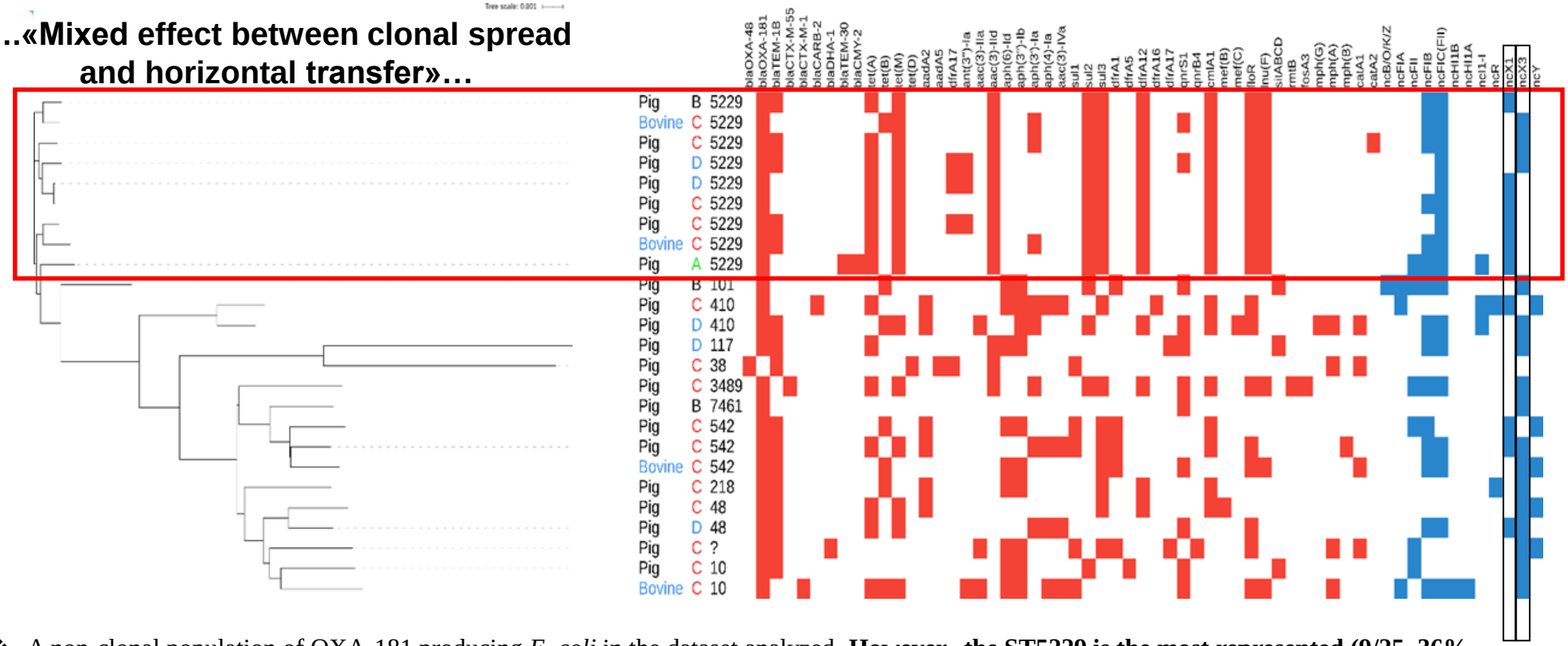
Epidemiological investigation: for >80% the positive (2021) EpiUnits that were **investigated and sampled at the farm of origin**, an OXA-48-like producing E. coli (OXA-181) **has been isolated**

Epidemiological Investigation: Case1 (Index case)



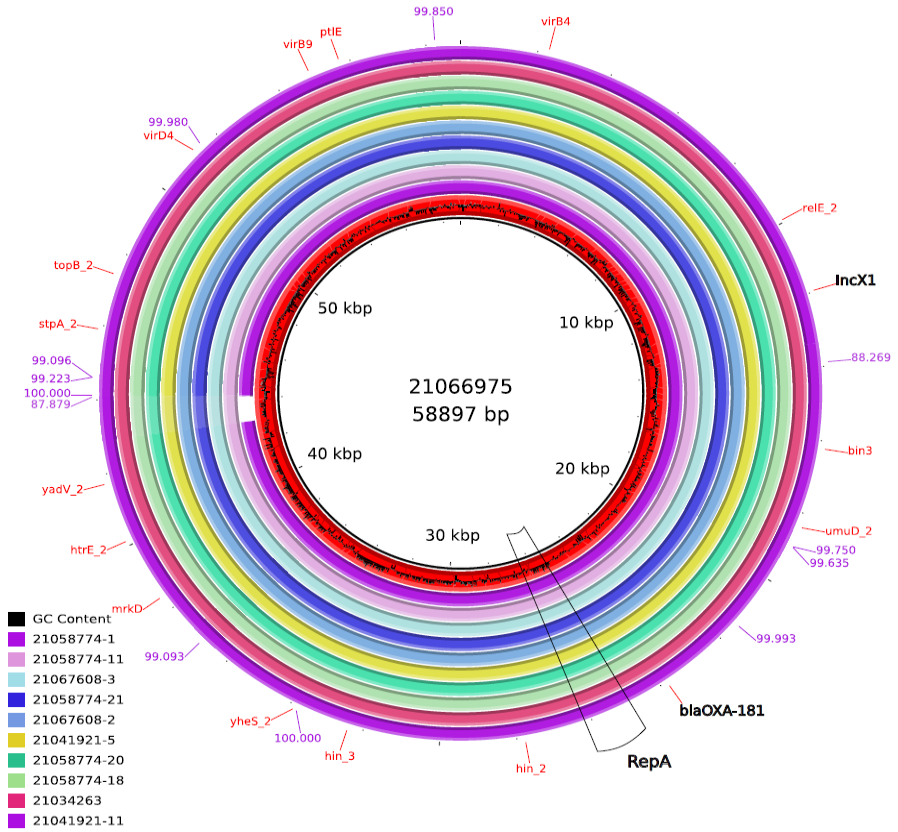
Results of the survey at slaughter (short-read): Mash clusterization of the WGS complete genome, resistome and plasmidome of the n=25 OXA181-producing Escherichia coli

….«Mixed effect between clonal spread and horizontal transfer»…



- A non-clonal population of OXA-181 producing *E. coli* in the dataset analyzed. **However, the ST5229 is the most represented (9/25, 36% isolates).**
- **IncX3, IncX1, (one IncF) the replicons most represented.**
- **IncX3 or IncX1 harboured the OXA-181 gene. No specific pathotype found.**
- The clusters were distributed according to the different Clonal Complexes (CCs) and STs.
- No clear region or host species correlation was observed.

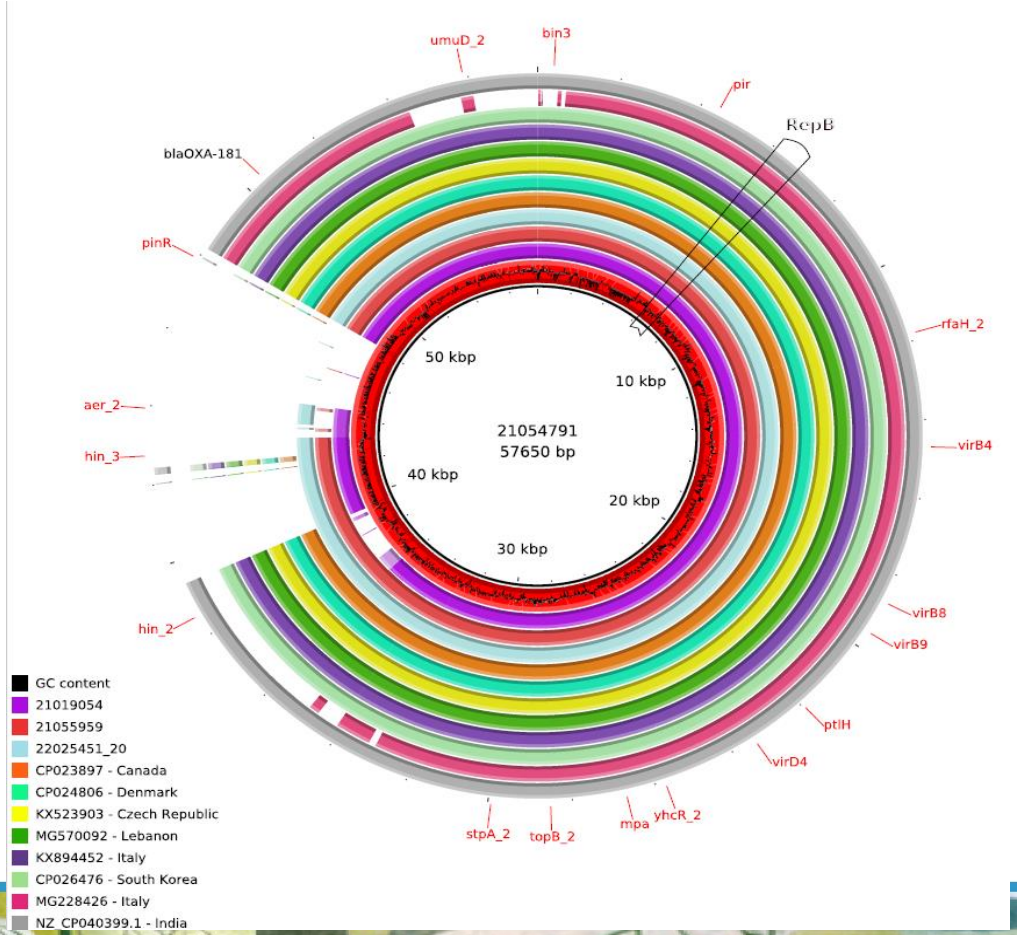
Full plasmid sequencing: ***IncX1*** *plasmids*



- ✓ All the 12 IncX1 resolved plasmids were almost identical with a 98-99% coverage and 99-100% sequence identity
- ✓ **Novelty: No similar IncX1 plasmids were found in public available databases (around 50% identity with other publicly available plasmids).**

IncX1 more stable than IncX3 because of the presence of the RelE/StbE toxin family and its antitoxin RelB?

Full plasmid sequencing: ***IncX3*** *plasmids*



- ✓ All three resolved plasmids IncX3 harboring $bla_{\text{OXA-181}}$ from *E.coli* were very similar with a 90-91% coverage and 100% identity
- ✓ They shared a similarity of 99% with 89% of the plasmid covered, when compared with publicly available IncX3 plasmids containing $bla_{\text{OXA-181}}$ (from *E. coli*, *C. freundii*, *K. pneumoniae*), from human cases
- ✓ 100% coverage and identity of the IncX3 plasmid from *E. coli* ID 21019054 with a $bla_{\text{OXA-181}}$-IncX3 plasmid of a *C. freundii* isolate (ID 22025451-20) from the same pig holding


21019054-OXA_ONT_MS.fasta
3
3
Go
51 kb
10 kb
20 kb
30 kb
40 kb
50 kb
21019054.gbk
Click and drag to zoom in.
bin3
pir
dnaJ
rfaH
virB4
virB8
ptlH
virD4
mpa
topB
stpA
hin
yheS
bla
21041921-11_sort.bam Coverage
21041921-11_sort.bam
21055959_sort.bam Coverage
21055959_sort.bam
21058774-11_sort.bam Coverage
21058774-11_sort.bam

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Resistome

Resfinder
Abricate

Geni Resfinder	Mutation	PATTERN RESISTENZA
mdf(A)_1 tet(A)_6 dfrA12_8 aadA2_1 cmlA1_1 sul3_2 mef(B)_1 tet(M)_8 blaTEM-1B_1 blaOXA-181_1		AMP,CHL,SMX,TET,TMP,ETP,TRM
floR_2 cmlA1_1 tet(A)_6 sul2_2 sul3_2 tet(M)_8 aac(3)-IId_1 dfrA12_8 lnu(F)_1 mdf(A)_1 blaOXA-181_1	gyrA p.D87N parC p.S80I	AMP,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,ETP,MER,TRM
blaOXA-181_1 mdf(A)_1 tet(A)_6 floR_2 sul3_2 lnu(F)_1 ant(3'')-Ia_1 sul2_2 aac(3)-IId_1 aph(3')-Ia_1	gyrA p.D87N parC p.S80I	AMP,FOT,TAZ,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,TAZ,ETP,MER,TRM
blaOXA-181_1 tet(M)_8 cmlA1_1 aadA2_1 dfrA12_8 mdf(A)_1 blaCTX-M-1_1 mph(A)_2 floR_2 sul2_2 sul3_2	gyrA p.D87N parC p.S80I	AMP,AZI,FOT,TAZ,CHL,CIP,NAL,SMX,TMP,FEP,TAZ,ETP,MER,TRM
qnrS1_1 blaOXA-181_1 sitABCD_1 dfrA5_1 sul2_3 aph(3'')-Ib_5 aph(6)-Id_1 mdf(A)_1 blaTEM-1B_1		AMP,FOT,CIP,SMX,TMP,FEP,FOT,ETP,IMI,MER,TRM
mdf(A)_1 blaOXA-181_1 qnrS1_1 tet(M)_8 cmlA1_1 aadA2_1 dfrA12_8 blaTEM-1B_1 sul3_2 tet(A)_6 floR_2	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,FOX,ETP,IMI,MER,TRM
aph(3')-Ia_1 blaOXA-181_1 dfrA17_1 aadA5_1 sul1_5 armA_1 aph(4)-Ia_1 aac(3)-IVa_1 mph(G)_1 aac(3)-IIa_		AMP,AZI,GEN,SMX,TMP,FOT,ETP,MER,TRM
blaOXA-181_1 tet(A)_6 floR_2 mdf(A)_1 sul2_2 sul3_2 ant(3'')-Ia_1 lnu(F)_1 aac(3)-IId_1 aph(3')-Ia_1	gyrA p.D87N parC p.S80I	AMP,CHL,CIP,GEN,NAL,SMX,TET,ETP,MER,TRM
mdf(A)_1 tet(B)_1 floR_2 aac(3)-IId_1 tet(M)_8 sul3_2 dfrA12_8 lnu(F)_1 aadA2_1 blaOXA-181_1 blaTEM-1B		AMP,CHL,GEN,SMX,TET,TMP,FEP,FOT,ETP,MER,TRM
blaOXA-181_1 sul2_2 floR_2 tet(A)_6 sul3_2 mdf(A)_1 ant(3'')-Ia_1 lnu(F)_1 aac(3)-IId_1 aph(3')-Ia_1	gyrA p.D87N parC p.S80I	AMP,CHL,CIP,GEN,NAL,SMX,TET,ETP,MER,TRM
blaOXA-181_1 mdf(A)_1 blaTEM-1B_1 sul3_2 tet(A)_6 floR_2 sul2_2 lnu(F)_1 ant(3'')-Ia_1 aac(3)-IId_1 aph(3	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,ETP,TRM
mdf(A)_1 blaOXA-181_1 blaTEM-1B_1 sul3_2 tet(A)_6 floR_2 sul2_2 ant(3'')-Ia_1 lnu(F)_1 aac(3)-IId_1 aph(3	gyrA p.D87N parC p.S80I	AMP,CHL,CIP,GEN,NAL,SMX,TET,ETP,TRM
blaOXA-181_1 mdf(A)_1 cmlA1_1 tet(M)_8 sul3_2 blaTEM-1B_1 tet(A)_6 floR_2 sul2_2 aac(3)-IId_1 dfrA12_	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,ETP,MER,TRM
blaOXA-181_1 sul3_2 blaTEM-1B_1 mdf(A)_1 floR_2 sul2_2 ant(3'')-Ia_1 lnu(F)_1 aac(3)-IId_1 aph(3')-Ia_1	gyrA p.D87N parC p.A56T par	AMP,AZI,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,ETP,MER,TRM
blaTEM-1B_1 mdf(A)_1 blaOXA-181_1 sul1_5 aadA5_1 dfrA17_1 catA1_1 floR_2 aac(3)-IId_1 aph(6)-Id_1 aph	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,ETP,MER,TRM
blaOXA-181_1 mdf(A)_1 tet(A)_6 sul3_2 blaTEM-1B_1 floR_2 sul2_2 lnu(F)_1 ant(3'')-Ia_1 aac(3)-IId_1 aph(3	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,ETP,MER,TRM
mdf(A)_1 blaOXA-181_1 qnrS1_1 tet(A)_6 tet(M)_8 floR_2 sul2_2 cmlA1_1 aac(3)-IId_1 dfrA12_8 lnu(F)_1	gyrA p.D87N parC p.S80I	AMP,CHL,CIP,GEN,NAL,SMX,TET,TMP,ETP,TRM
mdf(A)_1 blaOXA-181_1 tet(A)_6 aph(4)-Ia_1 aac(3)-IVa_1 blaTEM-1B_1 aph(3')-Ia_1		AMP,CHL,CIP,GEN,TET,ETP,TRM
blaTEM-1B_1 aac(3)-IIa_1 lnu(G)_1 mdf(A)_1 sul3_2 cmlA1_1 aadA2_1 dfrA12_8 blaOXA-181_1 tet(B)_2 floR_2		AMP,CHL,GEN,SMX,TET,TMP,ETP,MER,TRM
floR_2 aac(3)-IIa_1 blaTEM-1B_1 lnu(G)_1 mdf(A)_1 dfrA12_8 aadA2_1 cmlA1_1 sul3_2 blaOXA-181_1 tet(B)_2		AMP,CHL,GEN,SMX,TET,TMP,ETP,TRM
mdf(A)_1 blaTEM-1B_1 blaOXA-181_1 lnu(G)_1 tet(A)_6 dfrA1_10 sul3_2 floR_2 catA1_1		AMP,CHL,SMX,TET,TMP,ETP,MER,TRM
blaOXA-181_1 floR_2 dfrA12_8 aadA2_1 cmlA1_1 sul3_2 qnrS1_1 tet(A)_6 blaTEM-1B_1 mdf(A)_1		AMP,CHL,GEN,SMX,TET,TMP,ETP,MER,TRM
mdf(A)_1 blaOXA-181_1 aac(3)-IVa_1 aph(4)-Ia_1 floR_2 tet(A)_6 blaTEM-1B_1 aph(3')-Ia_1 qnrS1_1		AMP,CHL,CIP,GEN,NAL,SMX,TET,TMP,ETP,TRM
rmtB_1 mdf(A)_1 blaOXA-181_1 cmlA1_1 tet(M)_8 tet(A)_6 fosA3_1 floR_2 qnrS1_1 sul3_2 sul2_2 aac(3)-IId	parC p.S80I	AMP,FOT,TAZ,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,TAZ,ETP,MER,TRM
blaOXA-181_1 blaTEM-1A_1 tet(A)_6 aph(6)-Id_1 aph(3'')-Ib_5 dfrA1_8 aac(3)-IId_1 cmlA1_1 aadA2_1 mdf(		AMP,CHL,CIP,GEN,NAL,SMX,TET,TMP,ETP,TRM
dfrA12_8 aadA2_1 cmlA1_1 blaTEM-1B_1 qnrS1_1 tet(A)_6 blaOXA-181_1 mdf(A)_1 sul3_2		AMP,CHL,CIP,SMX,TET,TMP,ETP,MER,TRM
blaTEM-30_1 mdf(A)_1 blaOXA-181_1 qnrS1_1 tet(A)_6 cmlA1_1 aadA2_1 dfrA12_8		AMP,CHL,CIP,SMX,TET,TMP,ETP,MER,TRM
mdf(A)_1 blaOXA-181_1 floR_2 sul2_2 cmlA1_1 tet(M)_8 tet(A)_6 sul3_2 aac(3)-IId_1 dfrA12_8 lnu(F)_1	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,TMP,FEP,ETP,MER,TRM
blaOXA-181_1 mdf(A)_1 tet(A)_6 floR_2 sul2_2 sul3_2 ant(3'')-Ia_1 lnu(F)_1 aac(3)-IId_1 aph(3')-Ia_1	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,ETP,TRM
blaOXA-181_1 sul2_2 floR_2 mdf(A)_1 tet(A)_6 sul3_2 blaTEM-1B_1 lnu(F)_1 ant(3'')-Ia_1 aac(3)-IId_1 aph(3	gyrA p.D87N parC p.S80I	AMP,FOT,CHL,CIP,GEN,NAL,SMX,TET,ETP,MER,TRM

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OXA-181+ve isolates: ECOFFs and clinical breakpoints for carbapenems and temocillin with number (nR) and percentage (%) of resistant E. coli isolates

The only carbapenem in the first panel

	R (ECOFF)	R (CB)	nR (%) ECOFF	nR (%) CB	MIC Range mg/L (Mode)
ETP (ertapenem)	>0,06	>0,5	25/25 **(100%)**	7/25 (28%)	0.12-4 (0.5)
IMI (imipenem)	>0,5	>4	5/25 (20%)	5/25(20%)	0.12-1 (0.25)
MER (meropenem)	>0,125	>8	13/25 (52%)	0/25 (0%)	0.06-1(0.25)
TRM (temocillin)	>16	>16	25/25 **(100%)**	25/25 **(100%)**	128-256 (256)

The hazard of carbapenemase (OXA-181)-producing *Escherichia coli* spreading in pig and veal calf holdings in Italy in the genomics era: Risk of spill over and spill back between humans and animals

Virginia Carfora[1] Elena Lavinia Diaconu[1] Angela Ianzano[1] Paola Di Matteo[1] Roberta Amoruso[1] Elena Dell'Aira[1] Luigi Sorbara[1] Francesco Bottoni[1] Flavia Guarneri[2] Laura Campana[3] Alessia Franco[1] Patricia Alba[1*] Antonio Battisti[1]

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Isolation rates (prevalence) OXA-181 (OXA-48-like)-producing *E. coli* from caecal samples according to the National Monitoring Programme on AMR, Dec (EU) 2020/1729, Italy 2021-2023

2021

Species	Samples cultured	Samples positive	%	95% CI
Pigs	301	21	6,98%	4,37-10,47%
Bovines <12m	310	4	1,29%	0,35–3,27%

2022

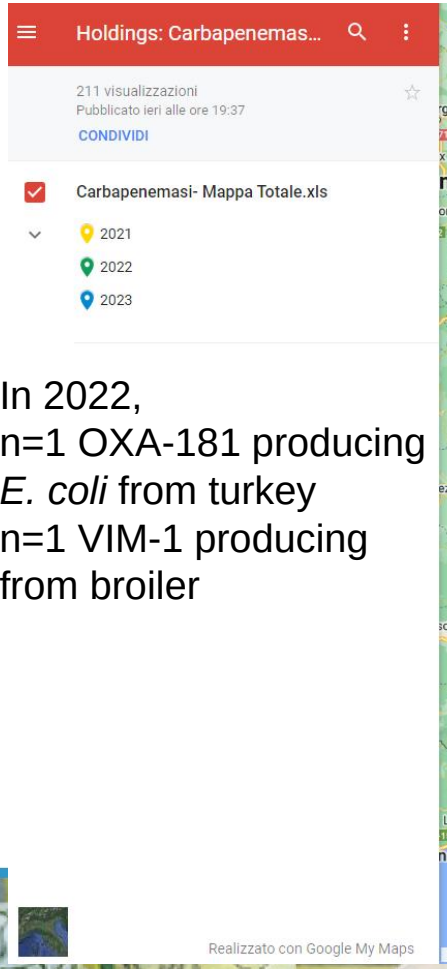
Species	Samples cultured	Samples positive	%	95% CI
Broiler chicken	479	0	0,00%	N.A.
Turkey	397	1	0,25%	0,01-1,40%

2023

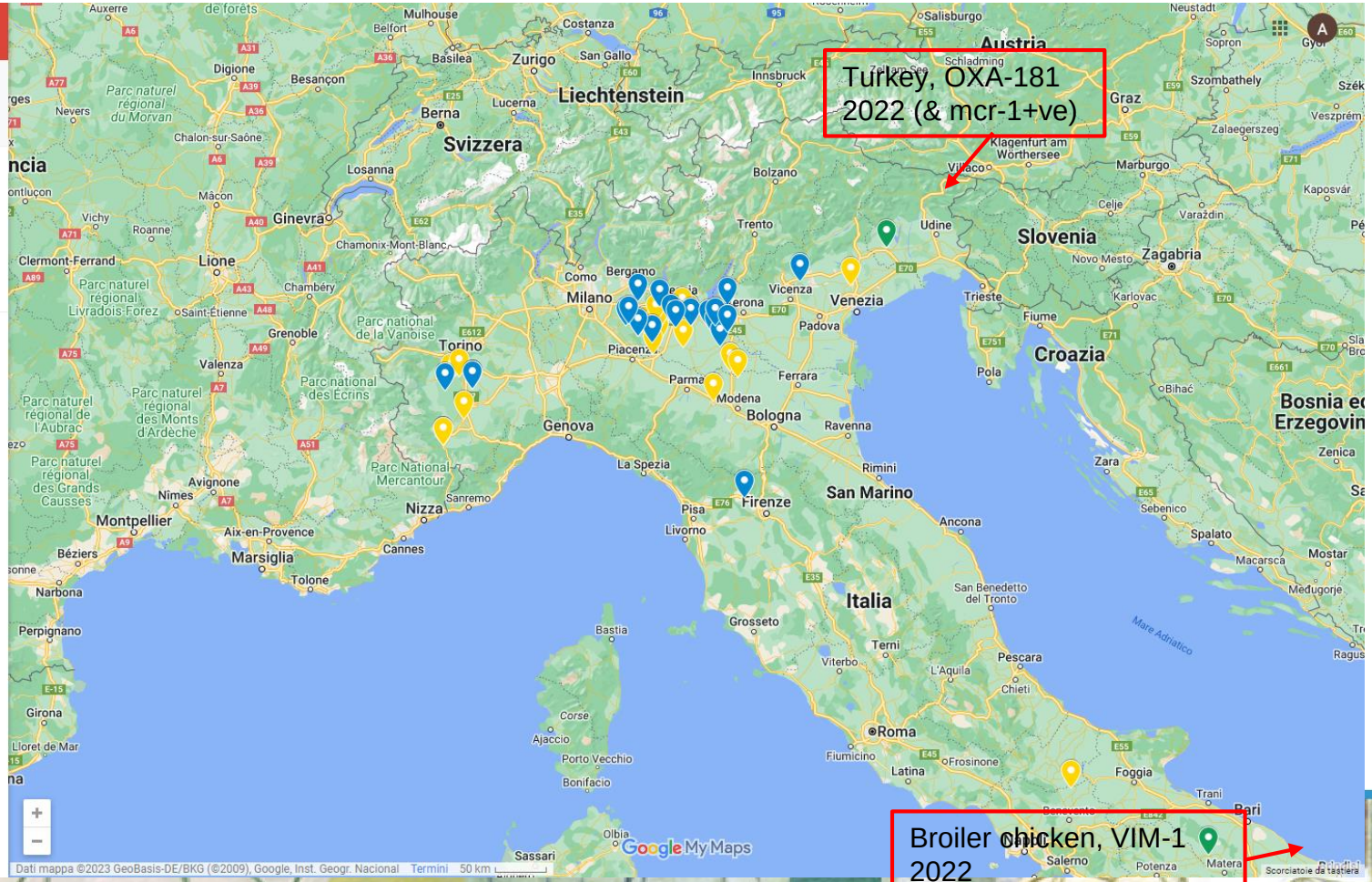
Species	Samples culture	Samples positive	%	95% CI
Pigs	300	19	6,33%	3,86-9,71%
Bovines <12m	301	4	1,33%	0,36-3,37%

Update: February 2024

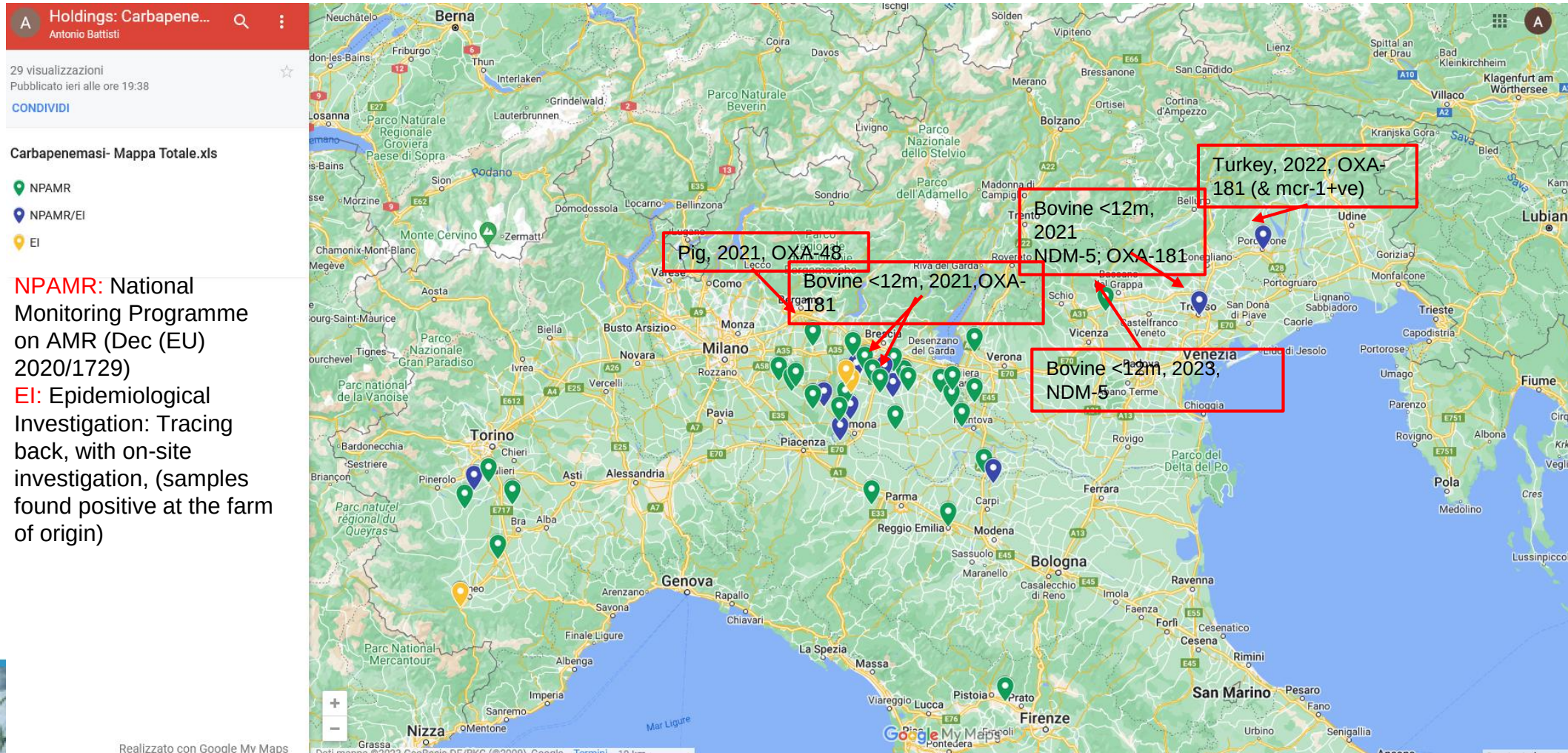
National Monitoring Programme on AMR, Dec (EU) 2020/1729, Italy 2021-2023: Holdings of origin of samples (slaughter batches) found positive at slaughter



In 2022,
n=1 OXA-181 producing *E. coli* from turkey
n=1 VIM-1 producing from broiler



2021-2023: Almost all CPE-producing E. coli isolates are OXA-181-positive except one OXA-48, two NDM-5 (bovine <12m), one VIM-1 (broiler chicken)



NPAMR: National Monitoring Programme on AMR (Dec (EU) 2020/1729)

EI: Epidemiological Investigation: Tracing back, with on-site investigation, (samples found positive at the farm of origin)

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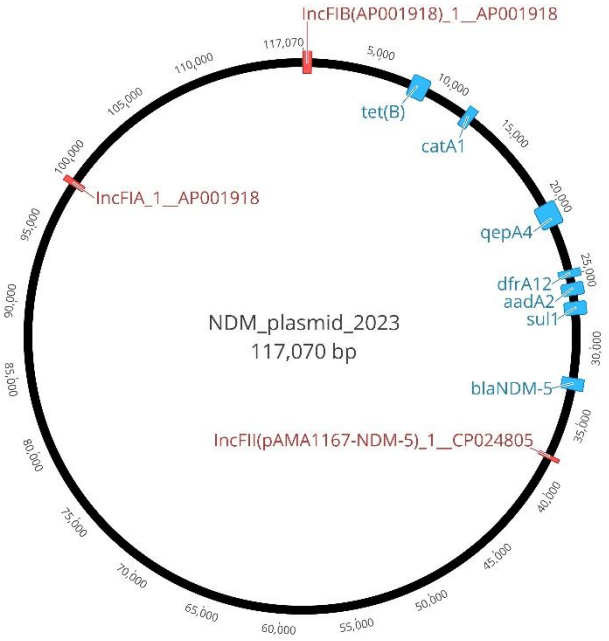
2021-2023: NDM-producing E. coli from bovine <12m
Sporadic isolation



NDM-2021: *aad*A2, *aad*A5, *aac*(6')-Ib-cr, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{NDM-5}, *cat*A1, *cat*B3, *dfr*A12, *dfr*A17, *mph*(A), *sul*1, *tet*(B)
IncFIA, IncFIB, IncI1-I, IncX4

NDM-2023: *aad*A2, *aph*(3'')-Ib, *aph*(6)-Id, $bla_{CTX-M-15}$, bla_{NDM-5}, bla_{TEM-1B}, *cat*A1, *dfr*A12, *qep*A4, *qnr*S1, *sul*1, *sul*2, *tet*(B)
IncFIA, IncFIB, IncFII, IncY

Take home messages

- Harmonized european monitoring of antimicrobial resistance in zoonotic and commensal bacteria in Italy indicates an emergent OXA-48-like producing *E. coli* issue in pigs and, to a lesser extent in bovines < 12 mo.

- Harmonized european monitoring of antimicrobial resistance reveled also the sporadic presence of NDM- and VIM-producing *E. coli* in Italy.

- The prevalence of OXA-181-producing *E. coli* from pig and bovine in 2023 has remained stable.

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A big thank you to all my colleagues of the Department of General Diagnostics and NRL-AR Italy (IZSLT), who have actually done all this amazing work

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Alessia Franco	Valentina Donati
Andrea Caprioli	Fabiola Feltrin
Virginia Carfora	Angelo Giacomi
Roberta Amoruso	Angela Ianzano
Franceso Bottoni	Manuela Iurescia
Carmela Buccella	Serena Lorenzetti
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