Carbapenemase-producing *E. coli* (OXA-181, NDM-5) from AMR monitoring in Italy, 2021
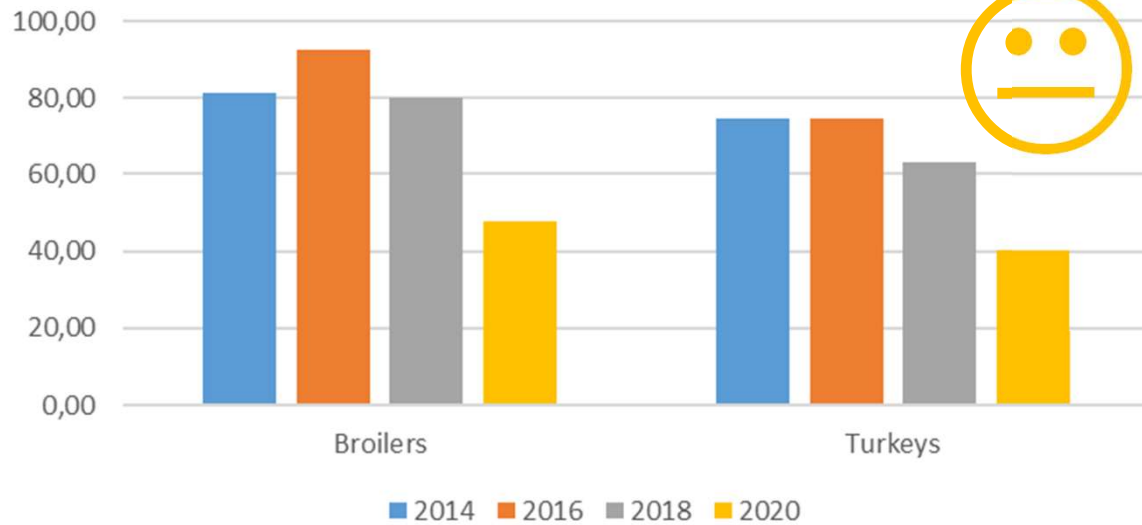
Virginia Carfora, Patricia Alba, Elena L Diaconu, Alessia Franco, Antonio Battisti

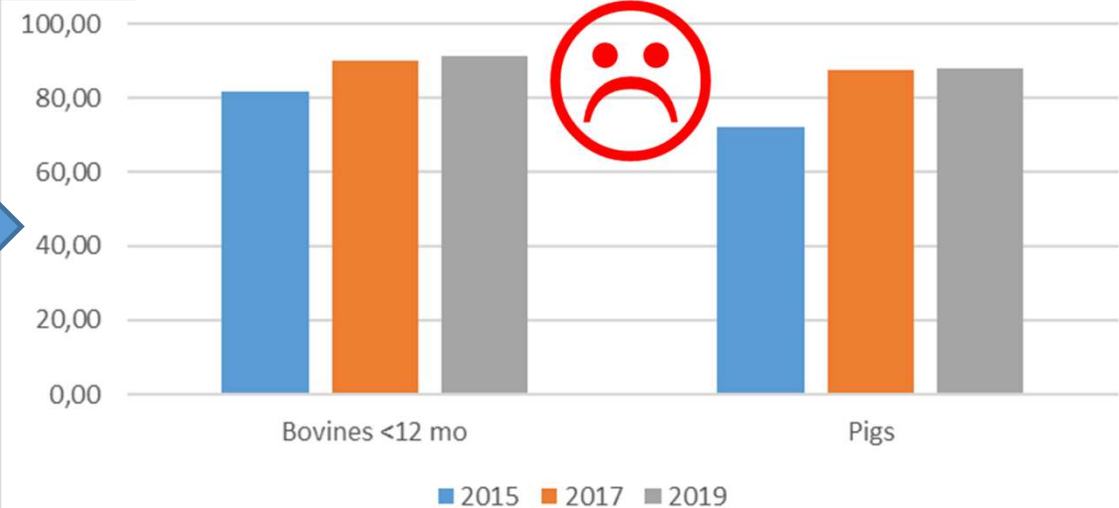IZSLT, Department of General Diagnostics,

National Reference Laboratory for Antimicrobial Resistance, Rome, Italy

Broilers & Turkeys: Prevalence epi units ESBL/AmpC producing Ecoli, Italy 2014-2020

Bovines <12 mo & Pigs: Prevalence epi units ESBL /AmpC producing Ecoli, Italy, 2015-2019

A clue that the selection pressure by all antimicrobials and especially by beta-lactams has not decreased enough in some Italian productions…

In pigs, this means mainly «selection by oral amoxicillin» for group treatment (beside individual 3°-4° cephs treatment)
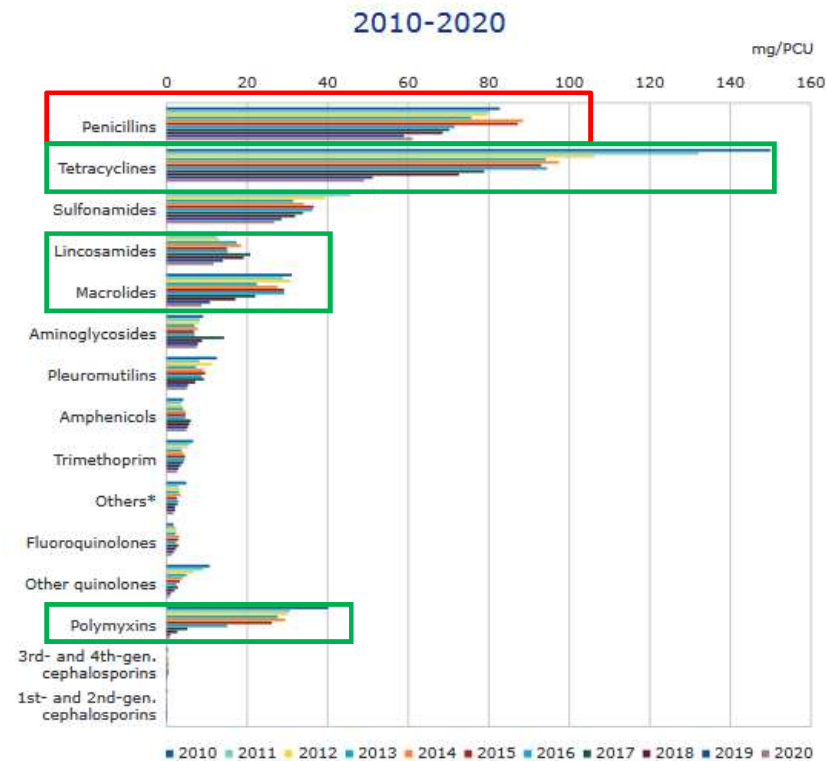
SALES TRENDS (MG/PCU) OF ANTIMICROBIAL VMPs FOR FOOD-PRODUCING ANIMALS, ITALY, 2010-2020

«Penicillins» in the EU and IT animal productions means «oral amoxicillin» and «oral clav+amoxicillin» (pigs)

Carbapenemase-producing
E. coli

😊

Since 2014, Italy implemented the specific Carba-producing E. coli (CPE) monitoring from caecal contents

ESBL-AmpC-producing
E. coli

☹

From «The European Union Summary Report on AMR, 2014»

Table 45: Prevalence of carbapenemase-producing *E. coli* from broilers and fattening turkeys collected within the specific carbapenemase-producing microorganisms monitoring in Italy in 2014

Poultry population	Number of caecal samples tested on selective culture media	Number of caecal samples tested positive for carbapenemase-producing *E. coli*	Prevalence (95% CI)
Broilers	300	0	0.0% (0.0, 1.2)
Fattening turkeys	300	0	0.0% (0.0, 1.2)

This study provides baseline information of utmost interest, as in Italy, CPE-R Enterobacteriaceae in humans are widespread and are currently considered a major burden among healthcare-associated infectious diseases.

Specific monitoring of ESBL-/AmpC-producing E. coli

ESC-R *E. coli* were confirmed as ESBL-/AmpC-producing *E. coli* by performing relevant Polymerase Chain Reaction (PCR) tests. Corresponding prevalence in broilers and fattening turkeys is shown in the table below.

Table 46: Prevalence of ESBL-/AmpC-producing *E. coli* from broilers and fattening turkeys within the specific ESBL-/AmpC-producing *E. coli* monitoring in Italy in 2014

Poultry population	Number of caecal samples tested on selective culture media	Number of caecal samples tested positive for ESBL-/AmpC-producing *E. coli*	Prevalence (95% CI)
Broilers	300	244[a]	81.3% (76.5, 85.6)
Fattening turkeys	300	224[b]	74.7% (69.5, 79.5)

(a): Nearly 86% were ESBL-producing *E. coli*, with 69% harbouring genes of the CTX-M family (mostly encoding the enzyme CTX-M-1). Transferable AmpC genes, encoding CMY-2, were found in 13.1% of isolates. All isolates had MICs indicating clinical resistance to cefotaxime or ceftazidime. Among these ESC-R isolates, 95.1% were multi-drug resistant.
(b): Nearly 96% were ESBL-producing *E. coli*, with 73% harbouring genes of the CTX-M family (mostly encoding the enzyme CTX-M-1). Transferable AmpC genes, encoding CMY-2, were found in 2.7% of isolates. All isolates had MICs above the Ecoffs and all isolates, except two, had MICs also in the range of clinical resistance for cefotaxime or ceftazidime. Among these ESC-R isolates, 90.2% were multi-drug resistant.

It should be noted that, when using selective culture methods, the occurrence of ESBL/AmpC-producing *E. coli* in broilers and fattening turkeys is assessed with much greater sensitivity than when using non-selective culture methods. Considering randomly selected isolates of indicator commensal *E. coli* (n=170) from the same caecal samples, cultured on non-selective media, the occurrence of

Figure 2. Comparative analysis of closely related plasmids pMOL412_FII and pM109_FII harbouring bla$_{NDM-4}$. ...

J Antimicrob Chemother, dkaa374, https://doi.org/10.1093/jac/dkaa374

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Article Contents

Abstract
Introduction
Materials and methods
Results
Discussion
Acknowledgements
References
Supplementary data

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 ...
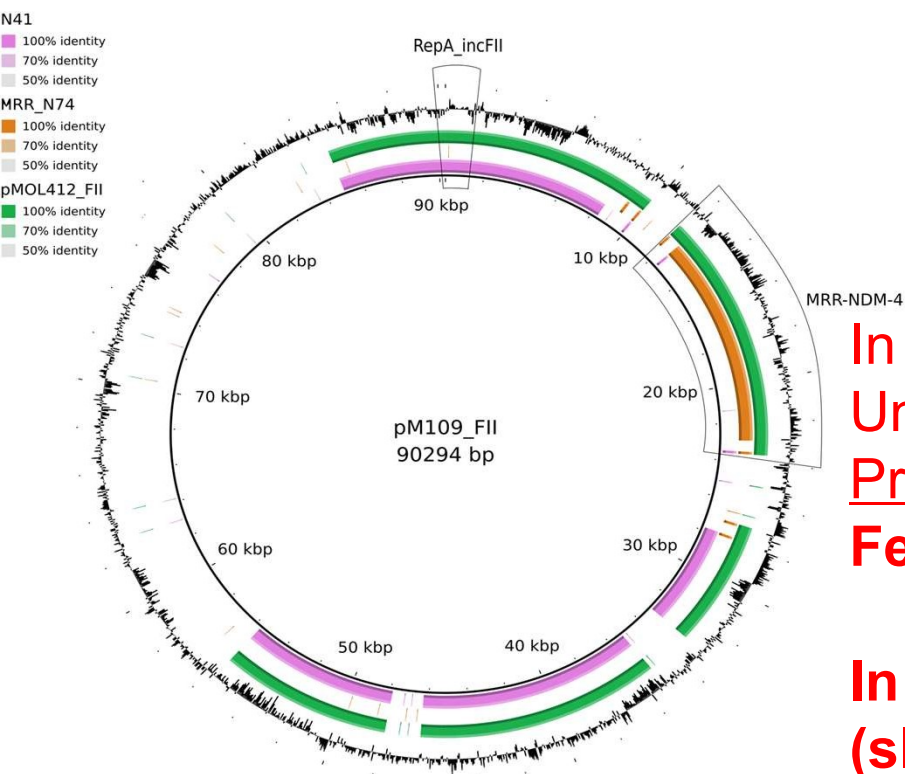Show more

📄 PDF   ⬛ Split View   66 Cite   🔧 Permissions   ◀ Share ▾

Abstract

In 2019, <u>first detection of</u> CRE (NDM-4) in one pig (Epi Unit: slaughter batch only) <u>in the Italian Animal Productions</u>
Few weeks before entering the COVID pandemic…

In 2021an NDM-5+ve E. coli in one veal calf EpiUnit (slaughter batch only):
<u>**BOTH are sporadic in Italy, SO FAR**</u>

OXFORD
UNIVERSITY PRESS

Update December 2021 → **25 isolates** OXA-48-like (24 OXA-181; 1 OXA-48) from different 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)

Lab Method: Same lab procedure since 2014:
Specific monitoring of CPE-producing E. coli:
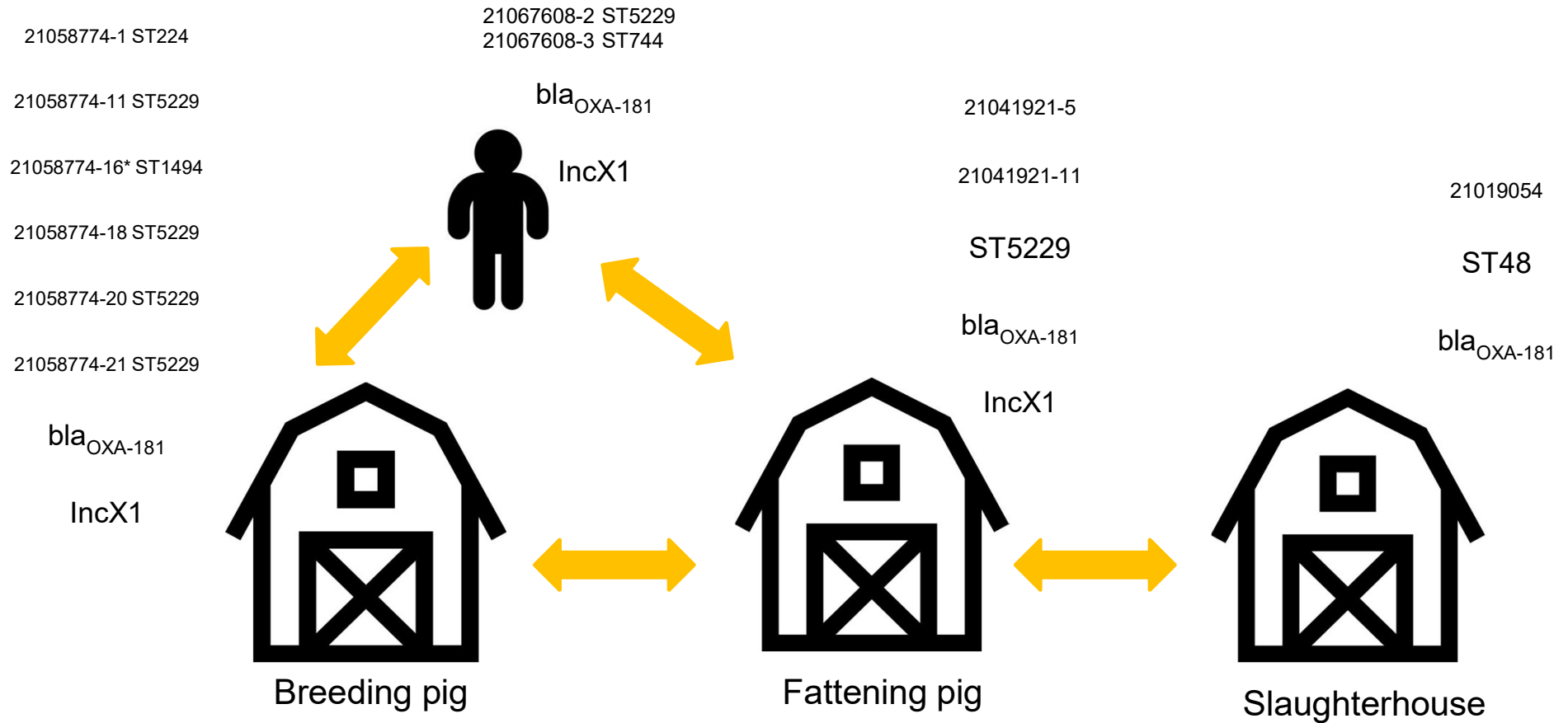The EURL-AR protocol (by using a commercial OXA/other Carbapenemases Biplate):
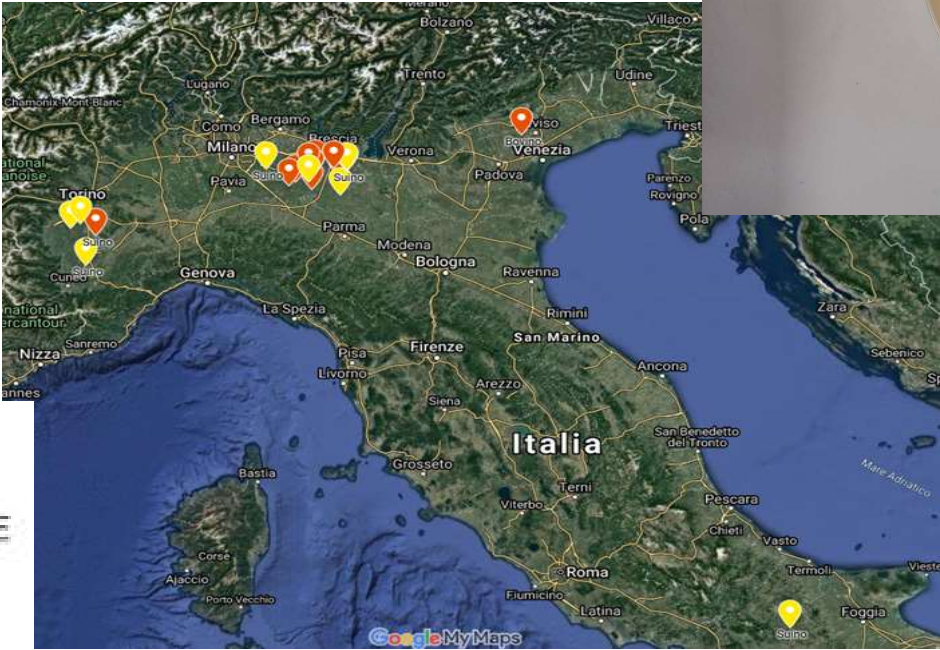
Epidemiological investigation: for >80%the positive EpiUnits investigated at slughter, an OXA-48-like producing E. coli (OXA-181) **has been isolated from samples taken at the farm of origin**

PNAMR
PNAMR/IE
IE

1. Improving Knowledge

"One Health" Investigations (epidemiological, microbiological)

- confirming the positive status at the fattening holding of origin;

(>80% of fattening holdings of origin investigated proved to be positive...)

a. Animal faecal samples (pools); water samples (well water supply), etc.

b. Workers'/farmers' faecal samples (voluntary, informed consent) collected by the Dept of Prevention of the Local Competent (Human) Health Authority

c. Questionnaire at holding level (epidemiological variables, including a section on workers)

- tracing back (and forward), thorough investigations on trades (animals, foodstuffs), other neighbouring holdings, and other risk factors etc;

(Where implemented, proved to be fruitful. New positives found among enrolled holdings)

Epidemiological Investigation: Case1

21058774-1 ST224

21058774-11 ST5229

21058774-16* ST1494

21058774-18 ST5229

21058774-20 ST5229

21058774-21 ST5229

bla$_{OXA-181}$

IncX1

21067608-2 ST5229
21067608-3 ST744

bla$_{OXA-181}$

IncX1

21041921-5

21041921-11

ST5229

bla$_{OXA-181}$

IncX1

21019054

ST48

bla$_{OXA-181}$

Breeding pig

Fattening pig

Slaughterhouse

Resistome 😞

| 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,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

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

	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 only carbapenem
In the First panel

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
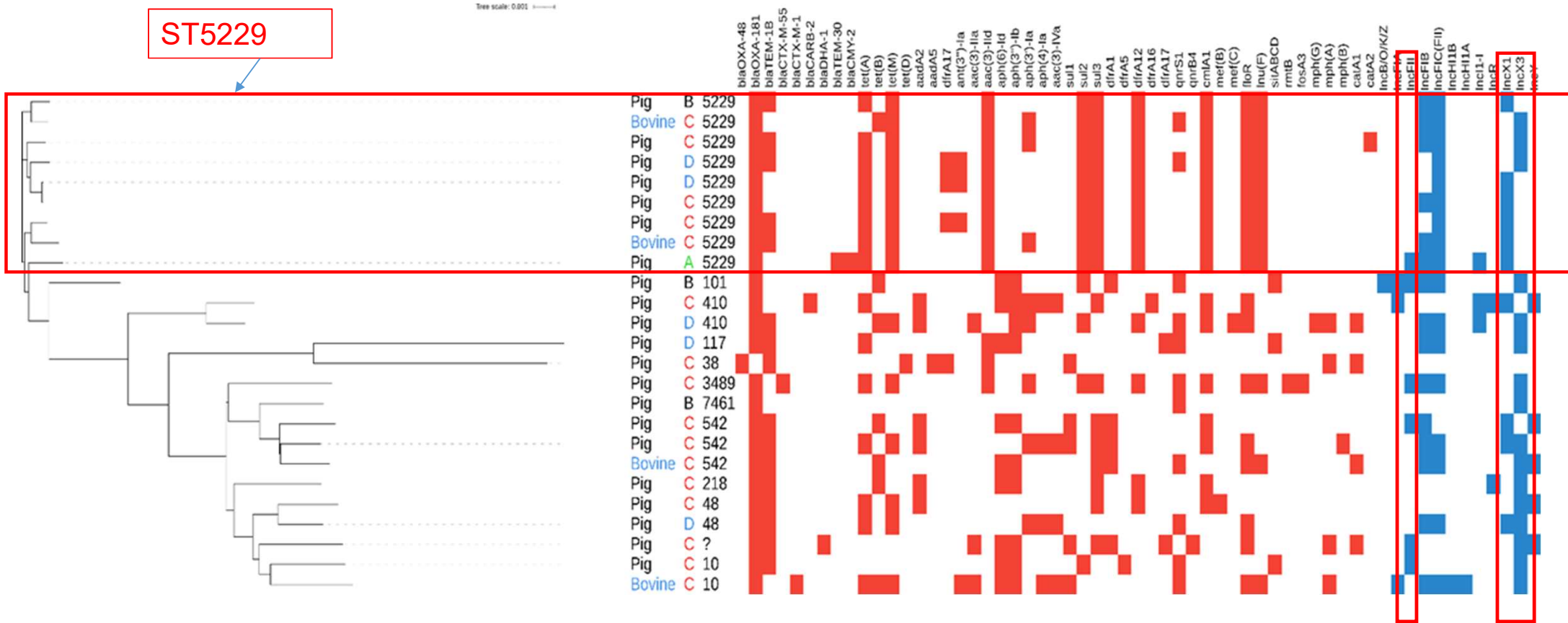
- ❖ A non-clonal population of OXA-48-like producing *E. coli* in the dataset analyzed. **However, …. (ST5229, 9/25, 36% isolates). IncX3, IncX1, IncF the replicons most represented.**
- ❖ **IncX3 or IncX1 harboured the OXA-181 gene. No specific pathotype found.**
- ❖ The clusters were distributed according to the different Clonal Complexes (CCs) and STs.
- ❖ No clear region or host species correlation was observed.

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

The complete sequence of plasmids from 16 selected OXA-181 producing isolates was obtained through the hybrid (Illumina–ONT) assembly approach

Unicycler

size range:
57,694–58,897 bp
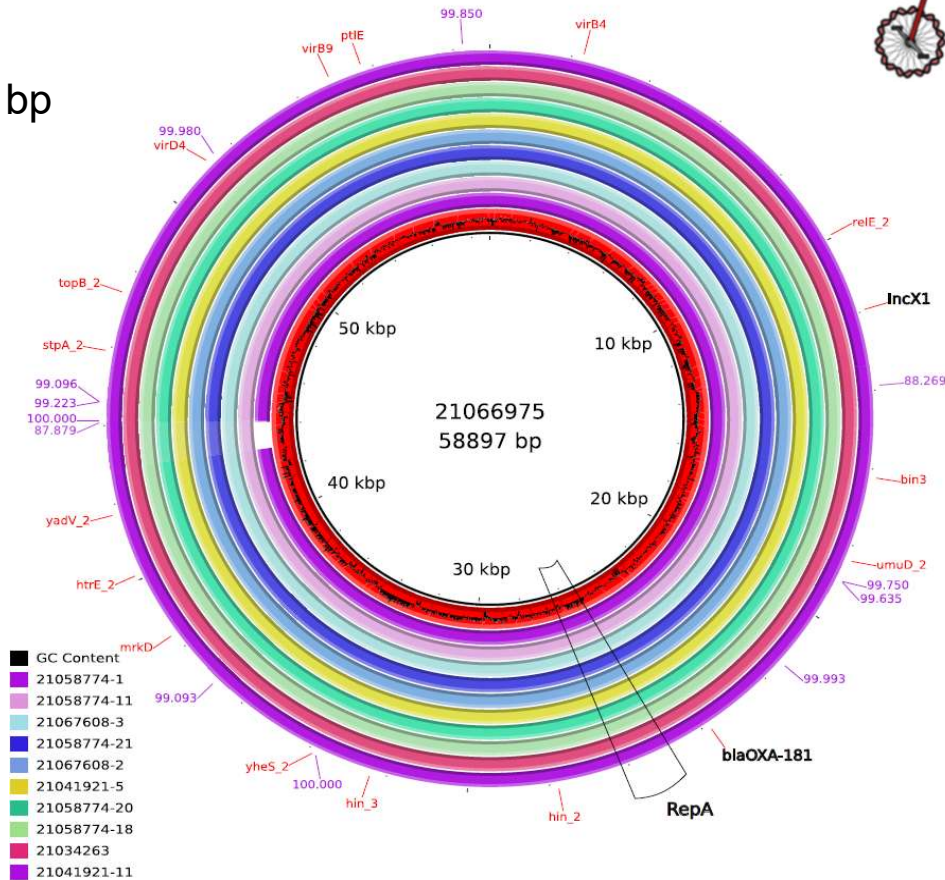
✓ All the 12 IncX1 resolved plasmids were almost identical with a 98-99% coverage and 99-100% sequence identity

✓ **No similar IncX1 plasmids were found in publicly available databases.**

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

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

Sizes:
51,982;
57,195,
57,650 bp
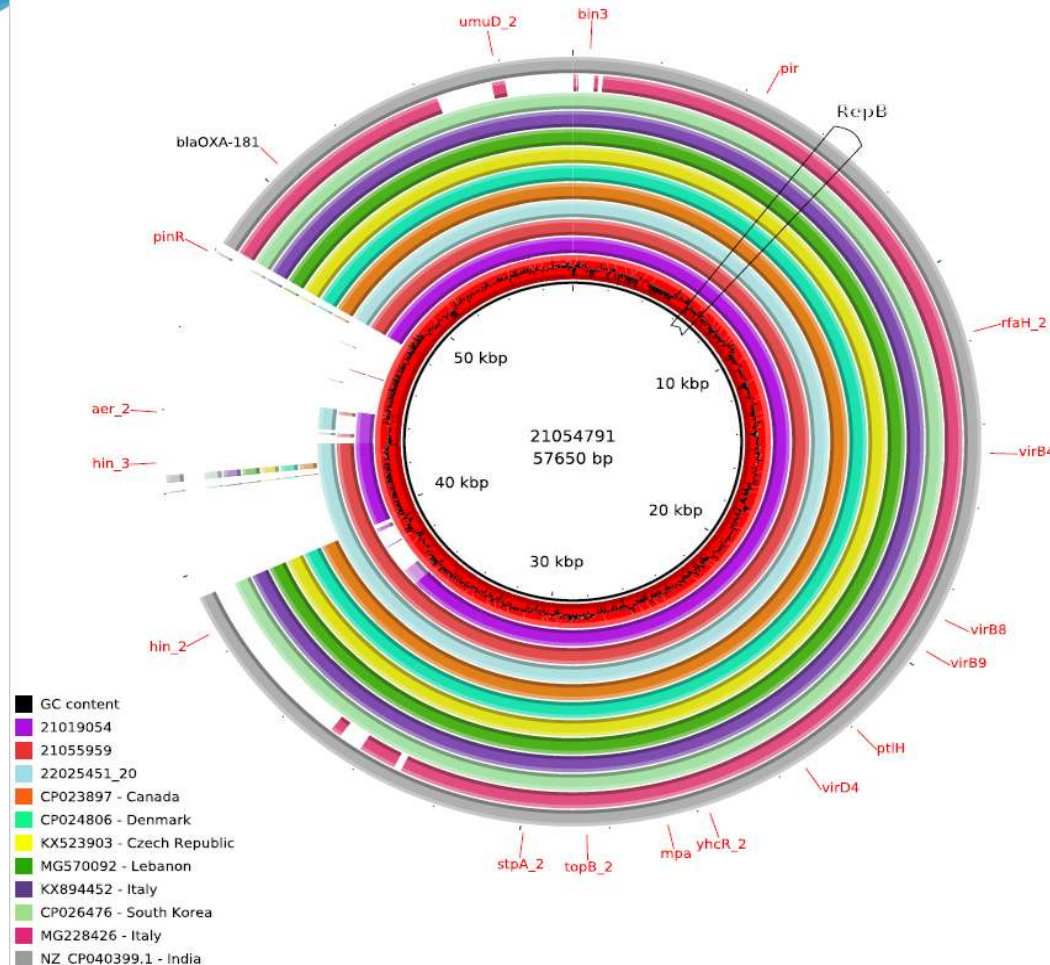
- ✓ All three resolved plasmids IncX3 harboring $bla_{OXA-181}$ from *E.coli* were very similar with a 90-91% coverage and 100% identity

- ✓ They shared a similarity of 99% with 89% of the plasmid covered, when compared with publicly available IncX3 plasmids containing $bla_{OXA-181}$ (from *E. coli, C. freundii, K. pneumoniae*)

- ✓ 100% coverage and identity of the IncX3 plasmid from *E. coli* ID 21019054 with a $bla_{OXA-181}$-IncX3 plasmid of a *C. freundii* isolate (ID 22025451-20) from the same pig holding

Full plasmid sequencing

Unicycler

The complete sequence of plasmids from 16 selected OXA-181 producing isolates was obtained through the hybrid (Illumina–ONT) assembly approach
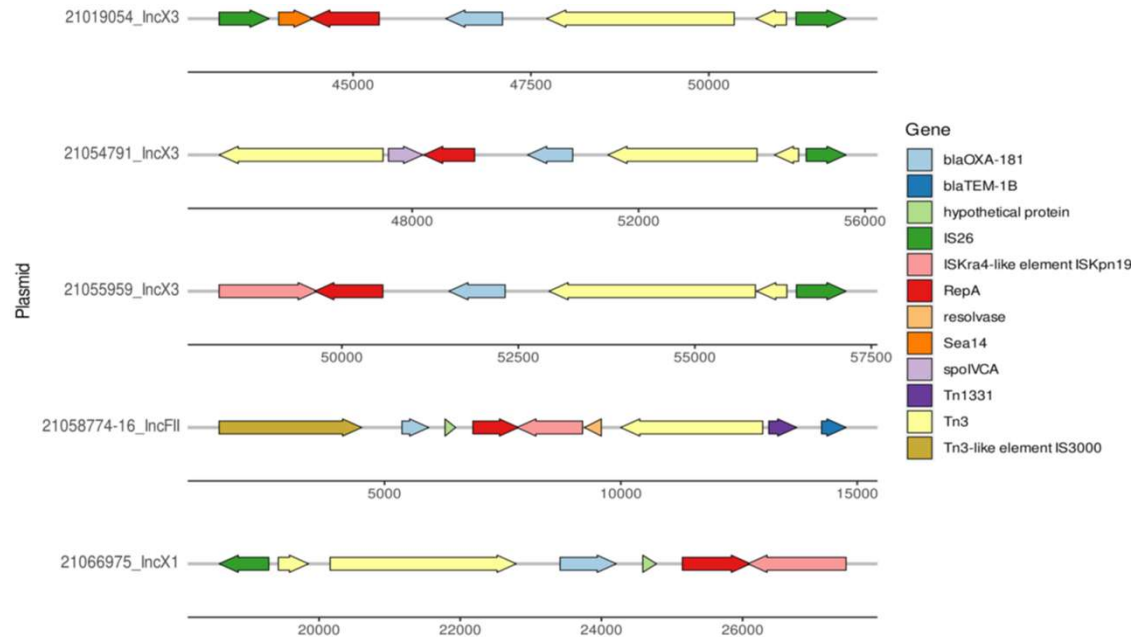
Graphical representation of the region where *bla*OXA-181 was located in the three IncX3 plasmids, one representative IncX1 (pMOL6975) from ID21066975 (accession number ERS12413440) and IncFII (21058774–16) plasmids.

$bla_{OXA-181}$ was part of a transposon with a similar general structure located in three different plasmid types:

- **IncX3** in three isolates from the survey at slaughter
- **IncX1** in two isolates from the survey at slaughter and 10 isolates from tracing-back activities
- **IncFII** in one isolate from tracing-back activities

✓ In all IncX1 plasmids, this composite transposon resulted identical

Risk Management (& Control) Options provided to the Central Competent Authority (IT MoH, DGSAF) and Regional CAs

To: The Directorate General for Animal Health and Veterinary Medicinal Products, ITMoH

-Descriptive epidemiology,
-main genomics, and provisional clustering results
-advice on how to improve prevention measures
-perspectives of possible RMOs
Discussed during the NRL-AR Italy annual Workshop (Nov 2021)
RMOs further discussed in a One Health perspective with the Public Health Sector in the Regions involved

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

Direzione Operativa Diagnostica Generale

Centro di Referenza Nazionale per l'Antibioticoresistenza (D. M. 4 ottobre 1999)

National Reference Laboratory for Antimicrobial Resistance (Reg.(EC) 2004/882 - Reg.(EU) 2017/625)

Roma, 29/09/2021

A: Ministero Salute

-DG Sanità Animale e Farmaci Veterinari

Via G. Ribotta 5, 00144;

ROMA

Prot.
All.

Oggetto: E. coli produttori di carbapenemasi e Piano Nazionale AMR: Proposta di protocollo generale per attività di approfondimento negli allevamenti di origine delle Unità Epidemiologiche positive al macello, e di opzioni di risk managament.

Si rimette uno schema generale, relativo alle modalità ed azioni in materia di approfondimento epidemiologico della rilevazione delle positività eventualmente riscontrate nelle unità epidemiologiche prelevate al macello ai sensi della Dec. (EU) 2020/1729.

Conclusions

- Epidemiological and lab investigations point to:

-a human source as the most likely cause for the introduction of the OXA-181 carrying plasmid (IncX1 type) in the breeding holding where initial tracing-back activities were conducted;

- Whatever the initial source, we have provided evidence that these CPE have been amplified within the intensive animal production systems, especially in pigs;

- A reflection on overall diagnostic sensitivity and accuracy of CPE lab protocols:

-OXA-48-like enzymes cannot be detected on MAC+1mg/L-CTX plates

-Only the specific CPE lab procedure allowed detection and an important change in trends in Italy;

-There is evidence for discussing and its maintenance across all MSs (to allow comparability across MSs is an important aim of the AMR monitoring legislation)

I wish to thank all my colleagues of the Department of General Diagnostics and NRL-AR Italy (IZSLT), for this amazing work

Alessia Franco	Paola Di Matteo
Andrea Caprioli	Valentina Donati
Virginia Carfora	Fabiola Feltrin
Patricia Alba	Angelo Giacomi
Manuela Iurescia	Angela Ianzano
Roberta Amoruso	Serena Lorenzetti
Franceso Bottoni	Ilaria Marani
Carmela Buccella	Roberta Onorati
Tamara Cerci	Luigi Sorbara
Gessica Cordaro	Fiorentino Stravino
Elena dell'Aira	Ilaria Congiu
Elena L. Diaconu	Daniele Smedile

https://www.frontiersin.org/articles/10.3389/fmicb.2022.1016895/full

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

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[3] Agenzia Tutela Della Salute, Brescia, Italy

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Centro di Referenza Nazionale
per l'Antibioticoresistenza

https://www.izslt.it/crab/