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

National human Campylobacter Sentinel Surveillance Reference Laboratory Service Quarter 1 Report 2022

Summary

- 76 clinical specimens received from 73 patients
- 29 isolates/76 (38%) samples were culture positive and characterized:
  - 20/59 stools (33.90%)
  - 9/17 isolate swabs (52.94%)
- 34.48% (n=10) susceptible to all three antimicrobials tested
  - 20.69% (n=6) isolates were resistant to ciprofloxacin only.
  - 17.24% (n=5) were resistant to tetracycline only.
  - 27.59% (n=8) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
- 29/29 passed WGS QC for analysis
  - 75.86% (n=22) *C. jejuni,* 24.14% *C. coli* (n=7)
  - 15 STs and 11 clonal complexes were detected in this *Campylobacter* dataset.
  - ST-828 clonal complex was the most prevalent 20.69% (n=6)
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected
- WGS identified 4 Q1 2022 potential clusters for public health alert.

Introduction

This is the 2022 Quarter 1 report of the human 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 2022 schedule began on January 1st 2022 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.

Specimen submission

The Quarter 1 period was from 01/01/2022 to 31/03/2022 we received:

- A total of 76 specimens from 73 patients comprising 59 stool specimens and 17 isolate swabs.
- A total of 29 (38%) *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 20/59 (33.90%) from PCR positive stool specimens and 9/17 (52.94%) from isolate swabs.

  This is a very poor culture yield, which we have audited & addressed in previous NRL reports. Consequently we recommend: Laboratories to send positive samples/isolates daily to NRL within your scheduled week for submission and not to delay by batching specimens. Consider your stool Ct result threshold when evaluating for clinical significance.

Speciation the samples/isolates

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 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=29) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline. 34.48% (n=10) susceptible to all three antimicrobials tested

- 20.69% (n=6) isolates were resistant to ciprofloxacin only
- 17.24% (n=5) were resistant to tetracycline only
- 27.59% (n=8) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
- No isolates were resistant to erythromycin

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

Whole Genome Sequence *Campylobacter* characterization

All 29 *Campylobacter* isolates were stored and available for batch 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. These genome assemblies were then assessed for quality using the metrics N50, contig length, total sequence length, percent core coverage. WGS analysis for speciation, genomic AMR and virulence determinants and cluster detection was completed for 29/29 isolates that passed the quality criteria.

C. jejuni accounted for 75.86% (n=22) of isolates and *C. coli* 24.14% (n=7). There was a diversity of sequence types (ST) with 15 STs found in total – 13 STs in *C. jejuni* and 1 unassigned ST, and 2 STs in *C. coli* and 1 unassigned ST.

The most prevalent STs were ST-10042 (17.24%) and ST-50 (13.79%). These STs resolved into 10 clonal complexes, with ST-828 clonal complex being the most prevalent at 20.69% (n=6). *A* clonal complex was not assigned to 2 isolates (6.90%) (Table1, Figure 1). 6/7 *C. coli* isolates belonged to the ST-828 clonal complex, but a clonal complex was not assigned to 1/7 *C. coli* isolates. The ST-828 clonal complex is exclusively associated with *C. coli.*

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. ST-21, ST-19, ST-50, ST-262, ST-6175) that match the central genotype *i.e.* ST-21 at four or more of the conventional MLST seven housekeeping gene alleles.

Table 1: Breakdown of 15 locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection Quarter *1* 2022 (n=29). STs with more than four representative isolates shown.

ST	clonal complex	N	%
10042	ST-828 complex	5	17.24%
50	ST-21 complex	4	13.79%
<4	N/A	20	68.97%

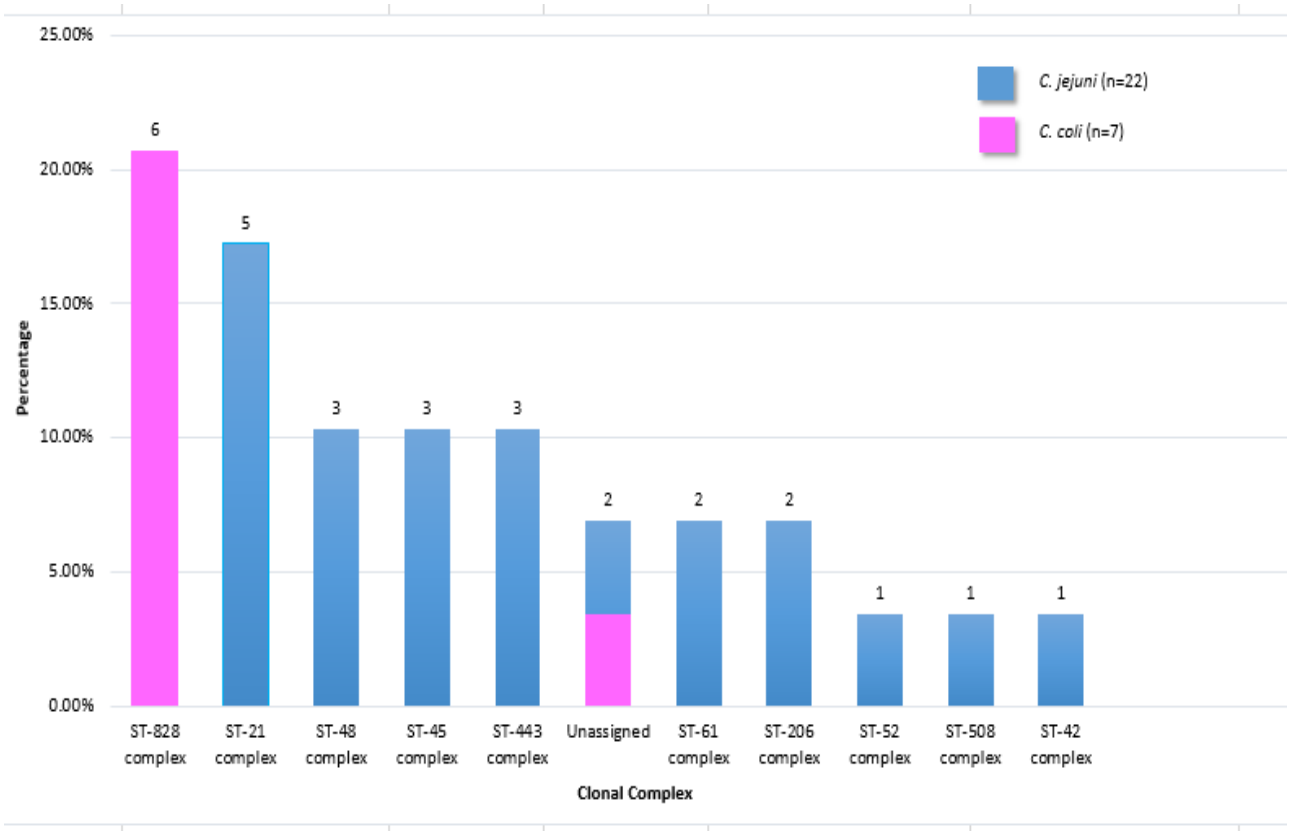

Figure 1: Quarter 1 2022 *Campylobacter* spp. isolates (n=29) by clonal complex.

AST phenotype and genotype comparison

Of the 14 isolates with phenotypic ciprofloxacin resistance, all 14 passed WGS validity criteria. Of these 14, all 14 contained the *gyrA* mutation Thr86Ile/Val. Of the 15 isolates that were susceptible to ciprofloxacin, 15 passed WGS validity criteria. Of these 15, 15 did not have the *gyrA* mutation. Therefore, there was 100% and 100% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 100% and negative predictive value of 100%. The sensitivity result is higher than that observed in Quarter 1, 2021.

Of the 13 isolates with phenotypic tetracycline resistance, 13 passed WGS validity criteria. Of these 13, 13 harboured the gene *tetO*. Of the 16 isolates that were susceptible to tetracycline, 16 passed WGS validity criteria. Of these 16, 16 did not harbour *tetO*. Therefore, there was 100% sensitivity and 100% specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 100% and negative predictive value of 100%. This result is aligned with that observed in Quarter 1, 2021.

No isolates were found to have phenotypic erythromycin resistance. The 23S rRNA gene/mutation and *ermB* gene associated with mediating macrolide resistance were not detected in any of the Campylobacter spp. isolates. This result is aligned with that observed in Quarter 1, 2021.

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

Table 2: *Campylobacter* resistance associated genes and phenotype concordance amongst isolates, Quarter 1 2022. n=29

	phenotype: resistant		phenotype: susceptible					
antibiotic class	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible	Sensitivity (%)	Specificity (%)	Positive Predictive Value	Negative Predictive Value
tetracycline	13	0	0	16	100.0	100.0	100.0	100.0
erythromycin	0	0	0	29	0.0	100.0	0.0	100.0
ciprofloxacin	14	0	0	15	100.0	100.0	100.0%	100.0

Virulence factors

There were a number of virulence factors found in all of the Campylobacter isolates (n=29) including the adherence and colonization associated factor genes *cadF, racR, dnaJ* and *flaA*.

cdtA was present in 28 isolates, *cdtB* was present in all 29 isolates, while *cdtC* was present in all of the 22 *C. jejuni* isolates but not present in any of the 7 *C. coli* isolates. The invasion associated *virB11* gene was not found in any isolates. The *iam* and *ciaB* genes were present in 29 isolates (Table 3).

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.

Table 3: Virulence factors presence detected by WGS among *Campylobacter* isolates Quarter 1 2022 (n=29)

mechanism	gene	no.	%
Cytotoxin production	*cdtA*	28	96.6
	cdtB	29	100
	cdtC	22	75.9
Adherence and colonization	*flaA*	29	100
	cadF	29	100
	dnaJ	29	100
	racR	29	100
Invasion	*virB11*	0	0
	iam	29	100
	ciaB	29	100

Cluster analysis

Isolate genomes were compared for relatedness by comparison at 1343 genes using core genome MLST (cgMLST) (**Figure 2**). A difference of five cgMLST alleles or fewer was used as an alert threshold to consider cluster investigation. Using this cluster criterion there were 4 sets of isolates that were closely related genomically and warranted a public health alert to consider investigation for potential epidemiological links.

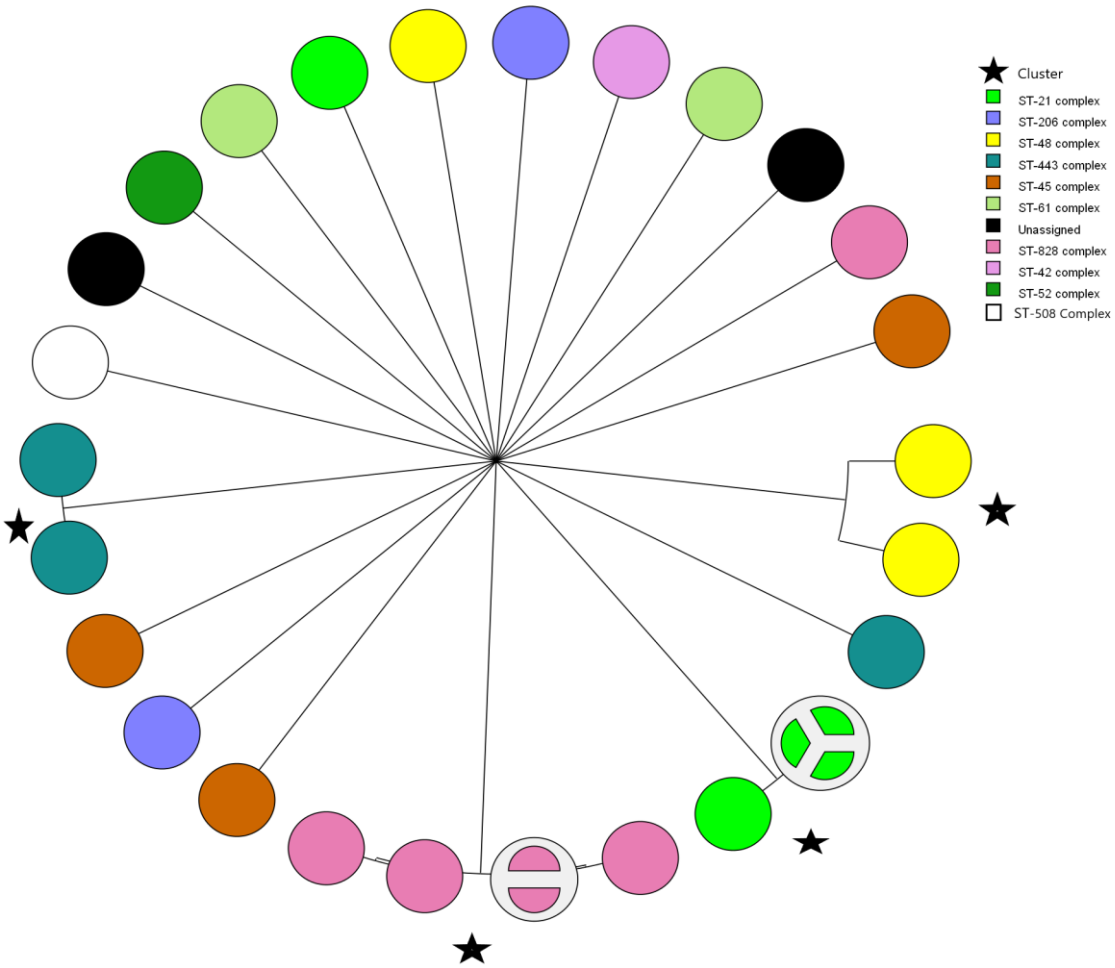

Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=29) Quarter 1 2022. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with black pentagon.

Conclusion

This is the Quarter1 2022 set of sentinel surveillance data for human 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.

On the basis of these Quarter 1 2022 data, human clinical *Campylobacter* in Ireland is still associated predominantly with *C. jejuni* with a range of virulence determinants evident & a diverse set of genotypes reflecting many of the major globally distributed lineages. Although *C. coli* with fewer virulence attributes contributed less infections in comparison, their presence is notable and may reflect their infection sources. 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 favour rarer *Campylobacter* species.

This data continues to support the current clinical guidelines for the use of macrolides for initial empiric treatment of severe campylobacteriosis. However monitoring AMR trends will continue as an important function of the NRL. As with many other pathogens, and those with zoonotic reservoirs in particular, increasing antimicrobial resistance is a threat and continued surveillance is imperative to detect trends or novel resistance mechanisms. Genomics has enabled a better understanding of the genetic mechanisms behind antibiotic resistance and here we have shown a strong correlation between genotype and phenotype. However, the correlation is not absolute and phenotypic antibiotic sensitivity testing will still be necessary in the short term at least.

Genomics also allowed for the detection of 4 Quarter 1 2022 exclusive 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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