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National *Campylobacter* Reference Laboratory Service provided by PHL HSE, Dublin

Summary

- 432 clinical specimens received from 415 patients:
    - 386 stools
    - 46 isolate swabs
- 214 isolates/432 (49.5%) samples culture positive:
    - 182/386 stools (47.1%)
    - 32/46 isolate swabs (69.6%)
- 54% (n=116) susceptible to all three antimicrobials tested:
    - 15.4% (n=33) isolates were resistant to ciprofloxacin only.
    - 8.9% (n=19) were resistant to tetracycline only.
    - 20.6% (n=44) were resistant to ciprofloxacin and tetracycline.
    - 0.9% (n=2) were resistant to ciprofloxacin, erythromycin and tetracycline.
- 213/214 were sequenced and passed WGS QC for analysis:
    - 84.0% (n=179) *C. jejuni,* 15.5% *C. coli* (n=33), 0.5% *C. fetus* (n=1)
    - 70 STs and 20 clonal complexes were detected within this *Campylobacter* dataset.
    - ST-21 clonal complex was the most prevalent 21.6% (n=46).
- High phenotypic-genotypic congruence for antibiotic sensitivity detected.
- WGS identified 20 potential clusters for public health alert in 2022.

National human *Campylobacter* Sentinel Surveillance Reference Laboratory Service Annual Report 2022

Introduction

This is the 2022 Annual report of the national *Campylobacter* Clinical Sentinel Surveillance Reference Laboratory Service provided by the Public Health Laboratory (PHL), HSE, Dublin. The national laboratory surveillance service was initiated in February 2019. The 2021 schedule began on January 1st 2021 and involved the participation of 24 clinical microbiology laboratories in HSE regions from across the country:

- 12 from HSE Dublin Mid-Leinster
- 5 from HSE South
- 4 from HSE West
- 3 from HSE Dublin North-East

As this is a sentinel surveillance service, the original 2019 sampling frame devised in collaboration with HPSC in order to provide a representative collection of specimens nationally was proposed for 2022. Consequently, a sampling schedule was established whereby laboratories sent their *Campylobacter* PCR positive stool specimens or confirmed *Campylobacter* isolates (on Amies transport swabs) to the PHL, HSE, Dublin processed on a single designated week (Monday to Sunday) of each month. The programme contributes data to the European Centre for Disease Prevention and Control (ECDC) surveillance programme for campylobacteriosis.

Specimen submission

From January 1st 2022 to December 31st 2022 we received:

- A total of 432 clinical specimens from 415 patients, comprising of 386 stool specimens and 46 isolate swabs.
- A total of 214 (49.5%) *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 182/386 (47.1%) from PCR positive stool specimens and 32/46 (69.6%) from isolate swabs.

Speciation

Campylobacter spp. were confirmed by culture contemporaneously, once receipted in PHL and reported to clients. Speciation by WGS was completed in batches later. The submitted specimens were processed as follows:

1. Specimens (stool/isolate swab) were cultured for 48 hours microaerophilically @ 42°C on CAMP (Preston agar);
2. Gram stain and oxidase test was performed on any suspect colonies *i.e.* mucoid with a slightly metallic sheen;
3. *Campylobacter* was present if Gram negative curved bacilli and oxidase positive;
4. Speciation and antimicrobial resistance (AMR) determinants were confirmed by whole genome sequencing (WGS) on the isolates and interrogation of genome data against the publicly available databases https://pubmlst.org/campylobacter/ and https://pubmlst.org/rmlst/.

Antimicrobial Sensitivity Testing - phenotypic

Antimicrobial susceptibility testing (AST) initially by disk diffusion was performed according to EUCAST guidelines on all retrieved cultured isolates (n=214) for susceptibility to the antimicrobials; ciprofloxacin (CIP), erythromycin (ERY) and tetracycline (TET). 54.2% (n=116) susceptible to all three antimicrobials tested:

- 15.4% (n=33) isolates were resistant to ciprofloxacin only;
- 8.9% (n=19) were resistant to tetracycline only;
- 20.6% (n=44) were resistant to ciprofloxacin and tetracycline only;
- 0.9% (n=3) were resistant to the three drugs (all *C. coli* isolates).

Phenotypic culture and AST results were reported contemporaneously to the referring laboratory on each specimen submitted.

Table 1 **– Antimicrobial susceptibility testing results, 2019 – 2022.**

Year	Total (n)	Susceptible n (%)	CIP resistance n (%)	TET resistance n (%)	ERY resistance n (%)
2022	**214**	**116 (54.2%)**	**84 (36.9%)**	**65 (30.4%)**	**2 (0.9%)**
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 testing.

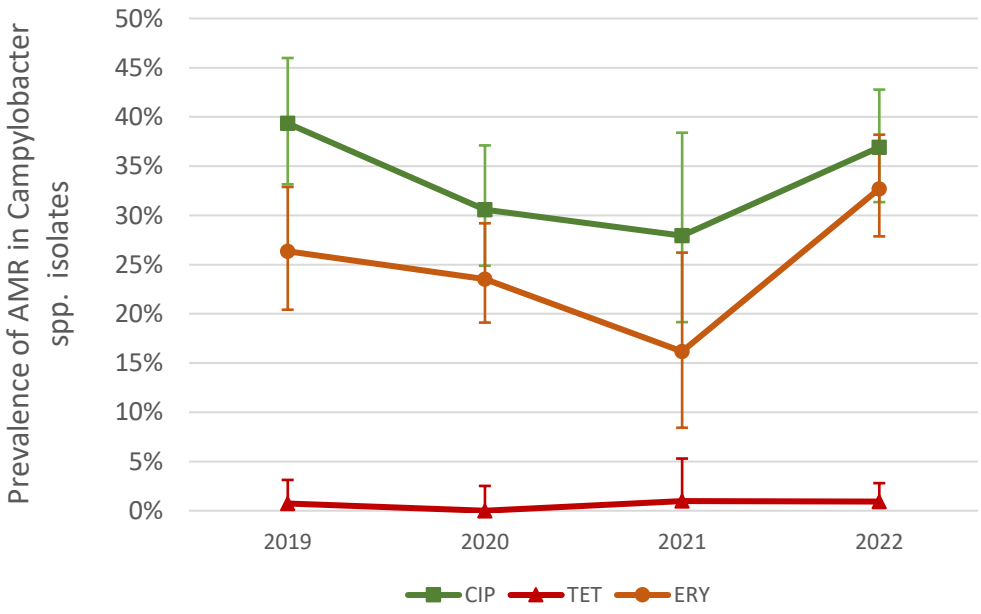

Figure 1- Trends in the prevalence of antimicrobial resistance in Campylobacter spp. Isolates received, 2019-2022.

Whole Genome Sequence *Campylobacter* characterization

A total of 213 *Campylobacter* isolates were available for WGS. One isolate could not be recovered after storage and was not characterized by WGS. High-quality DNA was extracted from confirmed isolates and DNA libraries were prepared using the Illumina DNA Prep kits and sequenced on an Illumina MiSeq instrument. Sequence yields that passed quality parameters (Q-score, GC content yield, coverage) were assembled *de novo* using the Bionumerics platform (version 8.1.1). These genome assemblies were then assessed for quality using the metrics N50, contig length, total sequence length, and percent core coverage. WGS analysis for speciation, genomic AMR and virulence determinants and cluster detection was completed for the 213 isolates that passed the quality criteria.

C. jejuni accounted for 84.0% (n=179) of isolates and *C. coli* 15.5% (n=33) *and C. fetus 0.5%* (n=1). There was a diversity of sequence types (ST) with 70 STs found in total – 53 STs in *C. jejuni* and 16 STs in *C. coli* and one ST assigned for the *C. fetus* isolate. Five isolates had no ST assigned, four *C. jejuni* and one *C. coli* isolate.

The 70 STs resolved into 20 clonal complexes (CC), with ST-21 CC being the most prevalent at 21.6% (n=46).The most prevalent STs were ST-21 (7.0%), ST-19 (6.1%) and ST48 (5.6%). A clonal complex was not assigned to 25 isolates (11.7%) (Table 3, Figure 2). 32/33 *C. coli* isolates belonged to the ST-828 CC, and one *C. coli* isolate had no CC assigned. The ST-828 CC is exclusively associated with *C. coli.*

Table 2 **- 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	**213**	**179**	84.0%	**33**	15.5%	**1**	0.5%	**0**	-
2021	200	181	90.5%	17	8.5%	-	-	2	1.0%
2020[†]	74	67	90.5%	7	9.5%	-	-	0	0.0%
2019	257	223	86.8%	29	11.3%	-	-	5	1.9%

[†] This dataset of sentinel surveillance was truncated due to SARS-CoV-2 testing.

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

Table 3 **- 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	**213**	15	7.0%	13	6.1%	12	5.6%	46	21.6%
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 testing.

* 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.

Note on clonal complexes: A clonal complex comprises a group of related STs. STs are grouped into clonal complexes by their similarity to a central genotype. For example, the ST-21 complex includes STs (e.g. here ST-19, ST-21, ST-47, ST-50, ST-53, ST-806, ST-7041) that matches the central genotype *i.e.* ST-21 at four or more of the conventional MLST seven housekeeping gene alleles.

Table 3 – Frequency of the 70 sequence types (STs) and clonal complexes (CCs) found in the *Campylobacter* sentinel collection 2022 (n=213). STs with more than four representative isolates shown.

Sequencing Type	Clonal Complex	N	%
21	ST-21	15	7.0%
19	ST-21	13	6.1%
48	ST-48	12	5.6%
45	ST-45	9	4.2%
61	ST-61	9	4.2%
10042*	ST-828	8	3.8%
50	ST-21	8	3.8%
257	ST-257	8	3.8%
441	Unassigned CC	8	3.8%
137	ST-45	7	3.8%
51	ST-443	6	3.3%
53	ST-21	6	2.8%
508	ST-508	6	2.8%
122	ST-206	5	2.8%
827*	ST-828	5	2.3%
206	ST-206	4	2.3%
436	Unassigned CC	4	1.9%
< 4	N/A	72	33.8%

* denotes sequence types/clonal complex composed entirely by *C. coli* isolates.

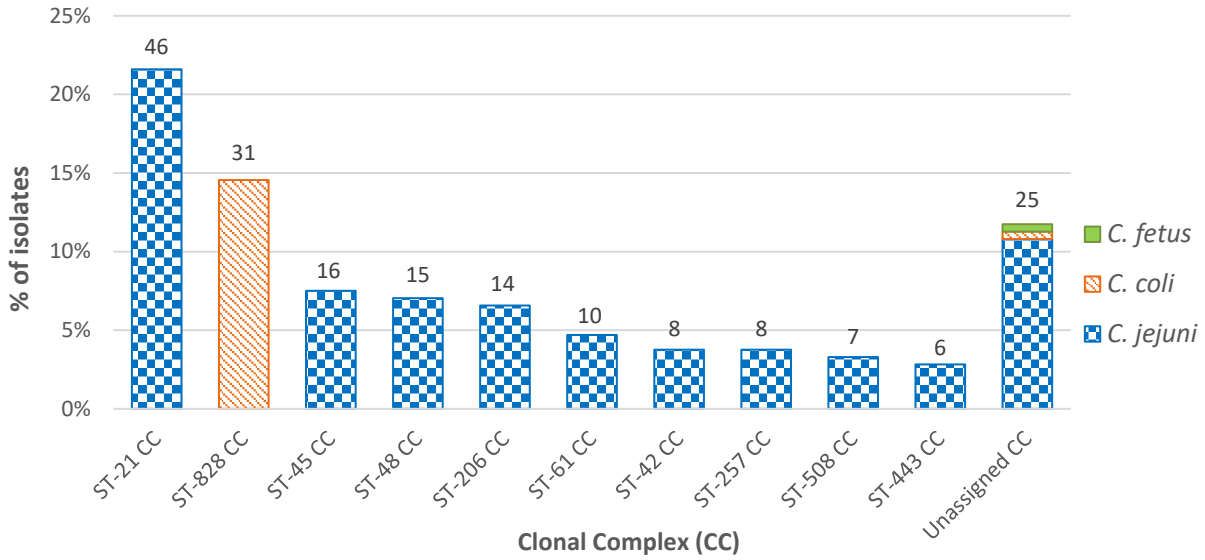

***Figure 2* - Distribution of the 213 sequenced *Campylobacter spp.* isolates by clonal complex.**

AST phenotype and genotype comparison

Of the 79 isolates with phenotypic ciprofloxacin resistance, 78 were sequenced and passed WGS validity criteria. Of these, 76 contained the *gyrA* mutation Thr86Ile/Val. Of the 135 isolates that were susceptible to ciprofloxacin, none had the *gyrA* mutation. Therefore, there was 97% and 100% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 100% and negative predictive value of 99% (Table 4). The sensitivity result is higher than that observed in 2021 (91%).

All 65 isolates with phenotypic tetracycline resistance were sequenced and passed WGS validity criteria. Of these, 62 harboured the gene *tetO*. Of the 149 isolates that were susceptible to tetracycline, 148 were sequenced and passed WGS validity criteria. Of these 148, 145 did not harbour *tetO*. Therefore, there was 95% sensitivity and 98% specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 95% and negative predictive value of 98% (Table 4). The sensitivity result is higher than that observed in 2021 (79%).

Two isolates were found to have phenotypic erythromycin resistance. The 23S rRNA gene mutation associated with mediating macrolide resistance mutation was detected in both isolates but not the *ermB* gene. On the other hand, the *erm*B gene was detected in a phenotypically susceptible isolate. (Table 4).

Based on the above observations, a monitoring of these trends is advised and will be maintained.

***Table 4* – Campylobacter resistance associated genes and phenotype concordance amongst isolates, 2022 (n=213).**

	phenotype: resistant		phenotype: susceptible					
antibiotic class	**genotype: R**	**genotype: S**	**genotype: R**	**genotype: S**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Tetracycline	62	3	3	145	95%	98%	95%	98%
Erythromycin	2	0	1	210	100%	100%	67%	100%
Ciprofloxacin	76	2	0	135	97%	100%	100%	99%

Virulence factors

The following is a breakdown of the virulence factors found in the *Campylobacter* isolates (n=213). The adherence and colonization associated factor genes *flaA, cadF, dnaJ* and *racR* were found in 211/213 isolates. *cdtA* was present in 205 isolates, *cdtB* was present in 208 isolates, while *cdtC* was present in 177 of the *C. jejuni* isolates and in four of the *C. coli* isolates. The invasion associated *virB11* gene was found in one *C. jejuni* and one *C. coli* isolates. The *iam* and *ciaB* genes were present in 195 and 194 isolates respectively (Table 5).

It must be noted that the Bionumerics and PubMLST databases were specifically developed for *C. jejuni* and *C. coli* and therefore not optimized for the analysis of non-*C. jejuni*/*coli* species like *C. lari* and *C. fetus*.

Table 5 **– Virulence factors presence detected by WGS among *Campylobacter spp.* isolates 2022 (n=213).**

Mechanism	Gene	No.	%
Cytotoxin production	*cdtA*	205	96.2
	cdtB	208	97.7
	cdtC	181	85.0
Adherence and colonization	*flaA*	211	99.1
	cadF	211	99.1
	dnaJ	211	99.1
	racR	211	99.1
Invasion	*virB11*	2	0.9
	iam	195	91.5
	ciaB	194	91.1

Cluster analysis

Isolate genomes were compared for relatedness by comparison at 1343 genes using core genome MLST (cgMLST) (Figure 3). A difference of five cgMLST alleles or fewer was used as an alert threshold to consider cluster investigation [1].

Using this cluster criterion there were 20 clusters of 2022 isolates, 17 of these exclusively composed of 2022 isolates, that were closely related genetically and warranted a public health alert to consider investigation for potential epidemiological links. This compared to 22 clusters in 2021, 31 clusters in 2019, and 7 clusters in 2020 (which was truncated due to SARS-CoV-2 testing). These sets of clusters ranged from 2 isolates per cluster up to eight isolates per cluster. Three of these sets also clustered with isolates from 2021, which were included in the public health alerts.

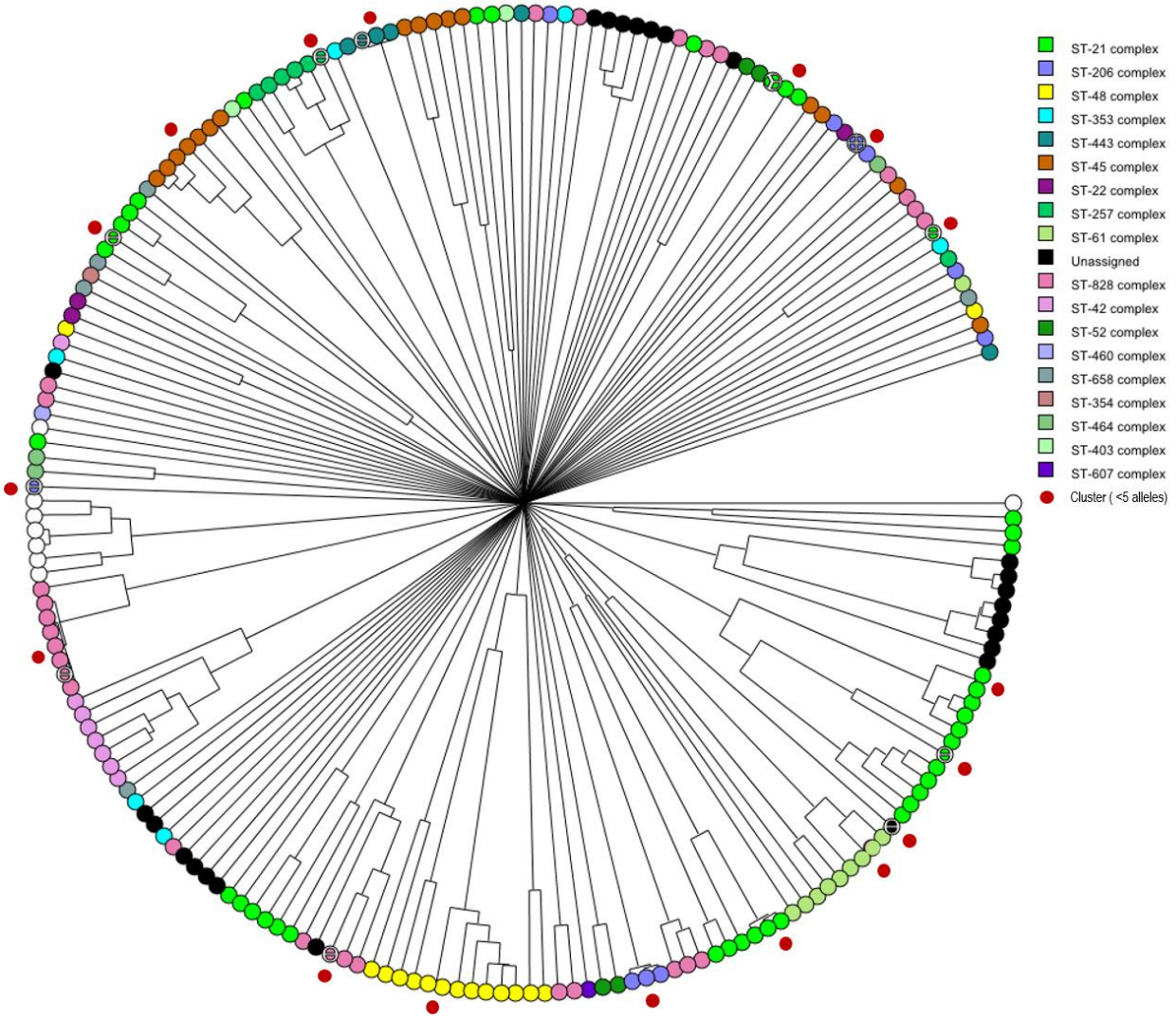

Figure 3 **– UPGMA tree of cgMLST differences amongst *Campylobacter spp.* isolates (n=213) from 2022.**

Legend: 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.

Conclusion

This is the 2022 annual report of sentinel surveillance data for clinical *Campylobacter* in Ireland. It expands the NRL human clinical *Campylobacter* laboratory database held at PHL, HSE Dublin, from where the human *Campylobacter* reference service is delivered.

Based on the data collected for the year of 2022, clinical *Campylobacter* in Ireland is still associated predominantly with *C. jejuni* (84%), with a range of virulence determinants evident & a diverse set of genotypes that reflect many of the major globally distributed lineages. However, this year an increase in the number of *C. coli* cases could be observed (15% *vs*. 9-11% in previous years) partly due to an outbreak involving eight individuals. It must be noted however that species retrieved from samples are in part a reflection of the testing and culture methods used that may omit or not favor rarer *Campylobacter* species.

The AMR data presented in this report continues to support the current clinical guidelines for the use of macrolides for initial empiric treatment of severe campylobacteriosis. However, increasing antimicrobial resistance is a threat and continued surveillance is imperative to detect trends or novel resistance mechanisms. With the ever evolving epidemiological landscape of bacterial pathogens, and those with zoonotic reservoirs in particular like *Campylobacter* spp., monitoring of AMR trends will continue as an important function of the NRL. Noteworthy, although with fewer virulence attributes, *C. coli* appears to be an important reservoir for antimicrobial resistance, as both isolates resistant to the three antimicrobials tested were *C. coli*.

Herein we have shown a good correlation between genotype and phenotype. However, the correlation is not absolute particularly in the case of erythromycin, and phenotypic antibiotic susceptibility testing will still be necessary in the short term at least.

Genomics also allowed for the detection of 20 clusters of potential public health interest. This emphasizes the value of this tool for further source investigations. Future NRL opportunities include relating clinical presentation with species, genotype and virulence factor profile. Also, collaborating with other partners in a 'One Health' framework would enable us to better explore sources of infection, to reduce disease burden and address the threat of increasing antimicrobial resistance in this pathogen.

We would like to sincerely thank all the participating laboratories that make this national human clinical *Campylobacter* surveillance possible. We would kindly remind you to adhere to the agreed sampling schedule.

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References

1. Brehony, C., Lanigan, D., Carroll, A., & McNamara, E. (2021). Establishment of sentinel surveillance of human clinical campylobacteriosis in Ireland. Zoonoses and public health, 68(2), 121–130. https://doi.org/10.1111/zph.12802.