

# Caratterizzazione di agenti zoonosici prioritari e MDR correlata: l'esempio di “*Salmonella* Infantis”

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Workshop annuale del CRN-AR e NRL-AR 2021



## IL CENTRO DI REFERENZA NAZIONALE E NATIONAL REFERENCE LABORATORY

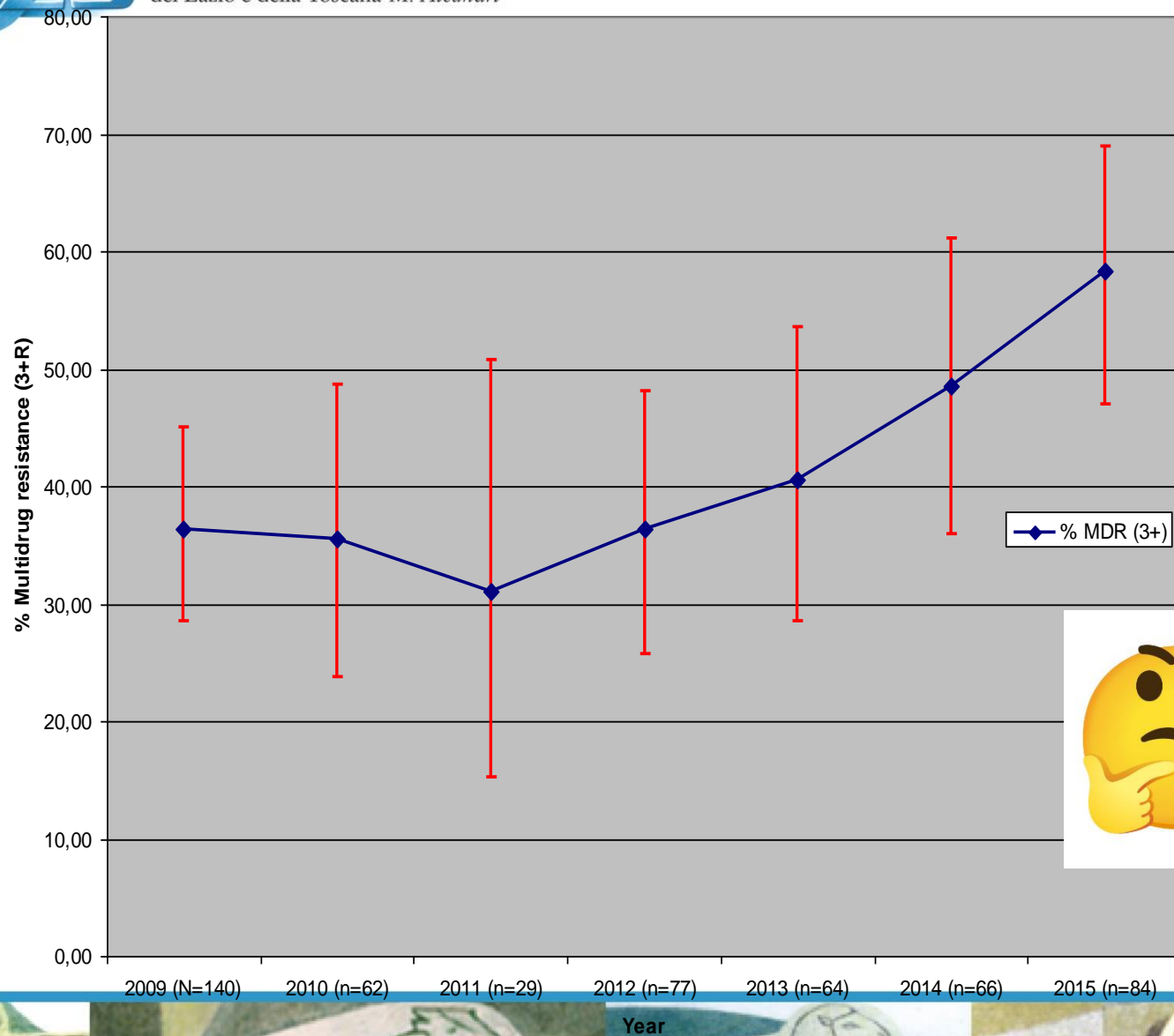
Il **Centro di Referenza Nazionale per l'Antibioticoresistenza (CRN-AR)** (*D.M. 8-5-2002, G. U. 22 maggio 2002, n. 118*), e **National Reference Laboratory for Antimicrobial Resistance (NRL-AR, Reg. CE 882/2004 - Reg. (EU) 2017/625)** per l'Italia dal 16 novembre 2006 (Prot. Uff. II DGVA/40778/P-I.4.d.a.), ha nel corso degli ultimi anni profuso impegno e prodotto risultati in termini di programmazione e di informazioni prodotte in materia di monitoraggio della resistenza agli antibiotici nel settore veterinario.

Opera a vantaggio della Sanità Animale e della Sanità Pubblica Veterinaria, in una prospettiva di **"One Health"**, avvalendosi della collaborazione di una rete nazionale, principalmente costituita dalla **rete degli Istituti Zooprofilattici Sperimentali (IIZZSS)**, dei **Centri di Referenza Nazionali** per gli agenti batterici zoonosici, ed in collaborazione con l'**Istituto Superiore di Sanità**.

Opera inoltre in un **network internazionale di monitoraggio ed armonizzazione di metodiche analitiche, di reporting ed interpretazione dei dati** di monitoraggio, a vantaggio dei fini di Sanità Pubblica dello Stato Membro EU Italia e della Commissione Europea.

Nell'ambito delle attribuzioni istituzionali, fornisce consulenza e supporto tecnici al **Ministero della Salute, Dipartimento della Sanità Pubblica Veterinaria**, della sicurezza alimentare e degli organi collegiali per la tutela della salute, agli **IIZZSS** ed altre istituzioni di Sanità Pubblica secondo quanto previsto dalla vigente normativa (*all'articolo 2 del D.M. 4 ottobre 1999*).

Il Centro di Referenza ha inoltre l'obiettivo di mantenere un sistema di monitoraggio sull'antibioticoresistenza in medicina veterinaria sul territorio italiano, in prospettiva di sorveglianza futura. Lo scopo è quello di individuare l'emergenza e la diffusione di resistenze (e multiresistenze) di particolare rilevanza in determinate categorie di batteri di origine animale (patogeni animali, zoonosici ed indicatori), e di comunicare le informazioni attraverso reportistica a carattere nazionale ed internazionale e pubblicazioni peer-reviewed su riviste internazionali.

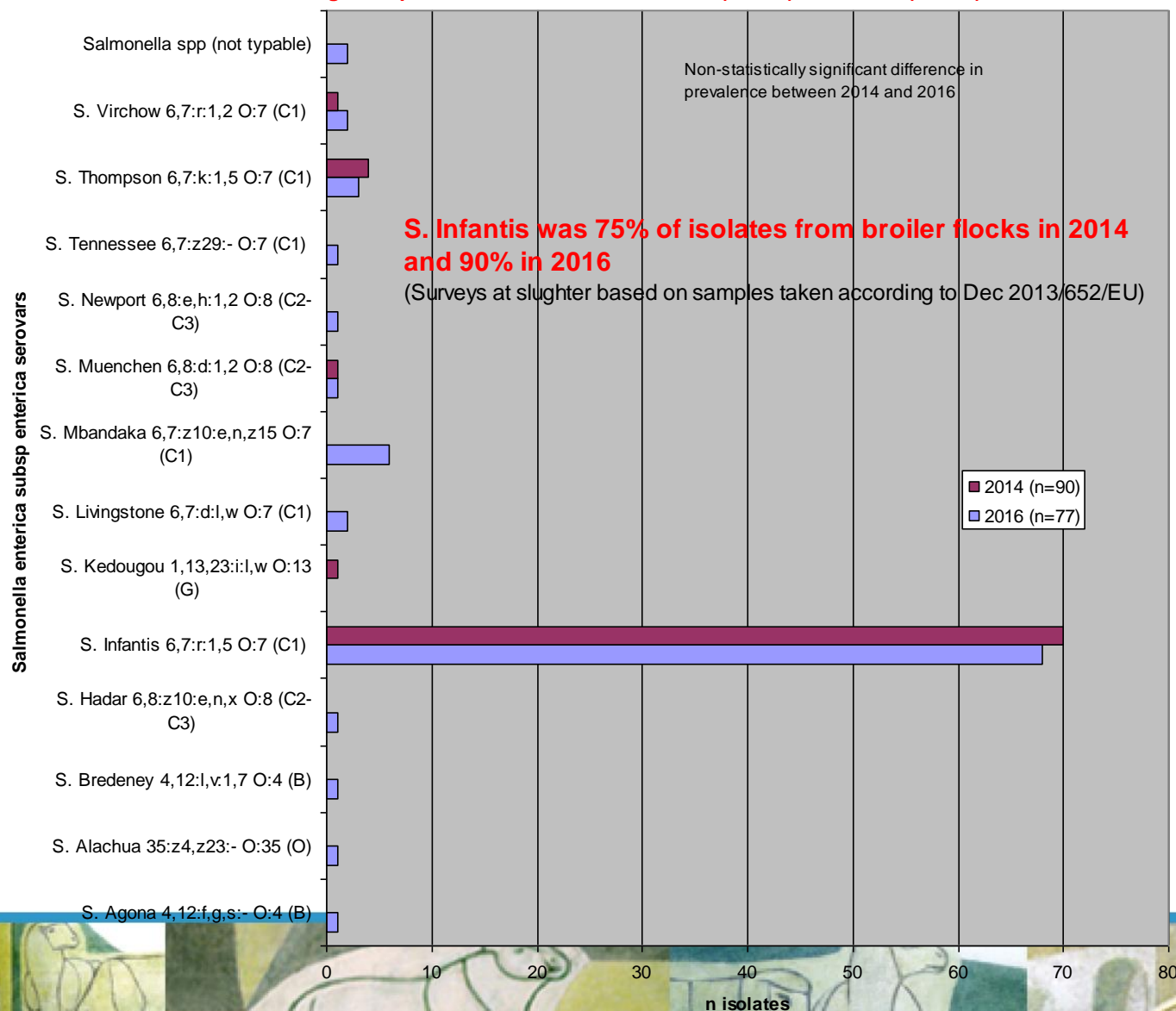




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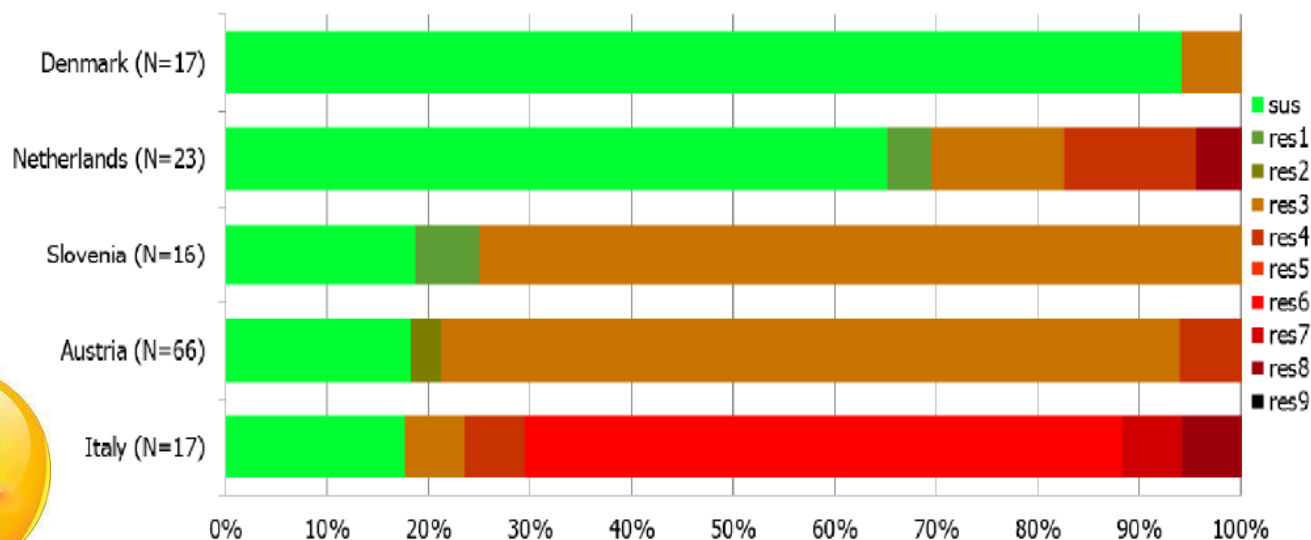
**Salmonella serovar distribution broiler chicken flocks, Italy, 2014 (n=90) and 2016 (N=77):**

**Among-flock prevalence S. Infantis 2014 9.6% (68/709); 2016 8.7% (70/807)**



# MDR *S. Infantis* is common in EU in humans, including Italy

**Figure 13:** Frequency distribution of *Salmonella* *Infantis* isolates from humans completely susceptible or resistant to one to nine antimicrobial classes in 2014



**Minimum  
common  
AMR  
pattern:  
TET+SUL+  
TMP+[CIP]**

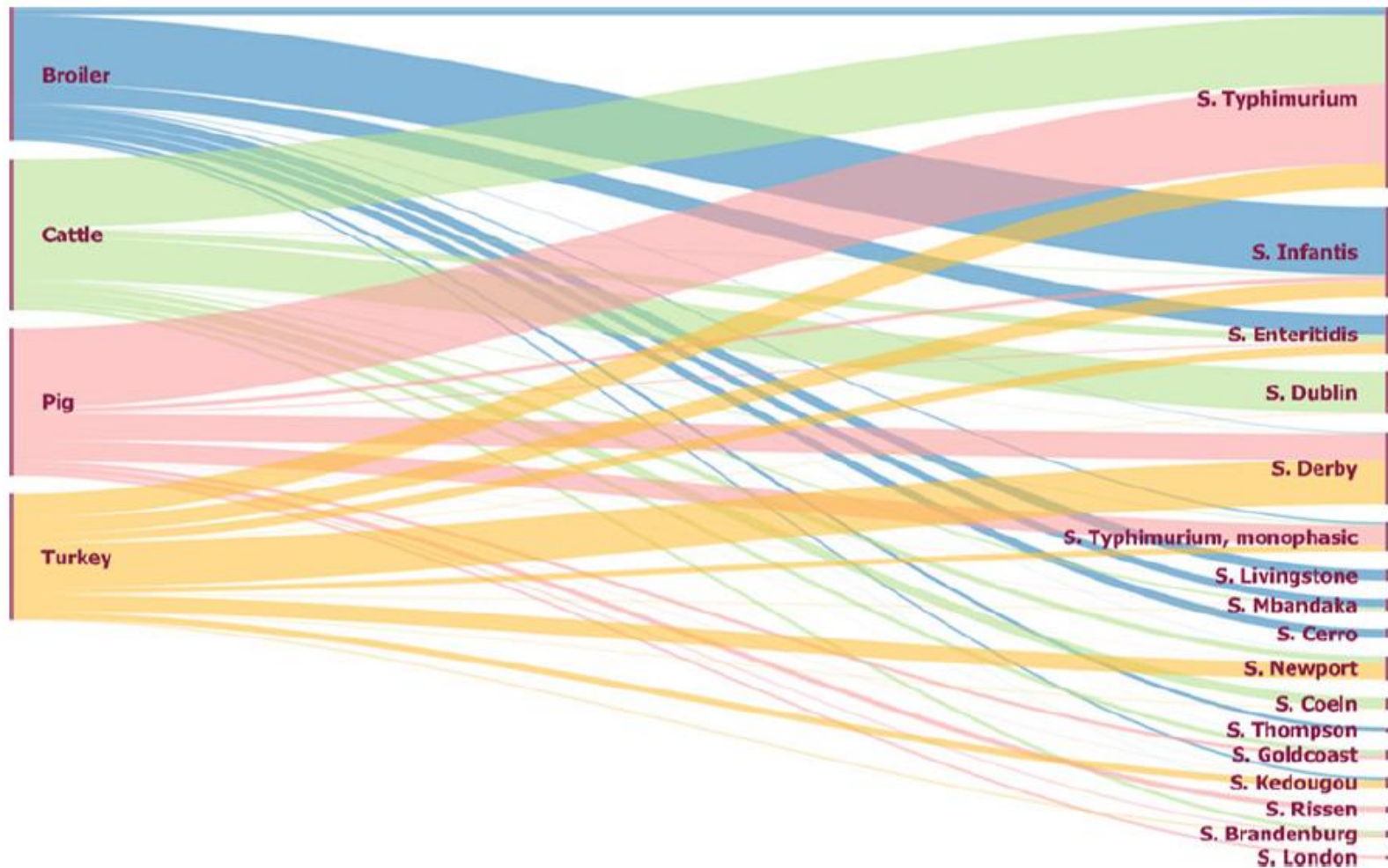


N: total number of isolates tested for susceptibility against the whole common antimicrobial set for *Salmonella*;

sus: susceptible to all antimicrobial classes of the common set for *Salmonella*;

res1-res9: resistance to one up to nine antimicrobial classes of the common set for *Salmonella*.





The left side of the diagram shows the sources considered: broiler (blue), cattle (green), pig (red) and turkey (yellow); animal and food data from the same source were merged (broiler includes isolates from chicken flocks and broiler meat, cattle includes data from bovine herds and bovine meat, pig includes data from pig herds and pig meat, turkey includes data from turkey flocks and turkey meat). On the right side, the list of the 17 most reported serovars from each matrix (combined animal and food) is reported. Included serovars are the outcome of a merging procedure involving the seven most reported serovars for each source. The width of the coloured bands linking sources and serovars is proportional to the percentage of isolation of each serovar in each source.



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Hi One Health!!



## Dal 2017, S. Infantis è il 4° serovar più ritrovato nel umono nella UE

**Table 15:** Distribution of reported confirmed cases of human salmonellosis in the EU/EEA, 2017–2019, by the 20 most frequent serovars in 2019

Serovar	2019			2018			2017		
	Cases	MSs	%	Cases	MSs	%	Cases	MSs	%
Enteritidis(*)	39,865	27	50.3	39,781	27	49.9	38,780	27	49.2
Typhimurium(*)	9,404	27	11.9	10,395	27	13.0	10,589	27	13.4
Monophasic Typhimurium 1.4.[5].12:i:-(*)	6,491	18	8.2	6,427	17	8.1	6,322	16	8.0
<b>Infantis(*)</b>	<b>1,924</b>	<b>26</b>	<b>2.4</b>	<b>1,859</b>	<b>26</b>	<b>2.3</b>	<b>1,803</b>	<b>26</b>	<b>2.3</b>
Newport	870	24	1.1	1,086	21	1.4	920	24	1.2
Derby	721	23	0.9	710	23	0.9	612	23	0.8
Stanley	560	19	0.7	521	22	0.7	554	21	0.7
Kentucky	545	24	0.7	663	22	0.8	617	19	0.8
Napoli	508	18	0.6	457	15	0.6	406	17	0.5
Agona	503	20	0.6	602	18	0.8	645	20	0.8
Virchow(*)	477	21	0.6	541	24	0.7	510	21	0.6
Coeln	455	18	0.6	443	20	0.6	265	21	0.3



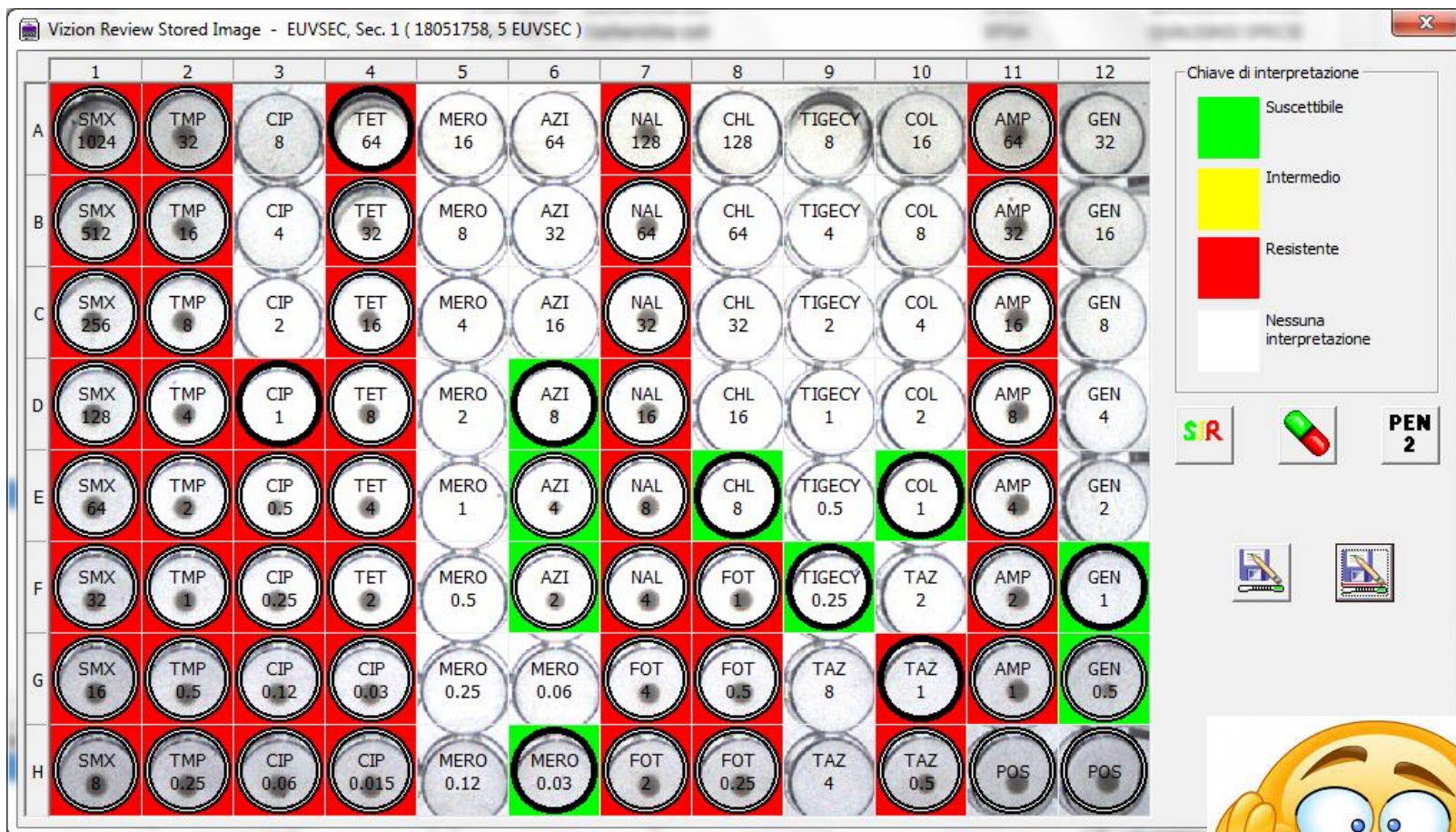
<https://www.ecdc.europa.eu/en/publications-data/european-union-one-health-2019-zoonoses-report>







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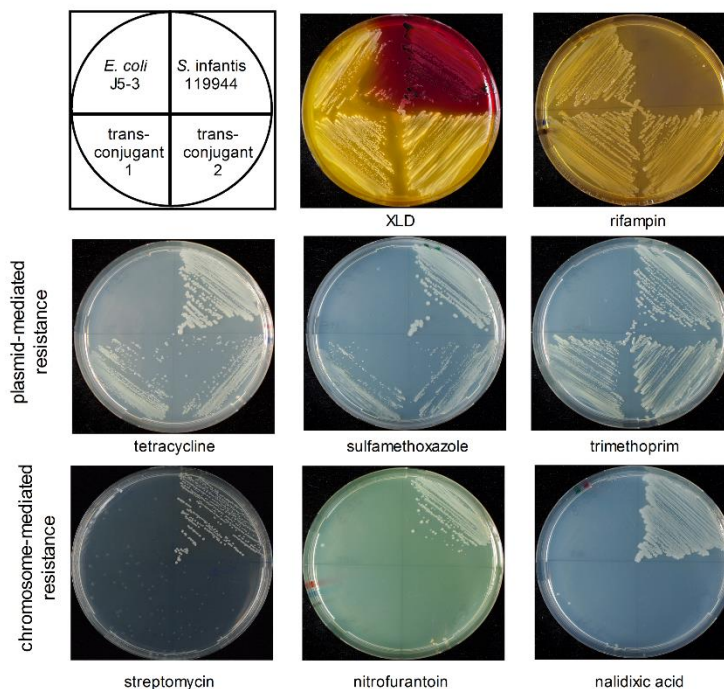
## A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent *Salmonella enterica* serovar Infantis strain

Gili Aviv, Katherine Tsyba, Natalie Steck, Mali Salmon-Divon, Antie Cornelius, Galia Rahav, Guntram A. Grassl, Ohad Gal-Mor 

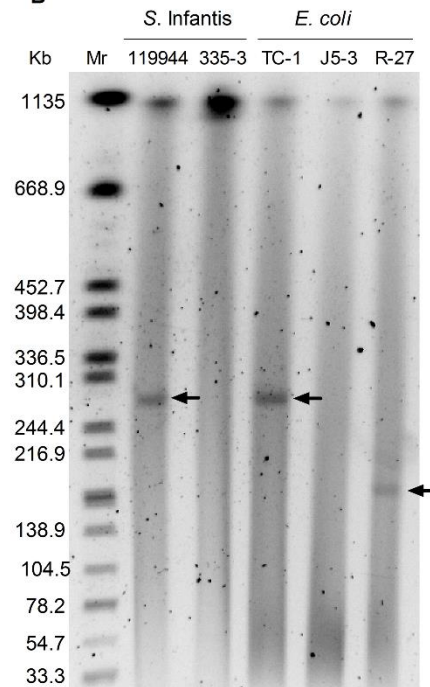
Descrivono un megaplasmide  
plasmide emergente: pESI

- Circa 300 Kb
- Contiene tet, sul, dfr
- qacE, operon mer
- Yersiniobactin (sideroforo)
- T/AT systems

A



B







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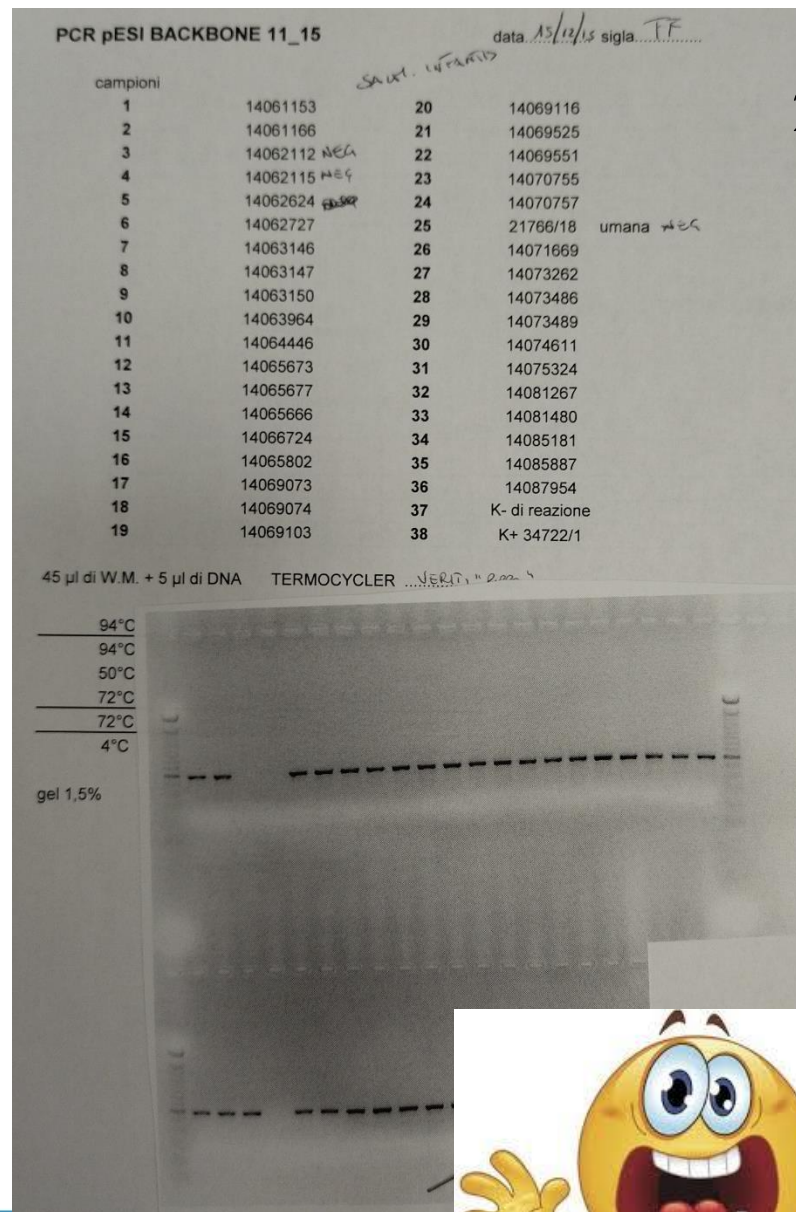
# E se in Italia circolasse una cosa simile?

→ Prove indirette

PCR dei markers di pESI  
(backbone, k88, Fim)

positive

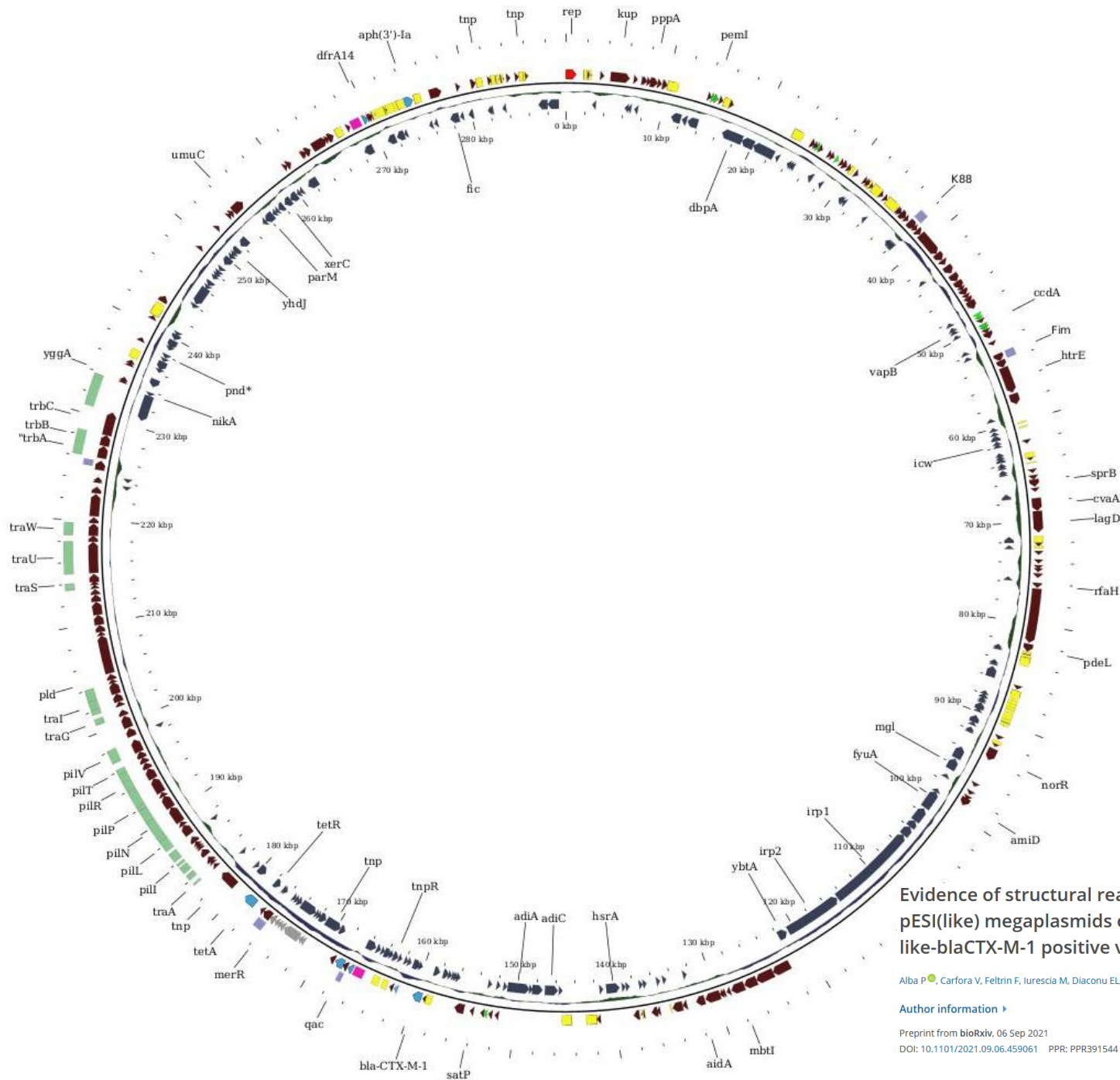
Fenotipicamente resistenti a:  
TET, SUL, STX, FOT  
Grande plasmide circolare  
... e trasferibile



2014



2021



Evidence of structural rearrangements and variability in pESI(like) megaplasmids of *S. Infantis* by resolving the pESI-like-blaCTX-M-1 positive variant

Alba P, Carfora V, Feltrin F, Iurescia M, Diaconu EL, Cordaro G, Dell'Aira E, Marani I, Giacomini A, Franco A, Battisti A

Author information

Preprint from bioRxiv, 06 Sep 2021

DOI: 10.1101/2021.09.06.459061 PPR: PPR391544

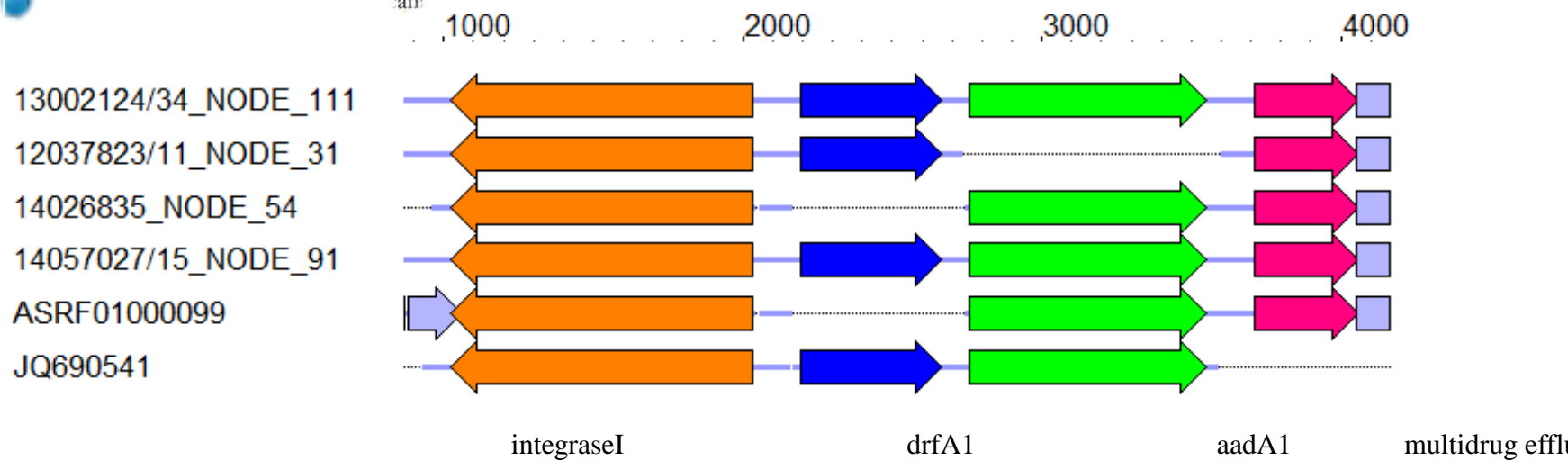


Questo megaplasmide è stato nominato pESI-like perché è una cosa simile ma non uguale...

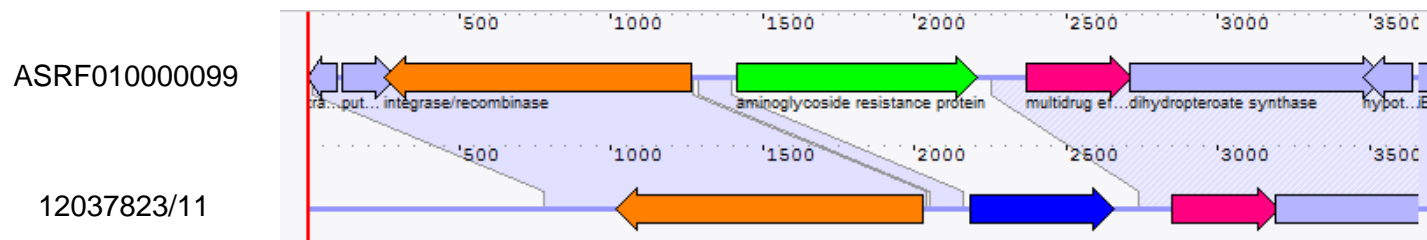
Presenza di *bla*<sub>CTX-M-1</sub> → Resistenti a cefalosporine di spettro esteso

Presenza di geni *dfr* diversi (*dfr1* o *dfr14*), probabilmente in posizione diversa





B



C

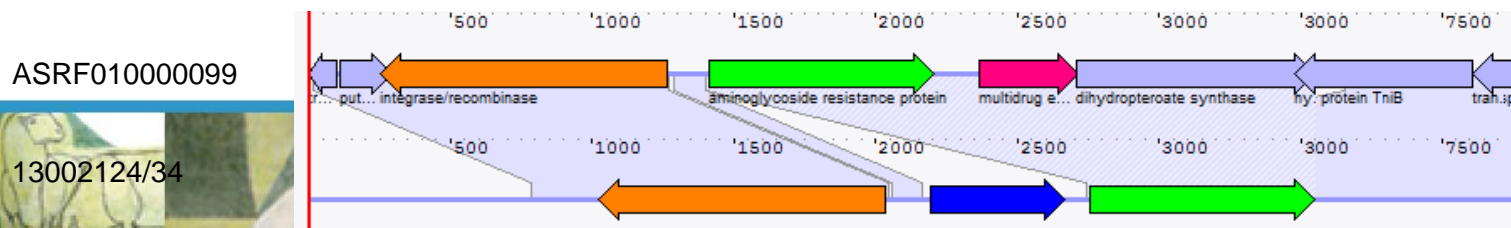
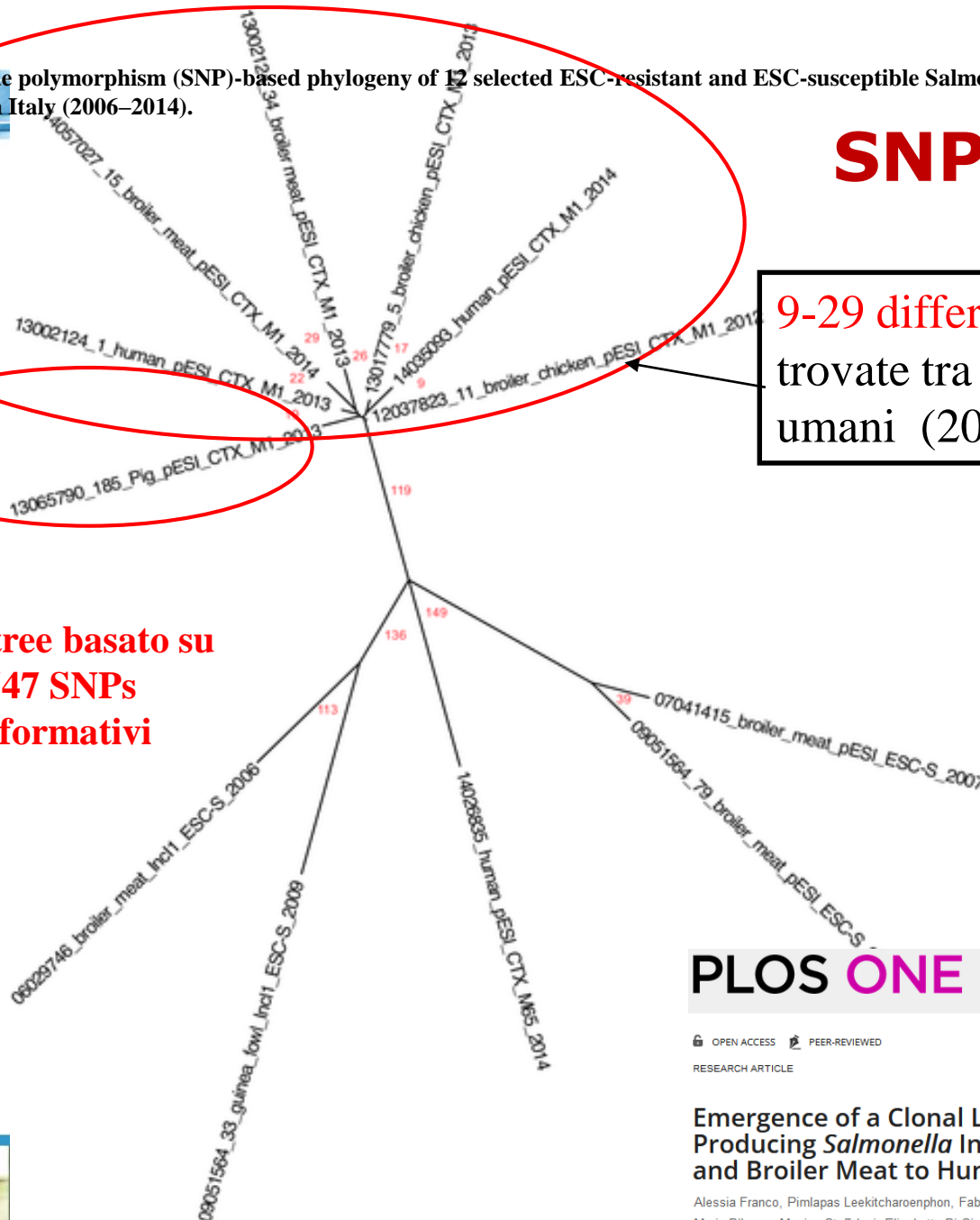


Fig 3. Single-nucleotide polymorphism (SNP)-based phylogeny of 12 selected ESC-resistant and ESC-susceptible *Salmonella* *Infantis* from poultry, meats, and humans, in Italy (2006–2014).

# SNPs Filogenia

9-29 differenze di SNP trovate tra isolati di pollo e umani (2012-2014)

SNP-tree basato su 547 SNPs informativi



PLOS ONE

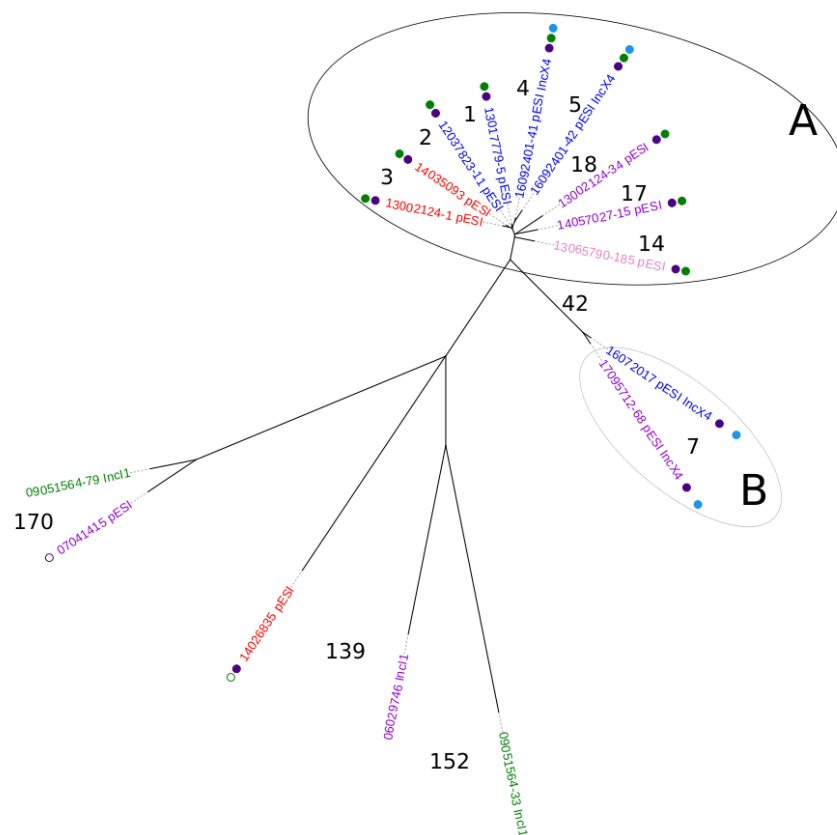
OPEN ACCESS PEER-REVIEWED  
RESEARCH ARTICLE

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

Alessia Franco, Pimlapas Leekitcharoenphon, Fabiola Feltrin, Patricia Alba, Gessica Cordaro, Manuela Iurescia, Rita Tolli, Mario D'Incau, Monica Staffolani, Elisabetta Di Giannatale, Rene S. Hendriksen, Antonio Battisti

# Incorporazione di altri geni di resistenza<sup>2018</sup>

Tree scale: 0.1



**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**

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

Virginia Carfora<sup>1</sup>, Patricia Alba<sup>1</sup>, Pimlapas Leekitcharoenphon<sup>2</sup>, Daniele Ballarò<sup>1</sup>, Gessica Cordaro<sup>1</sup>, Paola Di Matteo<sup>1</sup>, Valentina Donati<sup>1</sup>, Angela Ianzano<sup>1</sup>, Manuela Iurescia<sup>1</sup>, Fiorentino Stravino<sup>1</sup>, Tania Tagliaferri<sup>1</sup>, Antonio Battisti<sup>1\*</sup> and Alessia Franco<sup>1</sup>



E anche in Europa?

**Establishing Next Generation  
sequencing Ability for Genomic analysis  
in Europe – ENGAGE (EFSA call:  
GP/EFSA/AFSCO/2015/01)**



Denmark	56
Finland	3
Germany	38
Ireland	19
Italy	166
Luxembourg	17
Netherlands	26
Poland	16
United Kingdom	31
TOTAL	382

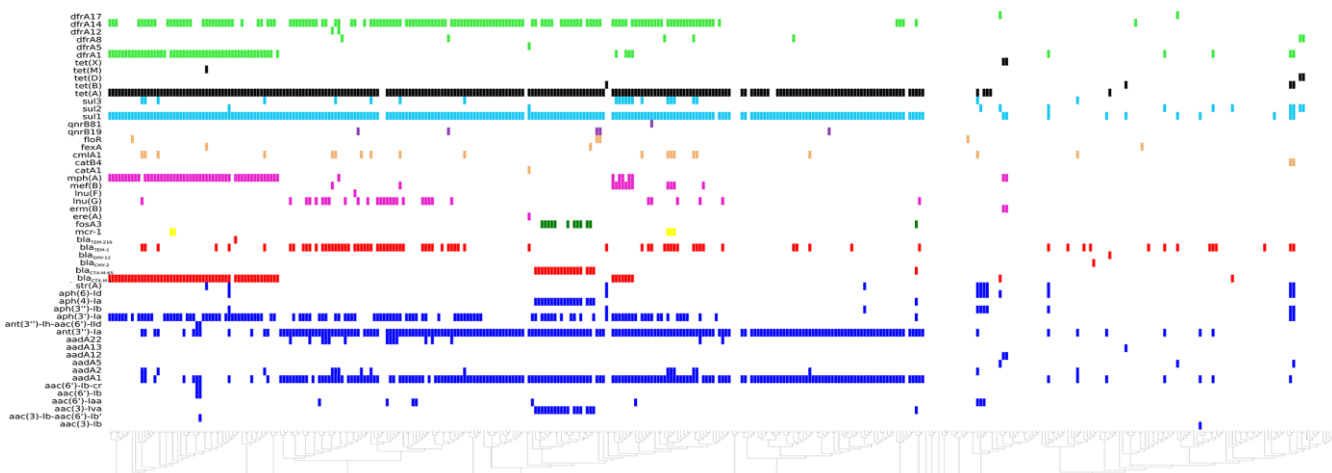
- Proof-of-concept: “**Salmonella  
Infantis in Italy and EU:  
phylogeny and plasmid  
carrying virulence, fitness and  
antimicrobial resistance (AMR)  
genes**”, headed by IZSLT in  
collaboration with DTU.





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# AMR fenotipo e genotipo



- trimethoprim
- tetracyclines
- sulphonamides
- phenicols
- macrolides
- fosfomycin
- polymyxins
- ESBL/AmpC genes
- aminoglycosides.

<b>MDR (3+R) S. Infantis</b>	<b>70.80%</b>	
concomitant resistance to TET, SUL and TMP	50.10%	
ESC-R	23.20%	17.2% <i>bla</i> <sub>CTX-M-1</sub>
		5.4% <i>bla</i> <sub>CTX-M-65</sub>
		0.26% <i>bla</i> <sub>CMY-2</sub>
		0.26% <i>bla</i> <sub>SHV-12</sub>
at least one gene encoding macrolide resistance ( <i>mph</i> , <i>mef</i> , <i>erm</i> (B)).	17.8 %	
(fluoro)quinolones resistance mediated by <i>gyrB</i> mutation	65.70%	43.6% D87G
		7.3% D87Y
		14.36% S83Y

# Molecular typing and characterization of pESI-like plasmid

pESI-like plasmid positives <i>S. Infantis</i>		63.90%
from 10 European countries:	60.70%	Denmark, Germany, Hungary, Italy, Luxembourg, Netherlands, Poland, Romania, UK and Ukraine.
same IncI1 pMLST profile ( <i>ardA2</i> , <i>pilL3</i> , <i>sogS9</i> , <i>trbA21</i> , <i>rep</i> absent)	55.80%	
pESI-like positives:		
T/AT system PemI/K	100%	
T/AT system CcdA/B	99.60%	
T/AT system HicA/B	17.60%	carried by IncX4 (associated to <i>bla</i> <sub>TEM-1</sub> )
<i>merA</i>	96.30%	
<i>qacEΔ</i>	80.90%	

Presenza di pESI-like è stata determinata utilizzando blast v2.2.31 (specific pESI markers), plasmidFinder e pMLST (CGE)

## 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<sup>1</sup>, Pimlapas Leekitcharoenphon<sup>2</sup>, Virginia Carfora<sup>1</sup>, Roberta Amoroso<sup>1</sup>, Gessica Cordaro<sup>1</sup>, Paola Di Matteo<sup>1</sup>, Angela Ianzano<sup>1</sup>, Manuela Iurescia<sup>1</sup>, Elena L. Diaconu<sup>1</sup>, ENGAGE-EURL-AR Network Study Group<sup>3</sup>, Susanne K. Pedersen<sup>2</sup>, Beatriz Guerra<sup>4</sup>, Rene S. Hendriksen<sup>2</sup>, Alessia Franco<sup>1</sup>, Antonio Battisti<sup>1</sup>

Tree scale: 0.01

#### Sample type

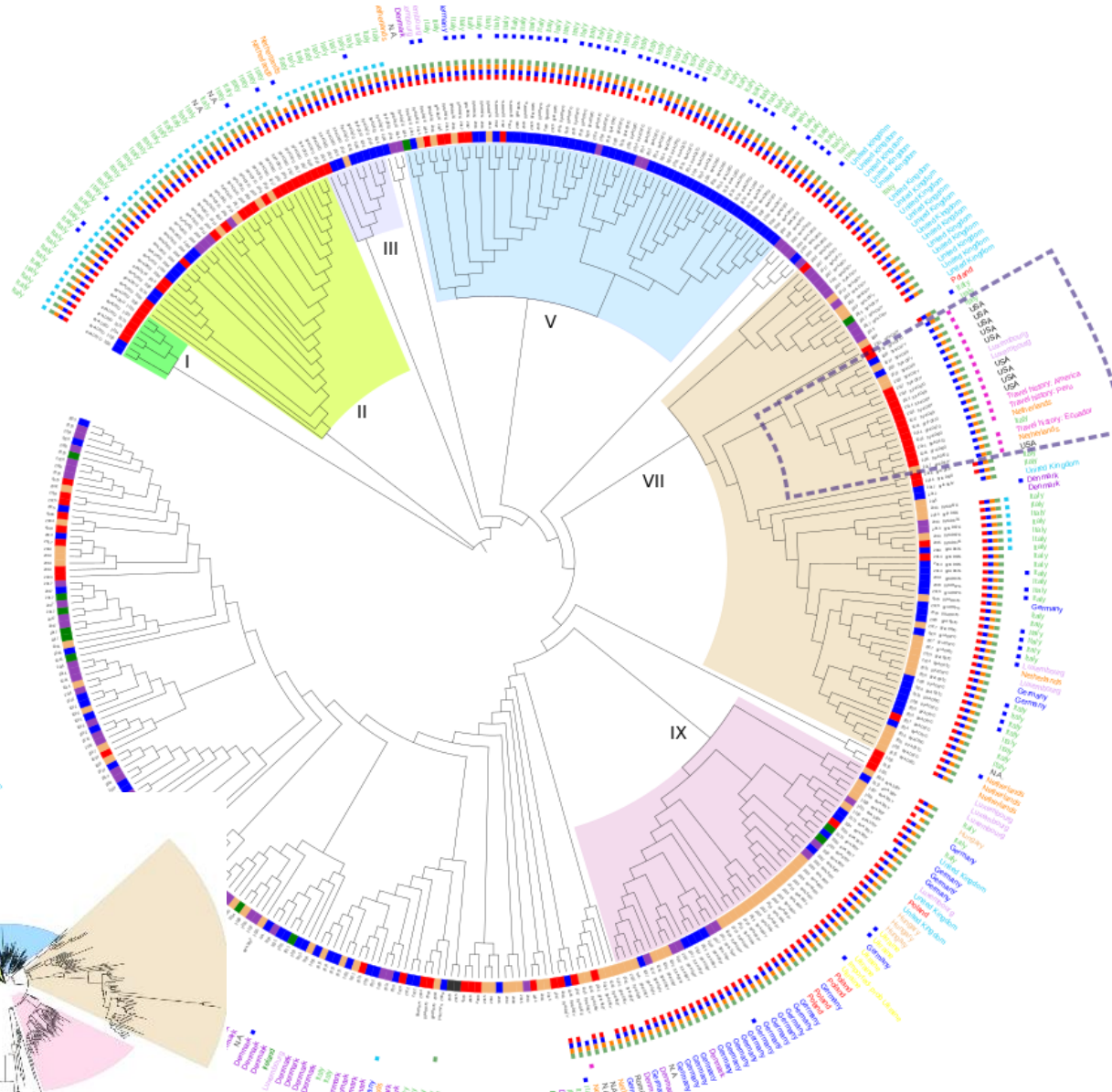
- Human
- Animal
- Food
- Environment
- Feed
- Other

#### pESI determinants

- backbone pESI
- incP
- K88
- Fim

#### beta-lactam resistance genes

- blaCTX-M-1
- blaCTX-M-65
- blaCMY-2
- blaSHV-12
- blaTEM



**MICROBIAL GENOMICS** Volume 6, Issue 5

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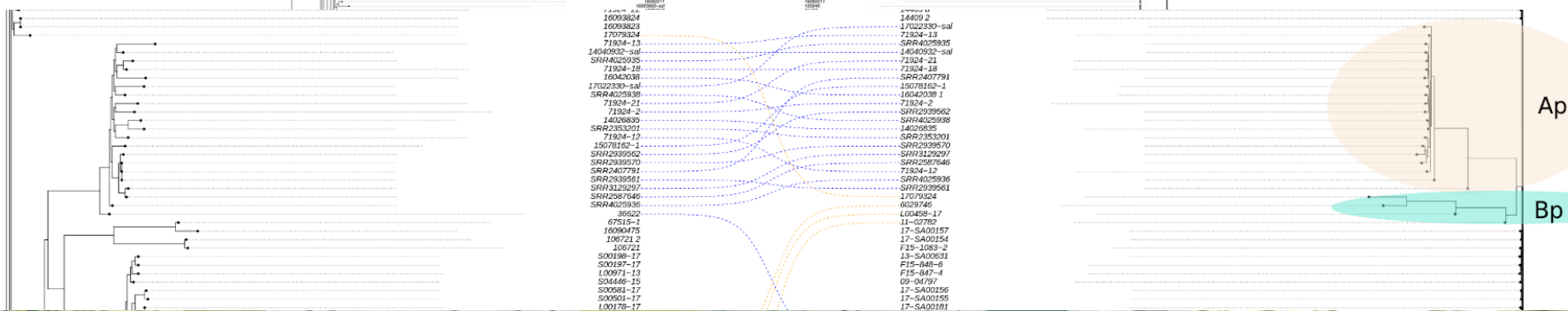
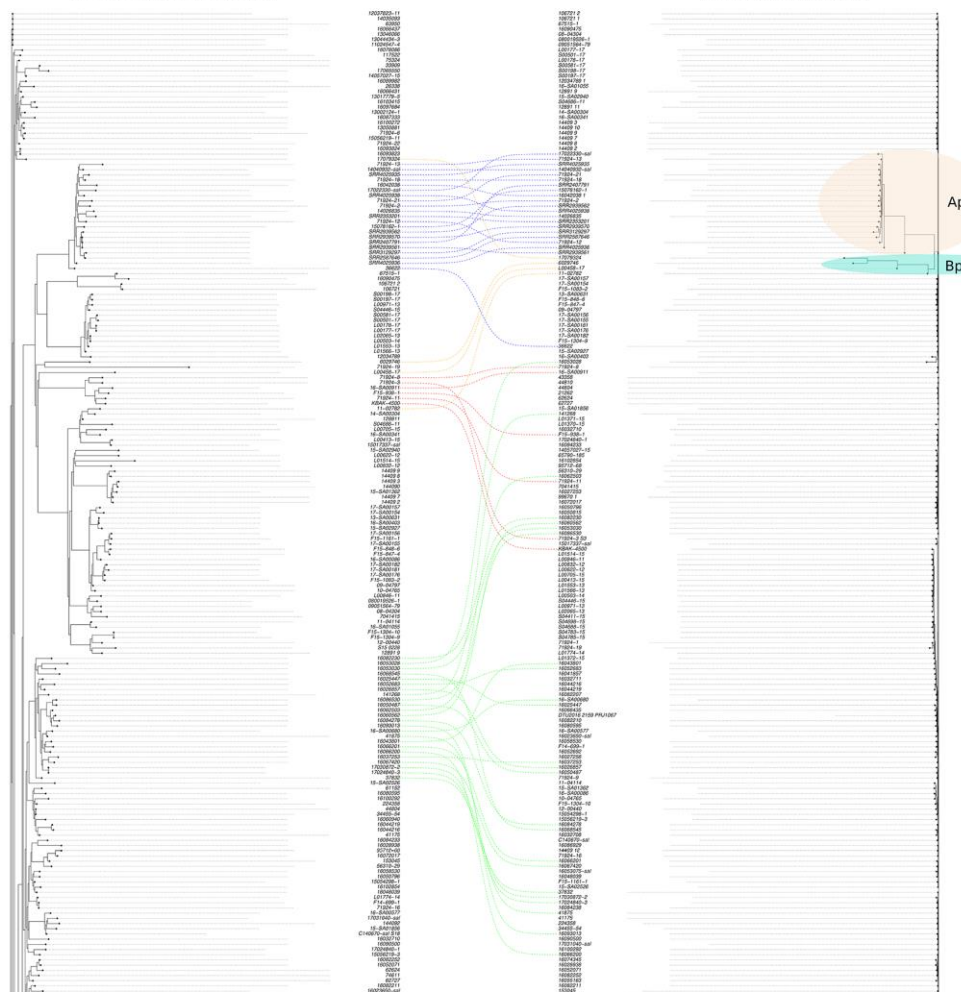
## Molecular epidemiology of *Salmonella* Infantis in Europe: insights into the success of the bacterial host and its parasitic pESI-like megaplasmid

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



Chromosome based reference

Plasmid based reference





# 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

Alessia Franco, Pimlapas Leekitcharoenphon, Fabiola Feltrin, Patricia Alba, Gessica Cordaro, Manuela Iurescia, Rita Tolli, Mario D'Incau, Monica Staffolani, Elisabetta Di Giannatale, Rene S. Hendriksen, Antonio Battisti

Ma quanto siamo sicuri che *bla*<sub>CTX-M-1</sub> sta su pESI-like?  
Contig < 300 kb

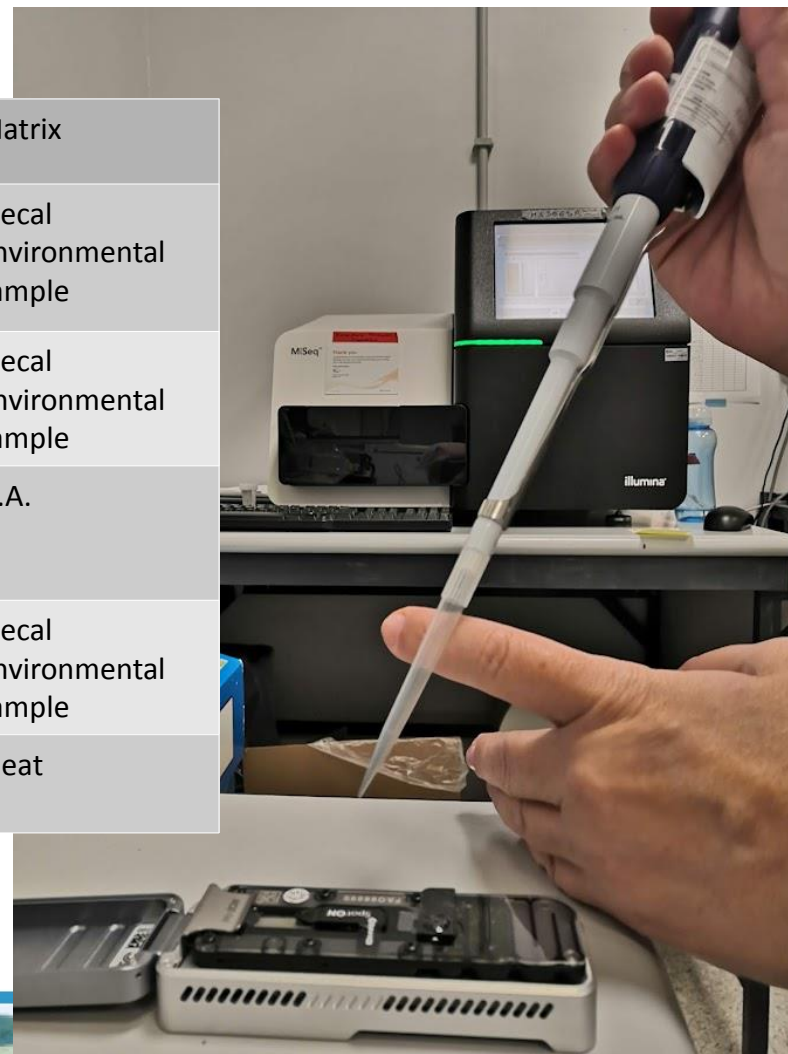
Esperimento di Trasmissione del carattere “di interesse”: Abbiamo fatto prove di coniugazione nel 2015 → *E. coli* riceventi sensibili a FOT (cefotaxime), diventarono resistenti e positivi ai marker genici di pESI (incluso *bla*CTX-M-1) di *Salmonella* Infantis “donatrici”

positive	Sample ID	12034722/1		12037823/11	
		Salmonella infantis	Transconjugant E. coli K12	Salmonella infantis	Transconjugant E. coli K12
aminoaminoglycoside resistance	aadA1_1	negative	negative	negative	negative
trimethoprim resistance	dfrA1_21	positive	positive	positive	positive
trimethoprim resistance	dfrA14_21	positive	positive	positive	positive
sulphonamide resistance	sul1_11	positive	positive	positive	positive
tetracycline resistance	tetA_11	positive	positive	positive	positive
beta lactam resistance	ctxM1_11	positive	positive	positive	positive

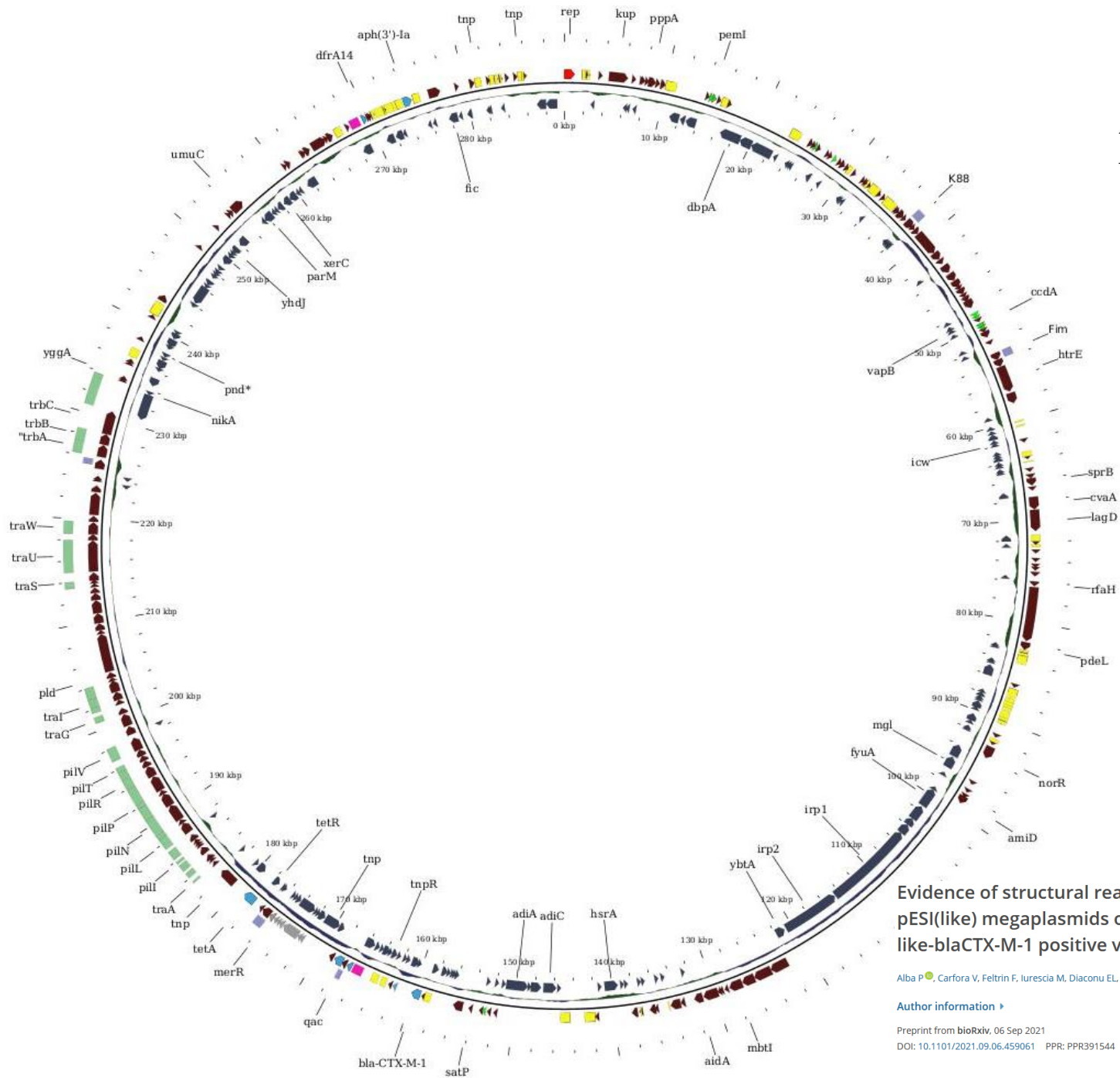


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Isolate ID	ENA acc. n	Country	Year	Origin	Matrix
12037823	ERR1014117	Italy	2012	Broiler chicken	faecal environmental sample
13065790	ERS2030348	Italy	2013	Broiler chicken	faecal environmental sample
14026835	ERR1014119	Travel history America	2014	Human, Diagnostic	N.A.
16092401	ERS2521096	Italy	2016	Broiler chicken	faecal environmental sample
11024547	ERS2030359	Italy	2011	Meat from pigs	meat



pESI-like



### Evidence of structural rearrangements and variability in pESI(like) megaplasms of *S. Infantis* by resolving the pESI-like-blaCTX-M-1 positive variant

Alba P , Carfora V, Feltrin F, Iurescia M, Diaconu EL, Cordaro G, Dell'Aira E, Marani I, Giacomini A, Franco A, Battisti A

[Author information](#) ▶

Preprint from bioRxiv, 06 Sep 2021

DOI: 10.1101/2021.09.06.459061 PPR: PPR391544

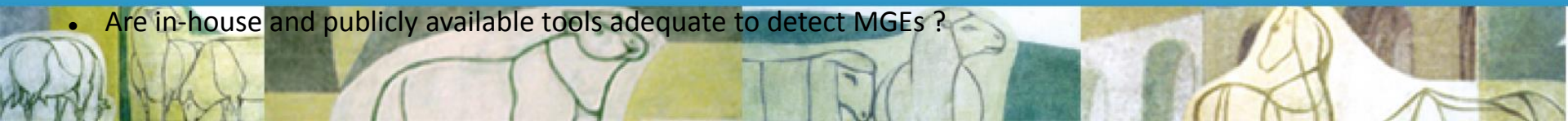


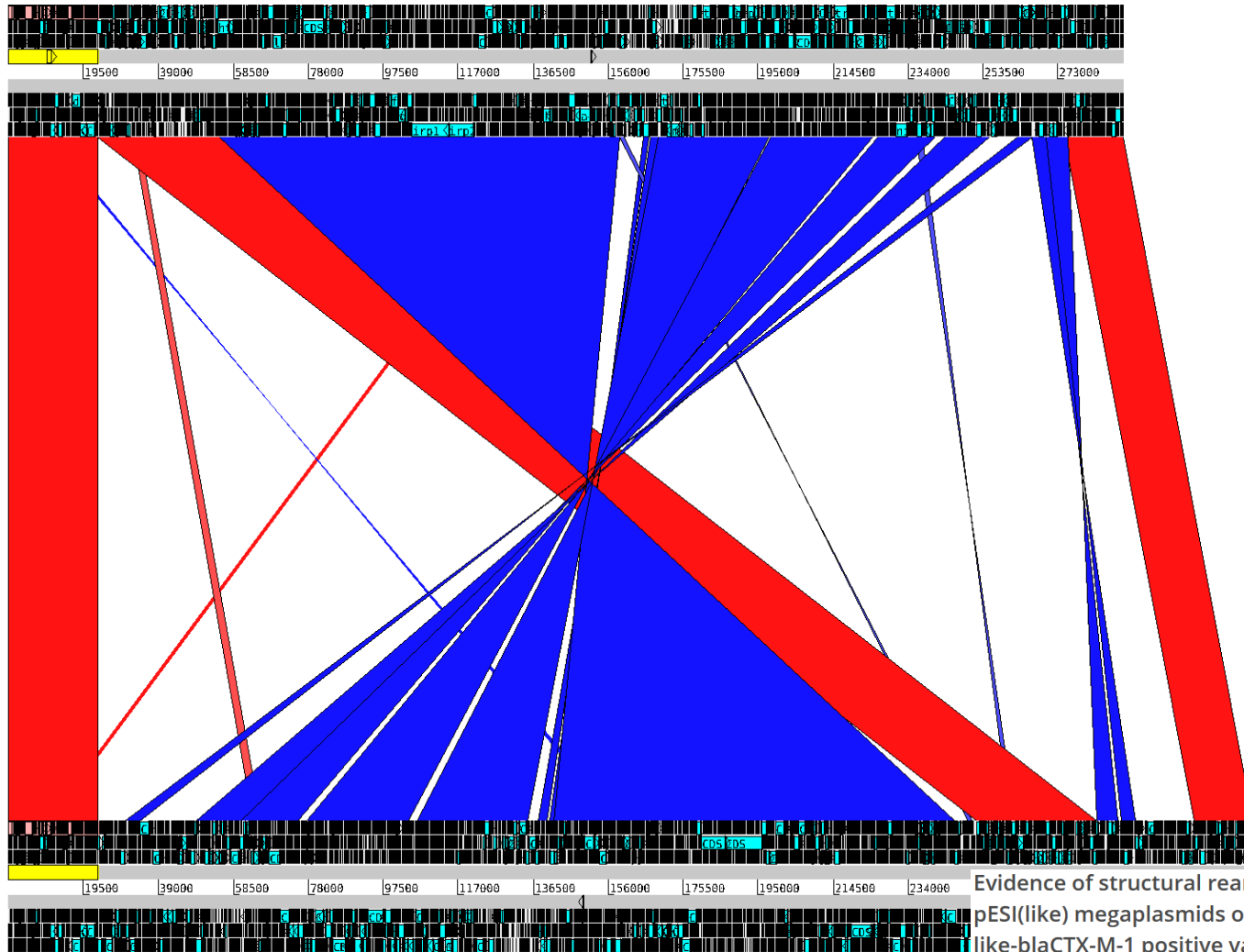
## Y en Europa?

Progetto EJP-One Health: Full-length sequencing for an enhanced EFFORT to map and understand drivers and reservoirs of antimicrobial resistance(FULL\_FORCE)

To apply this technology on a selection of multidrug resistant (MDR) *E. coli* strains collected from the EFFORT/COMPARE/ARDIG programs, and to Enterobacteriaceae resistant to critically important antimicrobials isolated in the context of different national monitoring programmes. More specifically, we will target the following research questions:

- How do MGEs evolve in longitudinal sample sets?
- Which genetic environment is associated with most common AMR in indicator *E. coli*?
- Do we find novel MGEs in *Klebsiella pneumoniae*, and would it mean this bacterium is a superior indicator organism?
- Which MGE are responsible for increasing resistance in horses?
- **What might explain the evolutionary success of MDR *Salmonella enterica* serovars Infantis and Kentucky in the EU?**
- Are in-house and publicly available tools adequate to detect MGEs ?







## Take home message:

- monitoraggio attivo serve per rilevare “anomalie” ed occorrenze non favorevoli
- aggiornato con le conoscenze: le “anomalie” bisogna studiarle nel contesto di conoscenza del momento, e con gli strumenti disponibili del momento
- aggiornamento continuo delle tecniche senza trascurare le informazioni (sia fenotipiche, che molecolari, per quanto “parziali”) acquisite con strumenti precedenti
- pESI di *S. Infantis* è un megaplasmide complesso e flessibile... con grande capacità di acquisizione di geni di resistenza, virulenza e aumento della fitness ed è molto difficile da eliminare.  
Conferisce vantaggio selettivo indubbio ai cloni di *S. Infantis* che infetta “nel contesto del sistema produttivo attuale”
- Fenotipo più frequente di **MDR *S. Infantis***: TET, SUL, TMP, FOT, TAZ, FQ



**Ringrazio a tutti i colleghi della  
D. O. Diagnostica Generale e CRAB (IZSLT),  
che hanno reso possibile questo lavoro**

Antonio Battisti  
Alessia Franco  
Andrea Caprioli  
Virginia Carfora  
Roberta Amoroso  
Francesco Bottoni  
Carmela Buccella  
Tamara Cerci  
Gessica Cordaro  
Elena dell'Aira  
Elena L. Diaconu  
Paola Di Matteo  
Valentina Donati  
Fabiola Feltrin  
Angelo Giacomi

Angela Ianzano  
Manuela Iurescia  
Serena Lorenzetti  
Ilaria Marani  
Roberta Onorati  
Luigi Sorbara  
Fiorentino Stravino

