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05.12.2019

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

Quarter 3 Report 2019

Summary

- 69/76 isolates characterized:
  - 59/112 (52.7%) stools;
  - 17/17 (100%) isolate swabs
- 84.1% (n=58) *C. jejuni*; 14.5% (n=10) *C. coli*; 1.4% (n=1) *C. lari*
- 37 STs and 16 clonal complexes
- ST-21 clonal complex most prevalent 24.6% (n=17)
- 43.4% (n=33) susceptible to all three antimicrobials tested
  - 47.3% (n=36) isolates were resistant to ciprofloxacin
  - 28.9% (n=22) were resistant to tetracycline
  - one isolate was resistant to erythromycin; this *C. coli* isolate was also resistant to tetracycline and ciprofloxacin
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected

Introduction

This is the Quarter 3 report of the human national *Campylobacter* Clinical Sentinel Surveillance reference laboratory service. The national laboratory surveillance service began in the week of February 4th 2019 and involves 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 Public Health Laboratory (PHL), HSE, Dublin processed on a single designated week (Monday to Sunday) of each month.

Specimen submission

The Quarter 3 period was from July 1st to September 30th inclusive. For this period we received:

- a total of 129 specimens comprising 112 stool specimens and 17 isolate swabs
- A total of 76 bacterial isolates were recovered from submitted specimens; 59/112 (52.7%) from stool specimens and 17/17 (100%) from isolate swabs

Speciation

Campylobacter spp. were identified and speciated from submitted specimens as follows:

1. Specimens (stool/isolate swab) plated for 48 hours microaerophilically @ 42°C on CAMP (Preston agar)
2. Gram stain and oxidase test on any suspect colonies *i.e.* mucoid with a slightly metallic sheen
3. *Campylobacter* is present if Gram negative curved bacilli and oxidase positive
4. Speciation was performed by whole genome sequencing (WGS) 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) by disk diffusion was performed according to EUCAST guidelines on all retrieved cultured isolates (n=76) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline.

- 43.4% (n=33) isolates were susceptible to all three antimicrobials tested
- 47.4% (n=36) isolates were resistant to ciprofloxacin
- 28.9% (n=22) were resistant to tetracycline

- 1.3% (n=1) isolates were resistant to erythromycin
- 18.4% (n=14) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
- One *C. coli* isolate was resistant to all three antimicrobials

Phenotypic culture and AST results were reported contemporaneously on each specimen submitted.

Whole Genome Sequence *Campylobacter* characterisation

All 76 *Campylobacter* isolates were stored and available for batch WGS at the end of Q3. 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 was completed for 69/76 isolates that passed the quality criteria.

C. jejuni accounted for 84% (n=58) of isolates, *C. coli* 14.4% (n=10) and one isolate (1.4%) speciated as *C. lari*. There was a diversity of sequence types (ST) with 37 STs found in total – 28 STs in *C. jejuni,* 8 in *C. coli* (Table 1). The most prevalent STs were ST-48 (14.5%) and ST-21 (13%). These STs resolved into 16 clonal complexes, with ST-21 clonal complex being the most prevalent at 24.6% (n=18) (Table 1, Figure 1). All ten *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 Q3 2019 (n=69). STs with more than one representative isolate shown. # denotes a new ST

ST	clonal complex	no.	%
48	48	10	14.5
21	21	9	13.0
50	21	6	8.7
61	61	3	4.3
827	828	3	4.3
6421	179	3	4.3
22	22	2	2.9
45	45	2	2.9
53	21	2	2.9
10074#	692	2	2.9
≤1 isolates	na	27	39.1

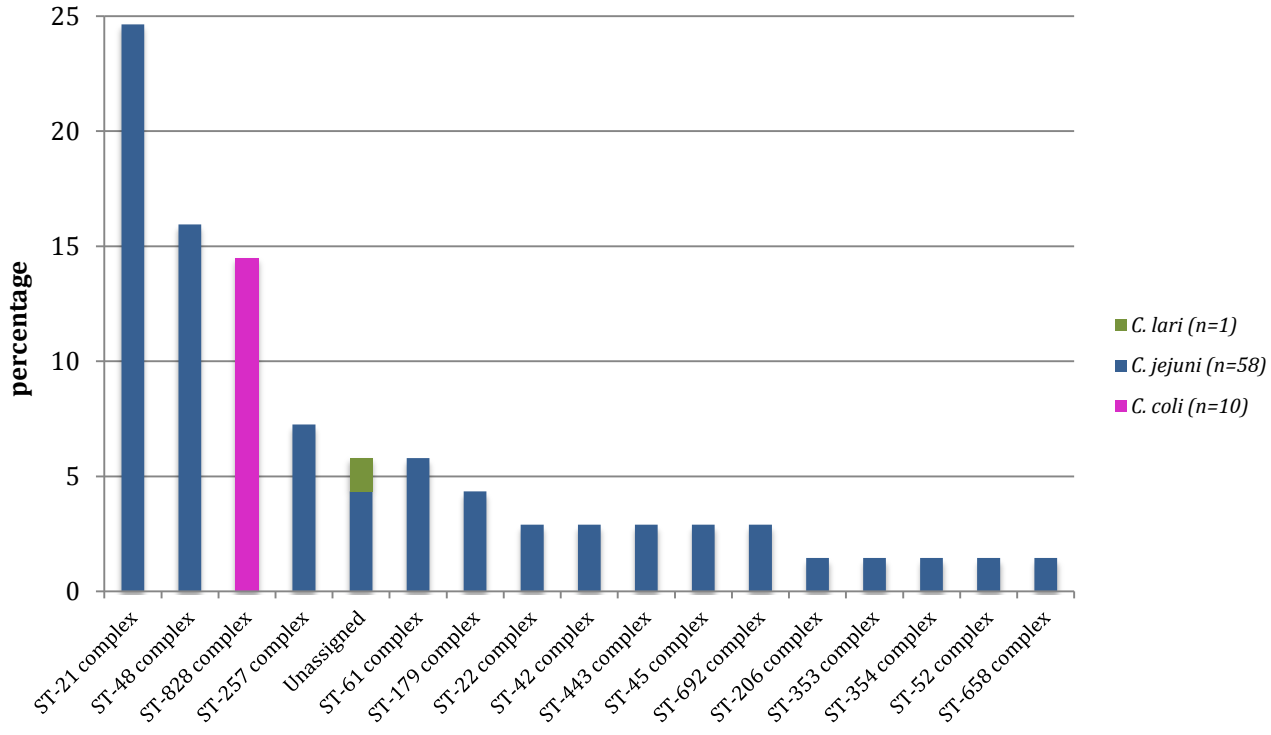

Figure 1: Q3, 2019 *Campylobacter* spp. isolates (n=69) by clonal complex.

AST phenotype and genotype comparison

Of the 69 isolates sequenced, 44.9% (31/69) displayed phenotypic resistance to ciprofloxacin (Table 2) and all contained the *gyrA* mutation Thr86Ile/Val. Therefore, there was 100% concordance between genotype and phenotype for resistance to tetracycline.

All isolates with tetracycline phenotypic resistance 28.9% (n=20/69) contained the *tetO* gene. Therefore, there was 100% concordance between genotype and phenotype for resistance to tetracycline.

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, Q3 2019. N=69

	phenotype: resistant		phenotype: susceptible					
antibiotic class	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
tetracycline	20	0	0	49	100	100	100	100
erythromycin	0	1	0	68	0	100	0	100
ciprofloxacin	31	0	0	38	100	100	100	100

Virulence factors

There were a number of virulence factors found in all of the *Campylobacter* isolates including the adherence and colonization associated factor gene *flaA* and cytotoxin genes *cdtA*, and *cdtB* (Table 3). *cdtC* was found in all *C. jejuni* but not in *C. coli* isolates. The invasion associated *virB11* gene was not found in any isolate. The *iam*, *cadF*, *dnaJ* and *racR* genes were present in all isolates except the single *C. lari* isolate (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. Q3 2019 (N=69)

mechanism	gene	no.	%
Cytotoxin production	*cdtA*	69	100
	cdtB	69	100
	cdtC	59	89
Adherence and colonization	*flaA*	69	100
	cadF	68	99
	dnaJ	68	99
	racR	68	99
Invasion	*virB11*	0	0
	iam	68	99
	ciaB	69	100

Cluster analysis

Isolate genomes were compared for relatedness by comparison at 1343 genes using core genome MLST (cgMLST) (Figure 2). A difference of 5 cgMLST alleles or fewer was used as an alert threshold to consider cluster investigation. Using this criterion there were four sets of isolates (all *C. jejuni*) that were closely related and warranted a public health alert to consider investigation for potential epidemiological links.

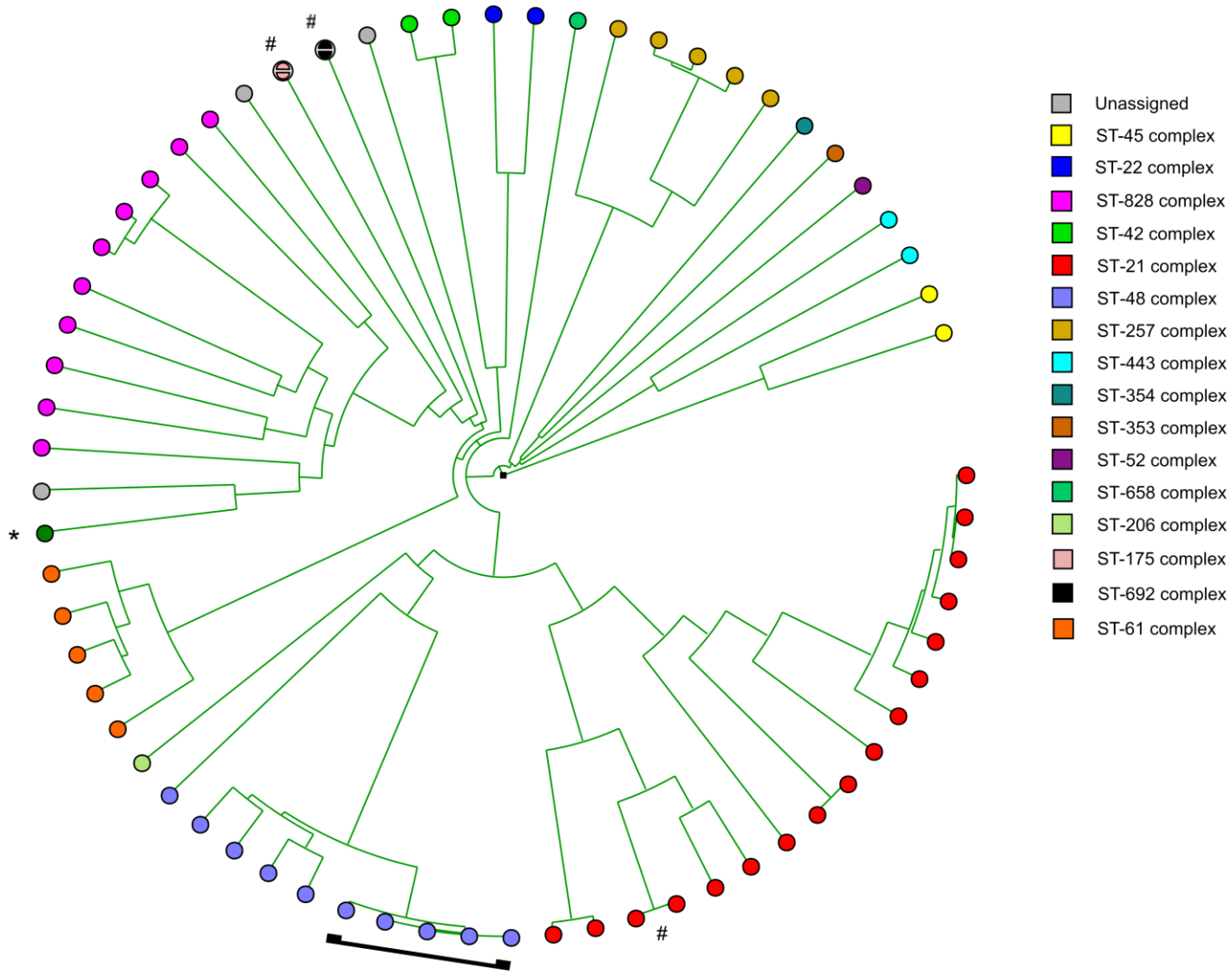

Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=69) Q3 2019. Each circle represents an isolate and they are coloured according to their clonal complex. *C. coli* isolates are ST828 complex (cerise pink). *C. lari* isolate indicated with an *. Isolates with ≤5 cgMLST allele differences are indicated with # or square bracket.