



IZSAM G. CAPORALE  
TERAMO



*Campylobacter*

Laboratorio Nazionale di Riferimento

## Diversità genetica del *Campylobacter* spp. negli uccelli selvatici

Francesca Marotta

Teramo, 13 dicembre 2017

Centro Internazionale per la Formazione e l'Informazione Veterinaria "Francesco Gramenzi"







Ospite primario del *Campylobacter*  
reservoir  
trasportatori

RISCHIO PER LA SALUTE UMANA E ANIMALE

## Dati contraddittori in letteratura

**THE LANCET**  
Online First | Current issue | All issues | Special issues | Multimedia | Information for Authors

Journal List > Appl Environ Microbiol > v.80(15); 2014 Aug > PMC4148789

< Previous

**WATER-  
GASTRO**

S.R. Palmer, P. Penner  
Published: 05 Fri  
Plum:XM:Mi

Applied Environmental Microbiology

environmental microbiology reports  
Environmental Microbiology Reports (2015) 7(5), 782-788

**Multilocus *Campylobacter jejuni* Peas**

Patrick S. L. Gardner, Pat

Alison J. Cody,<sup>1\*</sup> Noel D. McCarthy,<sup>1,2,3,4</sup> James E. Bray,<sup>1</sup> Helen M. L. Wimalaratne,<sup>1</sup> Frances M. Colles,<sup>1</sup> Melissa J. Jansen van Rensburg,<sup>1</sup> Kate E. Dingle,<sup>1</sup> Jonas Waldenström<sup>5</sup> and Martin C. J. Maiden<sup>1\*</sup>  
<sup>1</sup>Department of Zoology, University of Oxford, Oxford, UK

wild bird species representing a persistent source of campylobacteriosis, and contributing a distinctive seasonal pattern to disease burden. If Oxfordshire is representative of the UK as a whole in this respect, these data suggest that the national burden of wild bird-attributed isolates could be in the order of 10 000 annually.

**PLOS ONE** | A Peer-Reviewed Open Access Journal

Genomic Characterization of *Campylobacter jejuni* Strain M1

Genetic diversity of *Campylobacter jejuni* strains isolated from wild birds in Oxfordshire, UK

Prevalence and Pathogenic Potential of *Campylobacter jejuni* in Human, Commercial American Crows

Major Structural Differences and Novel Genomes of Multiple *Campylobacter* Species

Dynamics of *Campylobacter jejuni* in European host, *Sturmus vulgaris*

Prevalence and Pathogenic Potential of *Campylobacter jejuni* in Human, Commercial American Crows

Major Structural Differences and Novel Genomes of Multiple *Campylobacter* Species

# Materiali e Metodi-1 parte

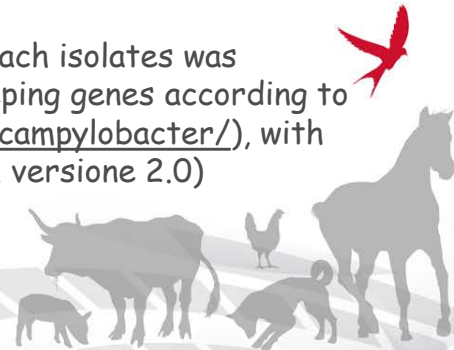
## 67 wb

M.G. CAPORALE  
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obacter  
stituto Nazionale di Riferimento

- Il DNA genomico è stato estratto usando il Maxwell
- Sequencing libraries were prepared with a Nextera XT DNA sample preparation kit (Illumina, Inc., San Diego, CA)
- *C. jejuni* sono stati sequenziati con Illumina NextSeq 500 e assemblati de novo with SPAdes 3.8.1.
- The quality control of the filtered reads was performed using the FastQC software
- Depth of coverage > 30 (30X) was required to ensure sequence quality.
- **MLST typing** è stata eseguita *in silico* (MLST of each isolates was performed using the sequences of the 7 housekeeping genes according to the scheme from PubMLST (<https://pubmlst.org/campylobacter/>), with the software srst2 (Short Read Sequence Typing, versione 2.0)

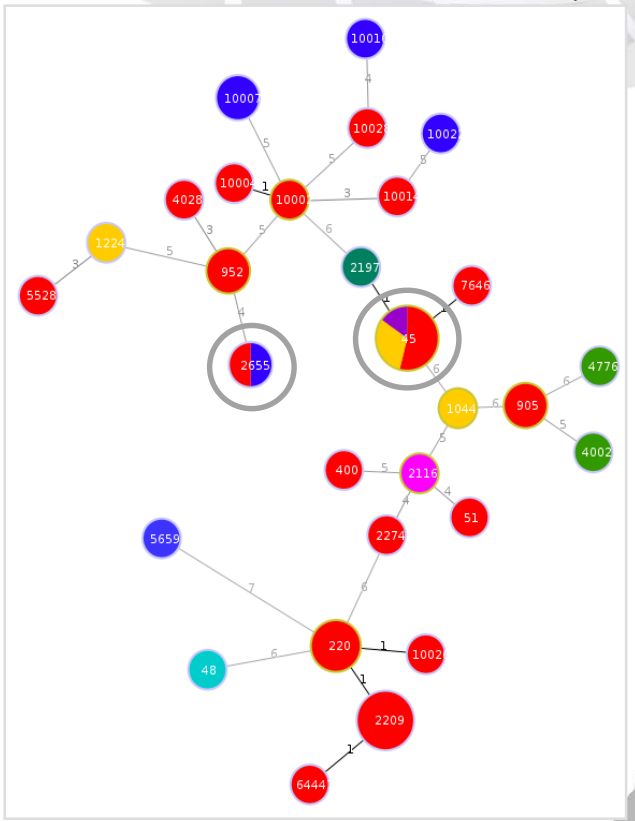
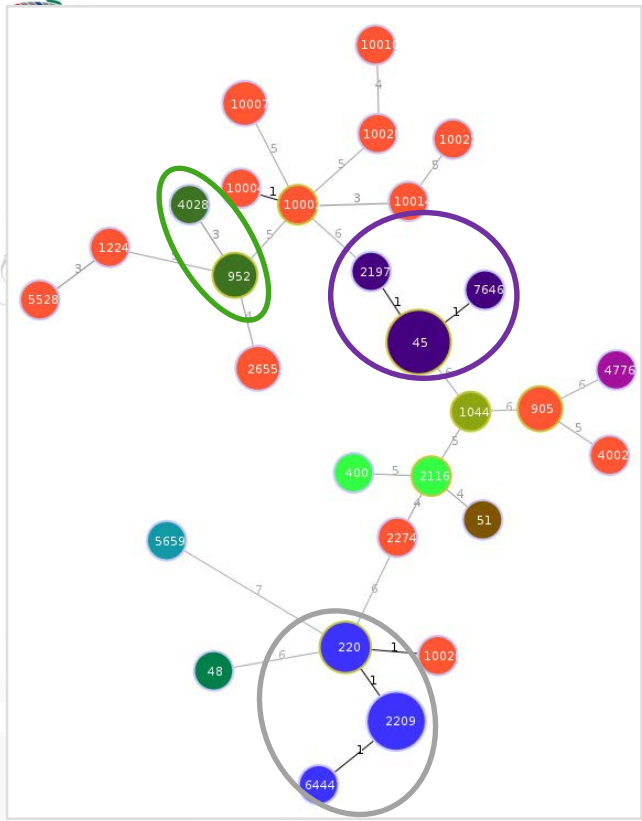


# Wild birds italiani-CCs

# Wild birds italiani-specie

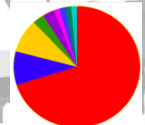
Phyloviz 2.0

Phyloviz 2.0



- CC:179 ( n = 25, 37.31% )
- CC: ( n = 17, 25.37% )
- CC:45 ( n = 15, 22.39% )
- CC:952 ( n = 3, 4.48% )
- CC:353 ( n = 2, 2.99% )
- CC:443 ( n = 1, 1.49% )
- CC:48 ( n = 1, 1.49% )
- CC:658 ( n = 1, 1.49% )
- CC:692 ( n = 1, 1.49% )
- CC:828 ( n = 1, 1.49% )

goeBURST full minimum spanning tree dei STs di Campylobacter isolati da selvatici

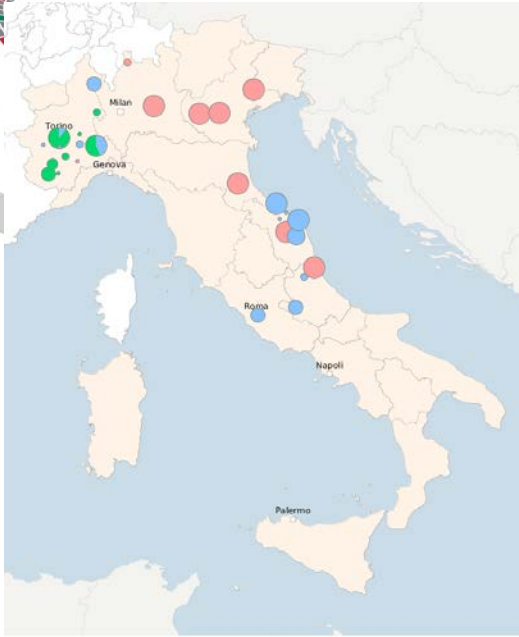


- piccione ( n = 47, 70.15% )
- corvi ( n = 6, 8.96% )
- gazza ( n = 6, 8.96% )
- germano reale ( n = 2, 2.99% )
- verdone ( n = 2, 2.99% )
- ballerina bianca ( n = 1, 1.49% )
- fagiano ( n = 1, 1.49% )
- gufo ( n = 1, 1.49% )
- storno ( n = 1, 1.49% )



# IN ALLEVAMENTO UOMO

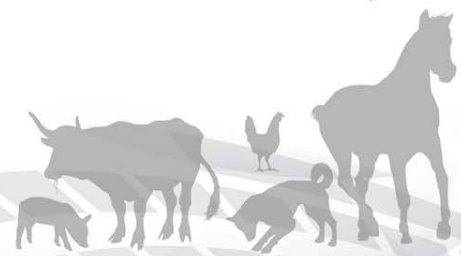




Color grouped by column "CC"

- ● ST-179 complex
- ST-206 complex
- ST-21 complex
- ST-22 complex
- ST-257 complex
- ST-283 complex
- ST-353 complex
- ST-354 complex
- ST-362 complex
- ST-42 complex
- ST-443 complex
- ST-446 complex
- ● ST-45 complex
- ST-460 complex
- ST-464 complex
- ST-48 complex
- ST-49 complex
- ST-52 complex
- ST-607 complex
- ST-61 complex
- ST-658 complex
- ST-677 complex
- ST-692 complex
- ST-952 complex
- no group assigned

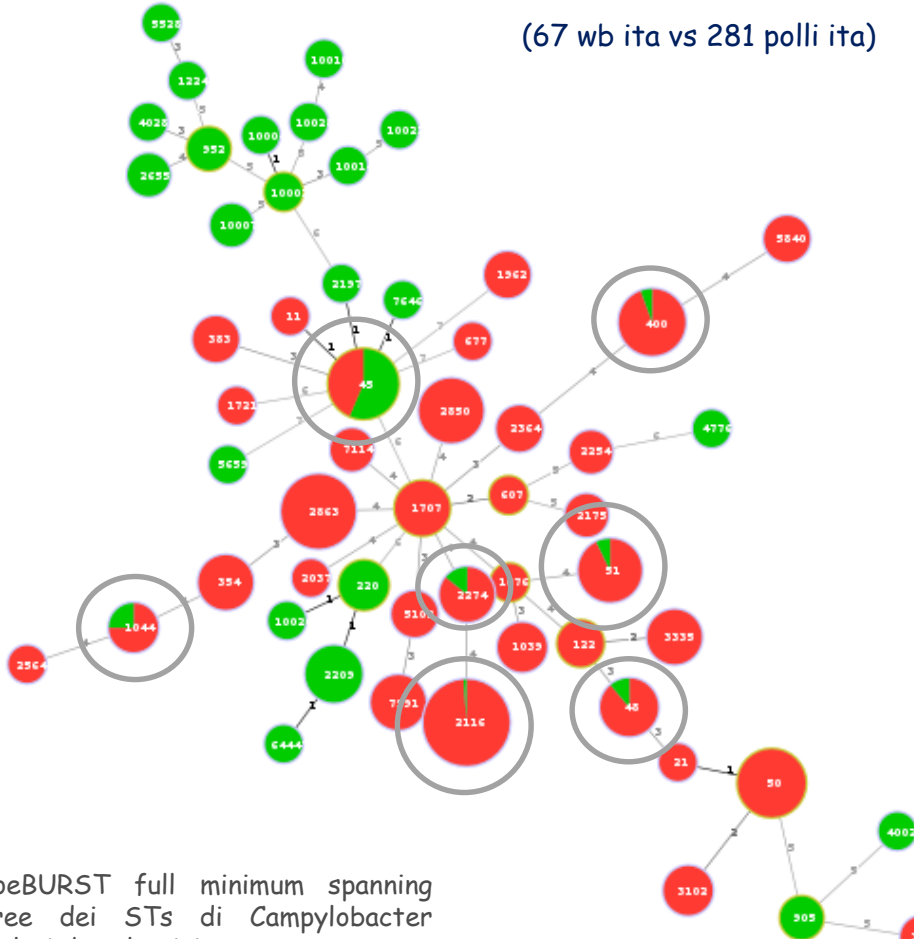
● Wild birds N= 67  
 ● Human N= 51  
 ● Chicken N= 281





# Wild birds italiani vs polli italiani

(67 wb ita vs 281 polli ita)



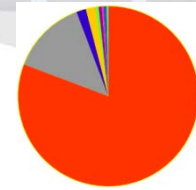
Legend	
<span style="color: red;">■</span>	Source: CHICKEN ( n = 281, 80.7
<span style="color: green;">■</span>	Source: wild bird ( n = 67, 19.25

goeBURST full minimum spanning tree dei STs di Campylobacter isolati da selvatici

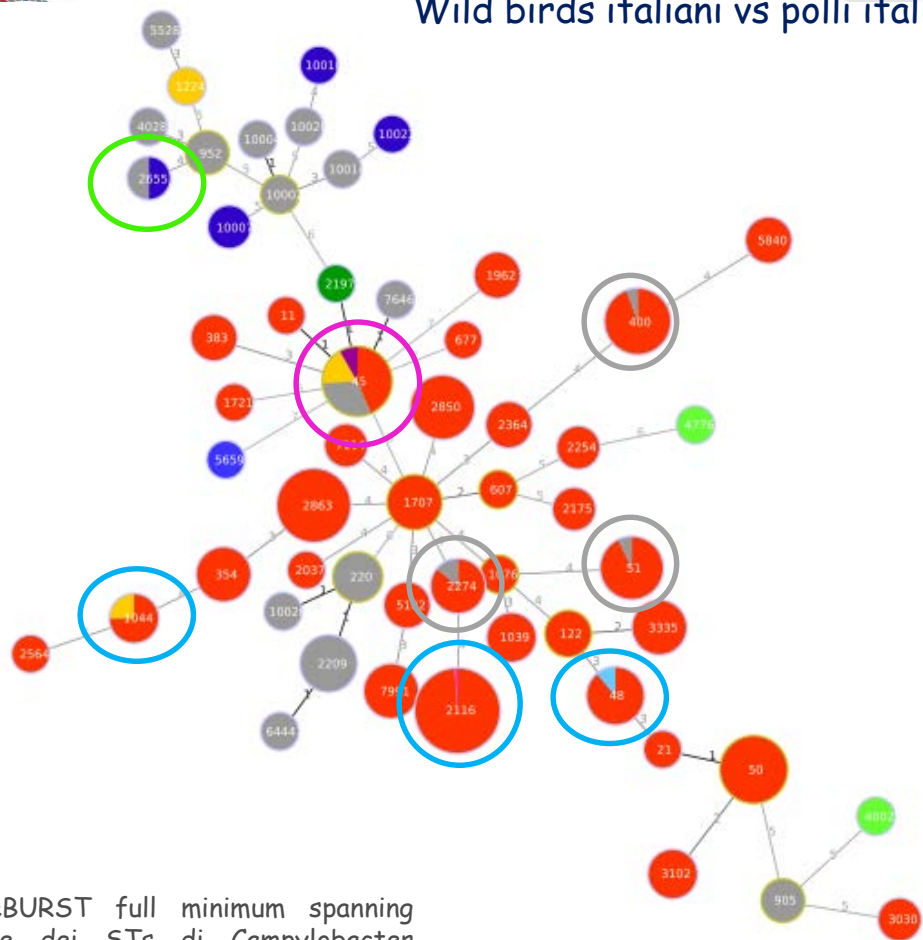




# Wild birds italiani vs polli italiani



- Epi Info:POULTRY ( n = 281, 80.0%)
- Epi Info:piccione ( n = 47, 13.51%)
- Epi Info:corvi ( n = 6, 1.72%)
- Epi Info:gazza ( n = 6, 1.72%)
- Epi Info:germano reale ( n = 2, 0.57%)
- Epi Info:verdone ( n = 2, 0.57%)
- Epi Info: ballerina bianca ( n = 1, 0.29%)
- Epi Info: fagiano ( n = 1, 0.29%)
- Epi Info:gufo ( n = 1, 0.29%)
- Epi Info:storno ( n = 1, 0.29%)

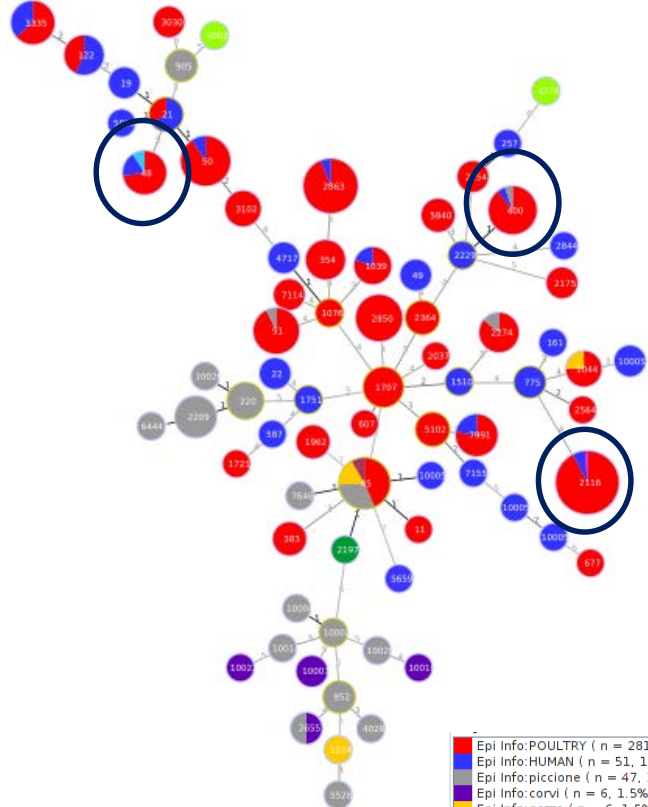
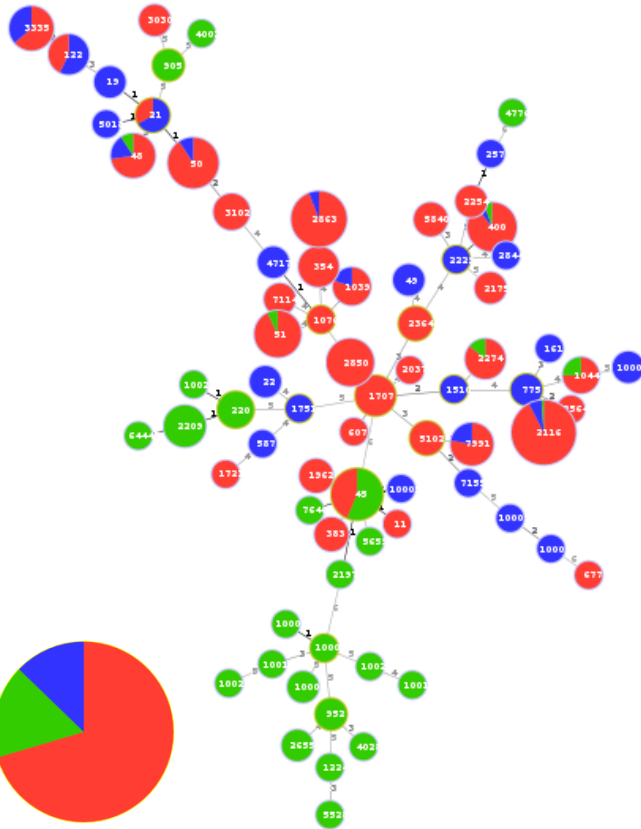


goeBURST full minimum spanning tree dei STs di Campylobacter isolati da selvatici



# Wild birds italiani vs polli italiani & uomo

(67 wb ita vs 281 polli ita vs 51 pazienti)

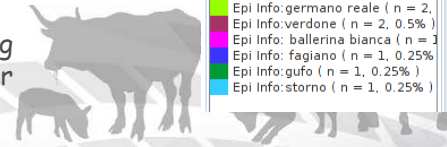


Legend

- Source:CHICKEN ( n = 281, 70.4
- Source:wild bird ( n = 67, 16.75
- Source:Human ( n = 51, 12.78%

goeBURST full minimum spanning tree dei STs di Campylobacter isolati da selvatici

- Epi Info:POULTRY ( n = 281, 70.4
- Epi Info:HUMAN ( n = 51, 12.78%
- Epi Info:piccione ( n = 47, 11.76
- Epi Info:corvi ( n = 6, 1.5%
- Epi Info:gazza ( n = 6, 1.5%
- Epi Info:germano reale ( n = 2,
- Epi Info:verdone ( n = 2, 0.5%
- Epi Info: ballerina bianca ( n = 1
- Epi Info: fagiano ( n = 1, 0.25%
- Epi Info:gufo ( n = 1, 0.25%
- Epi Info:storno ( n = 1, 0.25%



# Materiali e Metodi-2 parte

67 wb

- Il DNA genomico è stato estratto usando il Maxwell
- Sequencing libraries were prepared with a Nextera XT DNA sample preparation kit (Illumina, Inc., San Diego, CA)
- *C. jejuni* sono stati sequenziati con Illumina NextSeq 500 e assemblati de novo with SPAdes 3.8.1.
- The quality control of the filtered reads was performed using the FastQC software
- Depth of coverage  $> 30$  (30X) was required to ensure sequence quality.
- **cgMLST** → 949 loci (MLST target definer di Ridom SeqSphere+.)



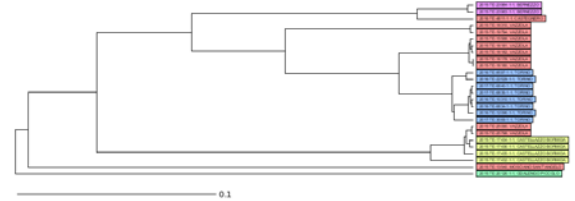
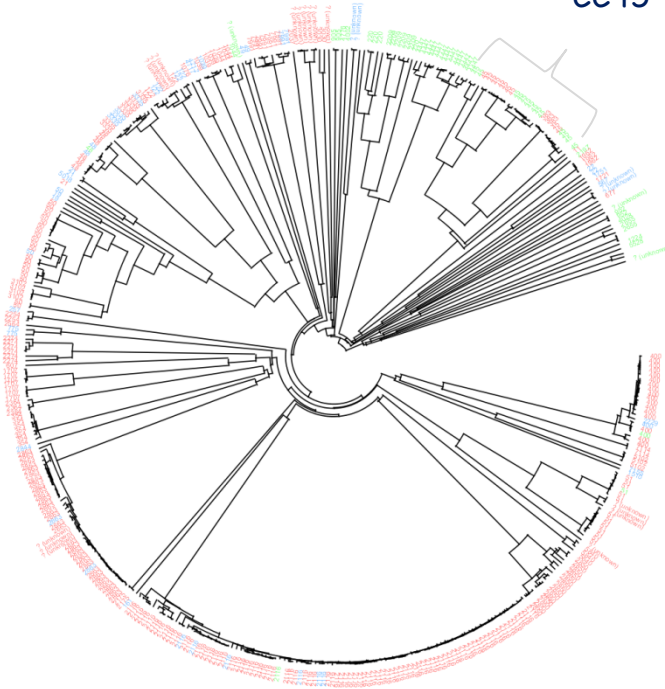
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CC45



cgMLST - CC45

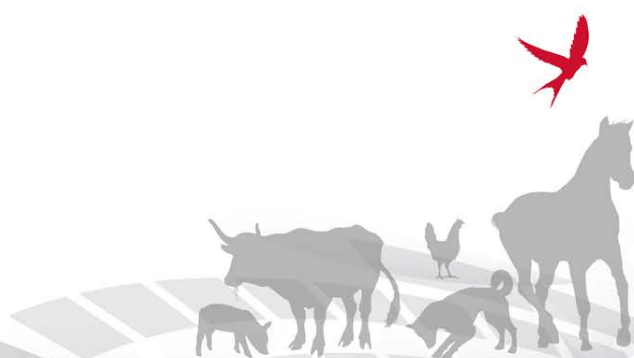


Projects: Campylobacter\_jejuni (Campylobacter jejuni/coli)  
Comparison Table Retrieval: Campylobacter\_jejuni [unstored]  
Task Templates: C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819, C. jejuni MLST, C. jejuni/coli MLST v1.1  
Comparison Table created: Nov 28, 2017 9:25 AM (v4.1.1\_(2017-08))  
Ridom SeqSphere+ UPGMA tree for 26 Samples based on 956 columns, pairwise ignoring missing values, % columns difference  
Distance based on columns from C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819 (949), C. jejuni MLST (7)

- CHICKEN
- GAZZA
- GLUFO
- PIGEON
- VERDONE

Projects: Campylobacter\_jejuni (Campylobacter jejuni/coli)  
Comparison Table Retrieval: Campylobacter\_jejuni [unstored]  
Task Templates: C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819, C. jejuni MLST, C. jejuni/coli MLST v1.1  
Comparison Table created: Nov 28, 2017 9:25 AM (v4.1.1\_(2017-08))  
Ridom SeqSphere+ UPGMA tree for 415 Samples based on 956 columns, pairwise ignoring missing values, % columns difference  
Distance based on columns from C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819 (949), C. jejuni MLST (7)

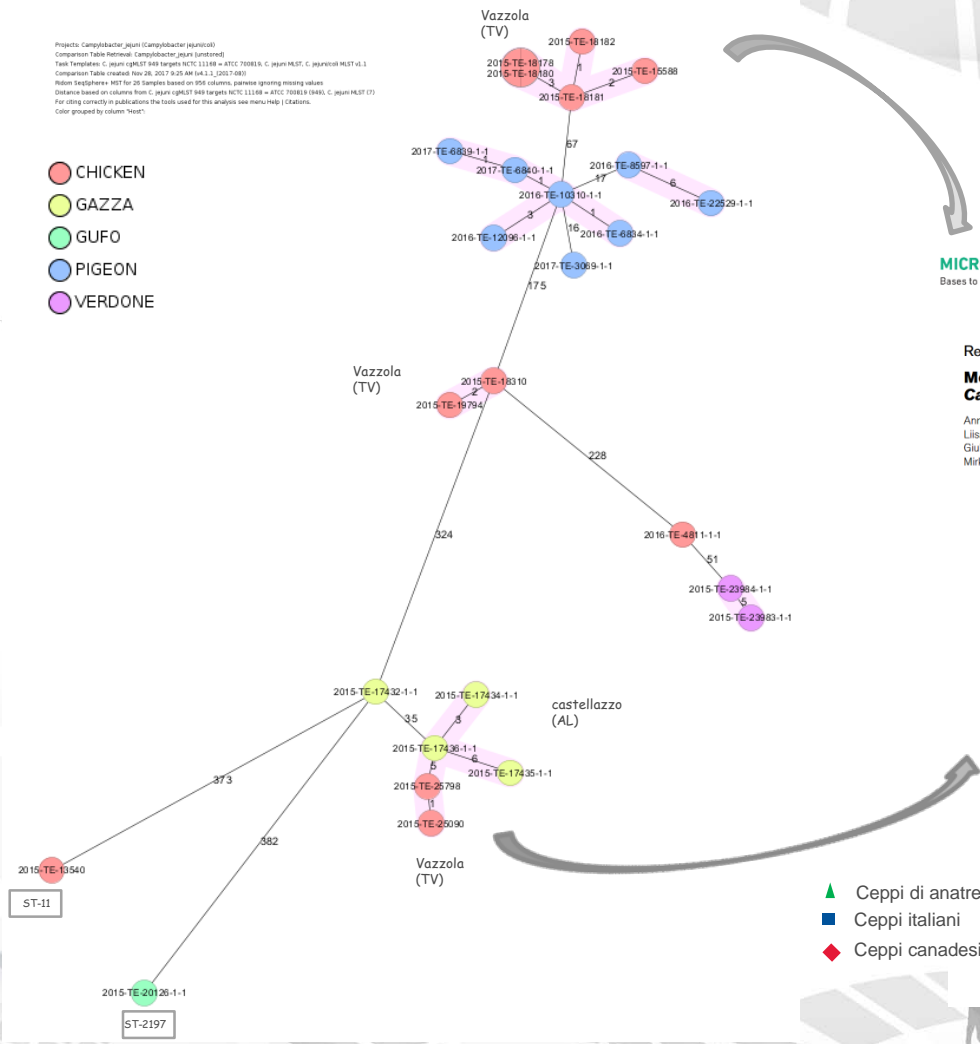
- Human
- Chicken
- Wild birds



# cgMLST - CC45

Project: *Campylobacter jejuni* (Campylobacter jejuni)  
 Comparison Table Retrieval: *Campylobacter jejuni* (unmerged)  
 Task Template: C: *jejun* cgMLST 849 targets NCTC 11189 v. ATCC 700819; C: *jejun* MLST; C: *jejun* MLST v.1  
 Comparison Table retrieved from: 26/10/2017 9:25 AM (url:3:200271900)  
 Relation length/char: MST for 28 samples based on 958 columns, pairwise ignoring missing values  
 Distance based on column: from: C: *jejun* cgMLST 849 targets NCTC 11189 v. ATCC 700819 (949); C: *jejun* MLST (7)  
 For citing correctly in publications the tools used for this analysis see metahelp | Citations  
 Color grouped by column 'host'

- CHICKEN
- GAZZA
- GUFO
- PIGEON
- VERDONE



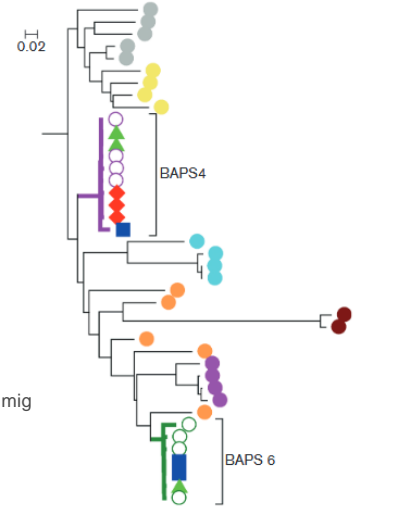
**MICROBIAL GENOMICS**  
 Bases to Biology



## Research Paper

### Monomorphic genotypes within a generalist lineage of *Campylobacter jejuni* show signs of global dispersion

Ann-Katrin Larena,<sup>1</sup> Ji Zhang,<sup>2</sup> Minna Vehkälä,<sup>3</sup> Niko Valimäki,<sup>4</sup> Marjaana Hakkinen,<sup>5</sup> Marja-Liisa Hänninen,<sup>1</sup> Mati Roasto,<sup>6</sup> Mihkel Maesaar,<sup>6,7</sup> Eduardo Taboada,<sup>8</sup> Dillon Barker,<sup>8</sup> Giuliano Garofolo,<sup>9</sup> Cesare Cammà,<sup>9</sup> Elisabetta Di Giannatale,<sup>9</sup> Jukka Corander<sup>10</sup> and Mirko Rossi<sup>1</sup>

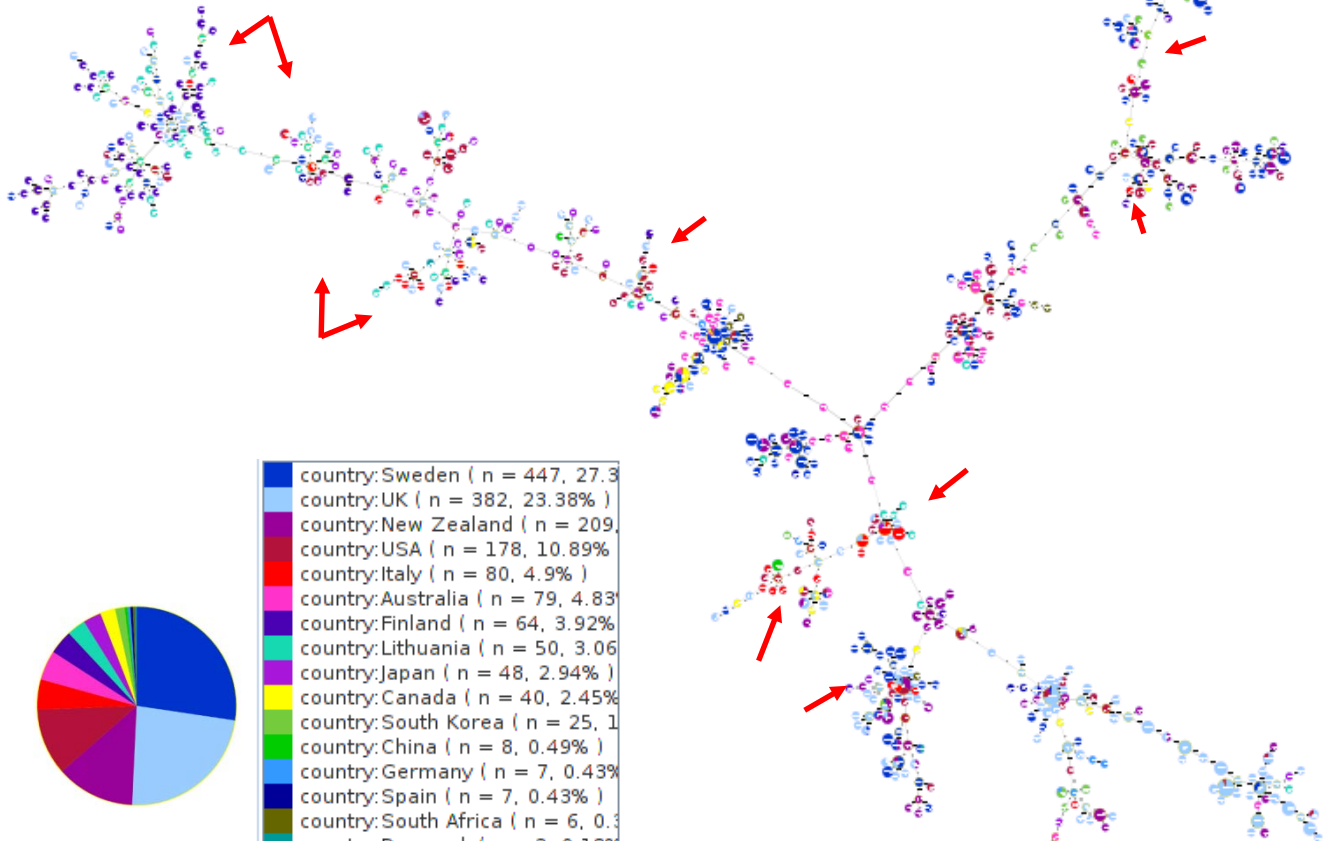


- ▲ Ceppi di anatre mig
- Ceppi italiani
- ◆ Ceppi canadesi

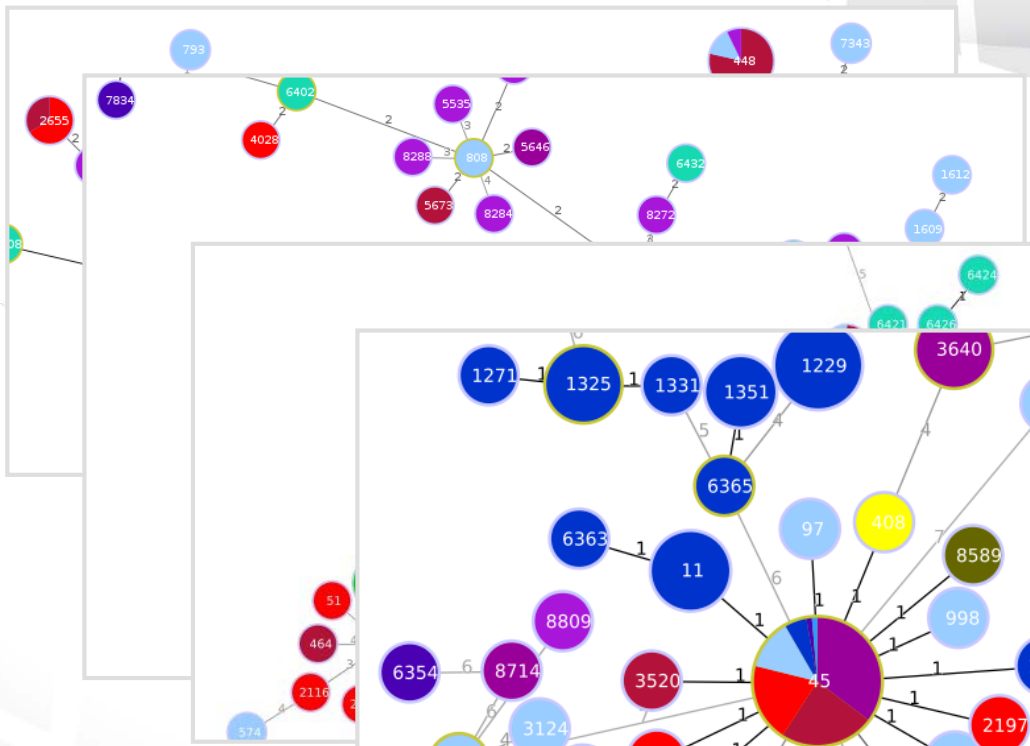


# Wild birds italiani vs GLOBAL

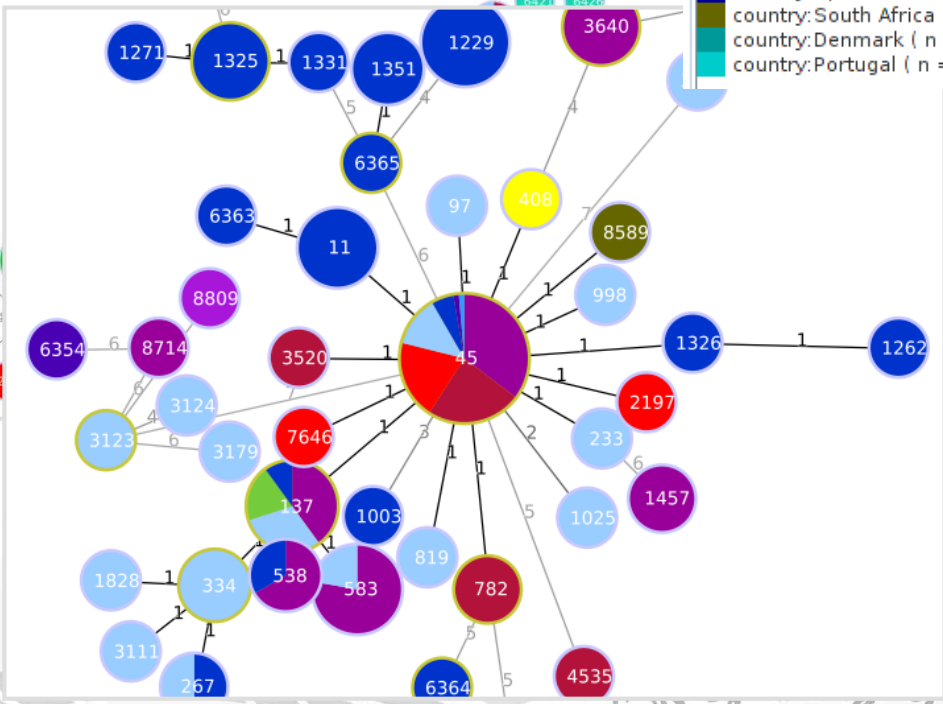
(67 wb ita vs 1565 wb global)

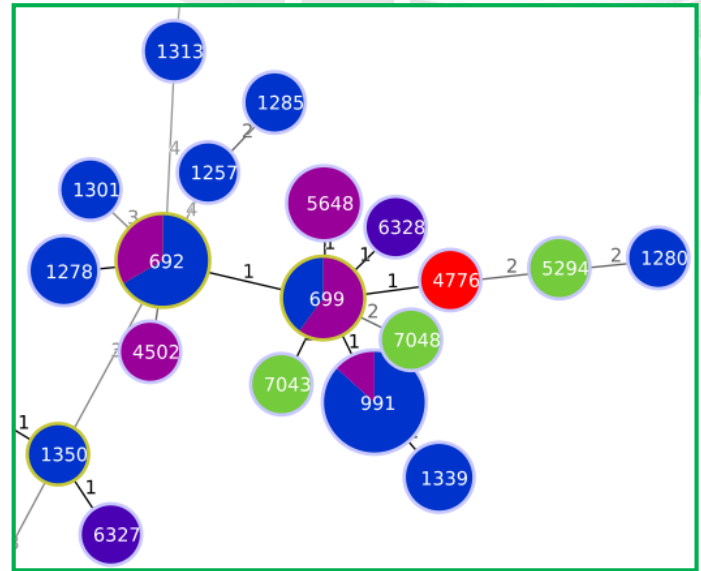
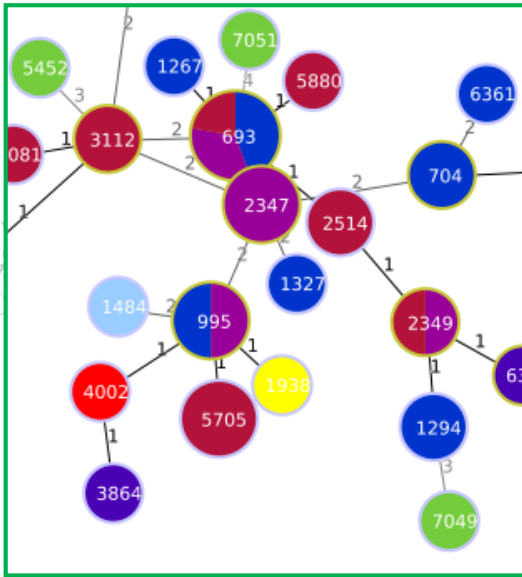


country:Sweden ( n = 447, 27.3%
country:UK ( n = 382, 23.38%
country:New Zealand ( n = 209, 13.35%
country:USA ( n = 178, 10.89%
country:Italy ( n = 80, 4.9%
country:Australia ( n = 79, 4.83%
country:Finland ( n = 64, 3.92%
country:Lithuania ( n = 50, 3.06%
country:Japan ( n = 48, 2.94%
country:Canada ( n = 40, 2.45%
country:South Korea ( n = 25, 1.53%
country:China ( n = 8, 0.49%
country:Germany ( n = 7, 0.43%
country:Spain ( n = 7, 0.43%
country:South Africa ( n = 6, 0.33%
country:Denmark ( n = 3, 0.18%
country:Portugal ( n = 1, 0.06%



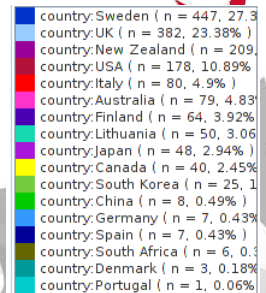
- country:Sweden ( n = 447, 27.3%
- country:UK ( n = 382, 23.38% )
- country:New Zealand ( n = 209,
- country:USA ( n = 178, 10.89%
- country:Italy ( n = 80, 4.9% )
- country:Australia ( n = 79, 4.83%
- country:Finland ( n = 64, 3.92%
- country:Lithuania ( n = 50, 3.06%
- country:Japan ( n = 48, 2.94% )
- country:Canada ( n = 40, 2.45% )
- country:South Korea ( n = 25, 1
- country:China ( n = 8, 0.49% )
- country:Germany ( n = 7, 0.43%
- country:Spain ( n = 7, 0.43% )
- country:South Africa ( n = 6, 0.3
- country:Denmark ( n = 3, 0.18%
- country:Portugal ( n = 1, 0.06%





1 differenza di un allele con un ceppo isolato dalla Svezia e dalla nuova Zelanda

1 differenza di 2 alleli con un ceppo isolato dalla Corea del Sud







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**Campylobacter**  
Laboratorio Nazionali

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## WHO Releases List of World's Most Dangerous Superbugs

The U.N. agency's first-ever such list names 12 bacterial threats as the highest-priority needs for antibiotics

By Helen Branswell, STAT on February 27, 2017

### Priority 1: Critical

1. *Acinetobacter baumannii*, carbapenem-resistant
2. *Pseudomonas aeruginosa*, carbapenem-resistant
3. Enterobacteriaceae, carbapenem-resistant, ESBL-producing

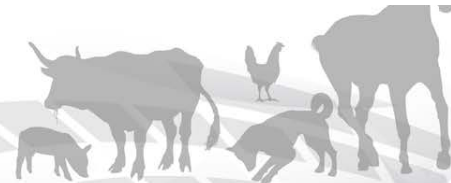
### Priority 2: High

4. *Enterococcus faecium*, vancomycin-resistant
5. *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
6. *Helicobacter pylori*, clarithromycin-resistant
7. *Campylobacter* spp., fluoroquinolone-resistant
8. Salmonellae, fluoroquinolone-resistant
9. *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

### Priority 3: Medium

10. *Streptococcus pneumoniae*, penicillin-non-susceptible
11. *Haemophilus influenzae*, ampicillin-resistant
12. *Shigella* spp., fluoroquinolone-resistant

*Republished with permission from STAT. This article originally appeared on February 27, 2017*



# Antibioticoresistenze.....



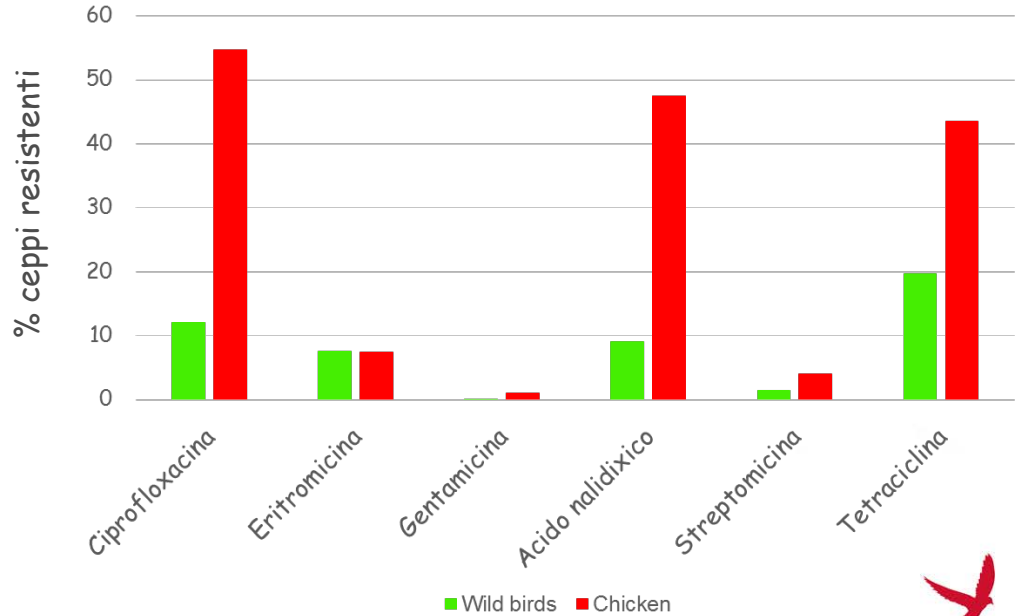
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TERAMO

 *Campylobacter*  
Laboratorio Nazionale di Riferimento

	1	2	3	4	5	6	7	8	9	10	11	12
A	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
B	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
C	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
D	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
E	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
F	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
G	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
H	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA
I	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA

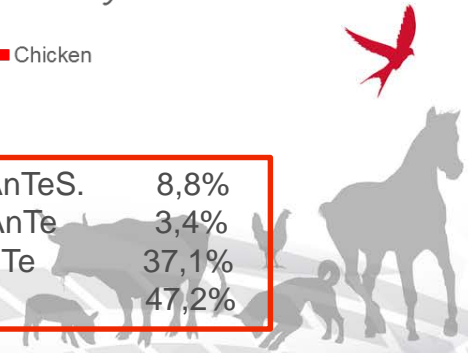
ANTIBIOTICI

GEN<sup>+</sup> Gentamicina  
STR<sup>+</sup> Streptomina  
CIP<sup>+</sup> Ciplofloxacina  
TET<sup>+</sup> Tetraciclina  
ERY<sup>+</sup> Eritromicina  
NAL<sup>+</sup> Acido Nalidixico  
COL<sup>+</sup> Colistina




Wb: CipEGenAnTeS.	1,51%
CipEAn	4,54%
CipEGen	3,03%

Chicken: CipEAnTeS.	8,8%
CipEAnTe	3,4%
CipAnTe	37,1%
CipAn	47,2%

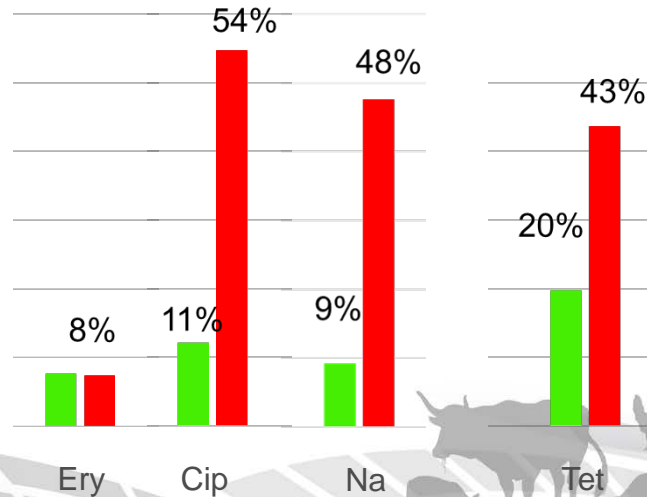


# Pipeline **ABRicate** - db CARD (Comprehensive Antibiotic Resistance database-2017-Aug-6)

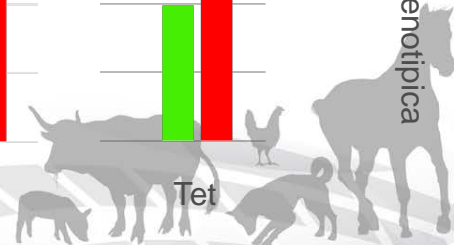
 <i>Campylobacter</i> Laboratorio Nazionale di Riferimento	OXA-184	OXA-61	<i>cmeA</i>	<i>cmeB</i>	<i>cmeC</i>	<i>cmeR</i>	TetO
Chicken	35,45%	58,53%	98,32%	97,99%	95,32%	95%	72,57%
Wild birds	81,66%	16,66%	98,33%	96,66%	95%	91,66%	8,3%

Resistenza genotipica


Torsten Seemann | @torstenseemann



Resistenza fenotipica



# Conclusioni




Nonostante sia ben noto che *C.jejuni* sia un batterio altamente ricombinante, esistono delle popolazioni di batteri con un tasso evolutivo molto lento che vivono in alcuni taxa di uccelli selvatici. Ciò indica l'esistenza di uno straordinario adattamento evolutivo di specifici genotipi a particolari specie di uccelli ospite, indipendente da un segnale filogeografico o temporale

2116ST-353 complex in un uccello selvatico; nessun 2863ST-354 complex in wb

Necessario investigare sulla funzione sinantropica che alcuni uccelli possono avere come trasportatori di batteri tra i wb selvatici e gli animali di allevamento/uomo

ATB indice della contaminazione ambientale

Mentre i pannelli tradizionali possono testare solo un numero limitato di farmaci, i dati di WGS possono rivelare tutti gli elementi genetici di resistenza. La conoscenza di quali geni di resistenza antimicrobica circolino nelle popolazioni batteriche è fondamentale per lo sviluppo di nuove strategie per combattere le resistenze ai farmaci, compreso il miglioramento programmi di sorveglianza delle malattie di origine alimentare.



# Grazie per l'attenzione

