WGS-based surveillance as a paradigm shift in outbreak detection, AMR monitoring and source attribution in *Campylobacter* spp in Portugal

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FWD AMR· RefLabCap

Welcome to the
3rd Multidisciplinary training workshop
June 2024

REPÚBLICA PORTUGUESA
SAÚDE

SNS SERVIÇO NACIONAL DE SAÚDE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

Surveillance of campylobacteriosis, Portugal:

Campylobacteriosis has been the most frequently reported zoonosis in humans across the EU since 2005, with a stable trend.

In Portugal:

Campylobacteriosis has been a notifiable disease since 2015.

Cases are (**should be!**) **reported by clinicians** in an electronic platform called SINAVE (National Epidemiological Surveillance System).

Since 2017, **laboratory notification**, using the same platform, has also been in place, and is mandatory.

A **sentinel laboratory-based surveillance** has been implemented in 2013; the lab network comprises primary laboratories mainly from hospitals, both public and private; participation in the surveillance network is voluntary.

Surveillance of campylobacteriosis, Portugal:

Laboratory-based surveillance:

Network includes laboratories from 3 of the 5 regions from mainland Portugal:

North (six hospital centres, one hospital),
Center (one hospital centre)
Metropolitan Lisbon Area (two hospitals centres, two hospitals)

NRL receives *Campylobacter* spp isolates; for each isolate, anonymized epidemiological, demographic, microbiological, and clinical data are also requested;

Species confirmation/determination (MALDI-TOF)

Antimicrobial susceptibility testing (**CIP; ERY, TET; GEN**; AMP*; AMOX+CL*; ERT*†)
Disk diffusion; †E-Test

Clinical breakpoints:

- ***The European Committee on Antimicrobial Susceptibility Testing*** **(EUCAST)**
- ***Comité de l'antibiogramme de la Société Française de Microbiologie*** **(CA-SFM)**

Surveillance of campylobacteriosis, Portugal:

Laboratory-based surveillance:

Whole-Genome Sequencing (since Jan 2022)

• High-quality DNA samples (MagnaPure, Roche), quantified using QubitTM (ThermoFisher Scientific)

• NextSeq 2000, Illumina; reads size 150

• INNUca v4.2.2 pipeline (https://github.com/B-UMMI/INNUca) for *de novo* assembly, species and ST, (includes Kraken and mlst softwares)

• Resfinder tool (ResFinder-4.4.2), ResFinder-2.2.1; PointFinder-4.0.1 databases

• INNUENDO wgMLST schemas

• Report tree for genetic clusters identification (https://github.com/insapathogenomics/ReporTree)

10.2903/sp.efsa.2018.EN-1498; Genome Med 15, 43 (2023). https://doi.org/10.1186/s13073-023-01196-1

Surveillance of campylobacteriosis, Portugal:

Notified cases (cases reported by the clinicians):

The European Union One Health 2022 Zoonoses Report

Country	2022				2021		2020		2019		2018	
	National Coverage[a]	Data format[a]	Confirmed cases and rate		Confirmed cases and rate		Confirmed cases and rate		Confirmed cases and rate		Confirmed cases and rate	
			Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Portugal	Y	C	868	8.4	973	9.4	790	7.7	887	8.6	610	5.9

A total of 1057 confirmed cases were notified in 2023.

EFSA Journal. 2023;21:e8442.
https://doi.org/10.2903/j.efsa.2023.8442

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Laboratory-based surveillance - some data:

	Year											Overall
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	
Total of cases received in the NRL	426	437	461	537	685	658	916	676	820	852	952	7420

>50% of the cases received in the NRL were not notified

Number of reported cases is likely underestimated in Portugal

Difficult to estimate the real number of *Campylobacter* infections in Portugal

Surveillance of campylobacteriosis, Portugal:

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Isolates with AST data	119	135	182	191	289	336	349	248	325	441	547	31/62
Isolates subjected to WGS*	0	0	0	20/17	23/30	27/42	21/32	22/53	23/70	82/181	79/446	297/871

*WGS numbers refer to *C. coli*/*C. jejuni* isolates with whole-genome sequencing;
WGS performed for projects (DiscOver- OHEJP)

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WGS coverage increased over time:

In 2022, 51% of *C. jejuni* and 95% of *C. coli*;

In 2023, 85% of *C. jejuni* and 98% of *C. coli*.

WGS-based surveillance, not continuously (2016-2021): *C. coli*

MLST-based genetic diversity:
CC ST-828 (89.7%), with 34 distinct STs,
CC ST-1150 (2.9%), with 3 distinct STs

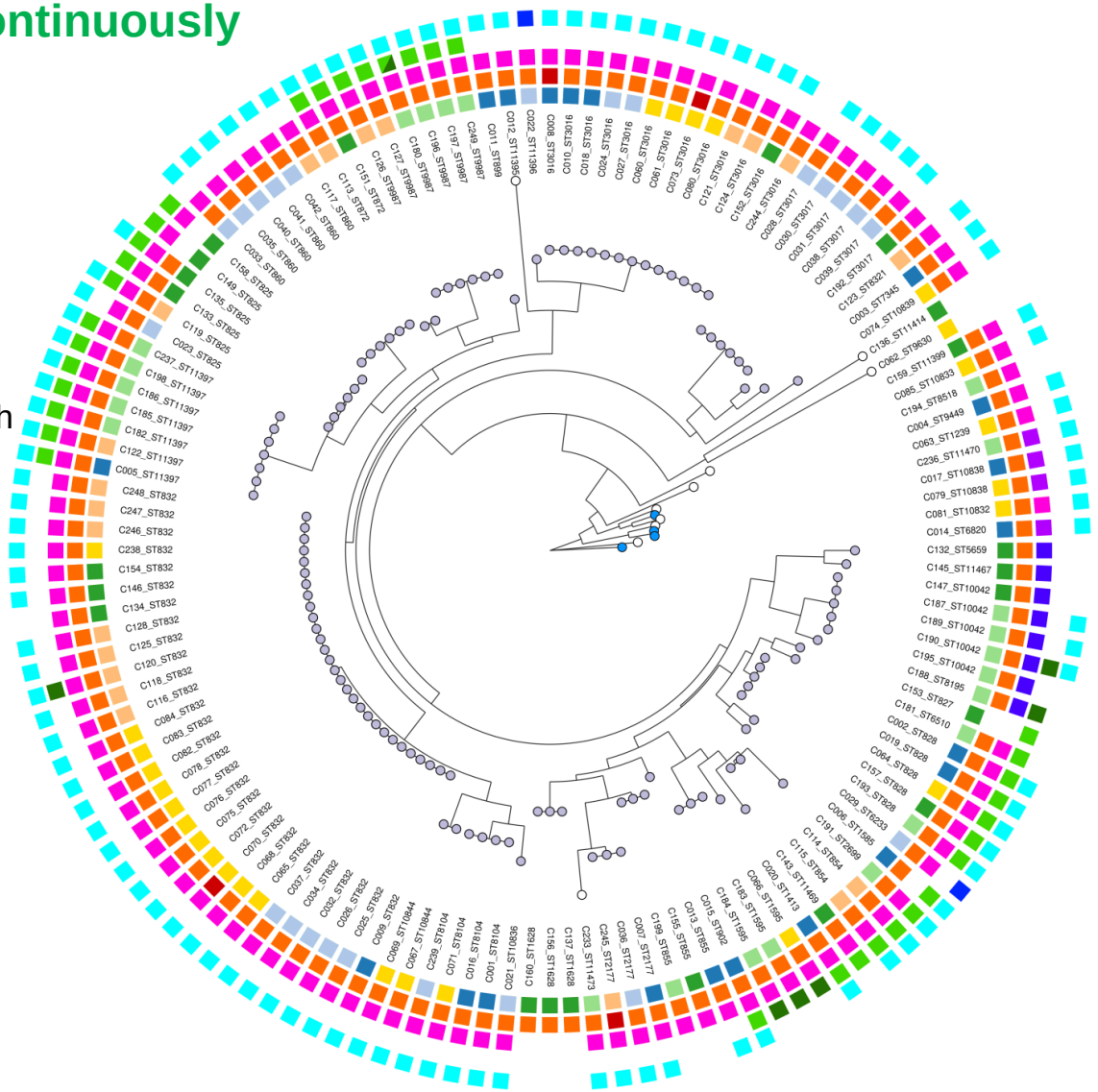
AMR-associated markers:
96.3% carried the *gyrA* mutations associated with CIP resistance (gyrA:p.T86I/gyrA:p.D90N)

94.9% were positive for *tetO* (or variants)

81.6% harbor the ERY resistance-associated mutation in the 23S rRNA gene (A2075G)

29.4% carried genes associated with streptomycin resistance (aadE, ant(6)-Ia)

Resistance markers were distributed among the different STs circulating over the studied time period, challenging the notion of a clonal origin for MDR isolates.



Clonal Complex
- ST-828 complex
- ST-1150 complex

Isolation year
- 2016
- 2017
- 2018
- 2019
- 2020
- 2021

Ciprofloxacin AMR
- gyrA T86I
- gyrA T86I + D90N

Tetracycline AMR
- tet(O)
- tet(O/32/O)
- tet(W)

Streptomycin AMR
- addE-Cc
- ant(6)-Ia

Erythromycin AMR
- 23SrRNA A2074N
- 23SrRNA A2075G

Pathogens 2024, 13, 147. https://doi.org/10.3390/pathogens13020147

WGS-based surveillance, not continuously (2016-2021): *C. jejuni*

MLST-based genetic diversity:
Higher genetic diversity that *C. coli*
CC ST-21 (16.8%), with 14 distinct STs,

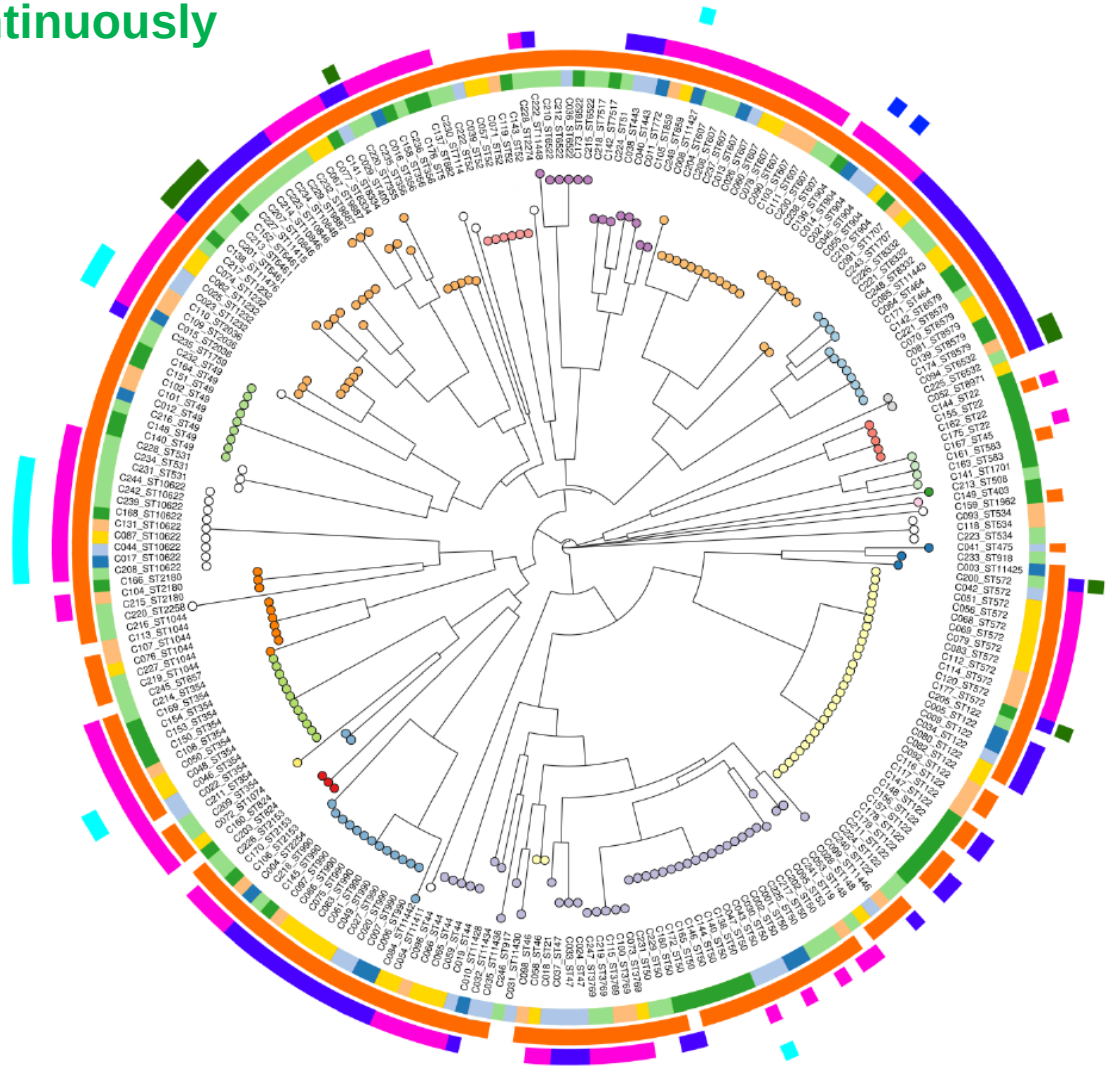
AMR-associated markers:
89.3%% carried the gyrA:p.T86I

67.6% were positive for tetO (or variants)

6.6% harbor the resistance-associated mutation in the 23S rRNA gene (A2075G) (much less than *C. coli*)

3.7% carried ant(6)-Ia) associated with streptomycin resistance

MDR isolates (ERY resistance with A2075G23S rRNA mutation) were less frequent than in *C. coli,* and were mainly associated with ST-10662



Clonal Complex

- ST-21 complex
- ST-22 complex
- ST-42 complex
- ST-45 complex
- ST-48 complex
- ST-206 complex
- ST-257 complex
- ST-353 complex
- ST-354 complex
- ST-403 complex
- ST-464 complex
- ST-508 complex
- ST-581 complex
- ST-607 complex
- ST-658 complex
- ST-49 complex
- ST-52 complex
- ST-443 complex
- ST-460 complex

Isolation year

- 2016
- 2017
- 2018
- 2019
- 2020
- 2021

Ciprofloxacin AMR

- gyrA T86I

Streptomycin AMR

- ant(6)-Ia

Tetracycline AMR

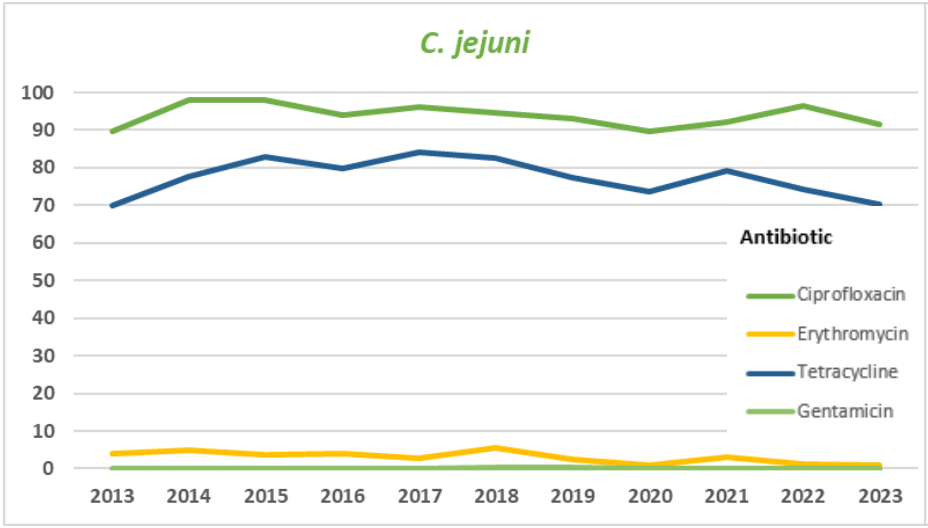
- tet(O)
- tet(O/32/O)

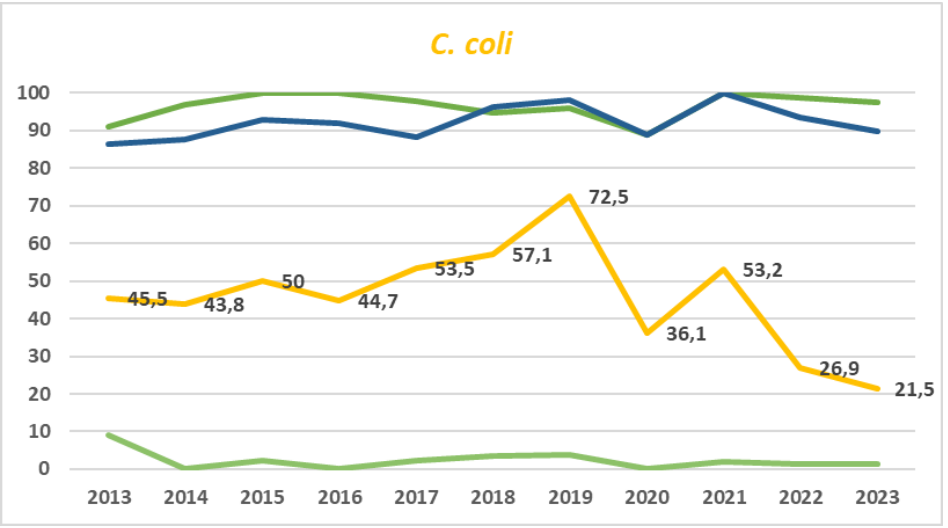
Erythromycin AMR

- 23SrRNA A2074N
- 23SrRNA A2075G

Pathogens 2024, 13, 147. https://doi.org/10.3390/pathogens13020147

AMR (phenotype):





Extremely high levels of resistance to CIP and TET, for both *C. jejuni* and *C. coli*, with a stable trend.

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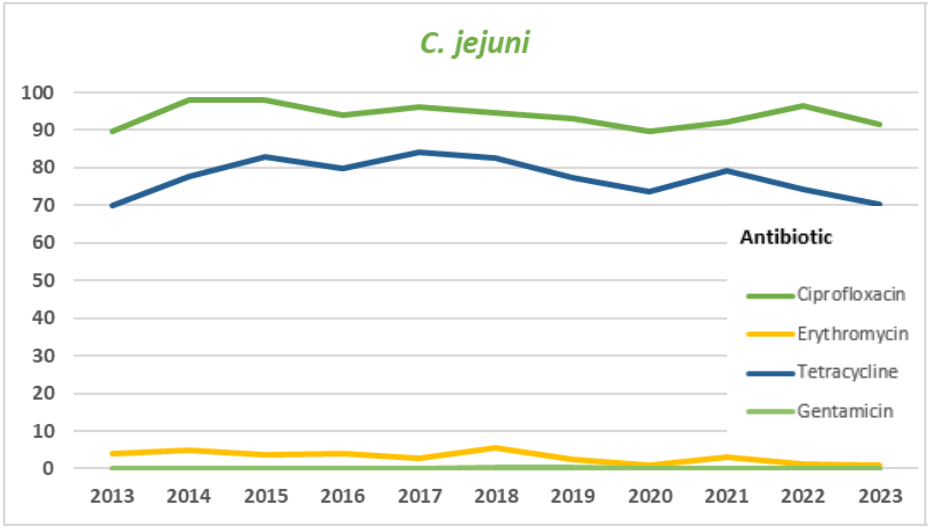

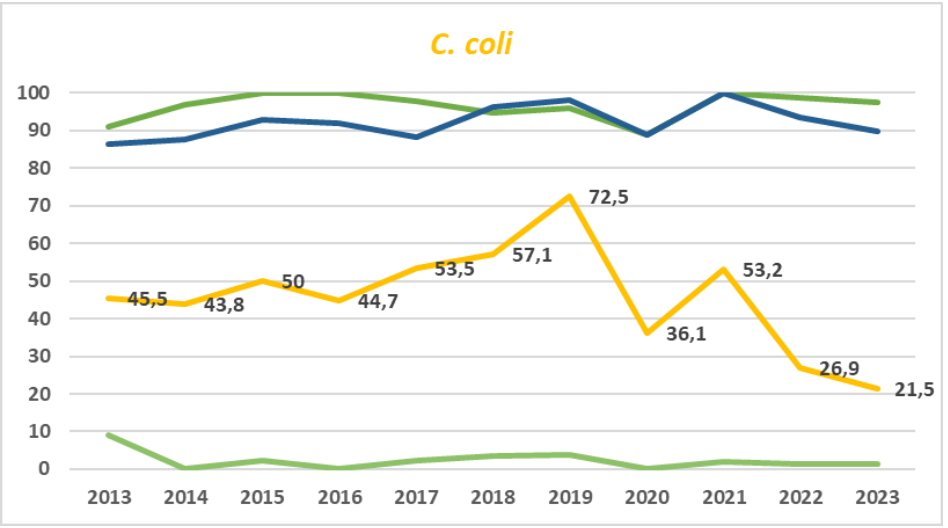

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Resistance to erythromycin:

The proportion of human isolates is significantly higher in *C. coli* than in *C. jejuni*, P-value <0.001; higher than the EU median for human isolates.

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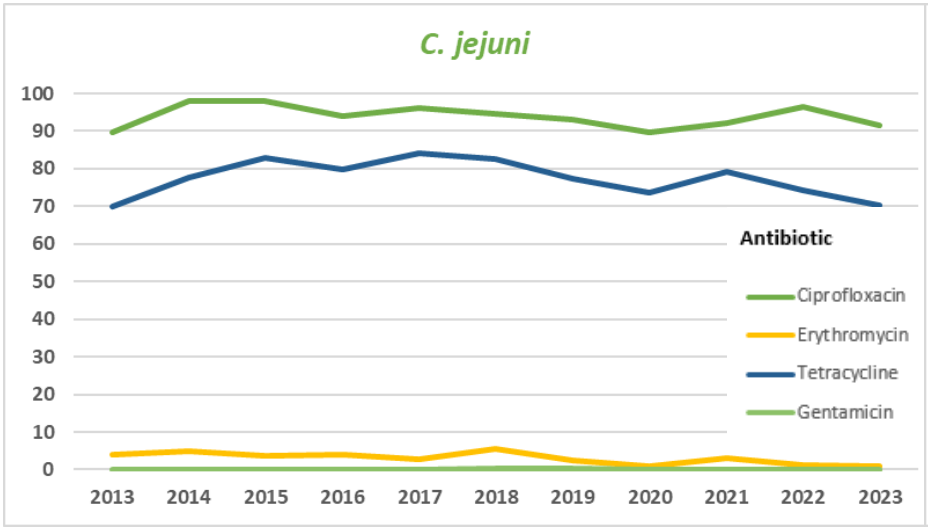

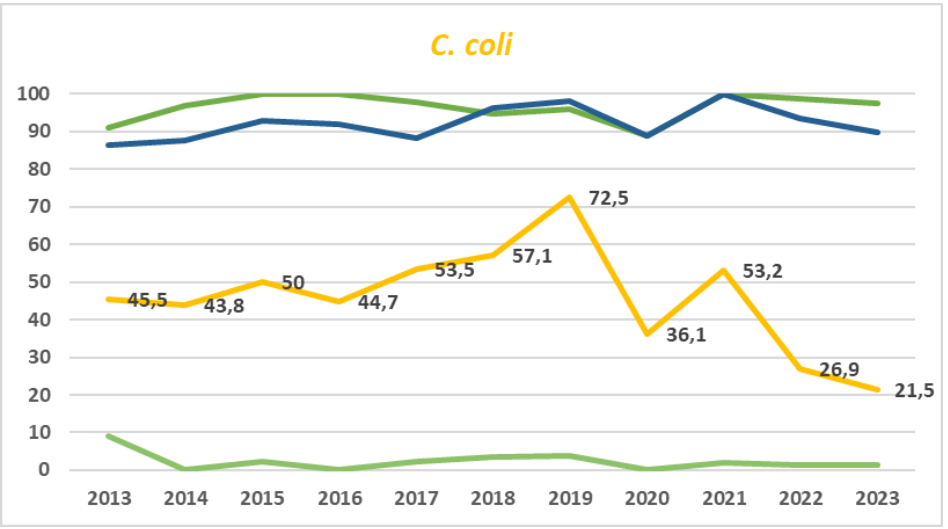

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Optional antibiotics:

AMP_R: ~ 71.5%; no difference between species
ERT_R: 0.7% in *C. jejuni*; 6.5% in *C. coli* (P-value <0.001)
AMC (intermediate): 0.2% in *C. jejuni*; 3.9% in *C. coli* (P-value <0.001)

AMR (genotype):

Antimicrobial class	Antimicrobial	Resistance determinants
Fluoroquinolones	Ciprofloxacin	***C. jejuni*: *gyrA*:p.T86I; *gyrA*:p.D90N** ***C. coli*: *gyrA*:p.T86I;** ***gyrA*:p.T86I + *gyrA*:p.D90N (double mutation)**
Tetracycline	Tetracycline	**tet(O), tet(W)**
Macrolides	Erythromycin	***C. jejuni*: 23S rRNA:2075A>G** ***C. coli*: 23S rRNA:2075A>G; *ermN* (5.1%)**
Aminoglycosides	Several	***aadB*** ***aadE-Cc*** ***ant(6)-Ia*** ***aph(3´)-III*** ***apmA**** **(gentamicin) in *C. coli***
Others	Several	***lnu(C)* (Lincosamide)** ***bla*$_{OXA-61}$/*bla*$_{OXA-193}$** ***G63T* in *bla*$_{OXA-61}$ promoter** (ampicillin; β-lactams)**

* *J Clin Microbiol* 56:e00390-18.https://doi.org/10.1128/JCM.00390-18; ***J. Antimicrob. Chemother.* 69, 1215–1223. doi: 10.1093/jac/dkt515

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- **ApmA**: aminocyclitol acetyltransferase; the gene found in *C. coli* revealed 100% identity with *apmA*, from MRSA, conferring resistance to apramycin;
- ApmA is associated with a low level of gentamicin hydrolysis, which is in line with the low gentamicin MIC found for the *C. coli* strain (16 mg/lL)

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AMR (phenotype vs genotype):

- Agreement between phenotypic resistance and the presence of known resistance genes and/or point mutations for the 4 priority antibiotics
- High diversity of resistance determinants
- Importance of dissemination of resistance gene among different bacterial species (*ermN* and *apmA)*
- For AMC and ERT: unknown mechanisms (*porA*; *cmeABC, cmeR*);

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AMR and STs:

Ery_R

C. jejuni

ST10622 23S rRNA:2075A>G (MIC>256 mg/L)

C. coli

ST828
ST832
ST3016
ST9987
ST13826
} 23S rRNA:2075A>G (MIC>256 mg/L)

ST6690 *ermN* (MIC=8-24 mg/L)

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C. coli

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ST6690 *ermN* (MIC=8-24 mg/L)

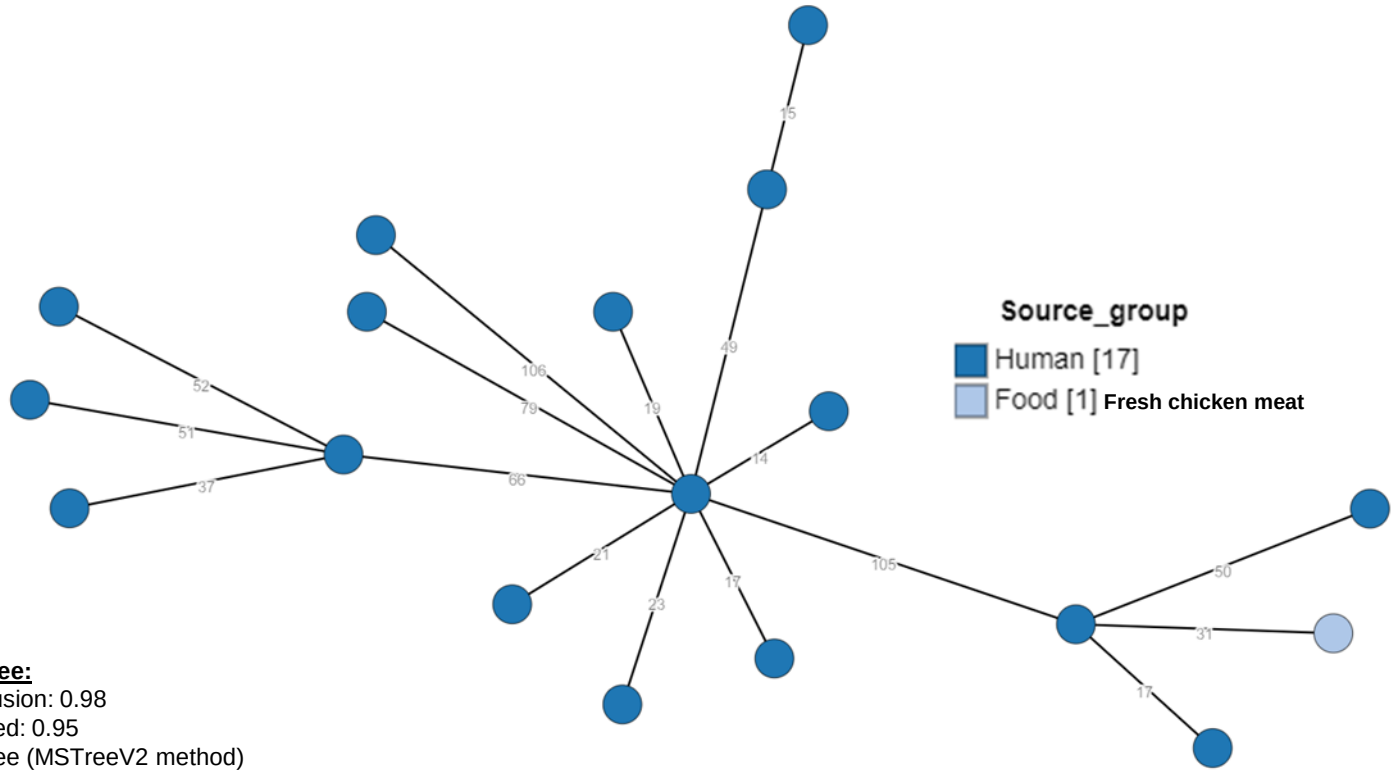
Ertapenem_R

ST354, (low level; MIC=1.5-3.0 mg/L)

AMR and STs:

Campylobacter jejuni

ST-10622 (1%); ERY_R; Mutation: **23S rRNA:2075A>G,** non-clonal (isolates collected from Y_2016-2024, 3 geographical regions)

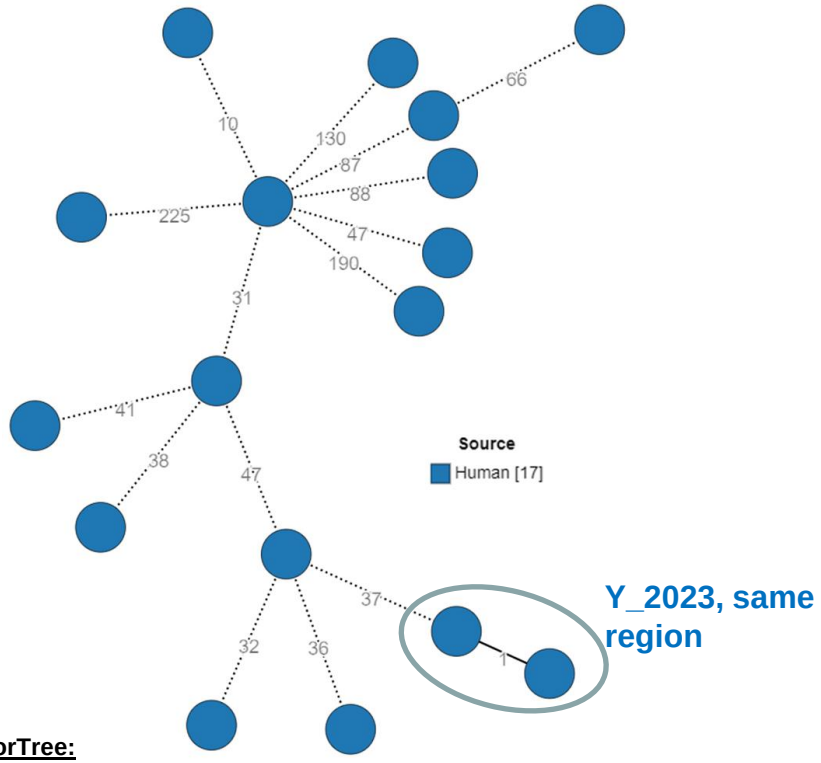


ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 18 samples x 765 allele-calls from a total of 2794 loci
Gene-by-gene analysis with the chewBBACA software and the INNUENDO *C. jejuni* wgMLST

AMR and STs:

Campylobacter coli

ST-3016 (CC828) (2.8%); ERY_R; **23S rRNA:2075A>G**; non-clonal (isolates collected from Y_2016-2023, 3 geographical regions)

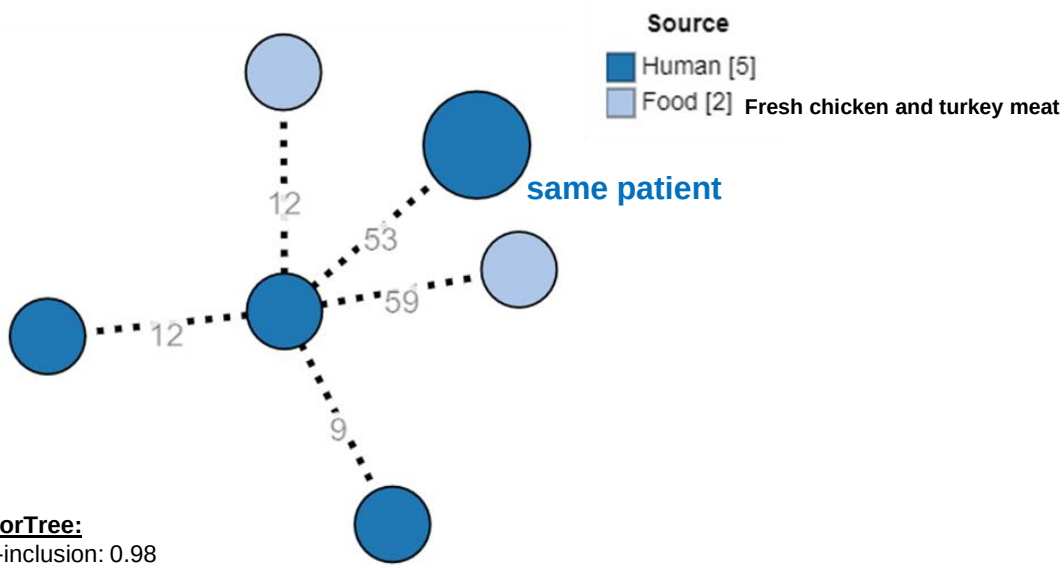


ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 17 samples x 799 allele-calls from a total of 2477 loci (gently provided by INNUENDO)
Gene-by-gene analysis with the chewBBACA software/*C. coli* wgMLST (unpublished)

AMR and STs:

Campylobacter coli

ST-6690 (CC828) (5.1%); ERY_R; ***ermN* positive**; non-clonal (all isolates from 2023, dispersed among the 3 regions)



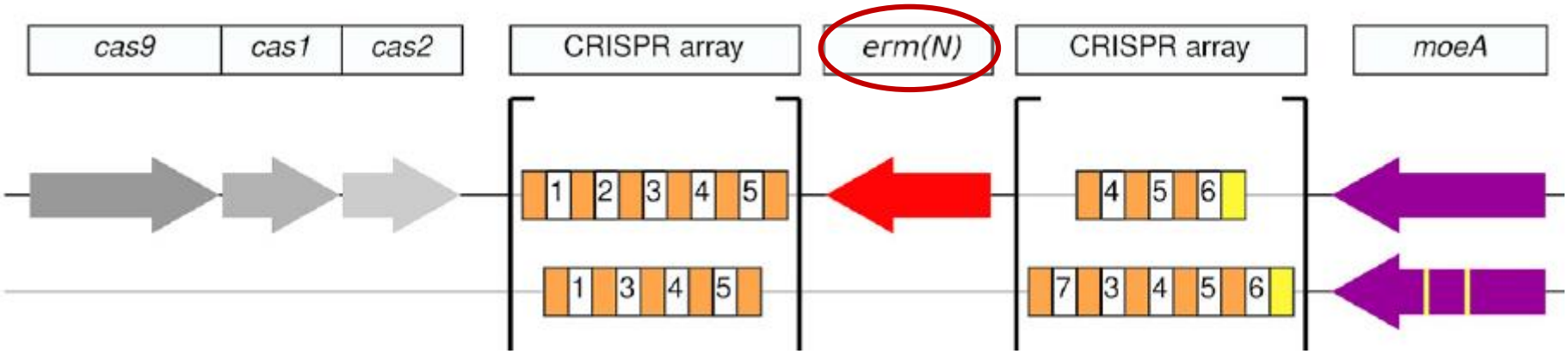
ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 7 samples x 721 allele-calls from a total of 2477 loci (gently provided by INNUENDO)
Gene-by-gene analysis with the chewBBACA software/*C. coli* wgMLST (unpublished)

AMR and STs:

Campylobacter coli

ST-6690 (CC828) (2.8%); ERY_R; ***ermN* positive**; non-clonal (all isolates from 2023, North region)

Region carrying *ermN* located within the CRISPR array of the CRISPR-cas9 operon



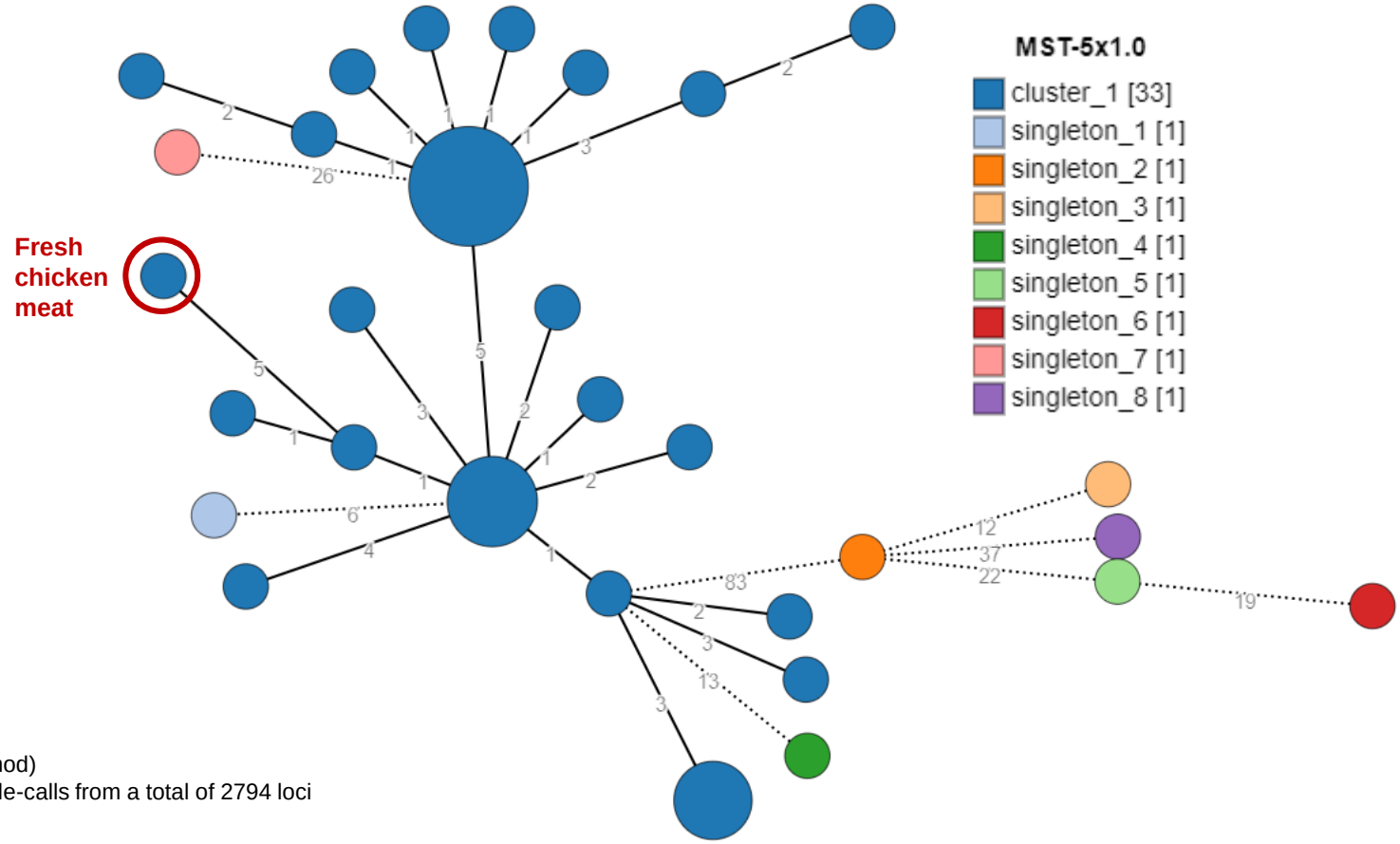
Different combinations of spacers within the **CRISPR array** (I-VII) (Quentin, J., CNRCH, unpublished data)

Antimicrob Agents Chemother. 2021 Oct 18;65(11):e0112421. doi: 10.1128/AAC.01124-21.

AMR and STs:

Campylobacter jejuni

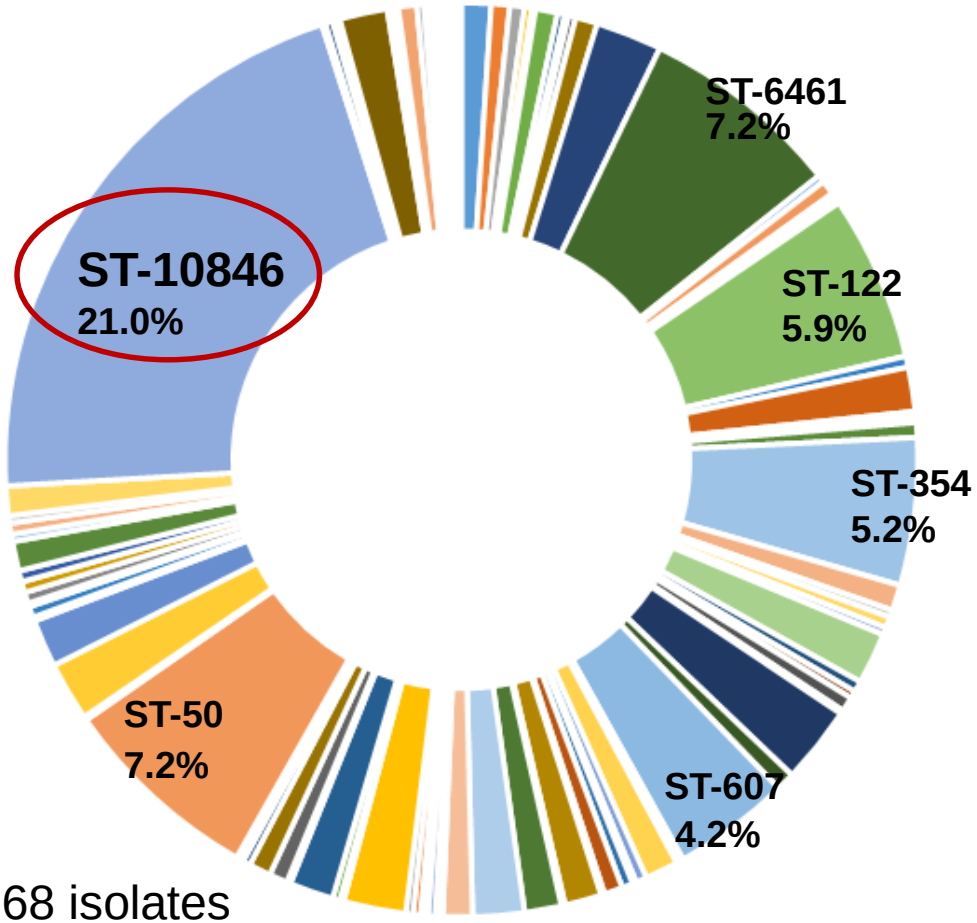
ST-354 (5.2%), CC ST-354; ERT_R (low level; MIC=1.5-3.0 mg/L); isolates collected from Y_2022-2024, mostly from North region



ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 41 samples x 586 allele-calls from a total of 2794 loci

WGS-based outbreak detection – *C. jejuni*

C. jejuni STs distribution (2022-2024)

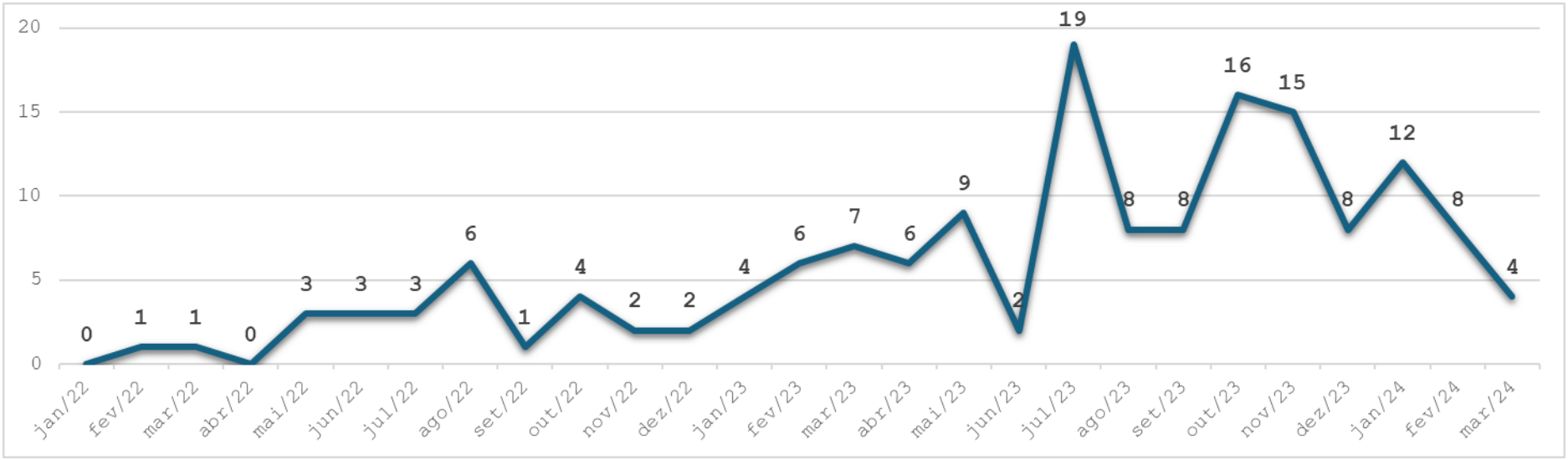


103 different STs in 768 isolates

6 frequent STs; CC ST-353 (ST-10846 + ST-6461) was the most prevalent.

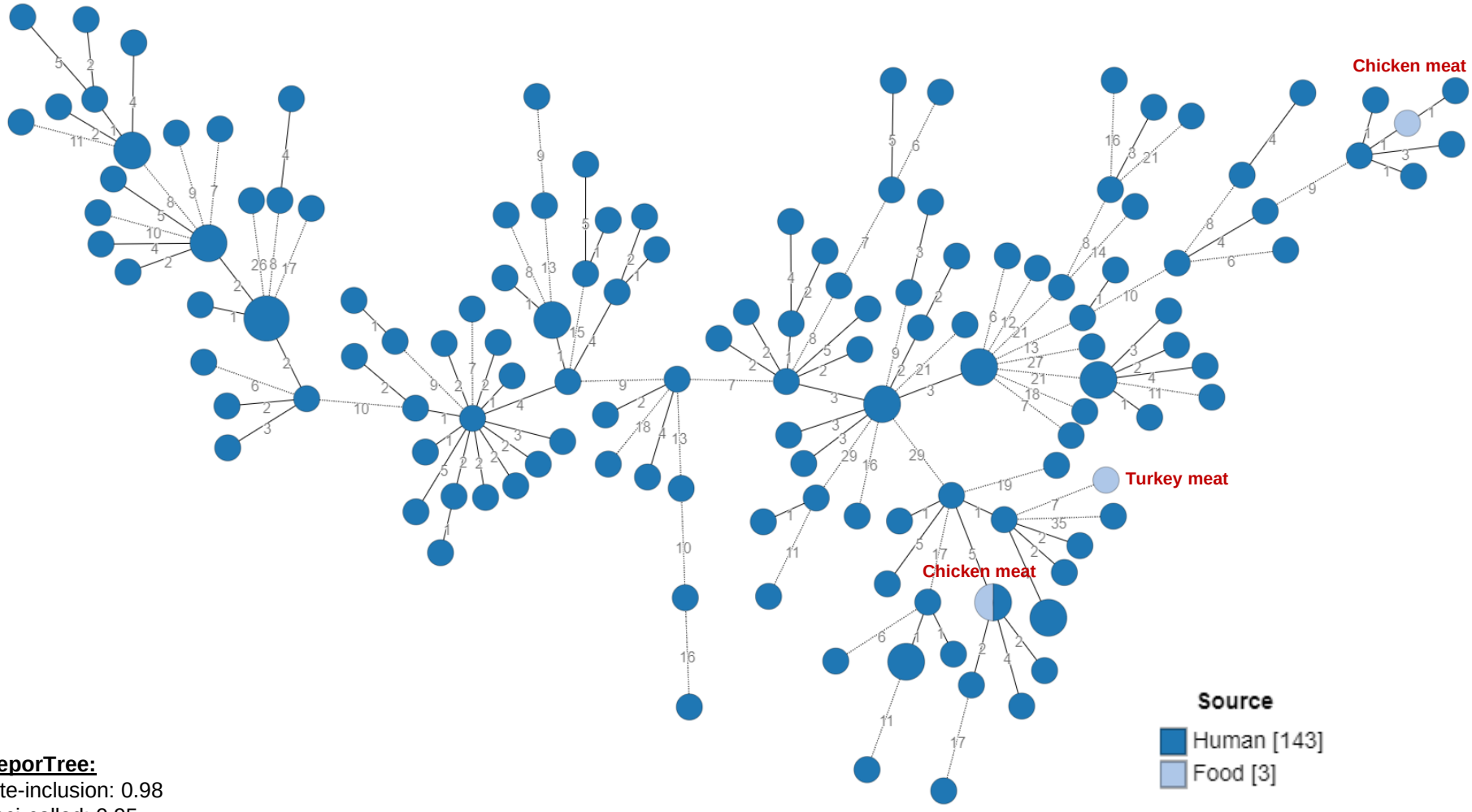
WGS-based outbreak detection – *C. jejuni* ST-10846

***C. jejuni* ST-10846 distribution (2022-2024)**



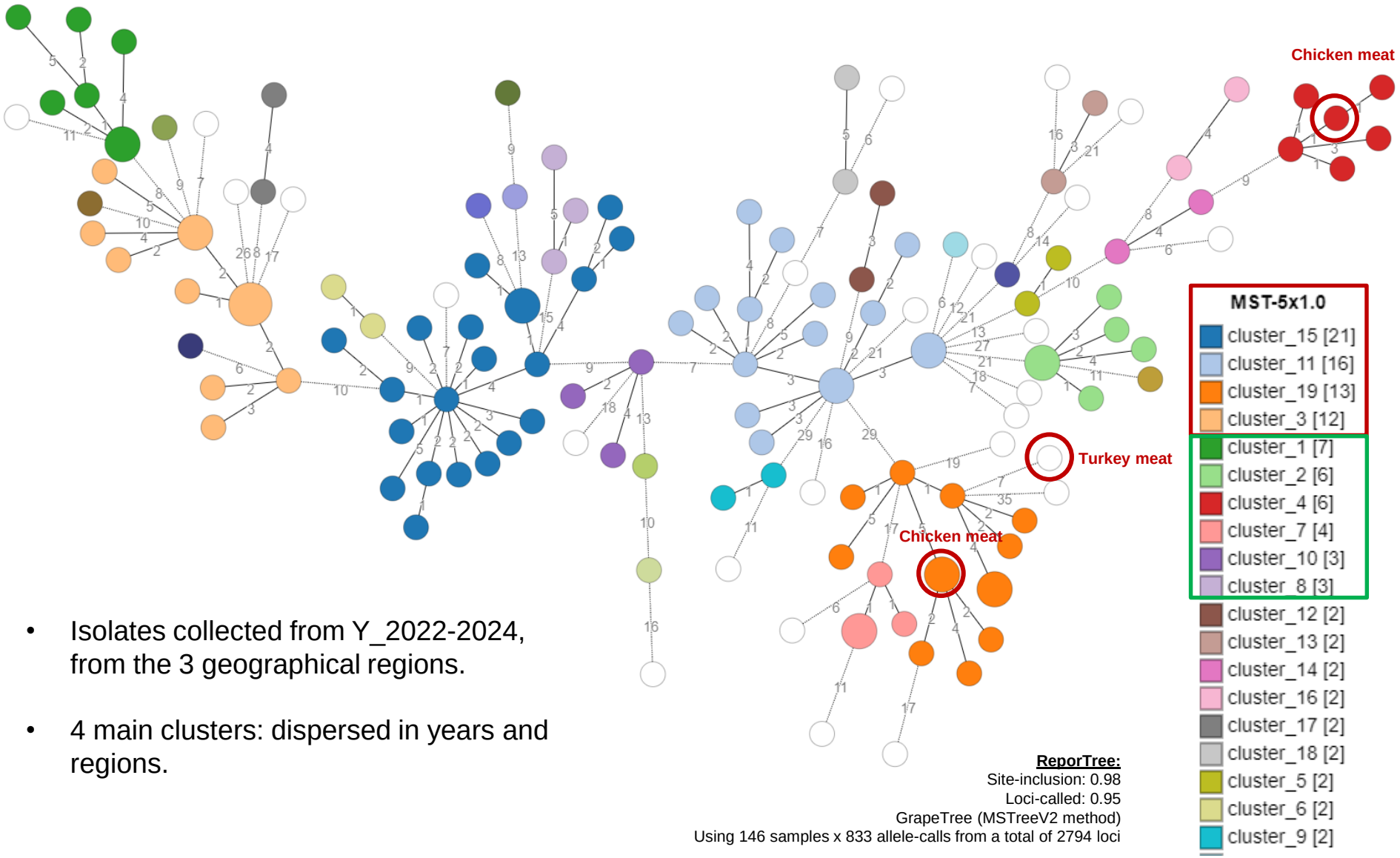
- Cases occurred throughout the years; peaks in summer and autumn.
- In **July-August 2023** there was a peak of infections (**n=27**).
- Cases peaked again in **October/November 2023, reaching the highest number (n=31)**, another peak in **January 2024**.

WGS-based outbreak detection – *C. jejuni* ST-10846



ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 146 samples x 833 allele-calls from a total of 2794 loci

WGS-based outbreak detection – *C. jejuni* ST-10846

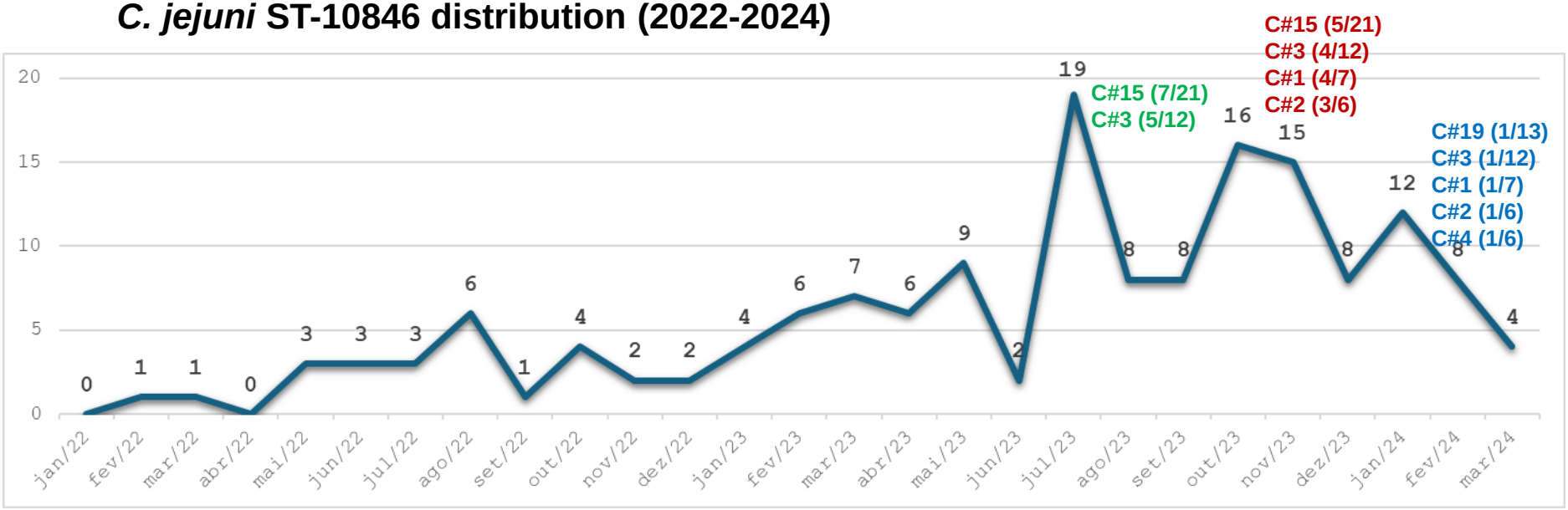


- Isolates collected from Y_2022-2024, from the 3 geographical regions.
- 4 main clusters: dispersed in years and regions.

ReporTree:
Site-inclusion: 0.98
Loci-called: 0.95
GrapeTree (MSTreeV2 method)
Using 146 samples x 833 allele-calls from a total of 2794 loci

WGS-based outbreak detection – *C. jejuni* ST-10846

***C. jejuni* ST-10846 distribution (2022-2024)**



- No association between peaks of infections and genetic clusters.

Summarizing

- *Campylobacter* AMR surveillance through WGS can be a valuable addition to the phenotypic surveillance, providing insights into the genetic basis of resistance mechanisms, and helping monitoring the emergence and spread of MDR clones
- A vast diversity of STs circulating in Portugal; huge diversity within the same ST
- MDR isolates within a single ST harboring the same genetic determinants (even rare ones, *ermN*) may belong to different genetic clusters
- *Campylobacter* outbreaks can span over several years and it's detection is challenging
- *Campylobacter* outbreaks often go unnoticed due to limited molecular typing in surveillance systems
- The data presented highlights the importance of WGS-based surveillance in the identification of potential *Campylobacter* outbreaks.
- Retail poultry meat is an important source of *Campylobacter*

THANK YOU FOR YOUR ATTENTION

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