La sorveglianza dell'antibiotico-resistenza nel settore animale in Italia ed in EU: strumenti consolidati e genomica

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Convegno: Antimicrobico-resistenza: cure e ambiente #6

L'eclettismo dell'antibiotico-resistenza

Auditorium di Sant'Apollonia, via S. Gallo, 25a - Firenze

Storia recente:
2014-2020 in vigore
Comm Dec 2013/652/EU
(Prima: Comm Dec 2007/407/EU di portata più limitata)

DECISIONS

The AMR Monitoring system in the EU, in food-producing animal populations… «ONE HEALTH perspective» (Repealed by 2021)

COMMISSION IMPLEMENTING DECISION

of 12 November 2013

on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria

(notified under document C(2013) 7145)

(Text with EEA relevance)

(2013/652/EU)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC [1], and in particular Article 7(3) and the fourth subparagraph of Article 9(1) thereof,

put in place a five-year action plan to fight against AMR based on 12 key actions, including strengthened surveillance systems on AMR.

(4) In the Council Conclusions of 22 June 2012 on the impact of antimicrobial resistance in the human health sector and in the veterinary sector — a One Health Perspective [3], that Institution calls upon the Commission to follow up on its Communication of 15 November 2011 through concrete initiatives to implement the 12 actions set out in that Communication, and to collaborate closely with the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) in strengthening the assessment and evaluation of the occurrence of AMR in humans, in animals and in food in the Union.

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Scientific Report | Open Access

Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food

European Food Safety Authority (EFSA), Marc Aerts, Antonio Battisti, René Hendriksen, Isabelle Kempf, Christopher Teale, Bernd-Alois Tenhagen, Kees Veldman, Dariusz Wasyl ... See all authors

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Approved: 30 April 2019

Abstract

Table 7: Combinations of bacterial species–food-producing animal populations/meat to be tested for antimicrobial susceptibility within the harmonised AMR monitoring

Animal population[(a)] / Meat[(b)]	*Salmonella* spp. (at the serovar level)	*C. jejuni*/ *C. coli*[(c)]	Indicator commensal *E. coli*	ESBL/ AmpC/CP-producing *E. coli*	CP-producing *E. coli*	*E. faecalis*/ *E. faecium*
Broilers	M: NCP, CSS	M: CSS	M: CSS	M: CSS	M/V[(d)]: CSS	M/V[(d)]: CSS
Laying hens	M: NCP	–	–	–	–	–
Fattening turkeys	M: NCP, CSS	M: CSS	M: CSS	M: CSS	M/V[(d)]: CSS	M/V[(d)]: CSS
Bovines, < 1 year old	M: CSS	M: CSS	M: CSS	M: CSS	M/V[(d)]: CSS	M/V[(d)]: CSS
Fattening pigs	M: CSS	M: CSS	M: CSS	M: CSS	M/V[(d)]: CSS	M/V[(d)]: CSS
Broiler meat	–	–	V: R	M: R	M/V[(d)]: R	–
Turkey meat	–	–	V: R	M: R	M/V[(d)]: R	–
Pig meat	–	–	V: R	M: R	M/V[(d)]: R	–
Bovine meat	–	–	V: R	M: R	M/V[(d)]: R	–

CSS: caecal samples from healthy animals at slaughter; M: mandatory monitoring; NCP: *Salmonella* national control plans; R: at retail; V: voluntary monitoring. CP: carbapenemase-producers.

(a): Domestically produced.

(b): Including imported and domestically produced products.

(c): For each animal species, and for each MS, the target is the more prevalent *Campylobacter* species; all isolates of the other *Campylobacter* species that are identified, considering the specification of one isolate per species and epidemiological unit, are to be included. However, for fattening pigs, only *C. coli* is considered.

(d): Mandatory on a 4-year rotational basis, voluntary intervening years.

In addition, it would be also desirable to specifically monitor AMR in indicator commensal *E. coli* from meat imported from third countries, for example poultry meat, at the EU level.

Additionally:
Baseline surveys on other type of Food of Animal Origin;
LA-MRSA Surveys
(at holding level, or at slaughter)
at least every 4-5 years

COMMISSION IMPLEMENTING DECISION (EU) 2020/1729

of 17 November 2020

on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU

(notified under document C(2020) 7894)

(1) Directive 2003/99/EC requires Member States to ensure that monitoring provides comparable data on the occurrence of antimicrobial resistance ('AMR') in zoonotic agents and, in so far they present a threat to public health, other agents.

(2) Directive 2003/99/EC also requires Member States to assess the trends and sources of AMR in their territory and to transmit a report every year covering data collected in accordance with that Directive to the Commission.

(8) Whole genome sequencing ('WGS') is a promising technique to replace conventional phenotypical testing in microbiology and is increasingly used worldwide. However, only a limited number of Member States are currently able to use WGS for AMR monitoring on a routine basis. It is therefore appropriate to authorise the use of WGS as an alternative to the conventional phenotypical techniques on a voluntary basis only, but to impose technical conditions on the WGS technique to ensure data comparability.

(9) AMR is a global threat that can easily spread across borders. Therefore, in order to improve coordination and gain a deeper understanding of how to help reduce the impact of AMR impact globally, it is essential that food products imported into the Union are also subjected to AMR monitoring requirements.

(10) In order to ensure continuity of the harmonised AMR monitoring and reporting by Member States after the period covered by Implementing Decision 2013/652/EU, this Decision should apply from 1 January 2021.

(11) For the sake of legal clarity, Implementing Decision 2013/652/EU should be repealed.

Article 1

Subject matter and scope

1. This Decision lays down harmonised rules for the period 2021-2027 for the monitoring and reporting of antimicrobial resistance ('AMR') to be carried out by Member States in accordance with Article 7(3) and 9(1) of Directive 2003/99/EC and Annex II (B) and Annex IV thereto.

2. The monitoring and reporting of AMR shall cover the following bacteria:

(a) *Salmonella* spp.;

(b) *Campylobacter coli* (*C. coli*);

(c) *Campylobacter jejuni* (*C. jejuni*);

(d) Indicator commensal *Escherichia coli* (*E. coli*);

(e) *Salmonella* spp. and *E. coli* producing the following enzymes:

(i) Extended Spectrum β-Lactamases (ESBL);

(ii) AmpC β-Lactamases (AmpC);

(iii) Carbapenemases (CP).

Isolates from National Control Programmes (NCPs) & Cross-sectional studies at slaughter

Isolates from cross-sectional studies at slaughter, at retail (meat), at BCPs (imported meat in the EU)

3. The monitoring and reporting of AMR may cover indicator commensal *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*).

Voluntary for EU MS…

4. The monitoring and reporting of AMR shall cover the following food-producing animal populations and food:

(a) broilers;

(b) laying hens;

(c) fattening turkeys;

(d) bovine animals under one year of age;

(e) fattening pigs;

(f) fresh meat from broilers;

(g) fresh meat from turkeys;

(h) fresh meat from pigs;

(i) fresh meat from bovine animals.

5. Member States shall monitor and report AMR in specific combinations of bacteria/antimicrobial substances/food-producing animal populations and fresh meat derived thereof in accordance with Articles 3 and 4.

Commission Decision(EU) 2020/1729

MAINLY "ACTIVE MONITORING" AT DIFFERENT STAGES…

Fresh meat (at BCPs) imported from non – EU Countries

Carbapenemase-producing E. coli

ESBL-AmpC-producing E. coli

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

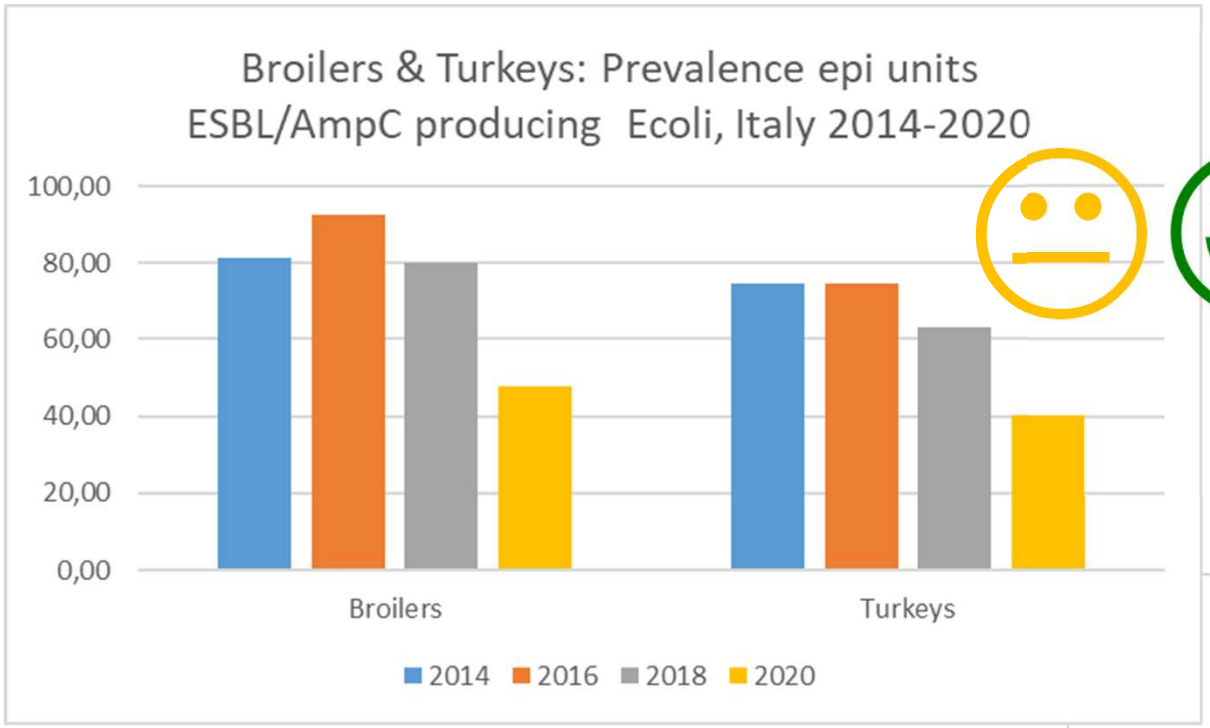
Poultry population	Number of caecal samples tested on selective culture media	Number of caecal samples tested positive for ESBL-/AmpC-producing *E. coli*	Prevalence (95% CI)
Broilers	300	244[a]	81.3% (76.5, 85.6)
Fattening turkeys	300	224[b]	74.7% (69.5, 79.5)

(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

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



A CLUE that the selection pressure by all antimicrobials and especially by beta-lactams has not decreased enough…
This means in the EU and IT amoxicillin (or anoxi-clav) by oral route and mass medication (beside injectable, individual, 3°-4° gen cephs usage

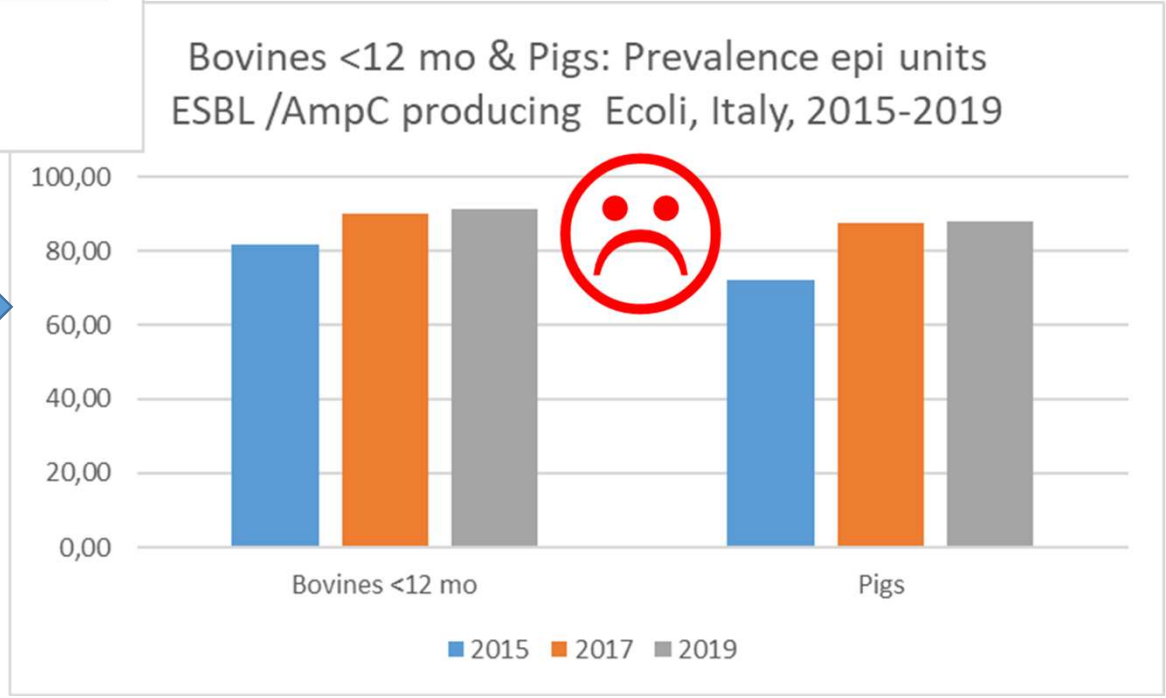


Table 2

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST thresholds for resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli* (First panel)

Antimicrobial	Class of antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
			ECOFF	Clinical breakpoint	
Amikacin	Aminoglycoside	*Salmonella*	> 4 *	> 16	4-128 (6)
		E. coli	> 8	> 16	
Ampicillin	Penicillin	*Salmonella*	> 8	> 8	1-32 (6)
		E. coli	> 8	> 8	
Azithromycin	Macrolide	*Salmonella*	NA	NA	2-64 (6)
		E. coli	NA	NA	
Cefotaxime	Cephalosporin	*Salmonella*	> 0,5	> 2	0,25-4 (5)
		E. coli	> 0,25	> 2	
Ceftazidime	Cephalosporin	*Salmonella*	> 2	> 4	0,25-8 (6)
		E. coli	> 0,5	> 4	
Chloramphenicol	Phenicol	*Salmonella*	> 16	> 8	8-64 (4)
		E. coli	> 16	> 8	
Ciprofloxacin	Fluoroquinolone	*Salmonella*	> 0,06	> 0,06	0,015-8 (10)
		E. coli	> 0,06	> 0,5	
Colistin	Polymyxin	*Salmonella*	NA	> 2	1-16 (5)
		E. coli	> 2	> 2	
Gentamicin	Aminoglycoside	*Salmonella*	> 2	> 4	0,5-16 (6)
		E. coli	> 2	> 4	
Meropenem	Carbapenem	*Salmonella*	> 0,125	> 8	0,03-16 (10)
		E. coli	> 0,125	> 8	
Nalidixic acid	Quinolone	*Salmonella*	> 8	NA	4-64 (5)
		E. coli	> 8	NA	
Sulfamethoxazole	Folate pathway antagonist	*Salmonella*	NA	NA	8-512 (7)
		E. coli	> 64	NA	
Tetracycline	Tetracycline	*Salmonella*	> 8	NA	2-32 (5)
		E. coli	> 8	NA	
Tigecycline	Glycylcycline	*Salmonella*	NA	NA	0,25-8 (6)
		E. coli	> 0,5	> 0,5	
Trimethoprim	Folate pathway antagonist	*Salmonella*	> 2	> 4	0,25-16 (7)
		E. coli	> 2	> 4	

NA: not available.
* tentative EUCAST threshold

Lievi modifiche ai panel di antibiotici da testarsi e del loro range:
Es. Inserimento Amikacina per Salmonella & E. coli

Panel of antimicrobial substances to be included in AMR monitoring, EUCAST interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni* and *C. coli*

Antimicrobial	Class of antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
			ECOFF	Clinical breakpoint	
Chloramphenicol	Phenicol	*C. jejuni*	> 16	NA	2-64 (6)
		C. coli	> 16	NA	
Ciprofloxacin	Fluoroquinolone	*C. jejuni*	> 0,5	> 0,5	0,12-32 (9)
		C. coli	> 0,5	> 0,5	
Ertapenem	Carbapenem	*C. jejuni*	NA	NA	0,125-4 (6)
		C. coli	NA	NA	
Erythromycin	Macrolide	*C. jejuni*	> 4	> 4	1-512 (10)
		C. coli	> 8	> 8	
Gentamicin	Aminoglycoside	*C. jejuni*	> 2	NA	0,25-16 (7)
		C. coli	> 2	NA	
Tetracycline	Tetracycline	*C. jejuni*	> 1	> 2	0,5-64 (8)
		C. coli	> 2	> 2	

NA: not available

Lievi modifiche ai panel di antibiotici da testarsi e del loro range:
Es. Inserimento Ertapenem per Campylobacter jejuni & C. coli

Table 5

Panel of antimicrobial substances, EUCAST epidemiological cut-off values (ECOFFs) and clinical resistance breakpoints and concentrations ranges to be used for testing only *Salmonella* spp. and *E. coli* isolates resistant to cefotaxime or ceftazidime or meropenem – (Second panel)

Antimicrobial	Class of antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
			ECOFF	Clinical breakpoint	
Cefepime	Cephalosporin	*Salmonella*	NA	> 4	0,06-32 (10)
		E. coli	> 0,125	> 4	
Cefotaxime	Cephalosporin	*Salmonella*	> 0,5	> 2	0,25-64 (9)
		E. coli	> 0,25	> 2	
Cefotaxime + clavulanic acid	Cephalosporin/ beta-lactamase inhibitor combination	*Salmonella*	NA	NA	0,06-64 (11)
		E. coli	> 0,25	NA	
Cefoxitin	Cephamycin	*Salmonella*	> 8	NA	0,5-64 (8)
		E. coli	> 8	NA	

(*) https://www.eurl-ar.eu/protocols.aspx

«Second Panel»: Ulteriore pannello AST (MIC) per presunta presenza di ESBL/AmpC/Carba-producing Salmonella & E. coli nel «First Panel»
Ovvero per tutti gli isolati che nel First panel risultano Resistenti a Ceftazidime, Cefotaxime, o Meropenem

Antimicrobial	Class of antimicrobial	Species	Interpretative thresholds of AMR (mg/L)		Range of concentrations (mg/L) (No of wells in brackets)
			ECOFF	Clinical breakpoint	
Ceftazidime	Cephalosporin	*Salmonella*	> 2	> 4	0,25-128 (10)
		E. coli	> 0,5	> 4	
Ceftazidime + clavulanic acid	Cephalosporin// beta-lactamase inhibitor combination	*Salmonella*	NA	NA	0,125-128 (11)
		E. coli	> 0,5	NA	
Ertapenem	Carbapenem	*Salmonella*	NA	> 0,5	0,015-2 (8)
		E. coli	NA	> 0,5	
Imipenem	Carbapenem	*Salmonella*	> 1	> 4	0,12-16 (8)
		E. coli	> 0,5	> 4	
Meropenem	Carbapenem	*Salmonella*	> 0,125	> 8	0,03-16 (10)
		E. coli	> 0,125	> 8	
Temocillin	Penicillin	*Salmonella*	> NA	NA	0,5-128 (9)
		E. coli	> 16	NA	

NA: not available

Introduzione della Genomica (Whole Genome Sequencing e Analisi Bionformatica) nella Normativa EU per Food Safety - Zoonoses

- Per la prima volta si introduce Genomica (WGS e analisi bioinformatica), metodologia impiegata, modalità di reportistica e dettagli di esecuzione e di analisi dei dati, nella normativa EU relativa a Zoonoses e Food Safety (Dir. 99/2003/EC, Regulation (EU)

6. **Alternative method**

Member States may decide to authorise the use of Whole Genome Sequencing ('WGS') as an alternative method to broth micro dilution using the testing panels of antimicrobial substances of Tables 2 and 5 when carrying out the specific monitoring of ESBL- or AmpC- or CP-producing *E. coli* as referred to in point 5. They may also authorise WGS as an alternative method to broth micro dilution using the testing panel of antimicrobial substances of Table 5 when further testing, in accordance with point 4.2, *E. coli* and *Salmonella* isolates showing resistance to cefotaxime or ceftazidime or meropenem.

Laboratories implementing WGS as an alternative method shall use the protocols of the EURL for AMR (6).

WGS e Analisi Bioinformatica nella nuova Dec. (EU) 2020/1729

- Per ora Voluntary nei MS e per i NRL-AR che intendono:

a) sostituire metodiche fenotipiche (Broth microdilution 1° e 2° panel) nella caratterizzazione di ESBL/AmpC- e Carbapenemase-producing E. coli

oppure

b) sostituire metodiche fenotipiche (Broth microdilution solo 2° panel) nella conferma di caratterizzazione di ESBL/AmpC- e Carbapenemase-producing E. coli & Salmonella risultati sospetti ESBL/AmpC/Carbapenemasi produttori nel primo panel AST

https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7867

https://www.efsa.europa.eu/es/data-report/biological-hazards-reports

IZS Roma

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Scientific Report | Open Access

The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2020/2021

European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC)

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Requestor European Commission
Question number EFSA-Q-2021-00768
Declarations of interest If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.
Acknowledgements EFSA and ECDC wish to thank the members of the Scientific Network for Zoonoses Monitoring Data (EFSA) and the Food- and Waterborne Diseases and Zoonoses Network (ECDC) who provided the data and reviewed the report and the members of the Scientific Network for Zoonoses Monitoring Data, for their endorsement of this scientific output. Also, the contribution of EFSA staff members: Raquel García-Fierro, Pierre-Alexandre Beloeil, Giusi Amore, Valentina Rizzi, Beatriz Guerra Roman, Ernesto Liébana, Anca-Violeta Stoicescu, Alexandra Papanikolau, Kenneth Mulligan, and the contributions of ECDC staff members: Therese Westrell and Hanna Merck, and the contributions of ECDC and EFSA contractor: AUSVET Europe (Angus Cameron, Skye Badger, Hester Tang, Ana Belen García, Céline Faverjon, Angela Fanelli, Alison Hillman, Anders Dalsgaard, Ute Wolff Sönksen) SOLADIS (Florian Kroell, Frédéric Chavanel, Thomas Briére, Omraam Agbohouto, Catherine Pahon) and EFOR-CVO-SOLADIS Group (Patrick Etiévant). Also, the support from the EURL-AR, specifically, Jette Sejer Kjeldgaard, Birhte S. Rosenqvist Lund, Jacob Dyring Jensen and Rene S. Hendriksen for the confirmatory testing are gratefully acknowledged.

National zoonoses country reports: 2004-2021

National zoonoses country reports are used as a basis for the EFSA and ECDC European Union Summary Reports on Trends and Sources of Zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks in the European Union. The information given covers both zoonoses that are important for the public health in the whole European Union as well as zoonoses, which are relevant on the basis of the national epidemiological situation. The reports include information reported regarding animals, food, feeding stuffs and food-borne outbreaks.

Dove finiscono le informazioni circa tutti questi dati (fenotipici, genotipici)?

2.2. *Reporting WGS testing results*

The following information shall be included for each individual isolate:

— Unique identifier or code of the isolate

— Bacterial species

— Food-producing animal population or food category

— Stage of sampling

— Type of sample

— TRACES code of the border control post (for testing of imported meat only)

— CHED reference of the consignment (for testing of imported meat only)

— Country of origin of the consignment (for testing of imported meat only)

— Sampler

— The sampling strategy

— Date of sampling

— Date of start of analysis (isolation)

— Identifier or code of the isolate given by the laboratory

— Date of sequencing

— Version of the predictive tool

— AMR-conferring genes data

— Sequencing technology used

— Library preparation used

Anche la Reportistica WGS seguirà i criteri di Reporting del Monitoring AMR già in vigore dal 2010 (voluntary)
e nel 2014 con la Dec 2013/652/EU (mandatory)
Ovvero secondo Standard Sample Description 2.0 (at isolate – Epi Unit level)

Technical Specifications on Reporting aggiornate da EFSA annualmente (Data Dictionary)

In qualità di IZS (IZSLT): NON SIAMO STATI «EARLY ADOPTER» di High Throughput Sequencing & Bioinformatics… Come NRL-AR, dal 2015…

- Un certo ritardo nell'applicazione (anche «specifica» e «selettiva») di metodi di sequenziamento massivo > ritardo dell'Ente nel «sentire» la necessità di confrontarsi con grandi moli di dati genomici, e con il «mondo» degli aspetti (organizzativi, tecnico-scientifici, gestionali) che sono necessari anche per impostare e condurre le discipline genomiche
- Abbiamo recuperato…
- Come UOC Direzione Operativa Diagnostica Generale, CRN-AR e NRL-AR, **abbiamo iniziato già nel 2015**: Applicazione in Sorveglianza & Ricerca
- Progetti Ricerca EU (ENGAGE, EFFORT) già attivi nel 2015-2016

Advocacy – How to bring WGS to the attention of decision makers at the Institute

Antonio Battisti, DVM, Alessia Franco, DVM
Istituto Zooprofilattico Sperimentale del Lazio e Toscana,
National Reference Laboratory for Antimicrobial Resistance, Rome, Italy

The use of the Whole Genome Sequencing (WGS) in Monitoring of Antimicrobial Resistance
EURL- AR Training Course 2017
DTU, Kgs. Lyngby 27th September 2017

Prerequisites («protective variable…»)

Human factor:

- Try to «get in tune» with the General Director…

Ingredients:

-Reputation, credibility from your side…

-Wisdom and farsightedness on the General Director’s side

Usually both are of some help….

Reasons for purchasing at Department level

- The instrument is needed to **facilitate the mission (Accredited multisite laboratory following ISO 17025 rules) of the Department** (Institute) and allow:

- **deep molecular characterization** (ID, genetic basis of virulence **and AMR**, population structure, phylogeny etc.);

- **molecular epidemiology investigations** (tracing back, source attribution etc.) on major pathogens, zoonotic agents, **AMR determinants and their genetic environment**.

- Specifically, it is an instrument that can provide sequences of "whole genomes" of pathogenic agents (e. g. the entire genetic content of a pathogenic agent consisting of millions of nucleotides, known as "Whole Genome Sequencing") **in an accurate, fast, and cost-effective way**.
- **The above described is not feasible through traditional sequencing technology ("Sanger Sequencing“: we already have one machine with 8 capillaries)**. It should also be emphasized that the above-mentioned NGS technology is not currently available at our Institute.
- For a start, and for expected initial volume of activity, a machine with a medium/small output (e. g. min. 10 Gigabytes, with 25 million reads of at least 100-300 pairs of theoretical bases) would be sufficient, considering that the rapid evolution of in this field will provide a fairly fast "generational replacement” of technologies and related equipment.
- **Prudent approach to the initial investment on a new technology (in this case is around 100,000 Euro)**!!!

Global Microbial Identifier

ABOUT GMI PEOPLE WORKGROUPS NEWS & EVENTS CONTACT

GMI envisions a global system of DNA genome databases for microbial and infectious disease identification and diagnostics. Such a system will benefit those tackling individual problems at the frontline, clinicians, veterinarian, etc., as well as policy-makers, regulators, and industry. By enabling access to this global resource, a professional response on health threats will be within reach of all countries with basic laboratory infrastructure.

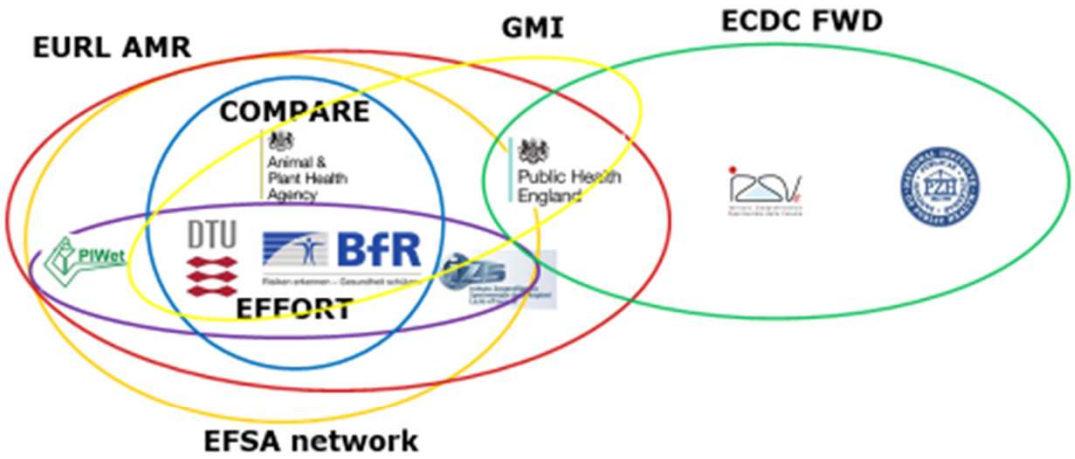
GMI11 Dates

GMI11 is being held in Geneva, Switzerland.
The dates are 16-18 May 2018.

Watch video on GMI

Video on GMI by David J. Lipman, NCBI, presented at an information meeting at the European Parliament on January 23, 2013.

Engage consortium



efsa European Food Safety Authority | DTU Food National Food Institute

REGIONE LAZIO

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

ENGAGE

Establishing **N**ext **G**eneration sequencing **A**bility for **G**enomic analysis in **E**urope

EFSA call "GP/EFSA/AFSCO/2015/01:

New approaches in identifying and characterizing microbiological and chemical hazards"

efsa European Food Safety Authority | DTU Food National Food Institute

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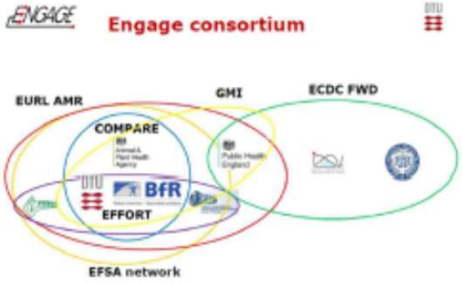
ENGAGE Training Course 2017 – 25-27 October 2017

"Training on NGS analysis based on command line tools"

LAZIOCREA (Regione Lazio),
Via del Serafico 104, Rome, Italy

The objective of ENGAGE is to establish collaboration between the Public Health, Food and Veterinary sectors across the European Union for building and enhancing the use of real-time Whole Genome Sequencing and analysis in Food Safety and Public Health protection.



Presente

- **Proficiency Testing Schemes annuali nel contesto GMI, EURL-AR (DTU-Food DK) e Biothreat**
- **Utilizzo continuo di NGS / HTS, specialmente WGS per scopi di Sorveglianza & Monitoraggio su agenti biologici** (zoonosici, patogeni animali) **nel contesto di studi di popolazione**
- **Priorità: sottoponiamo a NGS / HTS - WGS (short-read e long-read sequencing) & Bioinformatics ciò che è rappresentativo a livello di popolazione e di produzioni animali**
- **Per alcuni settori di studi di popolazione, sottoponiamo sistematicamente gli isolati a Whole Genome Sequencing dal 2019 (oltre 1000 genomi/anno per Dec. AMR Monitoring, Ricerca, etc);**
- **Amplicon sequencing & Shotgun (Metagenomica)**
- **Continuiamo attività di Public Service:**

-Interazione a livello EU:

-European Commission Working Group on AMR in Food;

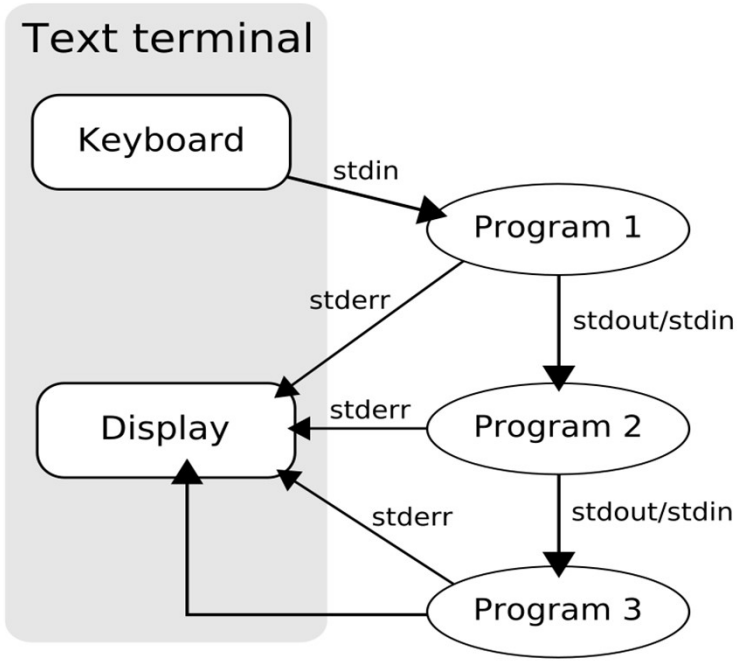
-EFSA EXPERTS for WGS & Scientific Network Zoonoses Monitoring Data: per implementare con dati genomici in WGS il Database EU Zoonoses – AMR;

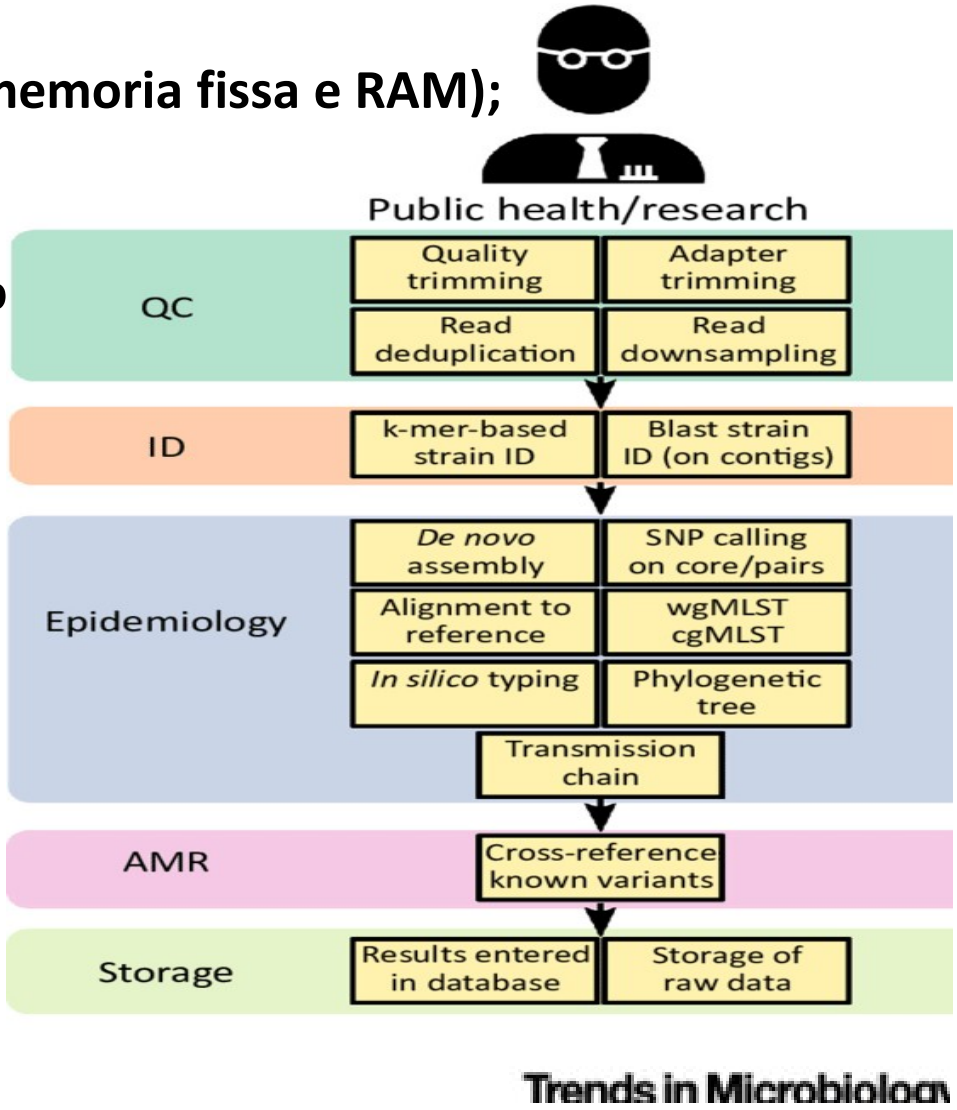
-Coautori delle EFSA Tech Specs on AMR Monitoring in EU (2019)

-Coautori delle EFSA Tech Specs on a survey for LA-MRSA in pig holdings

Indispensabile programmare e acquisire:
-Hardware (Server, capacità e tipologia HD per memoria fissa e RAM);
-Struttura e policies di Data Security

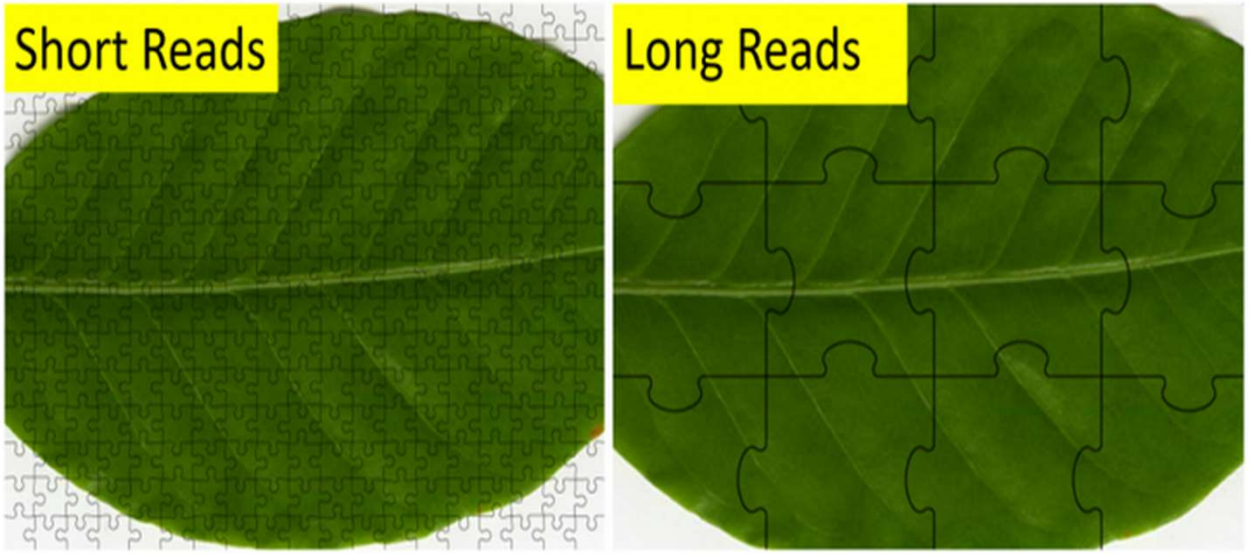
Costruzione di un flusso di analisi standardizzato



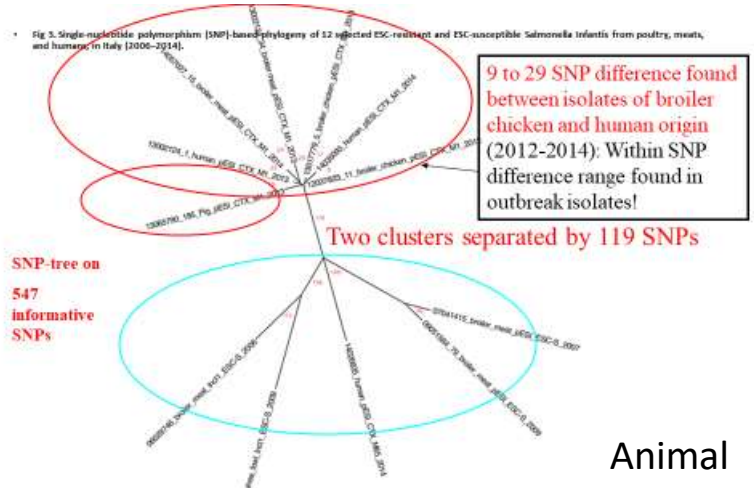


Trends in Microbiology

Introduzione di nuove tecnologie di High Throughput Sequencing



Oxford NANOPORE Technologies



Franco A, Leekitcharoenphon P, Feltrin F, Alba P, Cordaro G, et al. (2015) Emergence of a Clonal Lineage of Multidrug-Resistant ESBL-Producing Salmonella Infantis Transmitted from Broilers and Broiler Meat to Humans in Italy between 2011 and 2014. PLoS ONE 10(12): e0144802. doi:10.1371/journal.pone.0144802
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0144802

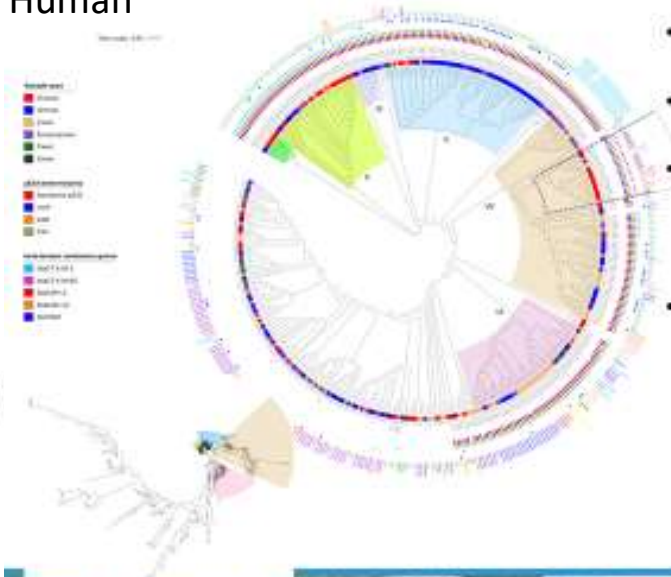
PLOS | ONE

High Resolution Studies (2015-present): Whole Genome Sequencing (WGS).
Bioinformatics for SNP-based Phylogeny of the Bacterial host and the Plasmid, and for virulence, fitness, AMR genes

Animal → Human

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri

Chromosome SNP-based phylogenetic tree



- Minimum and maximum SNPs differences were 7 and 1098.
- **Nine** main **clusters** were identified (branch length <0.05)
- Clusters I (n=7), II (n= 34) and III (n=9 isolates) consisted of pESI-like+ve harbouring $bla_{CTX-M-1}$, from **different sources**, mainly from **Italy**.
- Cluster V → (n=58 isolates) pESI-like+ve, from **different sources** and from **five EU countries** (Netherlands, Denmark, Luxembourg, Germany and Italy). 68.9 % harboured *bla*TEM-1 gene.

Transferable resistance to colistin (*mcr* genes) in the ESBL-producing Salmonella Infantis clone in the broiler industry, Italy

frontiers in Microbiology

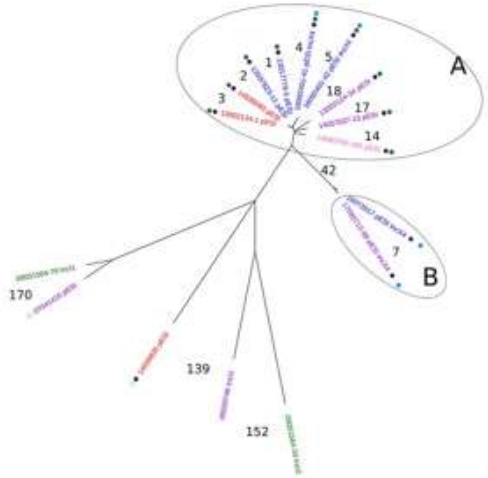
Colistin Resistance Mediated by *mcr-1* in ESBL-Producing, Multidrug Resistant *Salmonella* Infantis in Broiler Chicken Industry, Italy (2016–2017)

Virginia Carfora,[1] Patricia Alba,[1] Pimlapas Leekitcharoenphon,[2] Daniele Ballotti,[1] Gessica Cordaro,[1] Paola Di Matteo,[1] Valentina Donati,[1] Angela Iannetta,[1] Manuela Iannucci,[1] Fiorentino Stravino,[1] Tania Tagliabini,[1] Antonio Battisti,[1]* and Alessia Franco[1]

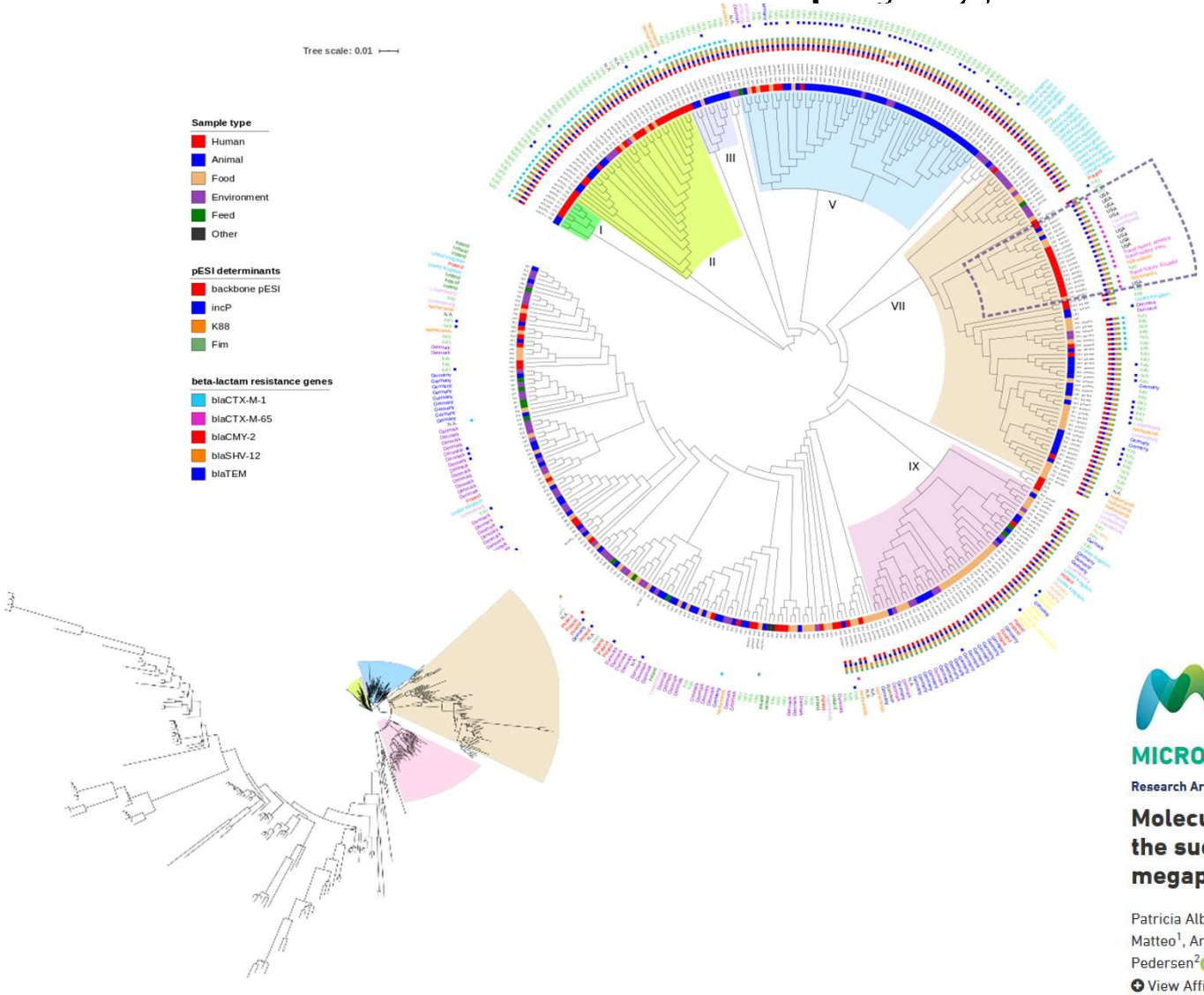
Abstract

Colistin-resistance mediated by mobilisable and plasmid-borne *mcr* genes has emerged worldwide, threatening the efficacy of colistin, a last resort antibiotic increasingly used for treating human invasive infections by multidrug-resistant or extensively drug-resistant *Enterobacteriaceae*. In this study, we report the first evidence of *mcr*-1-mediated colistin resistance in four multidrug resistant (MDR) out of 324 *Salmonella* infantis from the Italian antimicrobial resistance (AMR) monitoring (2001–2017) in broilers and broiler meat. Two were also Extended Spectrum Beta-Lactamases (ESBL)-producing isolates. Characterization by whole genome sequencing (WGS), located *mcr*-1.1 on an incX4 plasmid. Phylogenetic analysis of these isolates with selected Italian *S.* Infantis previously isolated from animals, meat and human clinical cases with unknown epidemiological relationship, demonstrated that ESBL-producing, *mcr*-1-positive isolates belonged to the emerging pESI-like-positive-ESBL-producing clone described in Italy in 2015.

Keywords: colistin resistance, *mcr* genes, ESBL (Extended Spectrum Beta-Lactamases), plasmids, whole genome sequencing, *Salmonella* Infantis, broilers, broiler meat



Chromosome SNP-based phylogenetic tree of S. Infantis in Europe



- Minimum and maximum SNPs differences were 7 and 1098.
- **Nine** main **clusters** were identified (branch length <0.05)
- Clusters I (n=7), II (n= 34) and III (n=9 isolates) consisted of pESI-like+ve harbouring ***bla*$_{\text{CTX-M-1}}$**, from **different sources**, mainly from **Italy**.
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MICROBIAL GENOMICS Volume 6, Issue 5

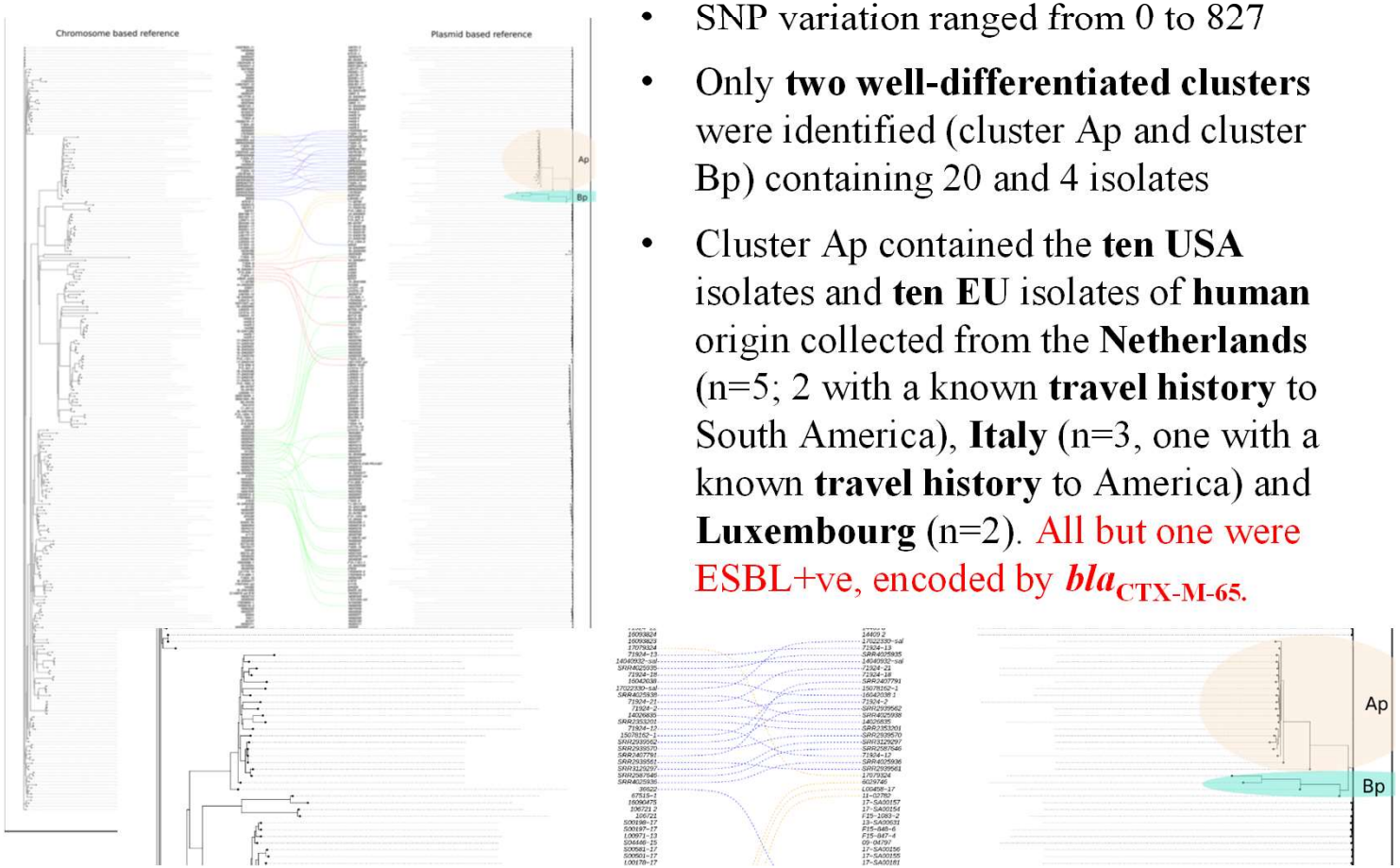
Research Article | Open Access

Molecular epidemiology of *Salmonella* Infantis in Europe: insights into the success of the bacterial host and its parasitic pESI-like megaplasmid

Patricia Alba[1], Pimlapas Leekitcharoenphon[2], Virginia Carfora[1], Roberta Amoruso[1], Gessica Cordaro[1], Paola Di Matteo[1], Angela Ianzano[1], Manuela Iurescia[1], Elena L. Diaconu[1], ENGAGE-EURL-AR Network Study Group[3], Susanne K. Pedersen[2], Beatriz Guerra[4], Rene S. Hendriksen[2], Alessia Franco[1], Antonio Battisti[1]

View Affiliations

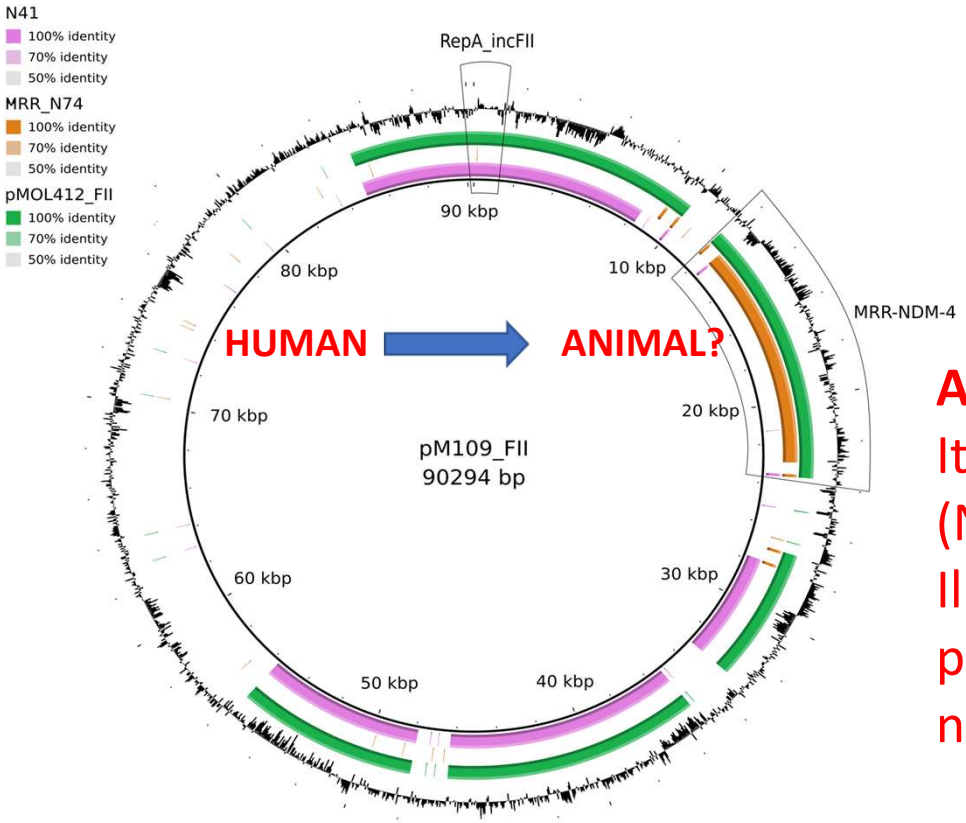
Plasmid SNP-based phylogenetic tree of S. Infantis in Europe



- SNP variation ranged from 0 to 827
- Only **two well-differentiated clusters** were identified (cluster Ap and cluster Bp) containing 20 and 4 isolates
- Cluster Ap contained the **ten USA** isolates and **ten EU** isolates of **human** origin collected from the **Netherlands** (n=5; 2 with a known **travel history** to South America), **Italy** (n=3, one with a known **travel history** to America) and **Luxembourg** (n=2). All but one were ESBL+ve, encoded by ***bla*$_{\text{CTX-M-65}}$.**

High Resolution Study: Whole Genome Sequencing and Bioinformatics (Bacterial Host and full resolution of the Plasmid)

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

Journal of Antimicrobial Chemotherapy

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Materials and methods
Results
Discussion
Acknowledgements
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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

Journal of Antimicrobial Chemotherapy, dkaa374, https://doi.org/10.1093/jac/dkaa374
Published: 24 August 2020 **Article history**

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Abstract

Allevamento suino: Nel 2019, primo riscontro in Italia e in EU di un E. coli resistente ai carbapenemi (NDM-4) nelle Produzioni Animali
Il Plasmide condivide 99,9% della sequenza con un plasmide isolato in infezione clinica da E. coli nell'Uomo in Myanmar

OXFORD UNIVERSITY PRESS

High Resolution Study: Whole Genome Sequencing and Bioinformatics (Bacterial Host and full resolution of the Plasmid)

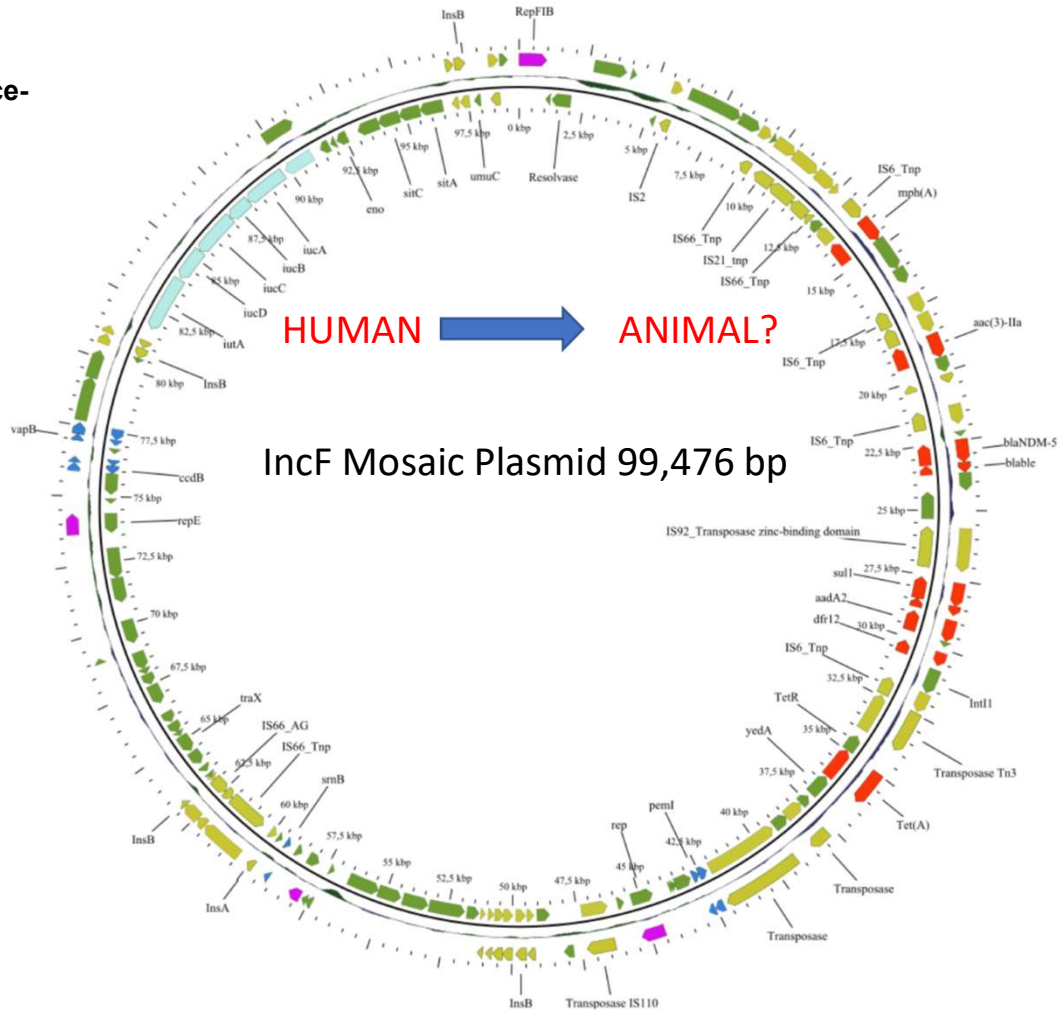
Veterinary Microbiology

Carbapenemase IncF-borne blaNDM-5 gene in the E. coli ST167 high-risk clone from canine clinical infection, Italy

Highlights

-First evidence in Italy of a NDM-5-producing *E. coli* in companion animals
-Full reconstruction of the blaNDM-5–carrying mosaic plasmid.
-Evidence of transmission of blaNDM-5 E. coli from humans to animals and vice-versa.

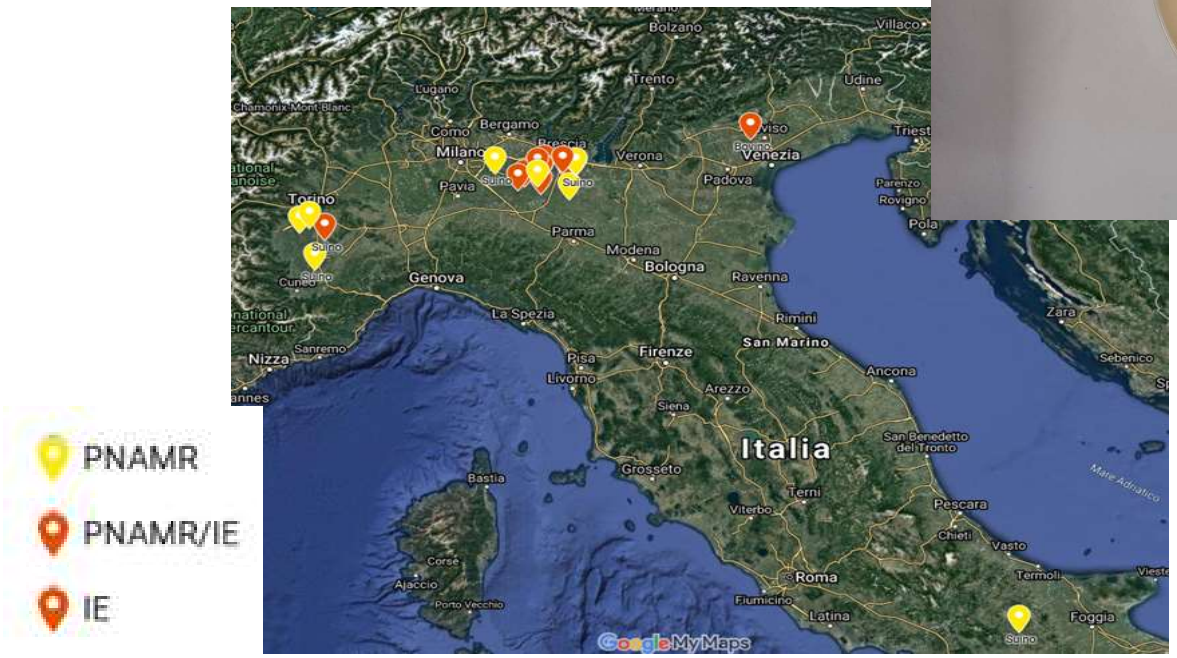
pMOL008: plasmide a mosaico con 4 diversi repliconi: due IncFII, IncFA e IncFB (F36:F31:A4:B1).
Rosso: geni antibioticoresistenza: aadA2, aac(3)-Iia, blaNDM-5, mph(A), sul1, tet(A) e dfrA12
Verde: IS o transposoni
Blue: sistemi T/AT; pemI/K, ccdA/B, vapC/B, srnB/C
Fucsia: origine di replicazione
Turchese: aerobactin operon.



The blaNDM-5-producing E. coli Sequence Type (ST)167 high-risk clone is emerging worldwide in human clinical cases, while its presence in companion animals is sporadic and has never been described in Italy. Using a combined Oxford Nanopore (ONT) long-reads and Illumina short-reads sequencing approach, an E. coli ST167 isolated from a hospitalized dog, was in-depth characterized by WGS and the plasmid containing bla NDM-5 was fully reconstructed. The complete sequence of the pMOL008 mosaic plasmid (F36:F31:A4:B1; pMOL008) harbouring bla NDM-5 , was resolved and characterized. Moreover, a (pro)phage and IncFII, containing bla CMY-2 and ermB , and IncI2 plasmid types were also identified. pMOL008 was identical to bla NDM-5 -containing plasmids from E. coli ST167 isolated from Italian human clinical cases and from a Swiss dog and colonized humans. bla NDM-5 was located in a class 1 integron together with aadA2 , aac(3)-IIa , mph (A), sul1 , tet (A) and dfrA12 . The risk of spill-over and spill-back transmission of carbapenem-resistance genes, related-plasmids and strains between humans and dogs, represents a Public Health threat and highlights the importance of the One Health approach for the AMR detection.

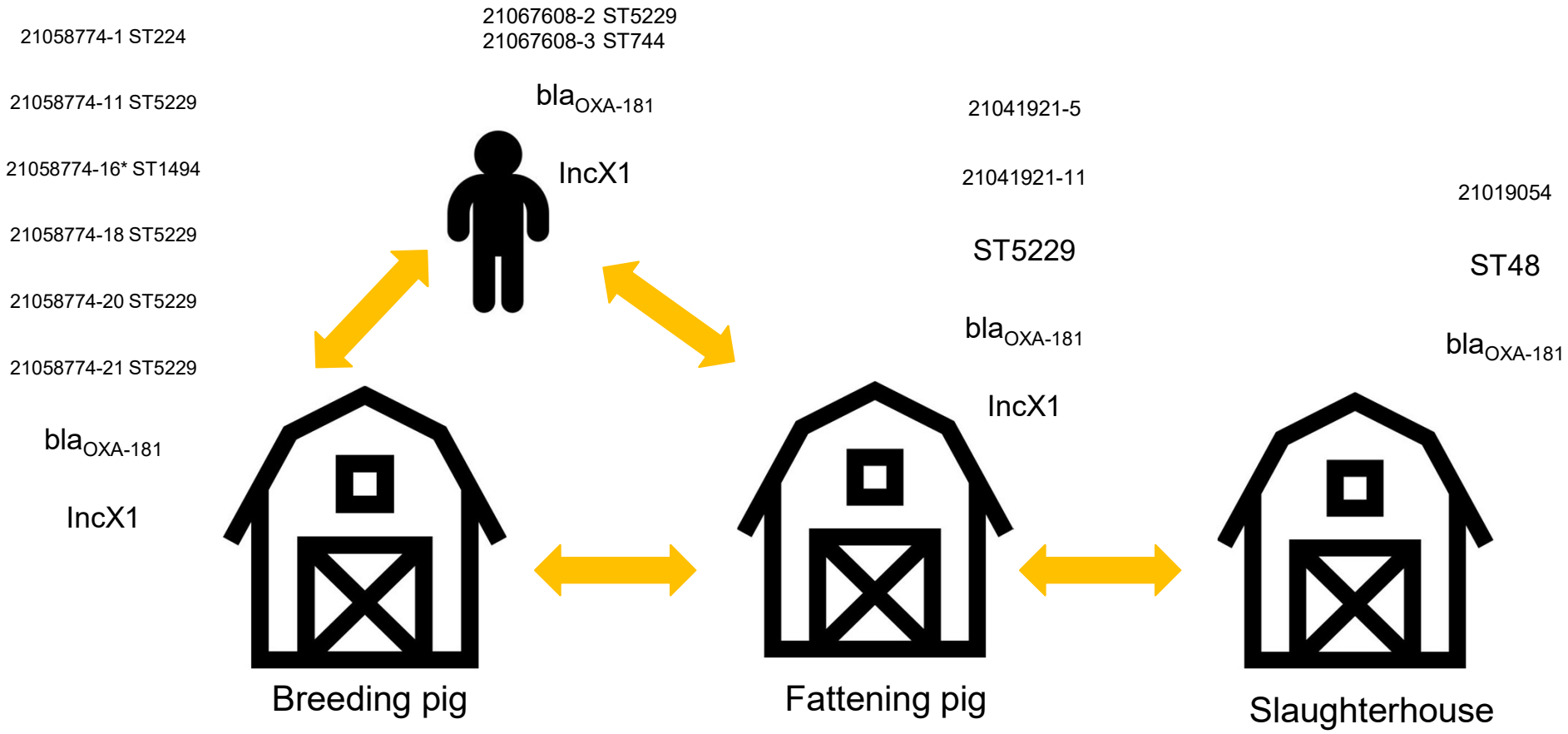
Update December 2021 → **25 isolates** OXA-48-like (24 OXA-181; 1 OXA-48) from different EpiUnits sampled at slautherhouse (Dec (EU) 2020/1729) in 11 provinces (5 Regions)
n=21 from pigs (**6.98%**; 95% CI 4.37-10.47%; 21/301) **n=4** from bovines <12 months (**1.29%;** 95% CI 0.35–3.27%, 4/310)

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):

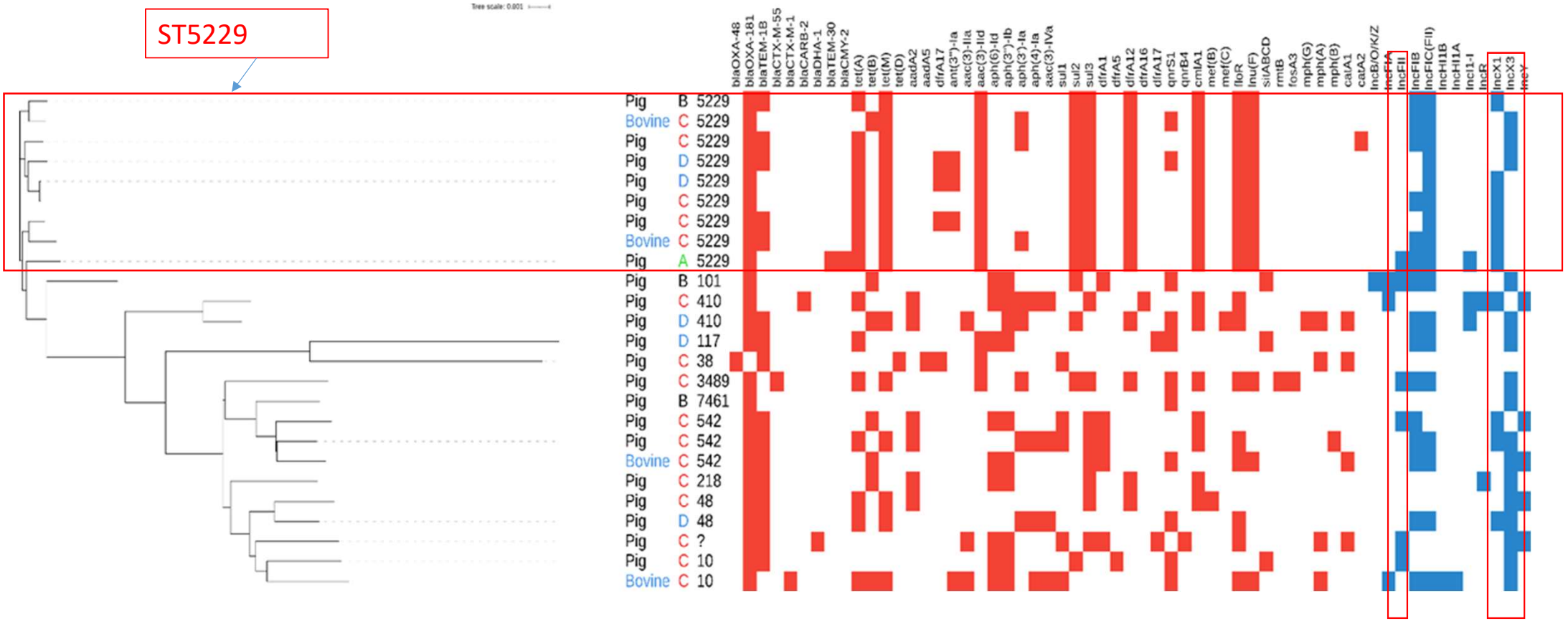


Epidemiological investigation: for >80%the positive EpiUnits investigated at slughter, an OXA-48-like producing E. coli (OXA-181) **has been isolated from samples taken at the farm of origin**

Epidemiological Investigation: Case1



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



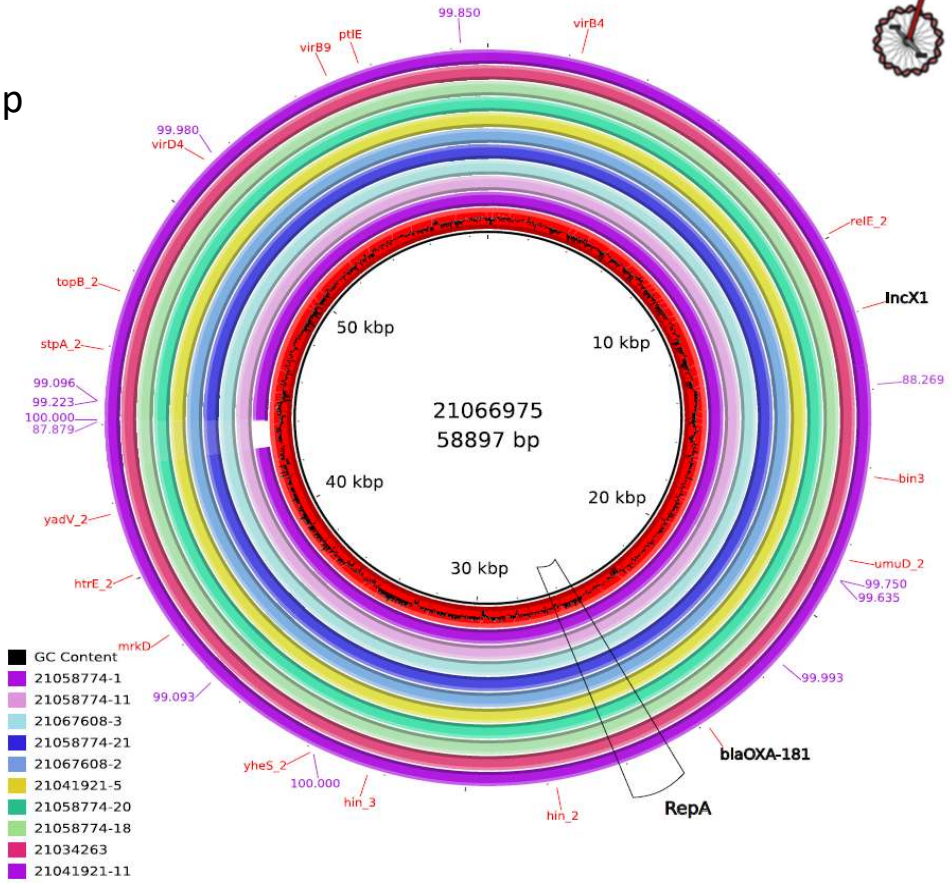
- A non-clonal population of OXA-48-like producing *E. coli* in the dataset analyzed. **However, …. (ST5229, 9/25, 36% isolates). IncX3, IncX1, IncF the replicons most represented.**
- **IncX3 or IncX1 harboured the OXA-181 gene. No specific pathotype found.**
- The clusters were distributed according 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

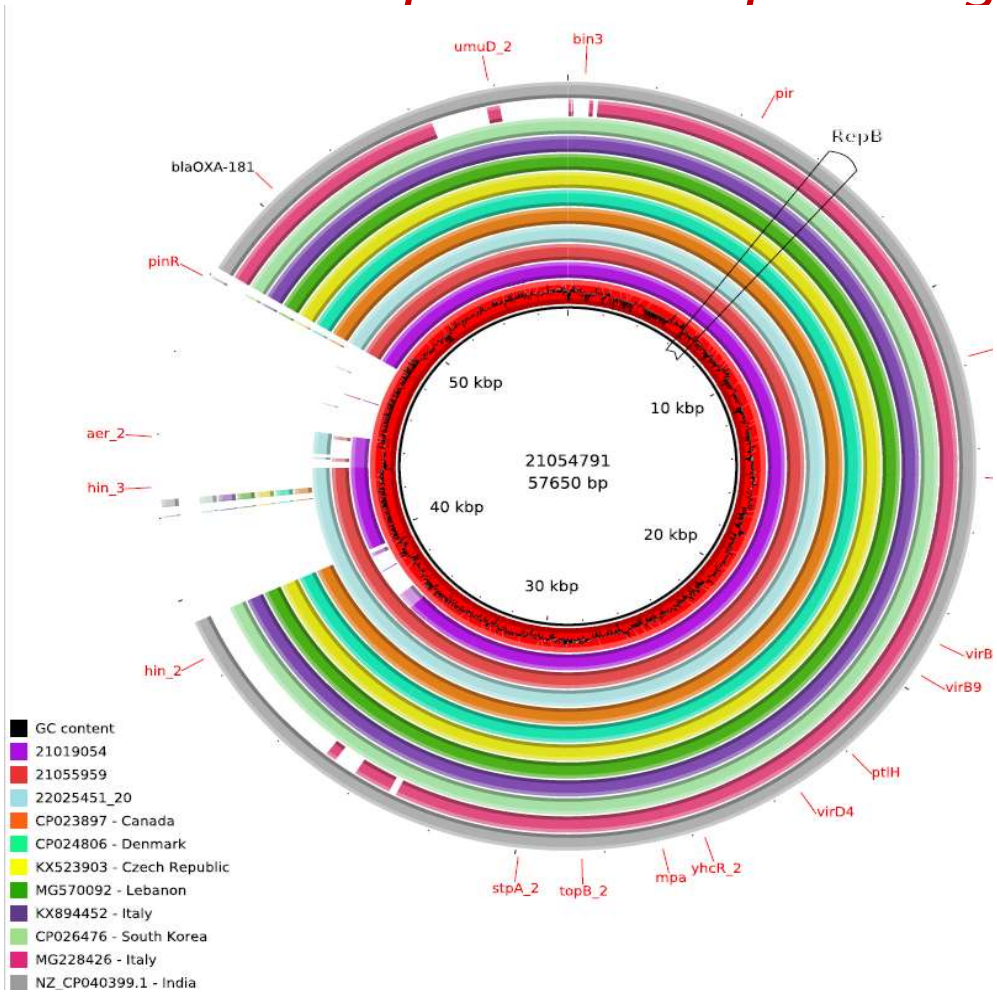
size range:
57,694–58,897 bp



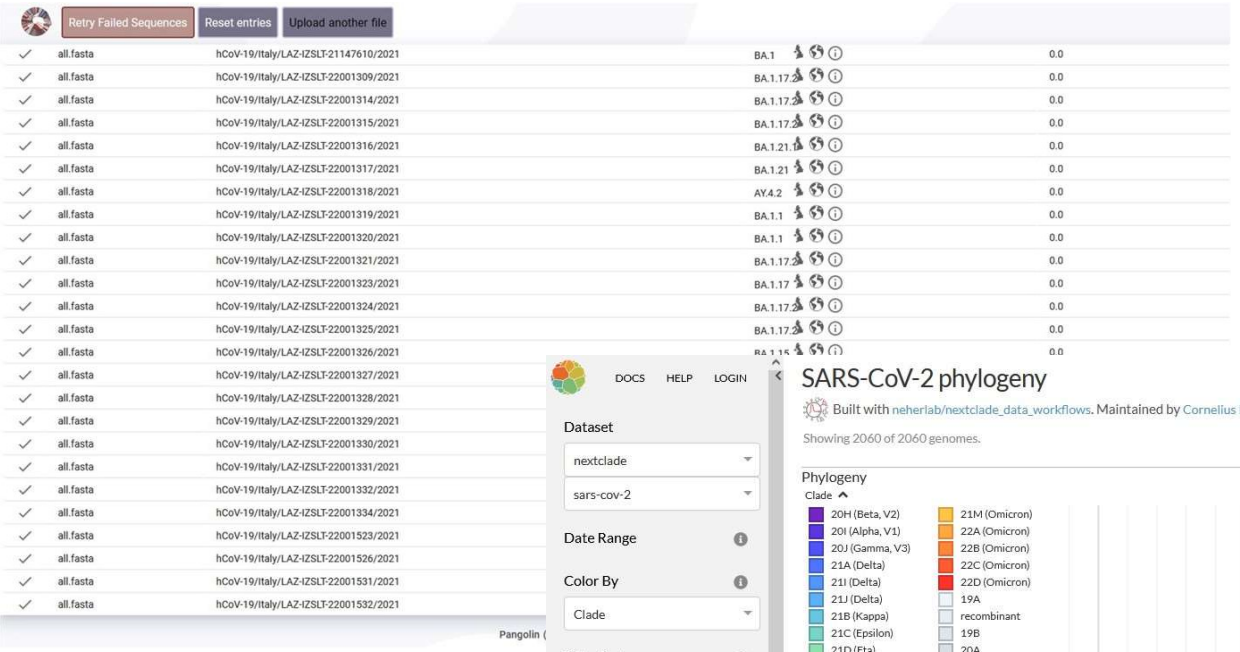
- ✓ All the 12 IncX1 resolved plasmids were almost identical with a 98-99% coverage and 99-100% sequence identity
- ✓ **No similar IncX1 plasmids were found in publicly available databases.**

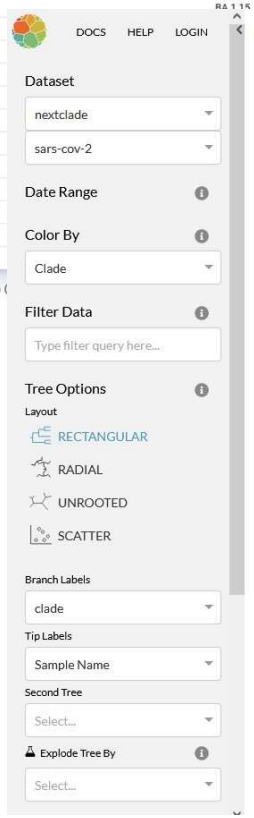
IncX1 more stable than IncX3 because of the presence of the RelE/StbE toxin family and its antitoxin RelB?

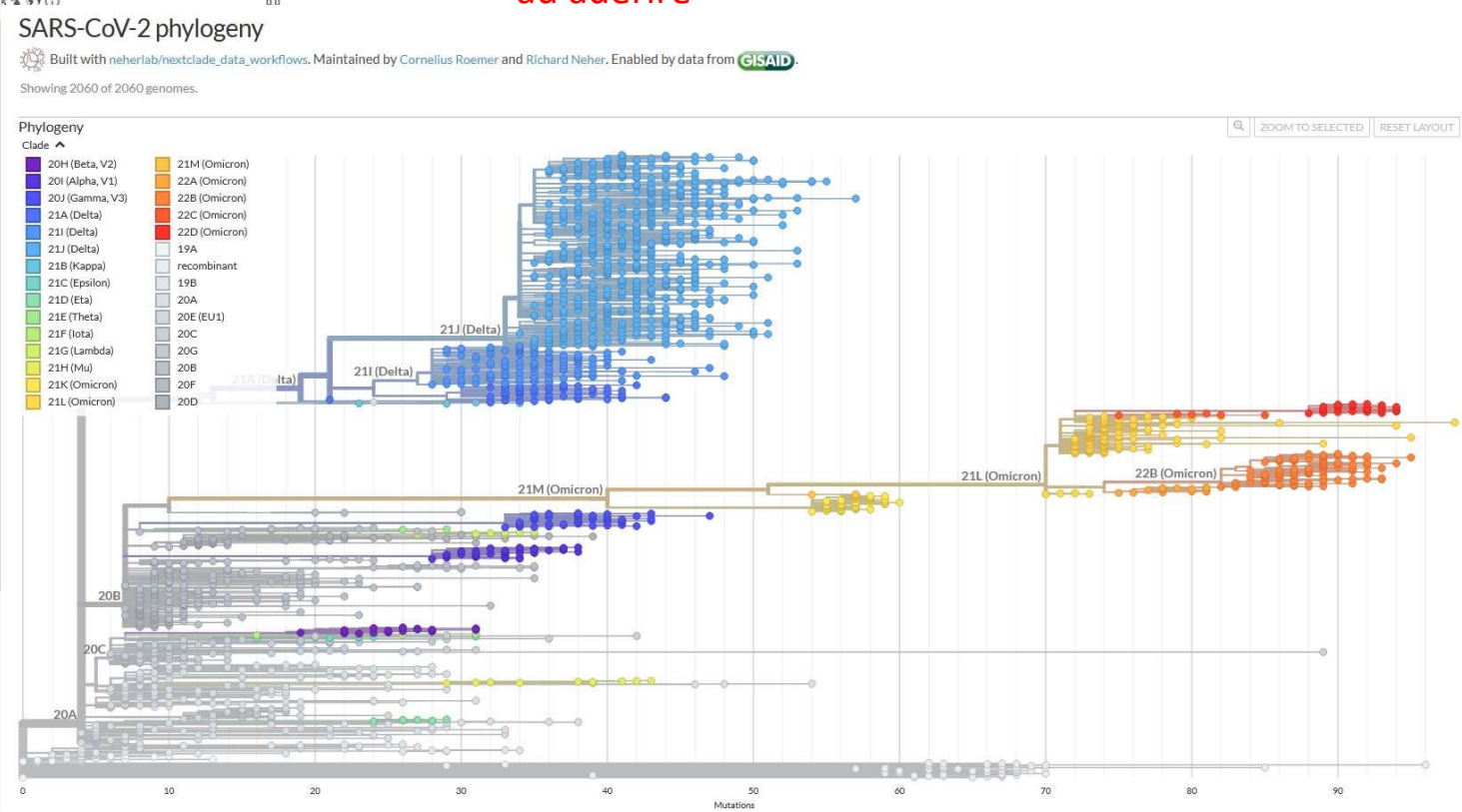
Full plasmid sequencing: ***IncX3*** *plasmids*



- ✓ 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*)
- ✓ 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







2021 - oggi: Il personale della UOC DO Diagnostica Generale (collab. con Virologia)continua a contribuire alla sorveglianza genomica (WGS) SARS-CoV-2 a livello regionale (WGS-Net, Regione Lazio), nazionale (Rete Sequenziamenti), internazionale: GISAID, EBI): IZSLT uno dei tre Enti (con INMI e IFO) nella regione Lazio ad aderire

Tracciare ed investigare trasferimento di determinanti AMR e agenti AMR in un'ottica «One Health»

- Le evidenze nel settore AMR sono indispensabili per orientare le raccomandazioni di Sanità Pubblica e orientare le Risk Management Options.
- Evidenze «di laboratorio» supportano le indagini epidemiologiche (e sempre più spesso orientano verso le ipotesi più plausibili da testare o confermare in modo prioritario)
- Studi di popolazione per valutare il grado di penetrazione di alcune Resistenze di cui è nota o «accettata» l'origine animale, o quella umana…
- **Low Resolution Methods, Medium Resolution Methods, High Resolution Methods si sono succeduti negli anni**
- **Con l'avvento della Genomica e del Sequenziamento Massivo (HTS), i primi non sono ritenuti più adeguati….**

- **Il focus sugli animali degli aspetti zoonosici dell'AMR non può essere l'unico, nell'approccio «One Health»… In alcuni casi è dimostrato il percorso contrario….**
- **Significato «bidirezionale» del termine zoonosi**
- Serbatoio animale (specialmente food-producing) può costituire un **amplificatore del fenomeno spill-over da Uomo ad Animale**

Un grazie particolare a tutti i miei collaboratori, UOC D. O. Diagnostica Generale, CRN-AR e NRL-AR

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

Arrivederci al prossimo Workshop annuale del CRN-AR e NRL-AR in novembre 2023

Grazie ad IZSLT, ed un grazie particolare a:
-Gli IIZZSS, e tutto il personale dei Servizi Veterinari che in Italia ha consentito di adempiere completamente a quanto previsto dal Piano Nazionale Monitoraggio AMR nel periodo 2020 -2022, in periodo pandemico, e anche quando altre attività di sorveglianza, controllo, monitoraggio, erano considerate «differibili» dal Ministero Salute…

CRAB
Centro di Referenza Nazionale per l'Antibioticoresistenza

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