The Establishment of the Irish human *Campylobacter* Reference Laboratory Service and Results from 2022 Annual Data

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Dr Tee Keat Teoh, Medical Microbiologist and Acting Director,
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incorporating NRL pathogen specific services

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Public Health Laboratory Dublin, Cherry Orchard Hospital

- Food and water microbiology laboratory
- Enteric pathogen diagnostic laboratory
- Incorporates national reference laboratory services for
  - *Campylobacter* species
  - *Clostridioides difficile*
  - Verotoxigenic *E.coli*

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Campylobacter NRL service

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- Sentinel surveillance programme
  - Started in February 2019
- Derived from work by EUPHEM fellow, Dr Carina Brehony and PHL Dublin team.
- 24 of 32 microbiology laboratories participating across Ireland
- Established testing schedule with HPSC to provide a representative collection of specimens nationally

Notified cases of *Campylobacter* infections in Ireland

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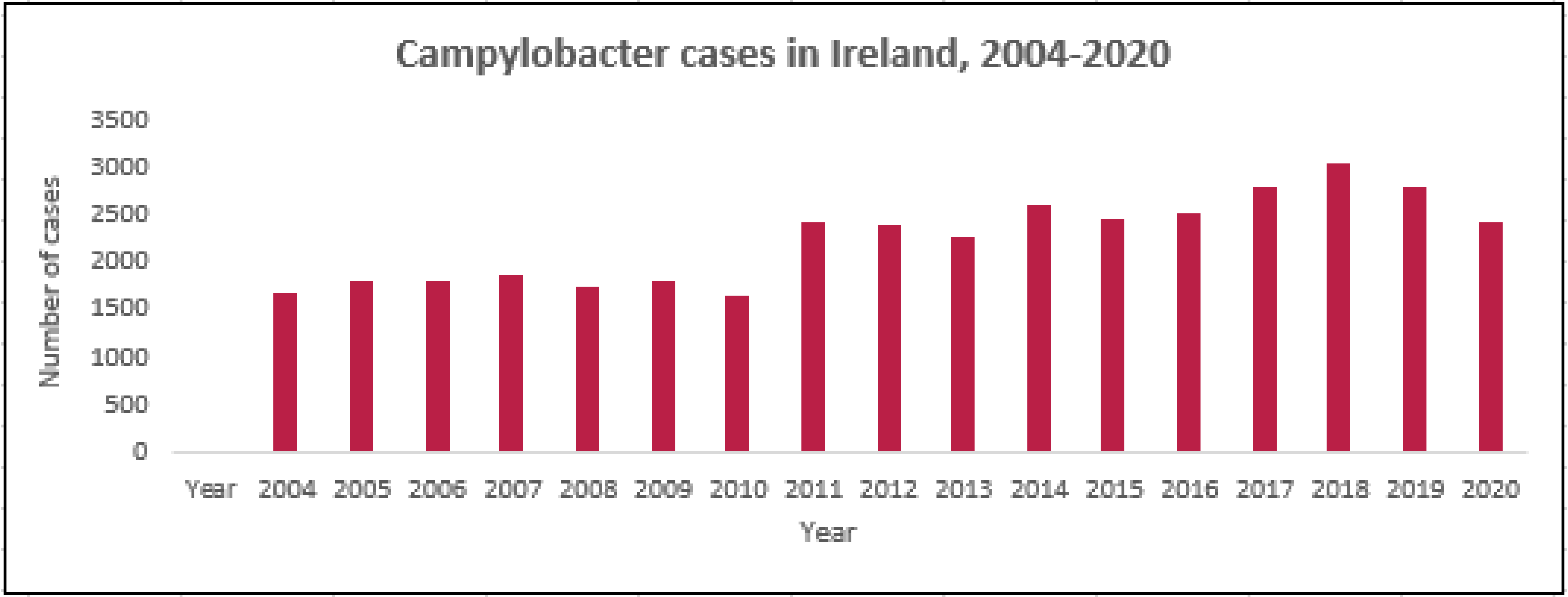

Campylobacter NRL Methodology

Isolates & Stools (PCR positive for *Campylobacter spp*)

- 48-hour culture microaerophilically 42°C on CAMP (Preston agar)
- Gram stain and oxidase test on suspect colonies
- MALDI-ToF being introduced

Phenotypic AMS by disc diffusion

- Ciprofloxacin, erythromycin and tetracycline
- EUCAST breakpoints applied

Genomic analysis

- Isolates referred for WGS
- Extraction and library preparation with Illumina Nextera kit
- Sequenced on Illumina MiSeq
- Bioinformatics performed using Bionumerics platform

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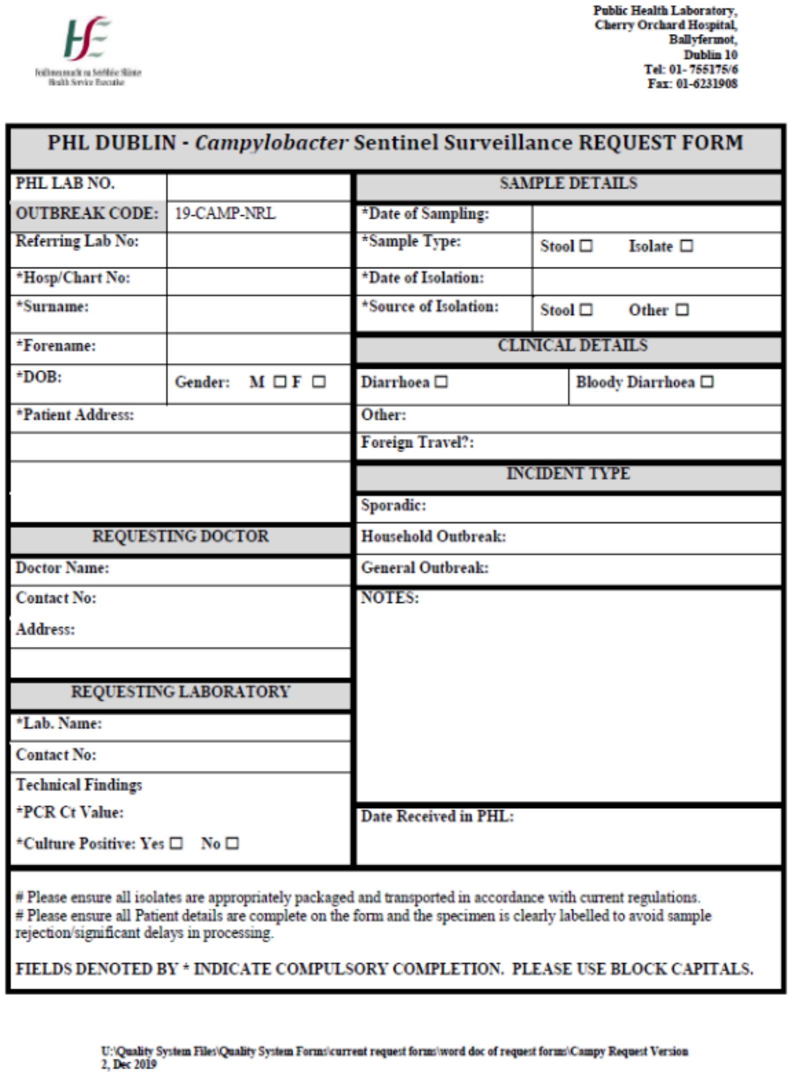
Sample referral



Public Health Laboratory,
Cherry Orchard Hospital,
Ballyfermot,
Dublin 10
Tel: 01- 755175/6
Fax: 01-6231908

PHL DUBLIN - *Campylobacter* Sentinel Surveillance REQUEST FORM

PHL LAB NO.	
OUTBREAK CODE:	19-CAMP-NRL
Referring Lab No:	
*Hosp/Chart No:	
*Surname:	
*Forename:	
*DOB:	Gender: M ☐ F ☐
*Patient Address:	

REQUESTING DOCTOR

Doctor Name:
Contact No:
Address:

REQUESTING LABORATORY

*Lab. Name:
Contact No:
Technical Findings
*PCR Ct Value:
*Culture Positive: Yes ☐ No ☐

SAMPLE DETAILS

*Date of Sampling:	
*Sample Type:	Stool ☐ Isolate ☐
*Date of Isolation:	
*Source of Isolation:	Stool ☐ Other ☐

CLINICAL DETAILS

Diarrhoea ☐ Bloody Diarrhoea ☐
Other:
Foreign Travel?:

INCIDENT TYPE

Sporadic:
Household Outbreak:
General Outbreak:

NOTES:

Date Received in PHL:

Please ensure all isolates are appropriately packaged and transported in accordance with current regulations.
Please ensure all Patient details are complete on the form and the specimen is clearly labelled to avoid sample rejection/significant delays in processing.

FIELDS DENOTED BY * INDICATE COMPULSORY COMPLETION. PLEASE USE BLOCK CAPITALS.

U:\Quality System Files\Quality System Forms\current request forms\word doc of request forms\Campy Request Version 2, Dec 2019

- ❑ Isolates and Stools – PCR pos for *Campylobacter spp.*
- ❑ Send PHL-NRL-*Campylobacter* Sentinel Surveillance form

Campylobacter NRL Reports

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1. Individual culture and phenotypic AMS results sent out contemporaneously to the sender
2. Fortnightly review of the *Campylobacter* WGS database for clusters
3. Quarterly NRL *Campylobacter* report
4. Annual NRL *Campylobacter* report

WGS Results: Cluster Reports

Core genome MLST

If clusters are identified ,

- allelic ≤ 5 difference cgMLST for transmission report.

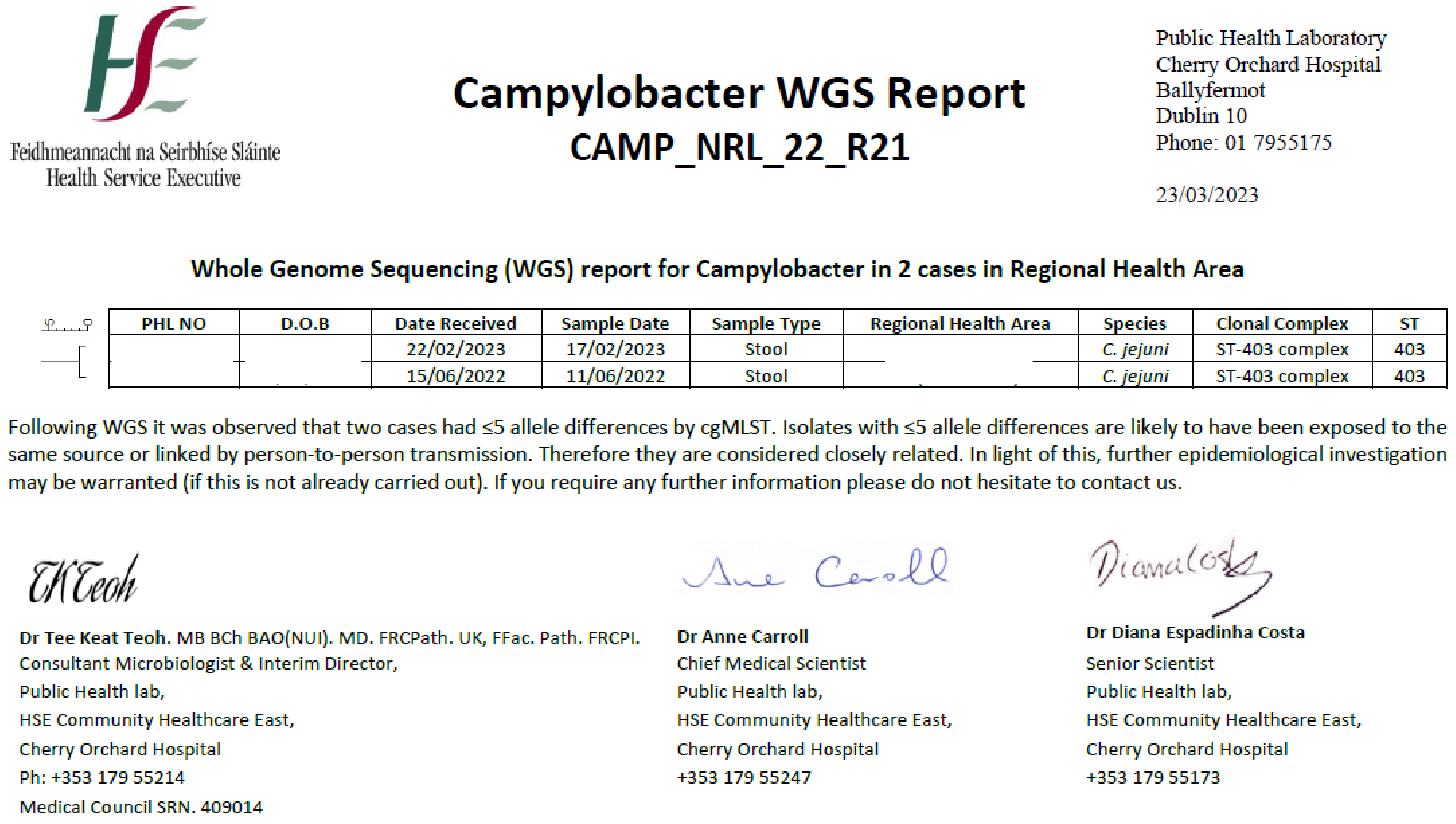
NRL issues a report to:

- Hospital lab. Senders (if relevant)
- Relevant Depts. of Public Health
- Health Protection and Surveillance Centre (HPSC)

Challenges:

Lack further epidemiological data and action plans

Example of a *Campylobacter* cluster report



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Campylobacter WGS Report
CAMP_NRL_22_R21

Public Health Laboratory
Cherry Orchard Hospital
Ballyfermot
Dublin 10
Phone: 01 7955175

23/03/2023

Whole Genome Sequencing (WGS) report for Campylobacter in 2 cases in Regional Health Area

PHL NO	D.O.B	Date Received	Sample Date	Sample Type	Regional Health Area	Species	Clonal Complex	ST
		22/02/2023	17/02/2023	Stool		*C. jejuni*	ST-403 complex	403
		15/06/2022	11/06/2022	Stool		*C. jejuni*	ST-403 complex	403

Following WGS it was observed that two cases had ≤5 allele differences by cgMLST. Isolates with ≤5 allele differences are likely to have been exposed to the same source or linked by person-to-person transmission. Therefore they are considered closely related. In light of this, further epidemiological investigation may be warranted (if this is not already carried out). If you require any further information please do not hesitate to contact us.

TKTeoh

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2022 WGS Results: Isolates

Table 1 - Breakdown of speciation of isolates sequenced from 2019 to 2022.

Year	WGS*	*C. jejuni*		*C. coli*		*C. fetus*		*C. lari*	
		n	%	*n*	%	*n*	%	*n*	%
2022	**218**	**181**	83%	**37**	17%	**1**	0.5%	**0**	**-**
2021	200	181	91%	17	9%	-	-	2	1%
2020†	74	67	91%	7	9%	-	-	0	0%
2019	257	223	87%	29	11%	-	-	5	2%

† This dataset of sentinel surveillance was truncated due to SARS-CoV-2 monitoring

* The number of isolates that pass the WGS QC analysis criteria

Table 2 - Breakdown of the most prevalent STs and clonal complexes 2019-2022

Year	WGS*	ST-21		ST-19		ST-48		ST-21 CC**	
		n	%	*n*	%	*n*	%	*n*	%
2022	**218**	15	6.9%	13	6.0%	12	5.5%	42	19.3%
2021	200	25	12.5%	13	6.5%	25	12.5%	54	27.0%
2020†	74	9	12.2%	5	6.8%	10	13.5%	27	36.5%
2019	257	31	12.1%	6	2.3%	26	10.1%	69	26.8%

† This dataset of sentinel surveillance was truncated due to SARS-CoV-2 monitoring

* The number of isolates that pass the WGS QC analysis criteria

** ST-21 clonal complex (CC) was the most prevalent CC for all four years

NRL *Campylobacter* Distribution by Clonal Complex

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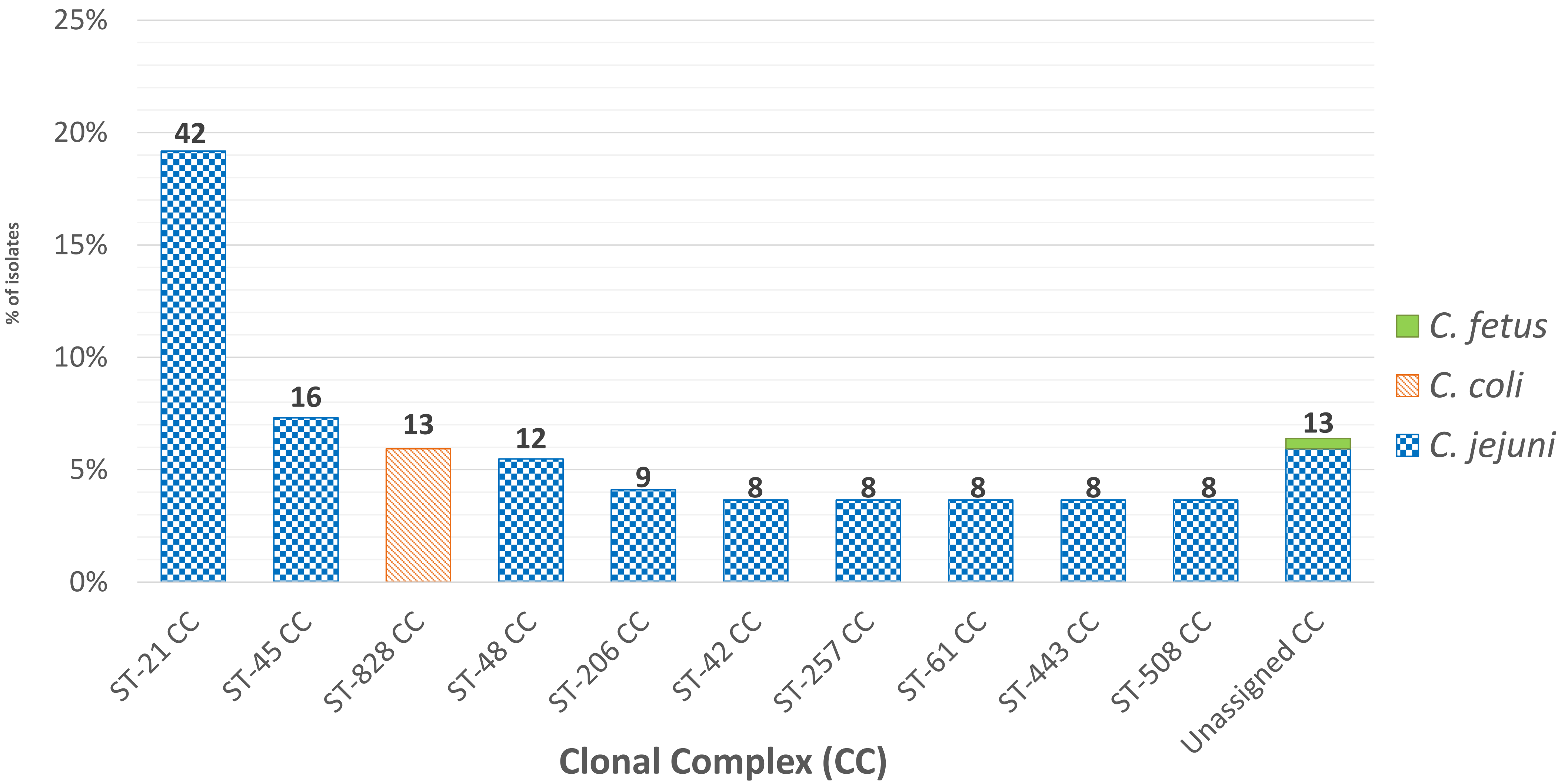

AST results

Table 1 – Antimicrobial susceptibility testing results, 2019 - 2022

Year	Total isolated	Susceptible n(%)	Ciprofloxacin resistance n(%)	Tetracycline resistance n(%)	Erythromycin resistance n(%)
2022	**220**	**116 (53.0%)**	**84 (38.4%)**	**70 (32.0%)**	**3** (1.4%)
2021	204	132 (64.7%)	57 (27.9%)	33 (16.2%)	2 (1.0%)
2020	85*	48 (56.5%)	26 (30.6%)	20 (23.5%)	0
2019	277	140 (50.5%)	109 (39.4%)	73 (26.4%)	2 (0.7%)

* This dataset of sentinel surveillance was truncated due to SARS-CoV-2 monitoring

Table 1 - Campylobacter resistance associated genes and phenotype concordance amongst isolates, 2022. N=218

	phenotype: resistant		**phenotype: susceptible**					
antibiotic class	**genotype: R**	**genotype: S**	**genotype: R**	**genotype: S**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Tetracycline	65	3	3	143	96%	98%	96%	98%
Erythromycin	3	0	1	210	100%	100%	75%	100%
Ciprofloxacin	79	2	0	133	98%	100%	100%	99%

Virulence factors

mechanism	**gene**	**no.**	**%**
Cytotoxin production	*cdtA*	210	96
	cdtB	213	98
	cdtC	186	85
Adherence and colonization	*flaA*	216	99
	cadF	216	99
	dnaJ	215	99
	racR	216	99
Invasion	*virB11*	2	1
	iam	200	92
	ciaB	199	91

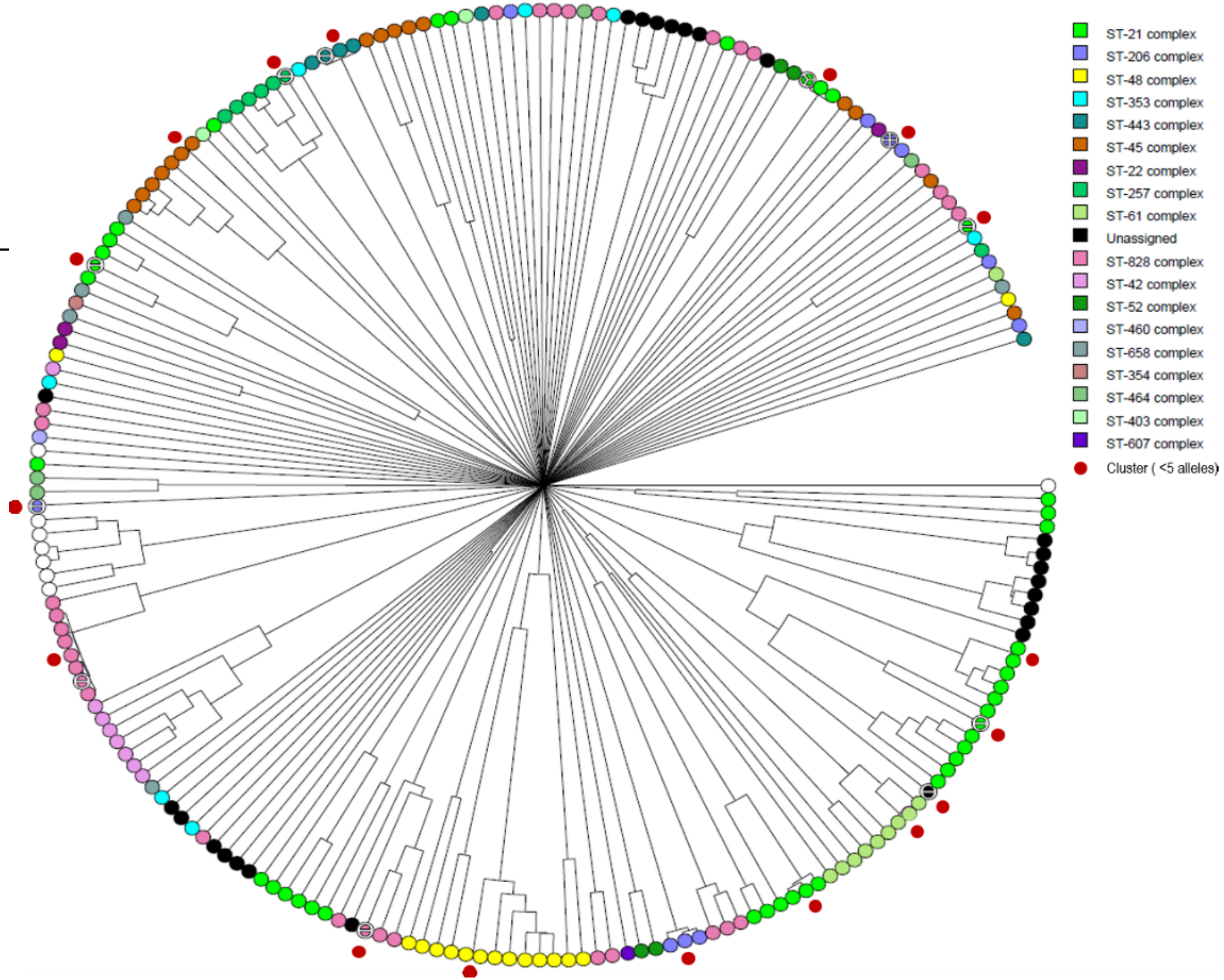

***Figure 2* –** UPGMA tree of cgMLST differences amongst *Campylobacter spp.* isolates (n=218) from 2022. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with a red circle**.**

- Elucidation of complex epidemiology strengthened by NRL service
- One Health Research with team from Department of Agriculture, Food and Marine
- Strengthen future public health measures in food bourne diseases

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