Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Public Health Laboratory
Health Services Executive
Dublin Mid-Leinster
Cherry Orchard Hospital
Ballyfermot
Dublin 10
Tel: 01-7955175/6
Fax: 01-6231908

15.04.2024

National Campylobacter Reference Laboratory Service provided by PHL HSE, Dublin

Key points

- 498 clinical specimens received from 489 patients in 2023:
  - 446 stools
  - 52 isolate swabs
- 273 isolates/498 (54.8%) samples culture positive:
  - 230/446 stools (51%)
  - 43/53 isolate swabs (81%)
- 67% (n=182) susceptible to all three antimicrobials tested:
  - 11.3% (n=31) isolates were resistant to ciprofloxacin only.
  - 8.0% (n=22) were resistant to tetracycline only.
  - 12.8% (n=35) were resistant to ciprofloxacin and tetracycline.
  - 0.4% (n=1) were resistant to ciprofloxacin, erythromycin and tetracycline.
- 271/273 were sequenced and passed WGS QC for analysis:
  - 85.0% (n=230) *C. jejuni,* 13% *C. coli* (n=35), 1.5% *C. fetus* (n=4), 0.4 % *C. peloridis* (n=1) and 0.4% *C. ureolyticus* (n=1).
  - 76 STs and 23 clonal complexes were detected within this *Campylobacter* dataset.
  - ST-21 clonal complex was the most prevalent 22.1% (n=60).
- Good phenotypic-genotypic congruence for antibiotic susceptibility detected, except for erythromycin.
- WGS identified 27 potential clusters for public health alert in 2023.

National human *Campylobacter* Sentinel Surveillance Reference Laboratory Service Annual Report 2023

Introduction

The national laboratory surveillance service was initiated in February 2019. The 2023 schedule began on January 2nd 2023 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 2023. 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 to be 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 2nd 2023 to December 31st 2023 we received:

- A total of 498 clinical specimens from 489 patients, comprising of 446 stool specimens and 52 isolate swabs.
- A total of 273 (54.8%) *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 228/446 (51%) from PCR positive stool specimens and 43/53 (81%) 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=273) for susceptibility to the antimicrobials ciprofloxacin (CIP), erythromycin (ERY) and tetracycline (TET):

- 66.7% (n=182) were susceptible* to all three antimicrobials tested:
- 11.3 % (n=31) isolates were resistant to ciprofloxacin only;
- 8.0 % (n=22) were resistant to tetracycline only;
- 12.8 % (n=35) were resistant to ciprofloxacin and tetracycline only;
- 0.4 % (n=1) were resistant to the three drugs (*C. coli* isolate).

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

*A total of 182 isolates were defined as "susceptible, increased exposure" according to EUCAST guidelines.

Table 1 **– Antimicrobial susceptibility testing results, 2019 – 2023.**

Year	Total (n)	Susceptible n (%)	CIP R n (%)	TET R n (%)	ERY R n (%)
2023	**273**	**182 (66.7%)**	**68 (24.9%)**	**58 (21.9%)**	**1 (0.4%)**
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.

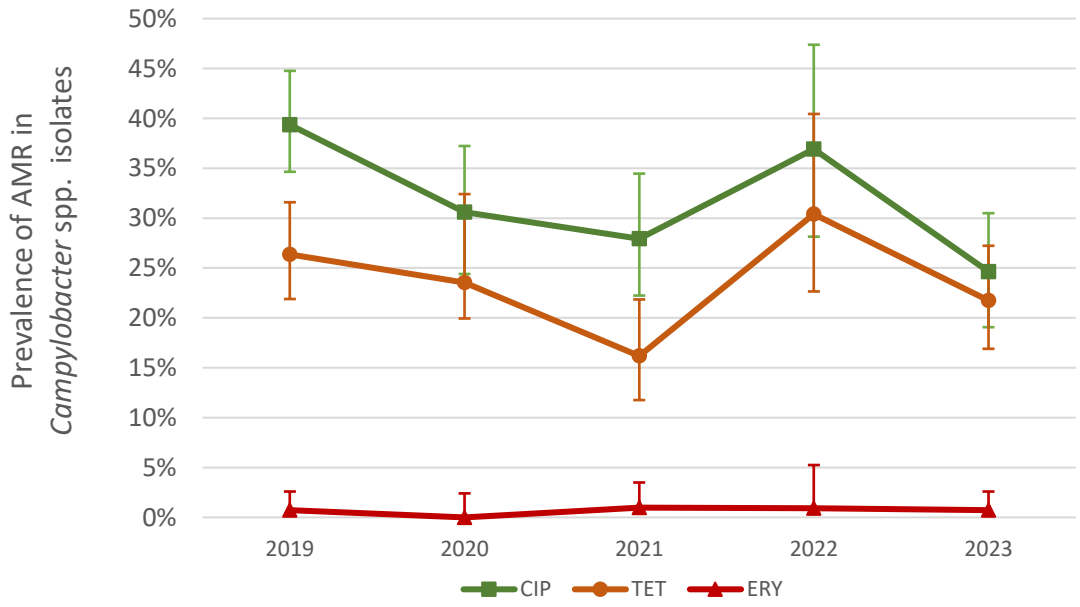

Figure 1- Trends in the prevalence of antimicrobial resistance in *Campylobacter* spp. Isolates received, 2019-2023.

Whole Genome Sequence *Campylobacter* characterization

A total of 271 *Campylobacter* isolates were sequenced by next-generation sequencing technology. 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 271 isolates that passed the quality criteria.

Five *Campylobacter* species were identified through WGS. *C. jejuni* accounted for the majority of the isolates (84.9%, n=230) followed by *C. coli* 12.9% (n=35), *C. fetus 1.5%* (n=4), 0.4 % *C. peloridis* (n=1) and 0.4% *C. ureolyticus* (n=1). There was a high diversity of sequence types (ST) with 76 STs found in total – 63 STs in *C. jejuni,* 12 STs in *C. coli* and one ST assigned to *C. fetus* isolates. Fifteen isolates had no ST assigned: eight *C. jejuni*, four *C. coli* isolates, one *C. fetus*, one *C. peloridis* and one *C. ureolyticus*.

The 76 STs resolved into 23 clonal complexes (CC), with ST21 CC being the most prevalent at 22.1% (n=60). The most prevalent STs were ST21 (9.6.0%), ST48 (6.3%), ST19 (5.2%) and ST827 (5.2%). A clonal complex could not be assigned to 40 isolates (14.8%) (Table 3, Figure 2). 31/35 *C. coli* isolates belonged to the ST-828 CC and the other four *C. coli* isolates had no CC assigned.

Table 2 **- Breakdown of speciation of isolates sequenced from 2019 to 2023.**

Year	WGS*	*C. jejuni*		*C. coli*		*C. fetus*		*C. lari*		*C. peloridis*		*C. ureolyticus*	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
2023	**271**	**230**	**84.9%**	**35**	**12.9%**	**4**	**1.5%**	**-**	**-**	**1**	**0.4%**	**1**	**0.4%**
2022	213	179	84.0%	33	15.5%	1	0.5%	-	-	-	-	-	-
2021	200	181	90.5%	17	8.5%	-	-	2	1.0%	-	-	-	-
2020†	74	67	90.5%	7	9.5%	-	-	-	-	-	-	-	-
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 passed the WGS QC analysis criteria.

Table 3 **- Breakdown of the most prevalent STs and clonal complexes 2019-2023.**

Year	WGS*	ST 21		ST 48		ST 19		ST 827		ST 21 CC**	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
2023	**271**	**26**	**9.6%**	**17**	**6.3%**	**14**	**5.2%**	**14**	**5.2%**	**60**	**22.1%**
2022	213	15	7.0%	12	5.6%	13	6.1%	5	2.3%	46	21.6%
2021	200	25	12.5%	25	12.5%	13	6.5%	7	3.5%	54	27.0%
2020†	74	9	12.2%	10	13.5%	5	6.8%	5	6.8%	27	36.5%
2019	257	31	12.1%	26	10.1%	6	2.3%	11	4.3%	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) has been the most prevalent CC since the sentinel surveillance program started.

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 4 - **Frequency of the 76 sequence types (STs) and clonal complexes (CCs) found in the Campylobacter sentinel collection 2023 (n=271). STs with five or more representative isolates shown.**

Sequencing Type	Clonal Complex	N	%
21	ST-21	26	9.6
48	ST-48	17	6.3
19	ST-21	14	5.2
827*	ST-828	14	5.2
42	ST-42	12	4.4
257	ST-257	10	4.4
50	ST-21	9	3.7
441	Unassigned	8	3.3
61	ST-61	7	3.0
11328	ST-48	7	2.6
206	ST-206	7	2.6
51	ST-443	6	2.6
508	ST-508	6	2.2
137	ST-45	5	2.2
436	Unassigned	5	1.8
403	ST-403	5	1.8
53	ST-21	5	1.8
45	ST-45	5	1.8
ST with ≤ 4 isolates	-	103	38

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

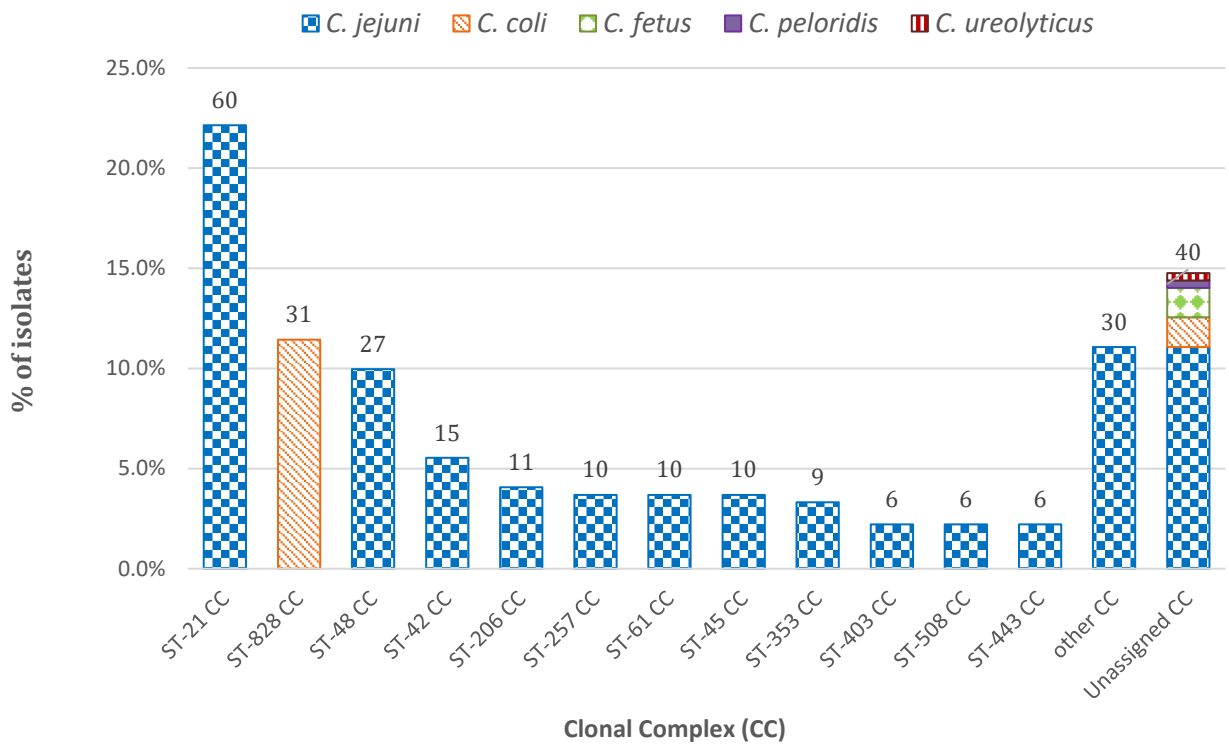

***Figure 2* - Distribution of the 271 sequenced *Campylobacter spp.* isolates by clonal complex.**

AST phenotype and genotype comparison

A total of 67 *Campylobacter spp.* samples phenotypically resistant to ciprofloxacin were sequenced and passed our WGS quality criteria. Of these, 64 carried the *gyrA* mutation Thr86Ile. One *C. peloridis* isolate had a Valine (GTT) in position 86, instead of a Threonin (ACT) but it is uncertain whether if this is a mutation or the wild type allele (conferring intrinsic resistance). Of the 204 isolates that were susceptible to ciprofloxacin, three had a *gyrA* mutation Thr86Ile. Therefore, there was a 97% sensitivity and 99% specificity for WGS to predict ciprofloxacin susceptibility with a corresponding positive predictive value of 96% and negative predictive value of 99% (Table 5).

All 58 isolates with phenotypic tetracycline resistance were sequenced and passed WGS validity criteria. Of these, 56 harboured the gene *tetO*. Of the 213 isolates that were susceptible to tetracycline, and passed WGS validity criteria, 195 did not harbour *tetO*. Therefore, there was 97% sensitivity and 92% specificity for WGS to predict tetracycline susceptibility with a corresponding positive predictive value of 76% and negative predictive value of 99% (Table 4).

One *C. coli* isolate was found to have phenotypic erythromycin resistance. However, neither the 23S rRNA gene mutation nor the *ermB* gene associated with mediating macrolide resistance were detected (Table 5).

Table 5 **– Campylobacter resistance associated genes and phenotype concordance amongst isolates, 2023 (n=271).**

	phenotype: resistant		phenotype: susceptible					
Antibiotic class	**genotype: R**	**genotype: S**	**genotype: R**	**genotype: S**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Tetracycline	56	2	18	195	97%	92%	76%	99%
Erythromycin	0	1	0	271	33%	100%	50%	99%
Ciprofloxacin	65	2	3	201	97%	99%	96%	99%

Virulence factors

The following is a breakdown of the virulence factors found in the *Campylobacter* isolates (n=271). The cytotoxin genes *cdtA* and *cdtB* were present in 95% and 98% isolates, respectively, while *cdtC* was present in 93% (n=214) of the *C. jejuni* isolates and in 26% (n=9) of the *C. coli* isolates. The adherence and colonization associated factor genes *flaA, cadF, dnaJ* and *racR* were found in ≈98% of the 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 265 and 251 isolates respectively (Table 6).

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. fetus*, *C. peloridis* or *C. ureolyticus*.

Table 6 **– Virulence factors presence detected by WGS among *Campylobacter spp.* isolates 2023 (n=271).**

Mechanism	Gene	No.	%
Cytotoxin production	*cdtA*	258	95.2
	cdtB	266	98.2
	cdtC	223	82.3
Adherence and colonization	*flaA*	264	97.4
	cadF	265	97.8
	dnaJ	264	97.4
	racR	265	97.8
Invasion	*virB11*	2	0.7
	iam	265	97.8
	ciaB	251	92.6

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 27 clusters of 2023 isolates, 23 of these exclusively composed of 2023 isolates, that were closely related genetically and warranted a public health alert to consider investigation for potential epidemiological links. This compared to 20 clusters in 2022, 22 clusters in 2021, 7 clusters in 2020 (which was truncated due to SARS-CoV-2 testing) and 31 clusters in 2019. These sets of clusters ranged from 2 isolates per cluster up to seven isolates per cluster. Four of these sets clustered with isolates from 2022, which were included in the public health alerts.

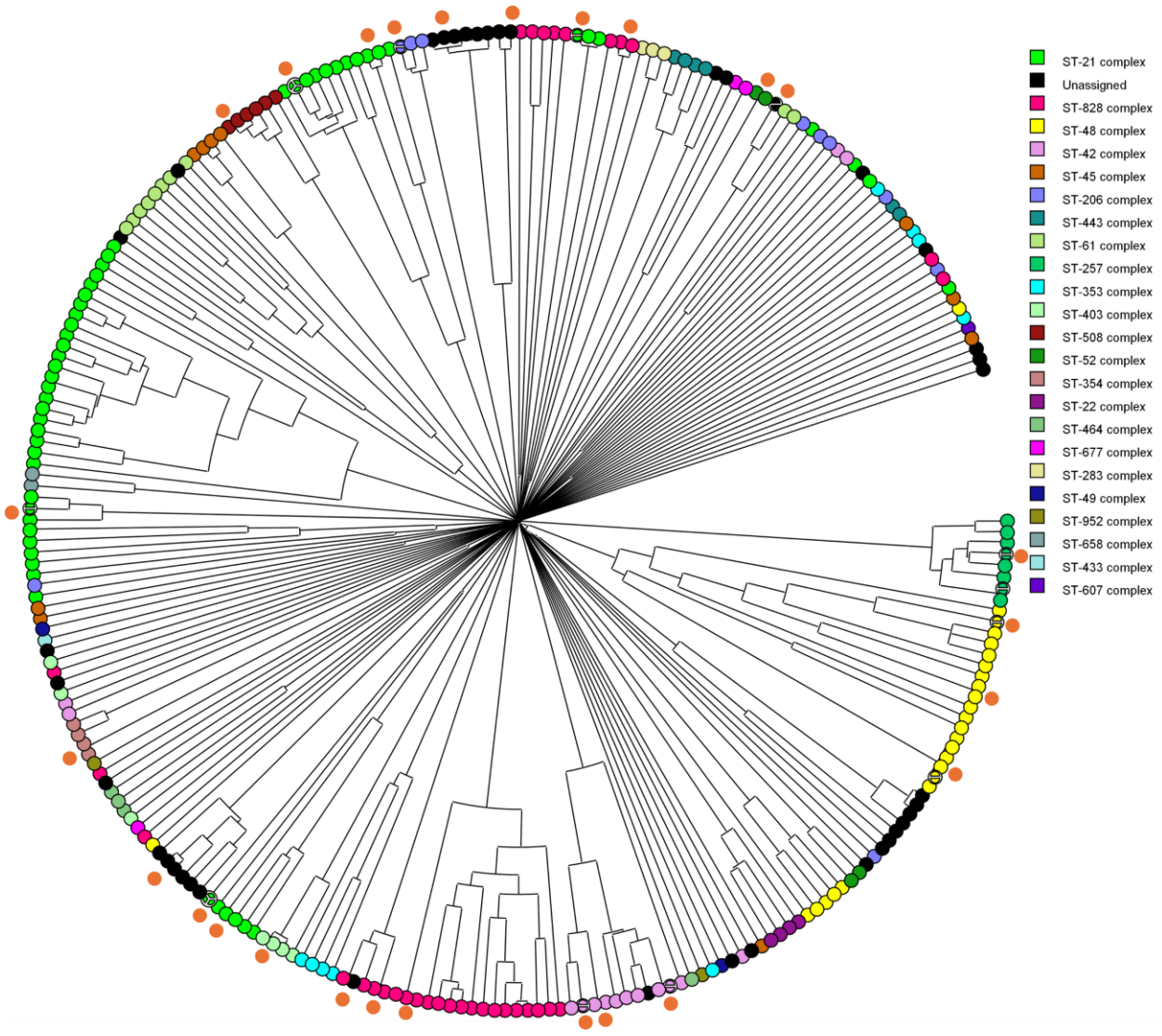

Figure 3 **– UPGMA tree of cgMLST differences amongst *Campylobacter spp.* isolates (n=271) from 2023.**

Legend: Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with an orange circle.

Conclusion

This is the 2023 annual report of sentinel surveillance data for clinical *Campylobacter* in Ireland. It expands the NRL 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 2023, clinical *Campylobacter* in Ireland is still predominantly associated with *C. jejuni* (85%), with a high diversity of genotypes associated and a rich set of virulence determinants that reflect many of the major globally distributed lineages. Similarly to previous years, *C. coli* cases were the second most frequent cause of campylobacteriosis in Ireland (13% *vs*. 9-16% in previous years), followed by just a few cases of *C. fetus* (1.5%), *C. peloridis* (0.4%) and *C. ureolyticus* (0.4%) detected. It is important to note that species retrieved from samples are in part a reflection of the testing and culture methods used in the laboratory that may not be optimised for the detection of these rarer *Campylobacter* species.

Even though a good correlation between genotypic and phenotypic antimicrobial susceptibility data was observed for ciprofloxacin (fluoroquinolone) and tetracycline, in the case of erythromycin (macrolide) no correlation was found for the single resistant isolate.

Nevertheless, the antimicrobial susceptibility 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.

Genomics also allowed for the detection of 27 clusters of potential public health interest, emphasizing the value of this tool for further source investigations. Future NRL opportunities include relating clinical presentation with species, genotype and virulence factor profile. Furthermore, we hope that a tighter collaboration with other partners in the 'One Health' framework will 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.

This report was prepared by Diana Espadinha Costa (Scientist), Rachell Kelly (Senior Medical Scientist), Anne Carroll (Chief Medical Scientist), and Tee Keat Teoh (Consultant Microbiologist & PHL Director).

For further information please contact at PHL HSE Dublin either:

Dr Tee Keat Teoh: teekeat.teoh@hse.ie
Dr Anne Carroll: anne.carroll@hse.ie
National Campylobacter Reference Laboratory @PHL HSE Dublin: campy.nrl@hse.ie

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.