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Laboratorio Nazionale di Riferimento

Caratterizzazione genomica di *Campylobacter* e lo sviluppo di un modello per la tracciabilità del microrganismo da diverse fonti

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Campylobacter in EU

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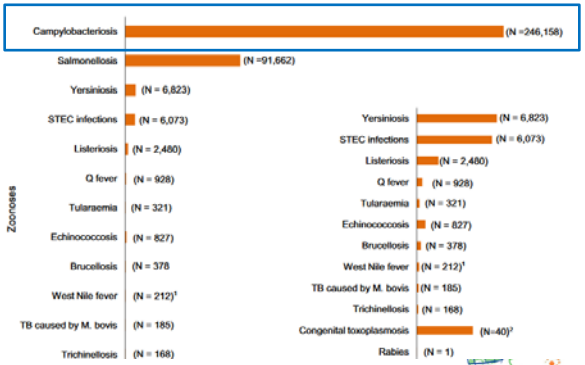


EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2017

doi: 10.2903/j.efsa.2018.5500

**The European Union summary report on
of zoonoses, zoonotic agents and food-b
2017**

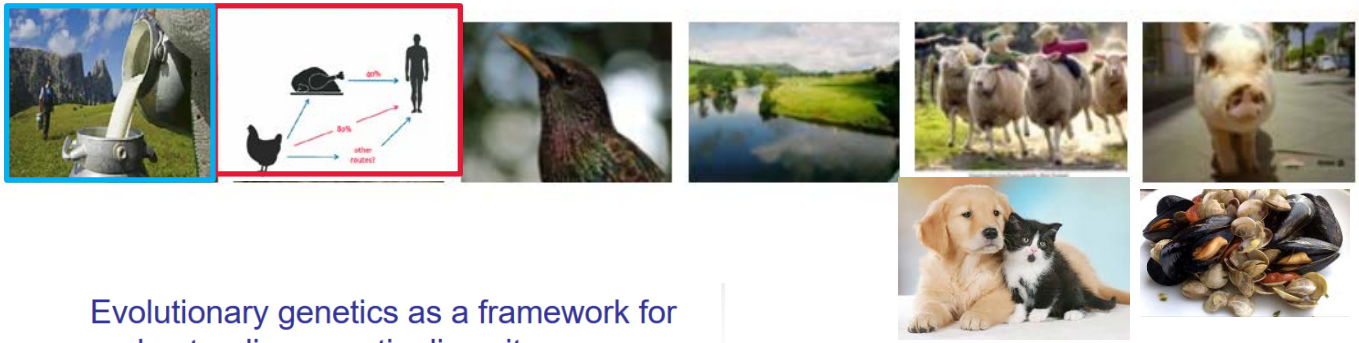
European Food Safety Authority and European Centre for Dise
(EFSA and ECDC)

Table 2: Reported hospitalisation and case fatalities due to zoonoses in confirmed human cases in the EU, 2017

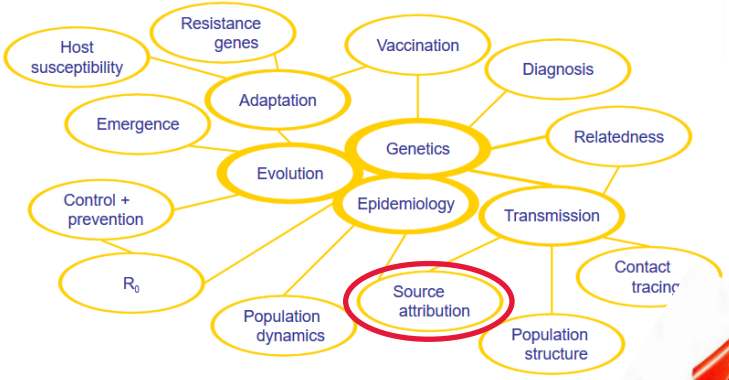
Disease	Number of confirmed[a]		Hospitalisation			Deaths			
	Human cases	Status available (%)	Number of reporting MS[b]	Reported hospitalised cases	Proportion hospitalised (%)	Outcome available (%)	Number of reporting MS[b]	Reported Deaths	Case Fatality (%)
Campylobacteriosis	246,158	27.6	17	20,810	30.5	72.8	16	45	0.04
Salmonellosis	91,662	43.1	14	16,796	42.5	67.8	17	156	0.25
Yersiniosis	6,823	27.1	14	616	33.4	65.5	15	3	0.07
STEC infections	6,073	41.0	18	933	37.5	66.1	21	20	0.50
Listeriosis	2,480	40.4	16	988	98.6	65.8	18	225	13.8
Q-fever	928	NA[c]	NA	NA	NA	56.0	10	7	1.35
Echinococcosis	827	31.2	14	140	54.3	30.1	14	1	0.40
Brucellosis	378	45.8	10	104	60.1	33.9	10	1	0.78
Tularaemia	321	38.3	9	76	61.8	51.1	9	1	0.6
West Nile fever[a]	212	72.2	8	134	87.6	98.6	9	25	12.0
Trichinellosis	168	44.6	9	56	74.7	40.5	9	0	0.0
Congenital toxoplasmosis	40	57.9	3	18	NA	63.2	3	0	0.0
Rabies	1	NA[c]	NA	NA	NA	0.0	0	NA	NA

(a): Exception: West Nile fever where total number of cases were included.
(b): Not all countries observed cases for all diseases.
(c): NA: Not applicable as the information is not collected for this disease.

Inferring host-host transmission: zoonotic transmission of *Campylobacter jejuni*



Evolutionary genetics as a framework for understanding genetic diversity

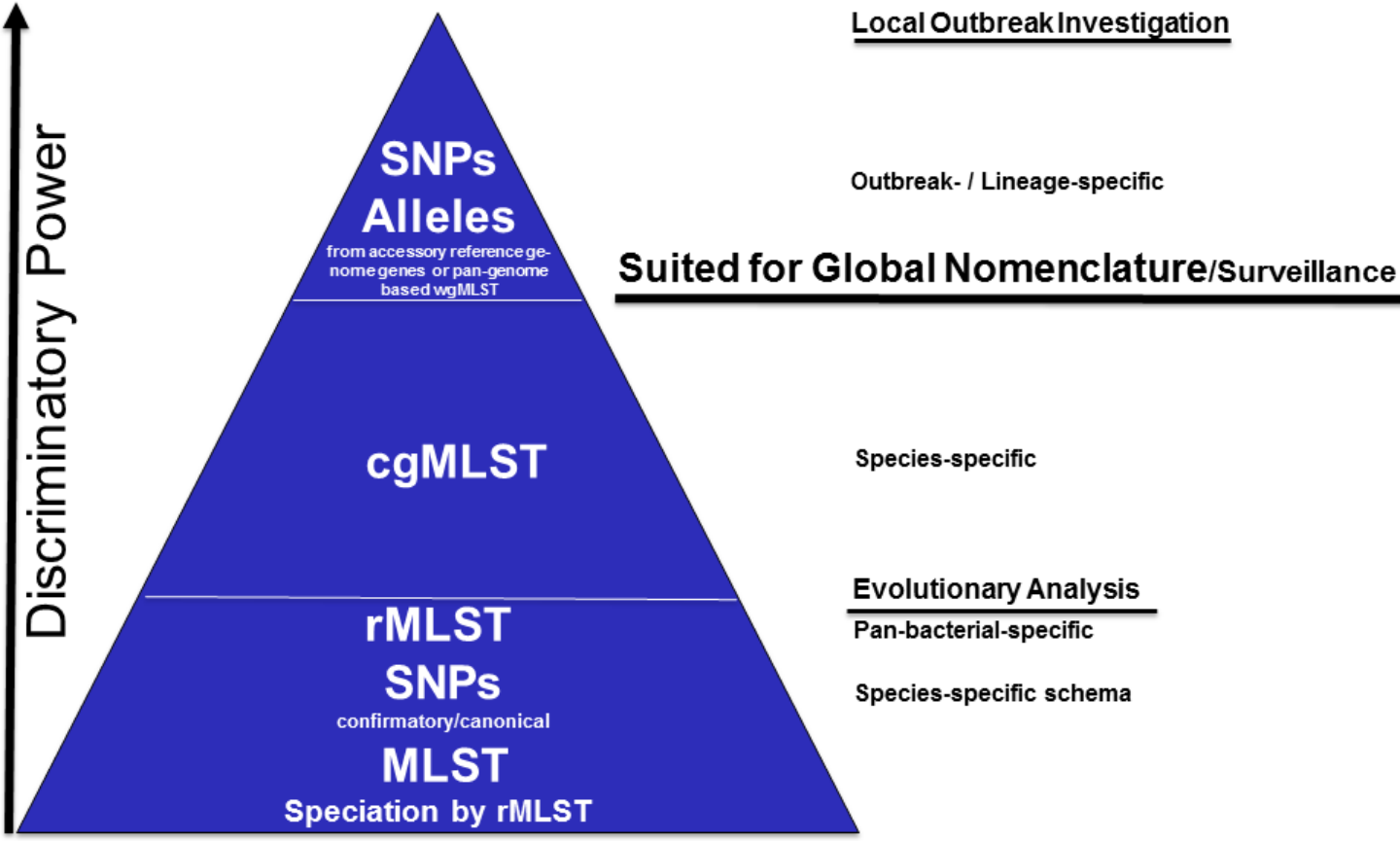


- Per stimare i contributi relativi di fonti differenti (animali, ambientali o alimentari) alla malattia nell'uomo;

-guidare degli interventi mirati e misurare il loro impatto;

-diversi approcci, tra cui

MICROBIAL SUBTYPING

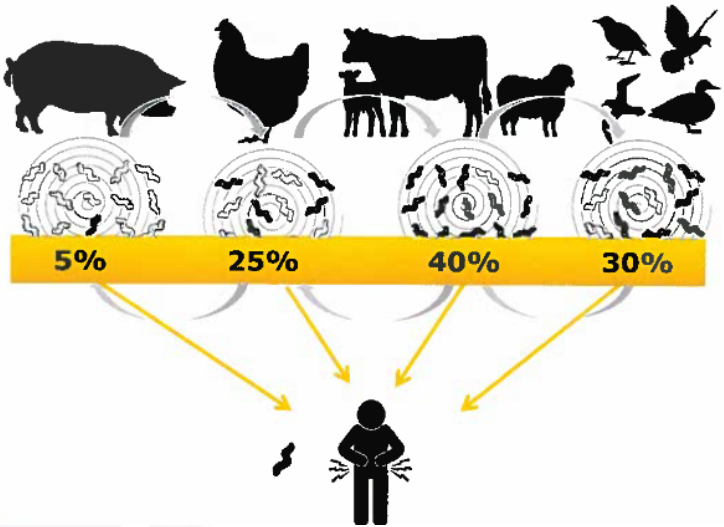


Bassa sensibilità
Molto robusta

Buona per analisi filogenetiche
Non abbastanza per investigare focolai

Analisi dell'attribuzione alle fonti

- **Asymmetric Island Model** (Wilson *et al.* PLoS Genet 2008;4:e1000203)
  - **Bayesian population genetics model** that uses MLST data for **probabilistic assignment of STs to sources**
  - ***Campylobacter* population** is seen as **separate islands** where **mutation, recombination** and **migration** occur

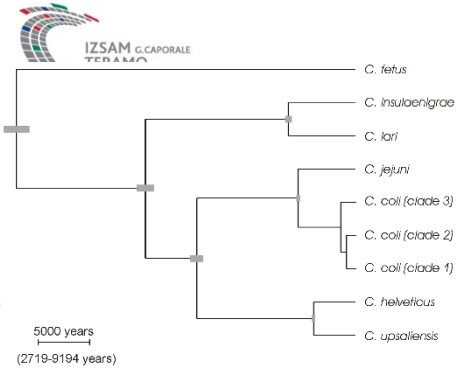


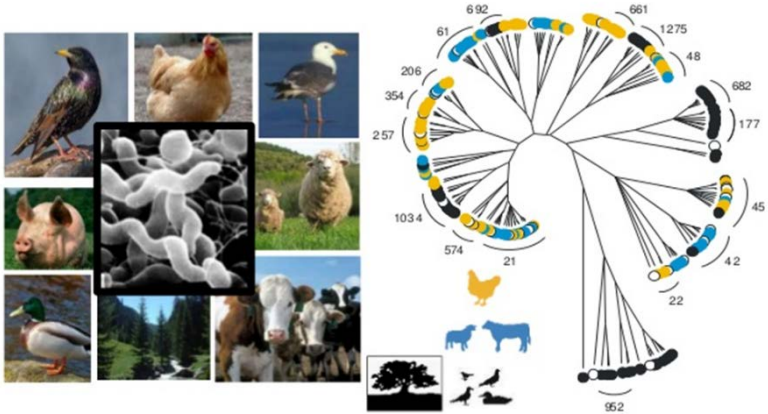
Evolutionary stage
- Only source data
- Estimates mutation, recombination and migration rates to be used in the second stage

Attribution stage
- Relies on sampling probabilities: probability that a given human ST is randomly sampled from each host population
- Estimates an assignment posterior probability for each ST

Storia evolutiva

C.jejuni

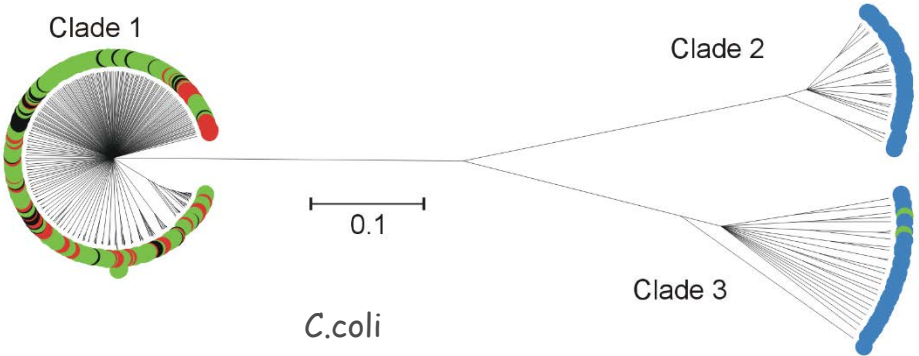




C. jejuni si caratterizza per la sua diversità genomica e per possedere una struttura di popolazione non clonale dovuta alla presenza di elevata ricombinazione genetica

Sheppard et al. (2009) *Clinical Infectious Diseases* 48:1072–1078
Sheppard et al. (2010) *Applied Environmental Microbiology* 76, 5269-5277

ST-828complex



Sheppard SK, Dallas JF, Wilson DJ, Strachan NJC, McCarthy ND, et al. (2010) Evolution of an Agriculture-Associated Disease Causing Campylobacter coli Clade: Evidence from National Surveillance Data in Scotland. PLOS ONE 5(12): e15708. https://doi.org/10.1371/journal.pone.0015708
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0015708

MLST data

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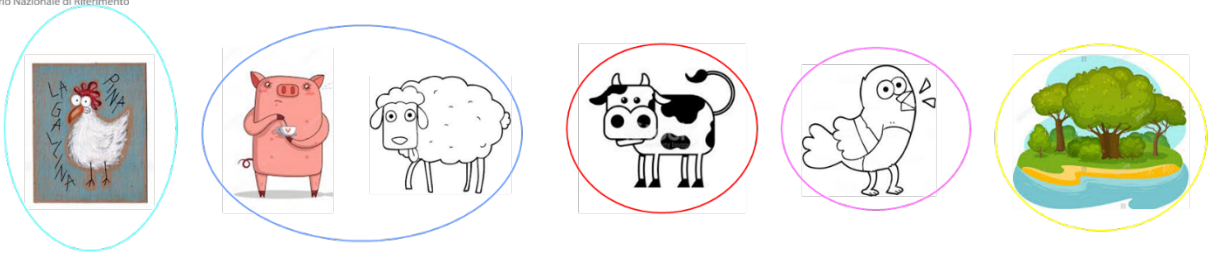
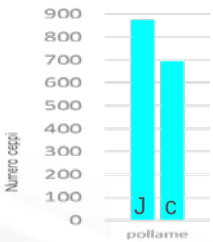
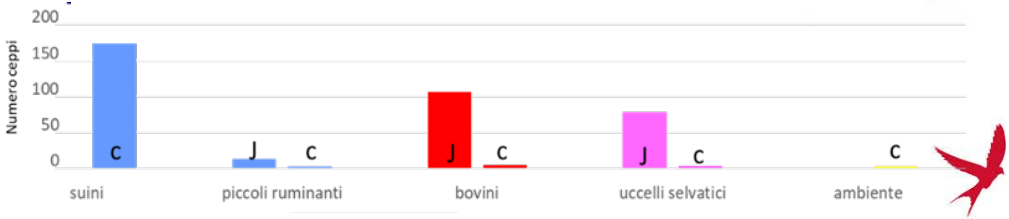
Materiali: reservoir → 8871 ceppi disponibili da 5 probabili reservoir



1569	174	16	112	84	3
3418	58	422	1221	1284	510





107 humans (75 c.jejuni-32 C.coli)

Analisi di genomica comparativa

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ridom BIOINFORMATICS

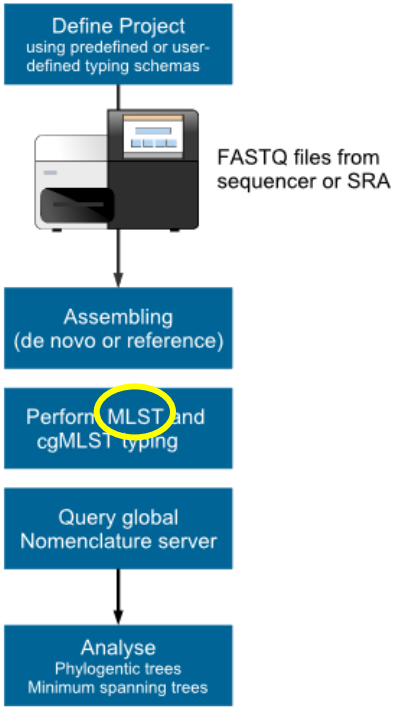
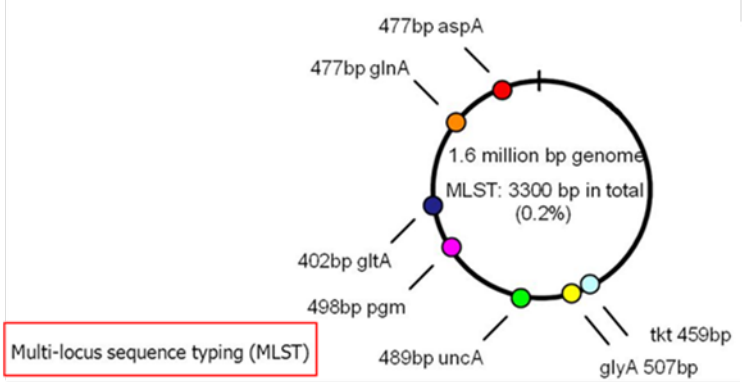
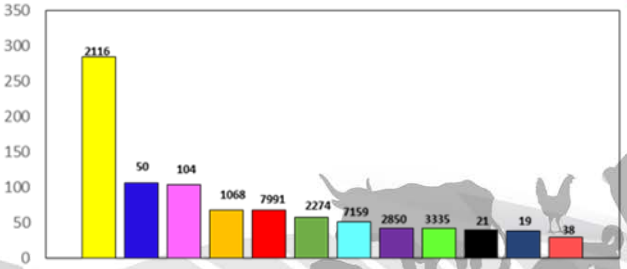


Figure. Microbial WGS next generation sequencing and genome-wide gene by gene (cgMLST) analysis workflow.

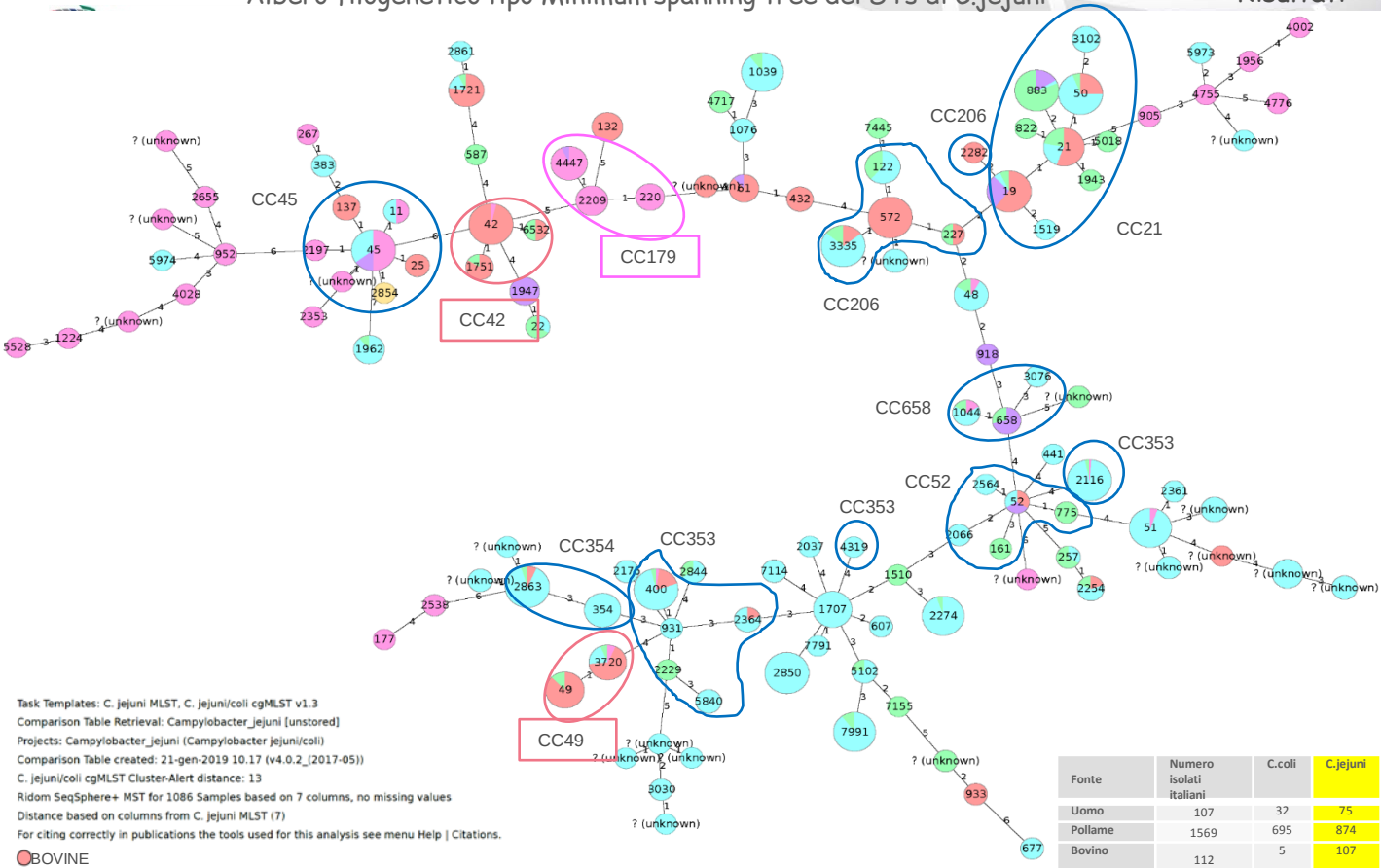


ST	aspA	glnA	glta	glya	pgm	tkt	uncA	freq
2116	7	17	52	10	89	3	6	284
50	2	1	12	3	2	1	5	106
104	2	1	1	3	7	1	5	104
1068	33	39	30	78	104	43	17	68



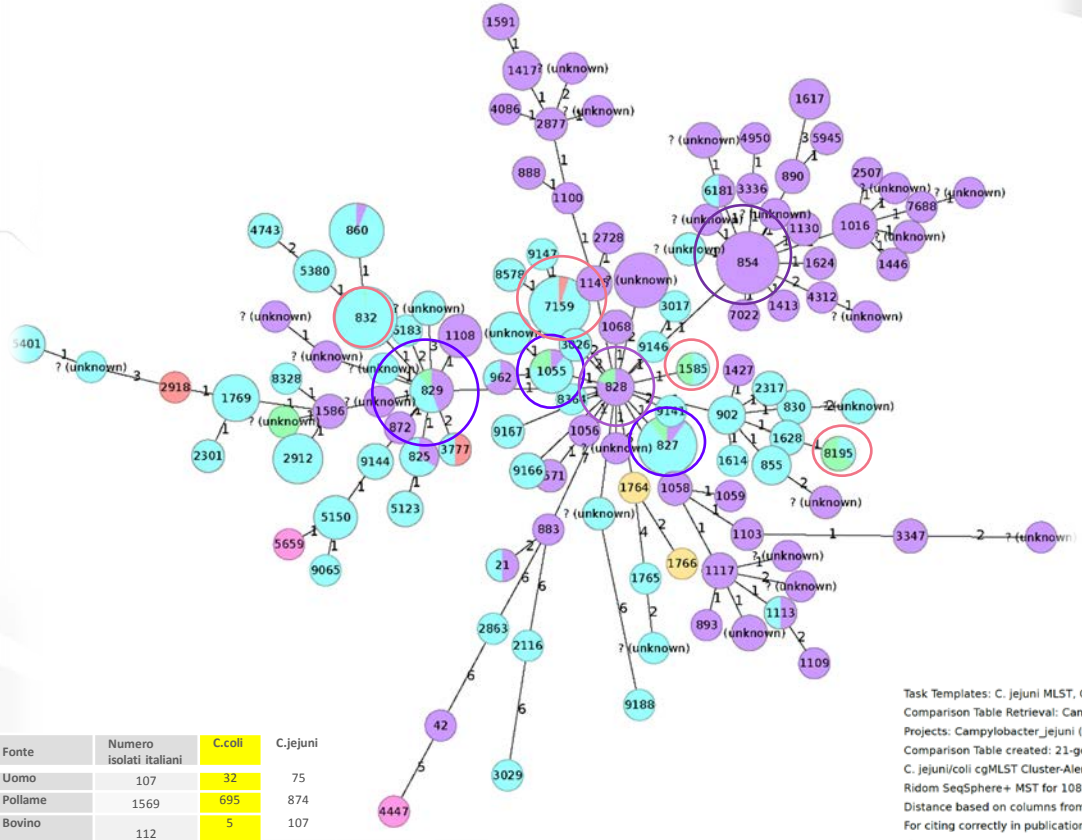
Multi-locus sequence typing C.jejuni

Albero filogenetico tipo Minimum spanning tree dei STs di C.jejuni



Fonte	Numero isolati italiani	C.coli	C.jejuni
Uomo	107	32	75
Pollame	1569	695	874
Bovino	112	5	107
Ambiente	3	3	-
Ucc. Selv.	84	4	79
Piccoli ruminanti	16	3	13
Suini	174	174	-

Albero filogenetico tipo Minimum spanning tree dei STs di C.coli



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Ambiente	3	3	-
Ucc. Seiv.	84	4	79
Piccoli ruminanti	16	3	13
Suini	174	174	-

Task Templates: C. jejuni MLST, C. jejuni/coli cgMLST v1.3
Comparison Table Retrieval: Campylobacter_jejuni [unstored]
Projects: Campylobacter_jejuni (Campylobacter jejuni/coli)
Comparison Table created: 21-gen-2019 10.17 (v4.0.2_(2017-05))
C. jejuni/coli cgMLST Cluster-Alert distance: 13
Ridom SeqSphere+ MST for 1086 Samples based on 7 columns, no missing values
Distance based on columns from C. jejuni MLST (7)
For citing correctly in publications the tools used for this analysis see menu Help | Citations.

- BOVINE
- ENVIRONMENT
- HUMAN
- POULTRY
- UNGULATES
- WILD BIRD

Source attribution

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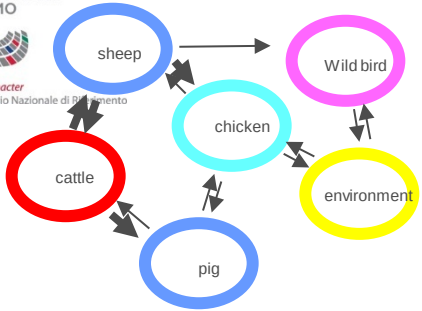
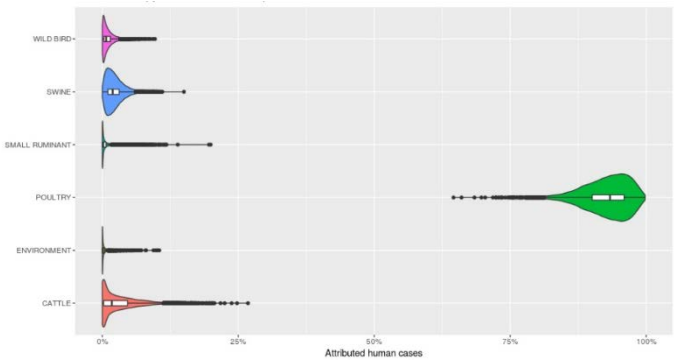


frontiers in Microbiology

ORIGINAL RESEARCH
published: 13 June 2016
doi: 10.3389/fmicb.2016.00887

Tracing Back Clinical *Campylobacter jejuni* in the Northwest of Italy and Assessing Their Potential Source

*Elisabetta Di Giannatale[1], Giuliano Garofolo[1], Alessandra Alessiani[1], Guido Di Donato[1], Luca Candeloro[2], Walter Vencia[3], Lucia Decastelli[3] and Francesca Marotta[1]**



islandR

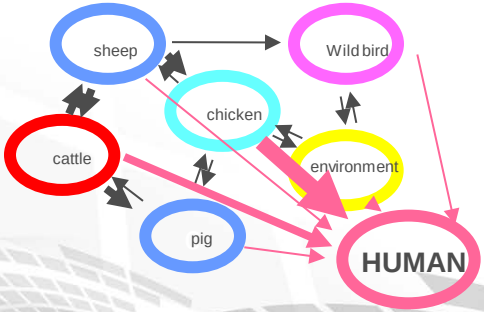
The islandR package allows source attribution using the island genomic model (and in the future, other genomic models).

This allows the attribution of cases of disease to their likely sources.

The assymmetric island model estimates the sampling distribution of genotypes on sources by using the genetic distance between isolates to infer mutation and recombination rates in addition to migration rates between sources. This allows improved estimation of the sampling distribution over and above that achieved using just the prevalence of each type. Further, it allows estimating the likely prevalence of unobserved genotypes.

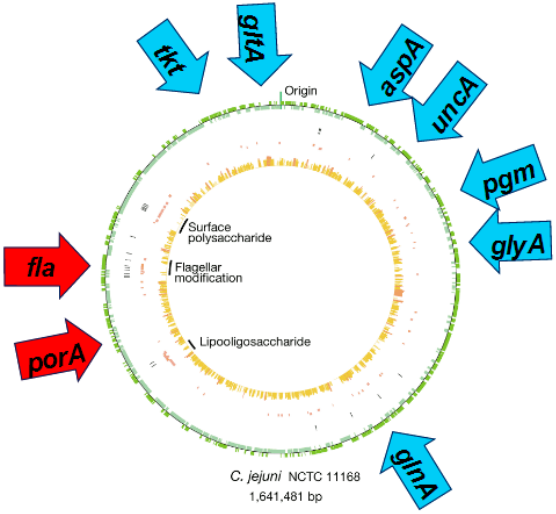
https://github.com/jmarshallnz/islandR

Wilson et al 2008



Fonte	Numero isolati italiani	Numero isolati europei
Uomo	107	-
Pollame	1569	3418
Bovino	112	1221
Ambiente	3	510
Uccelli selvatici	84	1284
Piccoli ruminanti	16	422
Suini	174	58

Campylobacter sequence typing



- *Campylobacter* Seven-locus ST summarises 3,309bp of data as an allelic profile: e.g. ST-45: 4-7-10-4-1-7-1
- This is 0.2% of the genome.
- 7,763 STs from 33,051 isolates in PubMLST database (May 2015).
- 400-744 alleles per locus.
- Many polymorphisms per locus.

Dingle, K. E., Colles, F. M., Wareing, D. R. A., Ure, R., Fox, A. J., Bolton, F. J., Bootsma, H. J., Willems, R. J. L., Urwin, R. & Maiden, M. C. J. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. *J Clin Microbiol 39, 14-23.*

Analisi di genomica comparativa

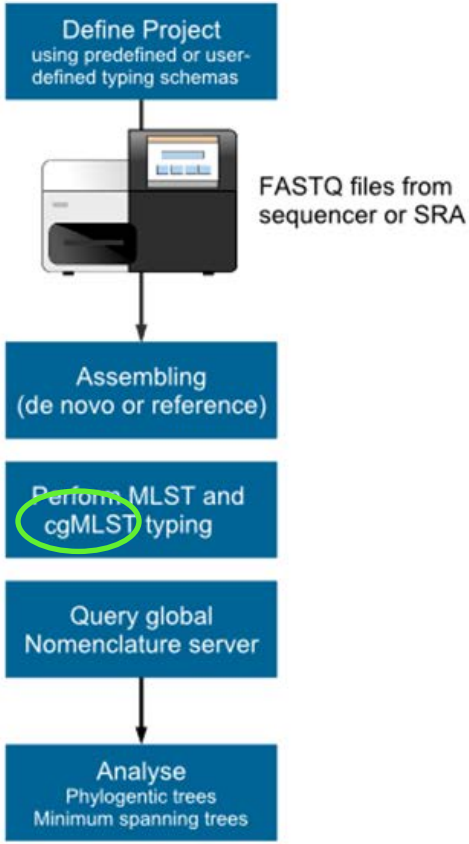
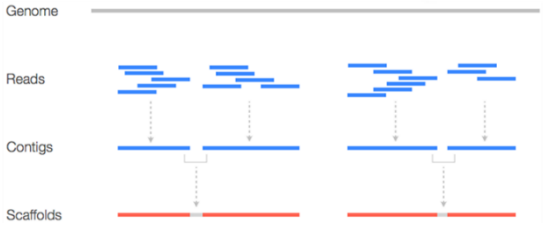
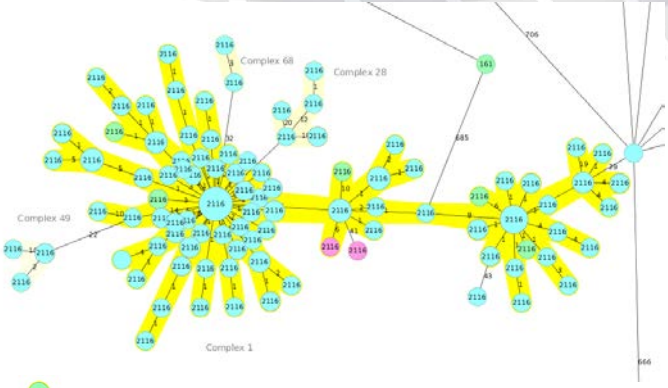


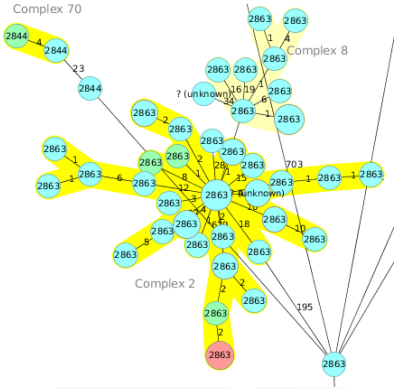
Figure. Microbial WGS next generation sequencing and genome-wide gene by gene (cgMLST) analysis workflow.

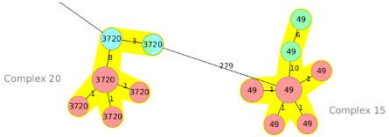
- Library prep (Nextera XT DNA Library Preparation Kit-Illumina)
- Sequencing (NextSeq500 –Illumina)
- De novo assembly (SPAdes)

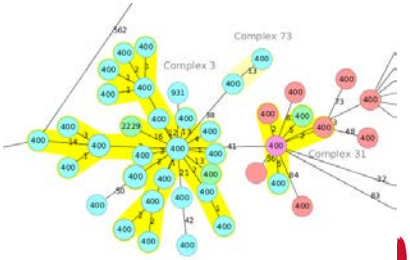


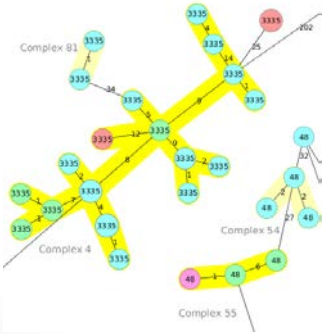
Core genome-MLST tree *C.jejuni*











Task Templates: C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819, C. jejuni MLST
Comparison Table Retrieval: Campylobacter_jejuni [unstored]
Projects: Campylobacter_jejuni (Campylobacter jejuni/coli)
Comparison Table created: 3-giu-2019 13.08 (v4.0.2_(2017-05))
Ridom SeqSphere+ MST for 1059 Samples based on 956 columns, pairwise ignoring missing values
Distance based on columns from C. jejuni cgMLST 949 targets NCTC 11168 = ATCC 700819 (949), C. jejuni MLST (7)
For citing correctly in publications the tools used for this analysis see menu Help | Citations.

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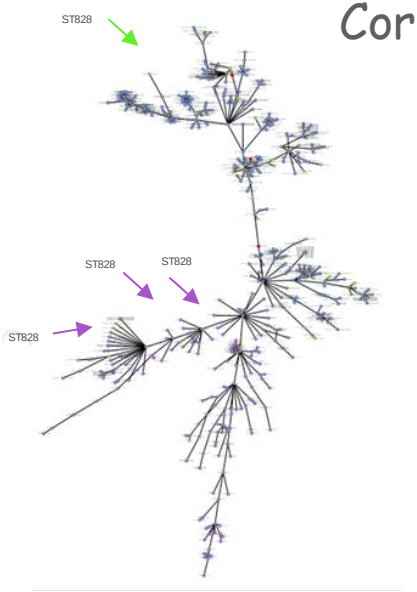
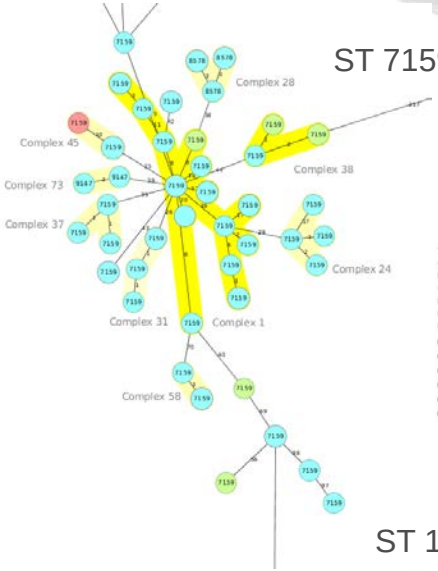
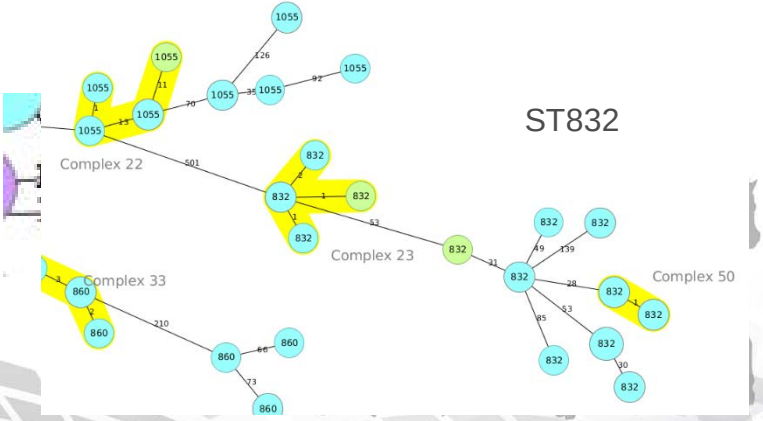
- WILD BIRD

20complessi

Core genome-MLST tree C.coli







Conclusioni

- Carni avicole: fonte primaria di infezione
- Bovini ed ovini una possibile fonte di infezione
- l'osservazione che le fonti ambientali sembrano poco importanti suggerisce che la via di trasmissione è soprattutto di origine alimentare
- interventi mirati sulla produzione primaria dei polli al fine di ridurre l'incidenza della malattia.
- grande variabilità esistente anche nei ceppi di Campylobacter isolati dagli umani, molteplici foci d'infezione.
- MLST e cgMLSTsono metodiche che si prestano largamente a questo scopo e recentemente sono state brillantemente impiegate per aumentare la risoluzione dell'analisi degli isolati associati ai focolai

WGS è un potente strumento per la sorveglianza di agenti patogeni di origine alimentare, stabilire un legame tra casi non correlati basati solo su livelli di prossimità della distanza genomica potrebbe essere fuorviante.

Elisabetta Di Giannatale

Giuliano Garofolo
Anna Janowicz
Katiuscia Zilli
Lisa Di Marcantonio
Alessandra Alessiani
Silvana Salvatore
Gabriella Di Serafino
Tiziana Persiani
Diana Neri
Romina Romantini
Lorena Sacchini
Daniela Paganico