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

05.06.2020

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

Annual Report 2019

Summary

- 277/453 isolates characterized:
  - 204/366 (55.7%) stools;
  - 73/75 (97.3%) isolate swabs
- 86.8% (n=223) *C. jejuni*; 11.3% (n=29) *C. coli*; 1.9% (n=5) *C. lari*
- One patient sample contained *C. coli* and *C. jejuni*
- 88 STs and 20 clonal complexes
- ST-21 clonal complex most prevalent 26.8% (n=69)
- 50.5% (n=140) susceptible to all three antimicrobials tested
  - 39.3% (n=109) isolates were resistant to ciprofloxacin
  - 26.3% (n=73) were resistant to tetracycline
  - two isolates were resistant to erythromycin; one *C. jejuni* and one MDR *C. coli*
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected
- WGS identified 31 potential clusters for public health alert

Introduction

This is the 2019 report of the first human national *Campylobacter* Clinical Sentinel Surveillance Reference Laboratory Service provided by the Public Health Laboratory (PHL), HSE, Dublin. The national laboratory surveillance service began in the week of February 4th 2019 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 project, a sampling frame was devised in collaboration with HPSC in order to provide a representative collection of specimens nationally for 2019. 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 February to December 2019 inclusive.

Specimen submission

From February 4th 2019 to December 31st 2019 we received:

- A total of 453 specimens comprising 366 stool specimens and 75 isolate swabs
- A total of 277 *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 204/366 (55.7%) from PCR positive stool specimens and 73/75 (97.3%) from isolate swabs

Speciation

Campylobacter spp. were identified and speciated from submitted specimens 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 was performed 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=277) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline.

- 50.5% (n=140) isolates were susceptible to all three antimicrobials tested
- 39.3% (n=109) isolates were resistant to ciprofloxacin
- 26.3% (n=73) were resistant to tetracycline
- 0.8% (n=2) isolates were resistant to erythromycin
- 17% (n=47) were resistant to two antimicrobials (ciprofloxacin and tetracycline n=46; erythromycin and tetracycline n=1)
- One *C. coli* isolate was resistant to all three antimicrobials

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

Whole Genome Sequence *Campylobacter* characterisation

All 277 *Campylobacter* isolates were stored and available for batch WGS at the end of each quarter. High-quality DNA was extracted from confirmed isolates and DNA libraries were prepared using the Illumina Nextera kit v3 and sequenced on an Illumina MiSeq instrument. Sequence yielded 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 257/277 isolates that passed the quality criteria.

C. jejuni accounted for 86.8% (n=223) of isolates, *C. coli* 11.3% (n=29) and *C. lari* 1.9% (n=5). There was a diversity of sequence types (ST) with 88 STs found in total (Table 1) – 68 STs in *C. jejuni,* 15 in *C. coli* and three in *C. lari*. Genomic speciation using the https://pubmlst.org/rmlst/ tool allowed for the identification of a mixed culture of *C. coli* (ST-828 complex) and *C. jejuni* (ST-45 complex) in one patient sample. The two isolates were purified, cultured from this sample and WGS was repeated for each species isolate.

The most prevalent STs were ST-21 (12.1%) and ST-48 (10.1%). These STs resolved into 20 clonal complexes, with ST-21 clonal complex being the most prevalent at 26.8% (n=69) (Table 1, Figure 1). All 29 *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-50, ST-806, ST-6175) 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 seven locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection 2019 (n=257). STs with more than four representative isolates shown. * denotes *C. coli* clonal complex

ST	clonal complex	N	%
21	ST-21 complex	31	12.1
48	ST-48 complex	26	10.1
50	ST-21 complex	14	5.4
827	ST-828 complex*	11	4.3
45	ST-45 complex	11	4.3
61	ST-61 complex	9	3.5
257	ST-257 complex	8	3.1
42	ST-42 complex	8	3.1
51	ST-443 complex	8	3.1
19	ST-21 complex	6	2.3
6209	ST-464 complex	5	1.9
806	ST-21 complex	5	1.9
53	ST-21 complex	5	1.9
122	ST-206 complex	5	1.9
<5	n/a	105	40.9

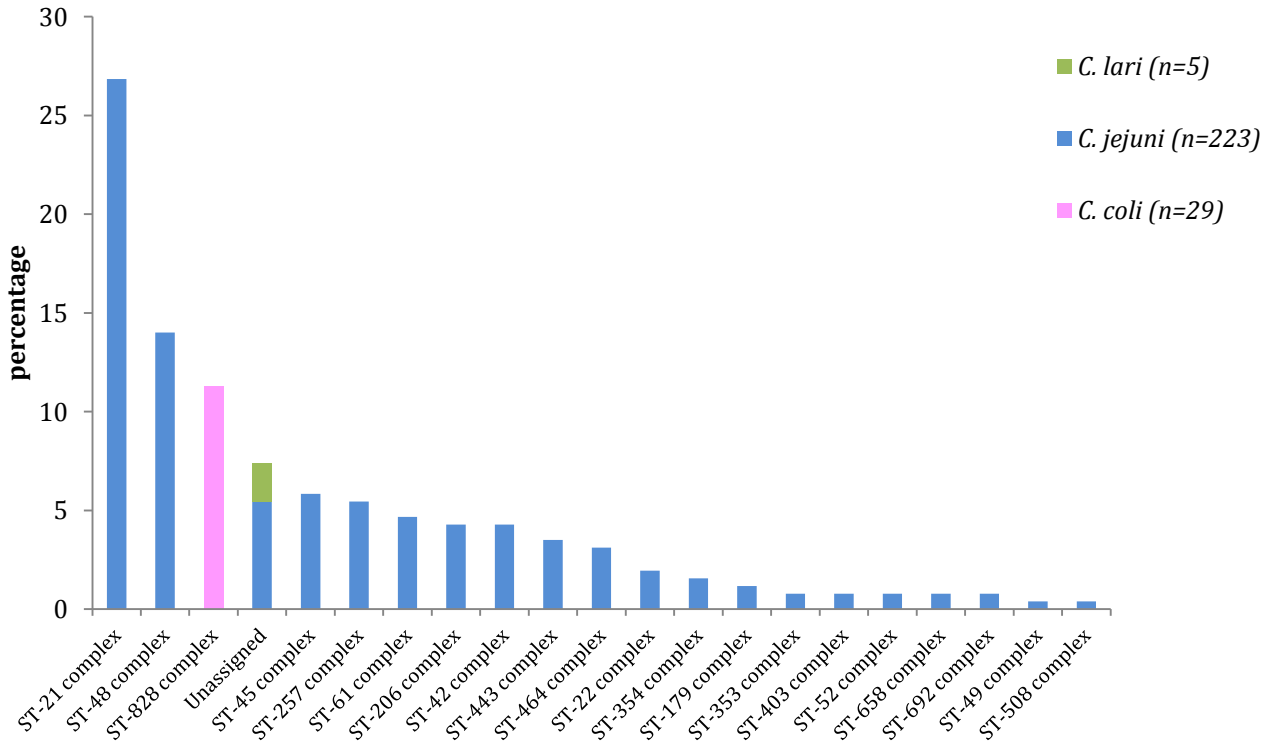

Figure 1: 2019 *Campylobacter* spp. isolates (n=257) by clonal complex.

AST phenotype and genotype comparison

Of the 98 isolates with phenotypic ciprofloxacin resistance, 94 contained the *gyrA* mutation Thr86Ile/Val. Of the 159 isolates that were susceptible to ciprofloxacin, 152 did not have the *gyrA* mutation. Therefore, there was 95.9% and 95.6% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 93.1% and negative predictive value of 97.4%.

Of the 73 isolates with phenotypic tetracycline resistance, 72 harboured the gene *tetO*. Of the 184 isolates that were susceptible to tetracycline, 183 did not harbour *tetO*. Therefore, there was 98.6% and 99.5% sensitivity and specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 98.6% and negative predictive value of 99.5%.

The 23S rRNA and *ermB* genes associated with mediating macrolide resistance were not detected in any of the *Campylobacter* spp. isolates including the multidrug resistant *C. coli* isolate. This may indicate that other resistant mechanisms were responsible for mediating macrolide phenotypic resistance.

Table 2: *Campylobacter* resistance associated genes and phenotype concordance amongst isolates, 2019. N=257

	phenotype: resistant		phenotype: susceptible					
antibiotic class	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible	Sensitivity (%)	Specificity (%)	Positive Predictive Value	Negative Predictive Value
tetracycline	72	1	1	183	98.6	99.5	98.6	99.5
erythromycin	0	2	0	255	0.0	100.0	n/a	99.2
ciprofloxacin	94	4	7	152	95.9	95.6	93.1	97.4

Virulence factors

There were a number of virulence factors found in all of the *Campylobacter* isolates including the adherence and colonization associated factor genes *flaA* and *racR,* the invasion gene *ciaB* and the cytotoxin gene *cdtB* (Table 3). *cdtA* was present in all but six isolates (3 *C. lari* and 3 *C. jejuni*). *cdtC* was absent in all *C. coli* and in three *C. lari* isolates. The invasion associated *virB11* gene was found in four isolates (all *C. jejuni*). The *iam*, *cadF*, and *dnaJ* genes were present in all isolates except the five *C. lari* isolates.

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 2019 (N=257)

mechanism	gene	no.	%
Cytotoxin production	*cdtA*	251	98
	cdtB	257	100
	cdtC	225	88
Adherence and colonization	*flaA*	257	100
	cadF	252	98
	dnaJ	252	98
	racR	257	100
Invasion	*virB11*	4	2
	iam	252	98
	ciaB	257	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 31 sets of isolates that were closely related genomically and warranted a public health alert to consider investigation for potential epidemiological links.

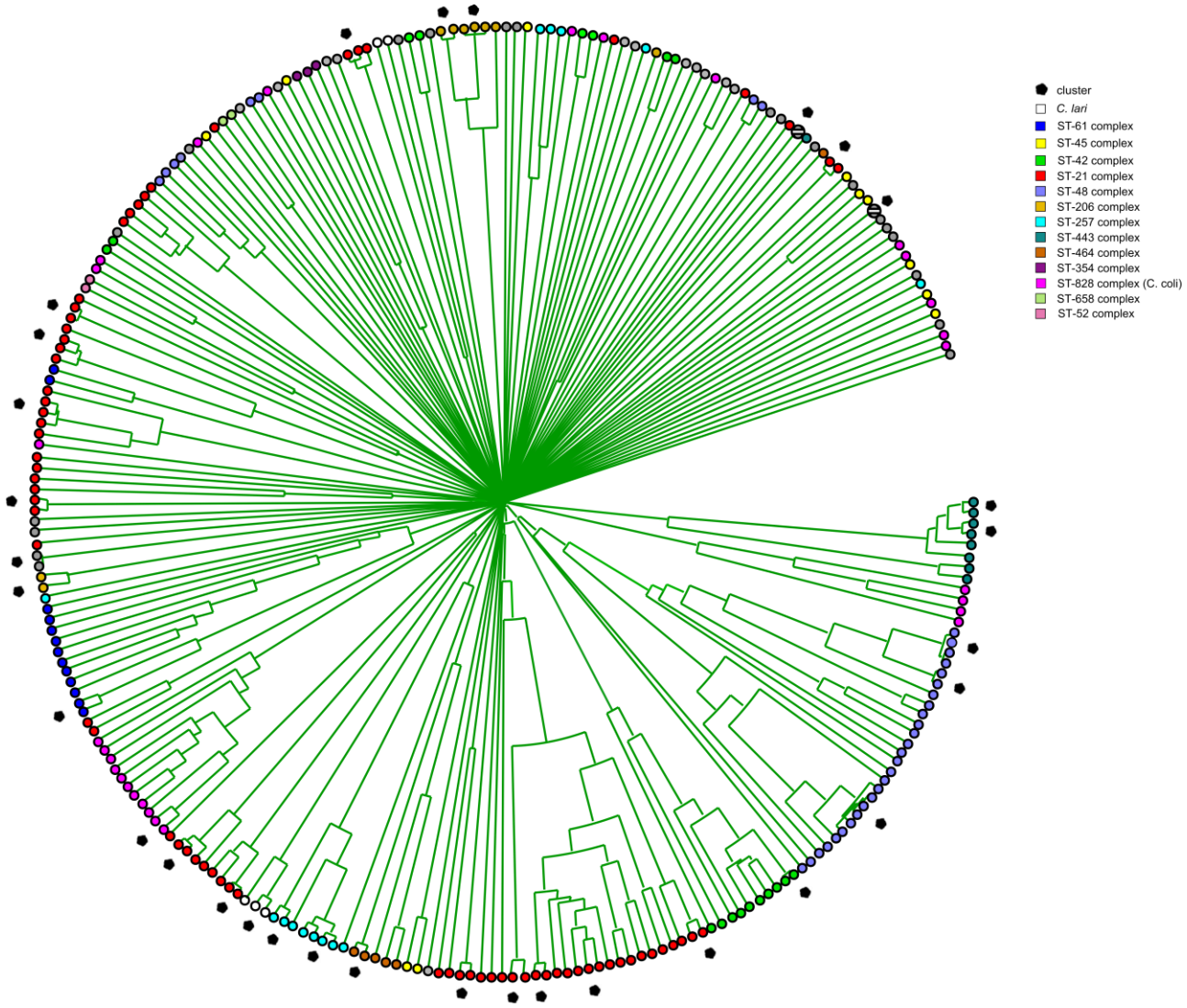

Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=257) 2019. Each circle represents an isolate and they are coloured according to their clonal complex. *C. coli* isolates are ST828 complex (cerise pink). *C. lari* isolates indicated with an open circle. Isolates with ≤5 cgMLST allele differences are indicated with black pentagon.

Conclusion

This is the first comprehensive set of sentinel surveillance data for human clinical *Campylobacter* in Ireland. It forms the basis of a national human *Campylobacter* reference service which will continue at the PHL, HSE, Dublin.

On the basis of these data human clinical *Campylobacter* in Ireland is associated predominantly with *C. jejuni* with a diverse set of genotypes reflecting many of the major globally distributed lineages. Although *C. coli* and *C. lari* contributed relatively less in comparison, their presence is notable and may reflect their infection source. It must be noted however that species retrieved from samples are in part are a reflection of the

testing and culture methods used that may omit or not favour rarer *Campylobacter* species.

These data support the current clinical guidelines for the use of macrolides for empiric treatment. The data also reflect what is seen elsewhere with *C. coli* having relatively more multidrug resistance, perhaps a reflection of higher antibiotic use in its predominant porcine reservoir. 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 not be completely forgone in the short term. Genomics however was shown to be absolutely superior to phenotypic testing for speciation and replaced the biochemical assay during the course of the year.

Genomics also allowed for the detection of 31 clusters of potential public health interest and alerts were raised for these with the relevant parties. Future opportunities include relating clinical presentation with species, genotype and virulence factor profile. Also, linking with other partners in a 'One Health' framework will help us better understand sources of infection to reduce disease burden and the threat of increasing antimicrobial resistance.

We would like to sincerely thank all the participating laboratories that make this national human clinical *Campylobacter* surveillance possible.

For further information please contact:

Dr Eleanor McNamara/Dr Anne Carroll

eleanor.mcnamara@hse.ie

anne.carroll@hse.ie

Public Health Laboratory
Health Services Executive
Dublin Mid-Leinster
Cherry Orchard Hospital
Ballyfermot
Dublin 10

Tel: 01-755175/6
Fax: 01-6231908