Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Public Health Laboratory
Health Services Executive
Dublin Mid-Leinster
Cherry Orchard Hospital
Ballyfermot
Dublin 10
Tel: 01-755175/6
Fax: 01-6231908

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

Annual Report 2020

Summary

- 90 clinical specimens received
- 85/90 isolates characterized:
  - 61/66 stools (92.4%)
  - 24/24 isolate swabs (100%)
  - *Campylobacter* spp could not be isolated from 5/90 specimens
- 56.5% (n=48) susceptible to all three antimicrobials tested
  - 18.8% (n=16) isolates were resistant to ciprofloxacin only
  - 11.8% (n=10) were resistant to tetracycline only
  - 11.8% (n=10) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
  - 1.2% (n=1) did not undergo AST
- 74/85 passed WGS QC for analysis
  - 4/85 viable cultures were not obtained from freezer beads
  - 7/85 did not pass WGS QC and were excluded from further analysis
  - 90.54% (n=67) *C. jejuni,* 9.46% *C. coli* (n=7)
  - 31 STs and 16 clonal complexes were detected in this Campylobacter dataset.
  - ST-21 clonal complex most prevalent 36.5% (n=27)
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected

Introduction

This is the 2020 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 2020 schedule began on January 2nd 2020 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 2020. 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 from January to December 2020 inclusive. However due to the onset of COVID-19 pandemic nationally in March 2020, the schedule was drastically curtailed. Thus we report a much reduced 2020 *Campylobacter* dataset (n=74) compared to that of 2019 (n=277).

Specimen submission

From January 2nd 2020 to December 31st 2020 we received:

- A total of 94 specimens comprising 66 stool specimens, 24 isolate swabs and 4 EQA samples
- The 4 EQA samples are not included in this report
- *Campylobacter* spp could not be isolated from 5 specimens
- A total of 85 *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 61/66 (92.4%) from PCR positive stool specimens and 24/24 (100.0%) from isolate swabs.

Speciation

Campylobacter spp. were confirmed by culture contemporaneously, once receipted in PHL and then speciated by WGS 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=85) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline. Please note that 1/85 sample in which viable cultures were obtained were not subjected to AST.

- 56.5% (n=48) isolates were susceptible to all three antimicrobials tested
- 18.8% (n=16) isolates were resistant to ciprofloxacin only
- 11.8% (n=10) were resistant to tetracycline only
- 11.8% (n=10) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
- 1.2% (n=1) did not undergo AST profiling

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 85 *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 74/85 isolates that passed the quality criteria.

C. jejuni accounted for 89.6% (n=67) of isolates and *C. coli* 10.4% (n=7). There was a diversity of sequence types (ST) with 31 STs found in total – 28 STs in *C. jejuni* and 3 STs in *C. coli*.

The most prevalent STs were ST-48 (13.5%) and ST-21 (12.2%). These STs resolved into 16 clonal complexes, with ST-21 clonal complex being the most prevalent at 36.5% (n=27). A clonal complex was not assigned to 1 isolate (n=1; 1.3%) (Table1, Figure 1). All 7 *C. coli* isolates belonged to the ST-828 clonal complex. 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. here ST-21, ST-19, ST-50) that matches the central genotype *i.e.* ST-21 at four or more of the conventional MLST seven housekeeping gene alleles.

Table 1: Breakdown of six locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection 2020 (n=74). STs with more than four representative isolates shown. * denotes *C. coli* clonal complex

ST	clonal complex	N	%
48	ST-48 complex	10	13.5%
21	ST-21 complex	9	12.2%
50	ST-21 complex	6	8.1%
19	ST-21 complex	5	6.8%
45	ST-45 complex	5	6.8%
827	ST-827 complex*	5	6.8%
<4	N/A	34	45.9%

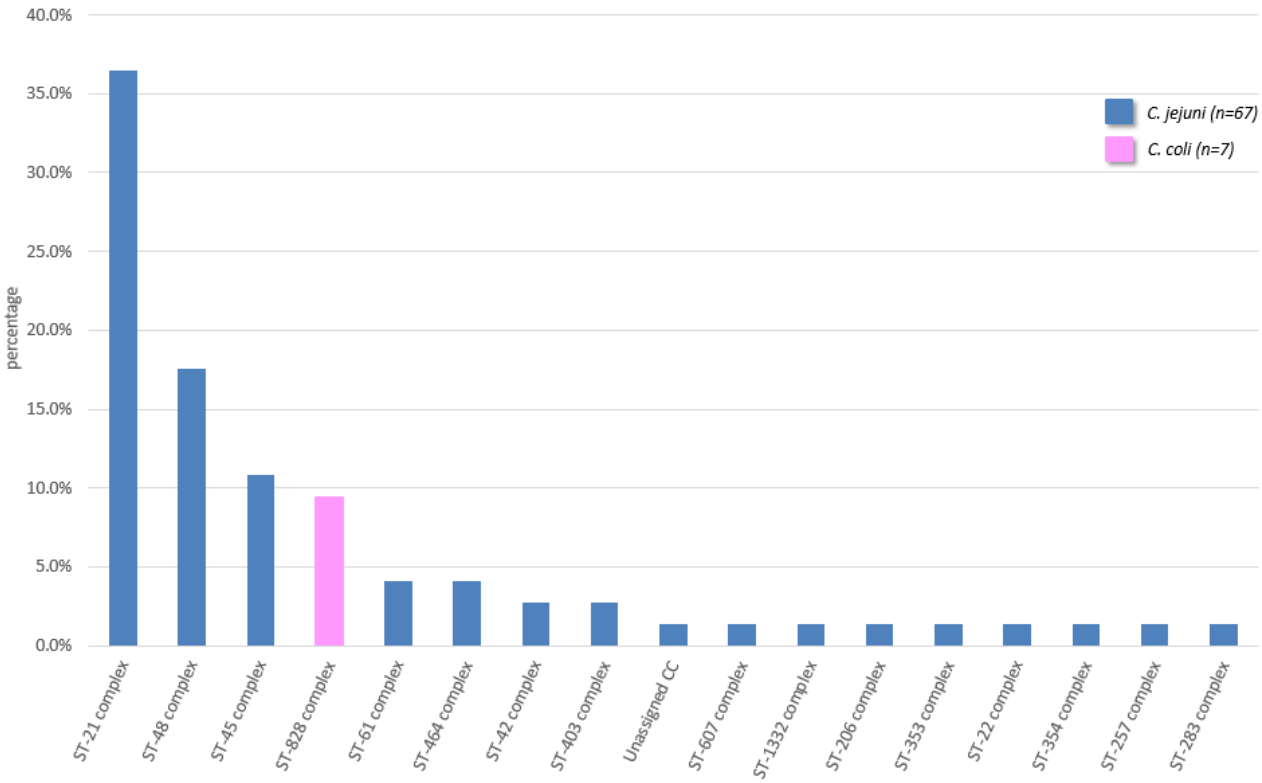

Figure 1: 2020 *Campylobacter* spp. isolates (n=74) by clonal complex.

AST phenotype and genotype comparison

Of the 26 isolates with phenotypic ciprofloxacin resistance, 21 passed WGS validity criteria. Of these 21, 18 contained the *gyrA* mutation Thr86Ile/Val. Of the 58 isolates that were susceptible to ciprofloxacin, 52 passed WGS validity criteria. Of these 52, 49 did not have the *gyrA* mutation. Therefore, there was 85.7% and 94.2% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 85.7% and negative predictive value of 94.2%. This result is slightly lower than that observed in 2019.

Of the 20 isolates with phenotypic tetracycline resistance, 17 passed WGS validity criteria. Of these 17, 16 harboured the gene *tetO*. Of the 64 isolates that were susceptible to tetracycline, 56 passed WGS validity criteria. Of these 56, 52 did not harbour *tetO*. Therefore, there was 94.1% and 92.9% sensitivity and specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 80.0% and negative predictive value of 98.1%. This result is slightly lower than that observed in 2019.

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 2019.

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

Table 2: *Campylobacter* resistance associated genes and phenotype concordance amongst isolates, 2020. N=73 (1 isolate did not undergo AST profiling)

	phenotype: resistant		phenotype: susceptible					
antibiotic class	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible	Sensitivity (%)	Specificity (%)	Positive Predictive Value	Negative Predictive Value
tetracycline	16	1	4	52	94.1	92.9	80.0	98.1
erythromycin	0	0	0	73	0.0	100.0	0.0	100.0
ciprofloxacin	18	3	3	49	85.7	94.2	85.7	94.2

Virulence factors

There were a number of virulence factors found in all of the *Campylobacter* isolates (n=74) including the adherence and colonization associated factor genes *flaA, cadF, racR* & *dnaJ*. *cdtA* and *cdtB* were also present in all 74 isolates. *cdtC* was present in the 67 *C. jejuni* isolates but not present in any of the 7 *C. coli* isolates. The invasion associated *virB11* gene was found in five isolates (all *C. jejuni*). The *iam* and *ciaB* were present in all 74 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 2020 (N=74)

mechanism	gene	no.	%
Cytotoxin production	*cdtA*	74	100
	cdtB	74	100
	cdtC	67	90.5
Adherence and colonization	*flaA*	74	100
	cadF	74	100
	dnaJ	74	100
	racR	74	100
Invasion	*virB11*	5	6.8
	iam	74	100
	ciaB	74	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 criterion there were 7 sets of isolates that were closely related genomically. Due to the COVID enforced delay in 2020 genomic analysis, these clusters are now placed in the NRL Campylobacter laboratory database to consider for future potential epidemiological links with 2021 isolates.

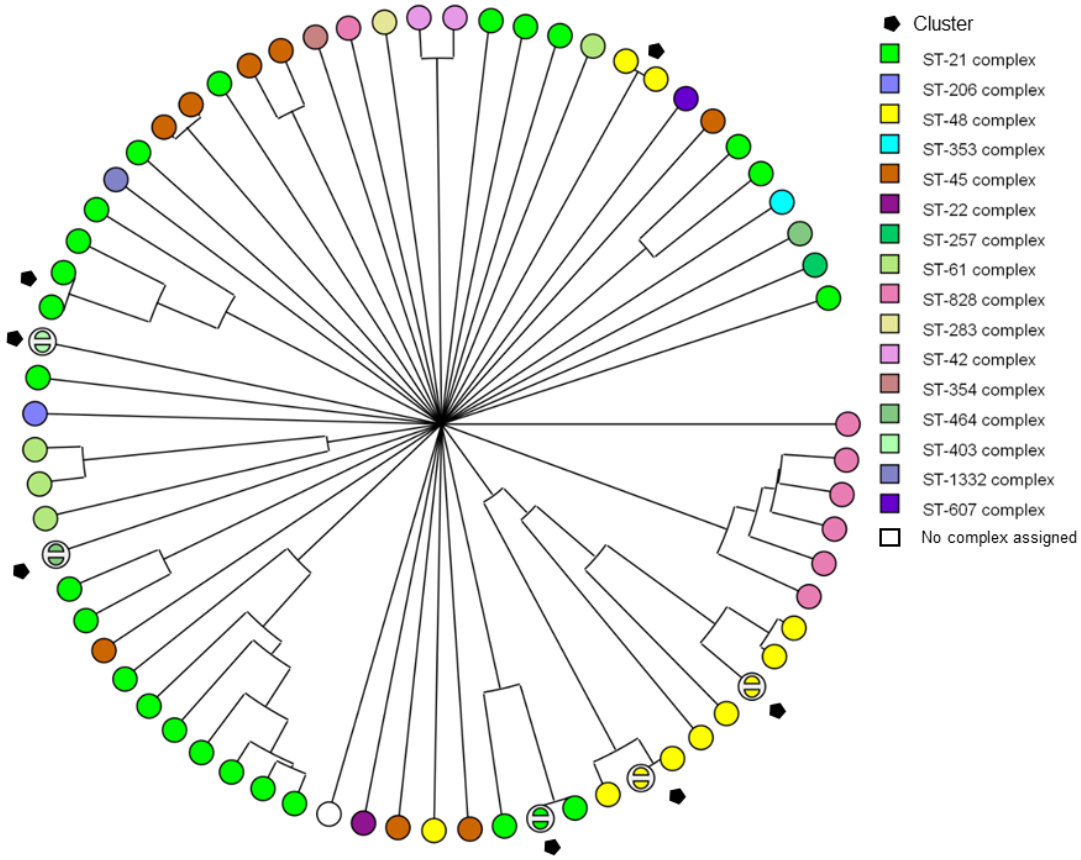

Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=74) 2020. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with black pentagon.

Conclusion

This is the second set (albeit truncated due to COVID) 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 will continue to be delivered.

On the basis of these 2020 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 7 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 like to reassure all participating laboratories that since January 2021 we are back working at full capacity to provide this NRL sentinel campylobacter surveillance service. We would kindly remind you to adhere to the agreed sampling schedule.

For further information please contact at PHL HSE Dublin either:

Dr Eleanor McNamara, Director
Dr Anne Carroll, Chief Medical Scientist,
Dr Leanne Murphy, Molecular Scientist.

eleanor.mcnamara@hse.ie
anne.carroll@hse.ie
leanne.murphy2@hse.ie
phl.dublin@hse.ie

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