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

Quarter 2 Report 2019

Summary

- 97 isolates:
  - 73/152 (48%) stools;
  - 24/24 (100%) isolate swabs
- 86% (n=71) *C. jejuni*; 12% (n=10) *C. coli*; 2% (n=2) *C. lari**
- 43 STs and 15 clonal complexes
- ST-21 clonal complex most prevalent 33% (n=27)
- 61% (n=59) susceptible to all three antimicrobials tested
  - 31% (n=30) isolates were resistant to ciprofloxacin
  - no isolates were resistant to erythromycin
  - 20% (n=19) were resistant to tetracycline
- Some phenotypic-genotypic discordance for antibiotic sensitivity detected

*WGS complete for n=83

Introduction

This is the Quarter 2 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 2 period was from April 1st to June 30th inclusive. For this period we received;

- a total of 176 specimens comprising 152 stool specimens and 24 isolate swabs
- A total of 97 bacterial isolates were recovered from submitted specimens; 73/152 (48%) from stool specimens and 24/24 (100%) from isolate swabs. The recovery of viable *Campylobacter* from PCR positive stools transferred (48%) is undergoing further investigation. Variables to be assessed are:
    - The date of sample and date of receipt in PHL Dublin.,
    - The Cp value detected at primary laboratory

  The results of this audit may aid us to optimize the recovery yield by culture.

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=97) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline.

- 61% of isolates (n=59) were susceptible to all three antimicrobials tested
- 31% (n=30) isolates were resistant to ciprofloxacin
- No isolates were resistant to erythromycin
- 20% (n=19) were resistant to tetracycline
- 11% (n=11) were resistant to two antimicrobials (all ciprofloxacin and tetracycline)

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

Whole Genome Sequence *Campylobacter* characterisation

All 97 *Campylobacter* isolates were stored and available for batch WGS at the end of Q2. High-quality DNA was extracted from confirmed isolates and DNA libraries were be 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 83/97 isolates which passed the quality criteria.

C. jejuni accounted for 86% (n=71) of isolates, *C. coli* 12% (n=10) and two isolates (2%) speciated as *C. lari*. There was a diversity of sequence types (ST) with 43 STs found in total – 35 STs in *C. jejuni,* 6 in *C. coli* (Table 1) and 2 in *C. lari*. The most prevalent ST was ST-21 (9.5%). These STs resolved into 15 clonal complexes, with ST-21 clonal complex being the most prevalent at 34% (n=28) (Table 1, Figure 1). All *C. coli* isolates belonged to the ST-828 clonal complex. This 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 Q2 2019 (n=83). N=number. * denotes *C. coli* clonal complex. # denotes ST from *C. lari* scheme.

ST	N	%	Clonal Complex
21	8	9.6	ST-21 complex
48	7	8.4	ST-48 complex
45	5	6.0	ST-45 complex
827	5	6.0	ST-828 complex*
50	4	4.8	ST-21 complex
19	4	4.8	ST-21 complex
53	3	3.6	ST-21 complex
806	3	3.6	ST-21 complex
22	2	2.4	ST-22 complex
38	2	2.4	ST-48 complex
42	2	2.4	ST-42 complex
44	2	2.4	ST-21 complex
51	2	2.4	ST-443 complex
61	2	2.4	ST-61 complex
137	2	2.4	ST-45 complex
6209	2	2.4	ST-464 complex
9401	2	2.4	Unassigned
5	1	1.2	ST-353 complex
52	1	1.2	ST-52 complex
122	1	1.2	ST-206 complex
206	1	1.2	ST-206 complex
230	1	1.2	ST-45 complex
257	1	1.2	ST-257 complex
262	1	1.2	ST-21 complex
270	1	1.2	ST-403 complex
447	1	1.2	ST-42 complex
474	1	1.2	ST-48 complex
475	1	1.2	ST-48 complex
575	1	1.2	ST-42 complex
825	1	1.2	ST-828 complex*
917	1	1.2	ST-21 complex
1044	1	1.2	ST-658 complex
1055	1	1.2	ST-828 complex*
1773	1	1.2	ST-828 complex*
3158	1	1.2	ST-48 complex
4766	1	1.2	Unassigned
6175	1	1.2	ST-21 complex
6543	1	1.2	ST-828 complex*
8044	1	1.2	ST-257 complex
9897	1	1.2	Unassigned
9896	1	1.2	ST-828 complex*
27#	1	1.2	Unassigned

new ST#	1	1.2	Unassigned

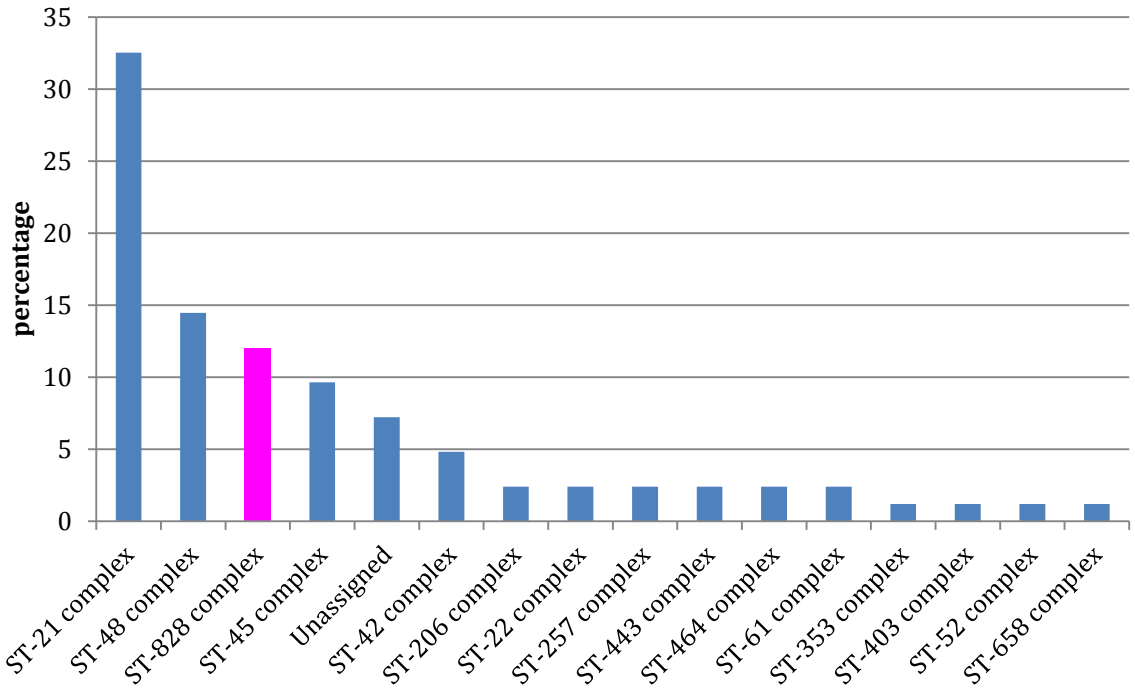


Figure 1: Q2, 2019 *Campylobacter* spp. isolates (n=83) by clonal complex. Blue indicates *C. jejuni* clonal complexes, pink indicates the *C. coli* ST-828 clonal complex

AST phenotype and genotype comparison

Of the 83 isolates sequenced, 31% (26/83) displayed phenotypic resistance to ciprofloxacin (Table 2) and all 26 contained the *gyrA* mutation Thr86Ile/Val. There was one isolate that had the *gyrA* mutation but was not phenotypically ciprofloxacin resistant. Thus the *gyrA* gene may have been 'switched off'. Therefore, there was 96% correlation between genotype and phenotype for ciprofloxacin resistance.

Of the isolates with tetracycline phenotypic resistance 19% (n=16/83), all contained the *tetO* gene. Therefore, there was 100% correlation 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.

Table 2: *Campylobacter* resistance associated genes and phenotype correlation amongst isolates, Q2 2019. N=83

resistance	mechanism	phenotypic resistance n	phenotype - genotype correlation (%)	gene absent but resistant phenotype (%)	gene present but susceptible phenotype (%)
tetracycline	*tetO*	16	16/16 (100)	0 (0)	0 (0)
ciprofloxacin	*gyrA* Thr86Ile/Val	26	26/27 (96)	0 (0)	1 (3)
erythromycin	*ermB/23S rRNA A2059G*	0	0 (0)	0 (0)	0 (0)

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* and the invasion associated gene *ciaB* (Table 3). The cytotoxin genes *cdtA*, and *cdtB* were also found in all isolates, while *cdtC* was found in all *C. jejuni*, but not in *C. coli* or *C. lari* isolates (n=12). The invasion associated *virB11* gene was found in just one *C. jejuni* isolate, while the *iam* gene was present in 98% (it was absent in the 2 *C. lari* isolates). Other genes such as *cadF* and *dnaJ* were found in the majority of 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. Q2 2019 (N=83)

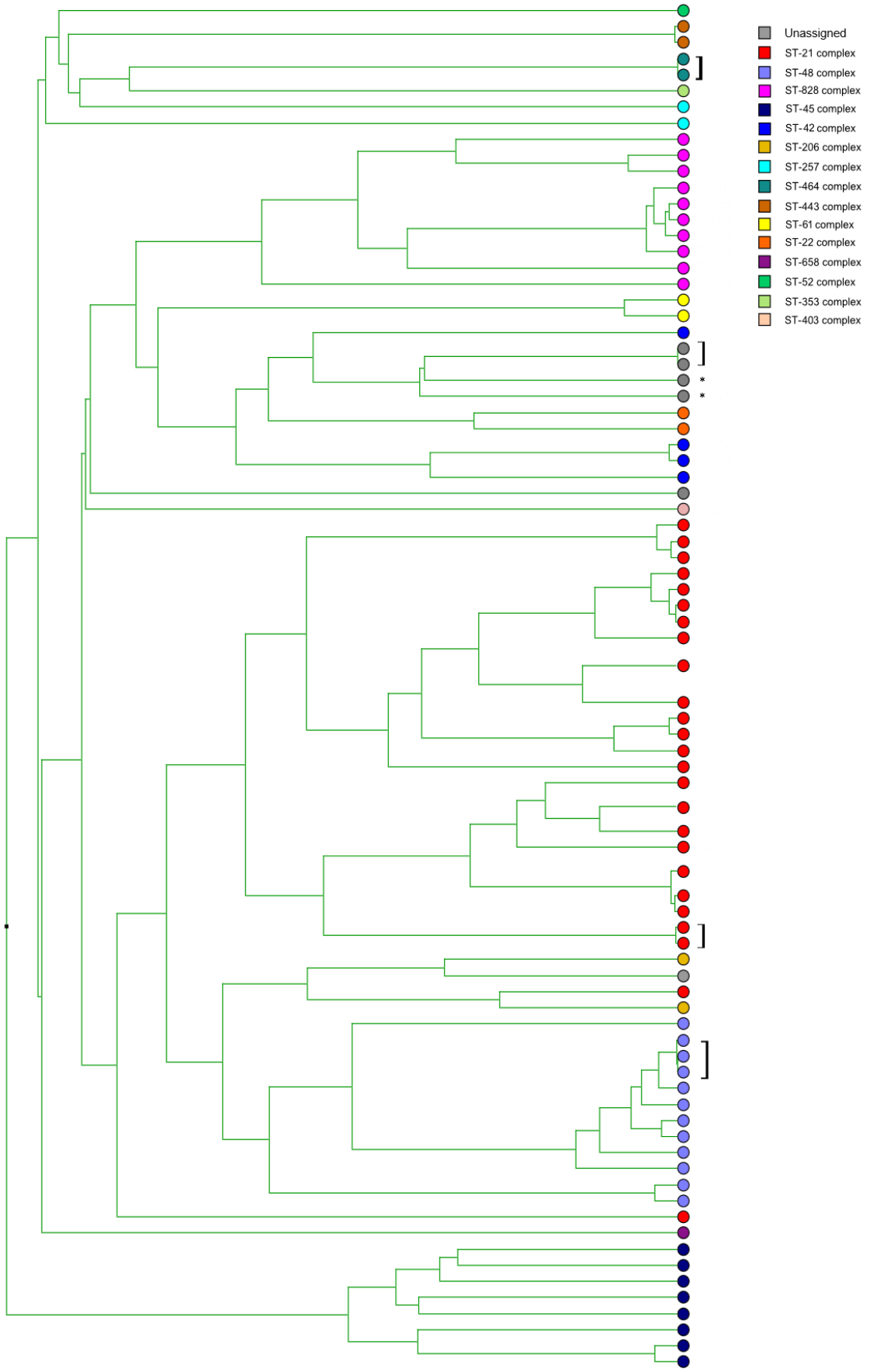
mechanism	gene	no.	%
Cytotoxin production	*cdtA*	83	100
	cdtB	83	100
	cdtC	71	86
Adherence and colonization	*flaA*	83	100
	cadF	81	98
	dnaJ	81	98
	racR	83	100
Invasion	*virB11*	1	1
	iam	81	98
	ciaB	83	100

Cluster analysis

Isolate genomes were compared for relatedness by comparison at 3529 genes using whole genome MLST (wgMLST) (Figure 2). A difference of 15-20 alleles or less is

suggested as an alert threshold to consider cluster investigation for *Campylobacter* (Cody *et al,* 2013, Schurch et al, 2018). Using the criterion of 15 or fewer wgMLST allele differences, there were four pairs of isolates (all *C. jejuni*) with between 1 and 7 allelic differences between them. If including isolates from 2019 Q1 database, there were 10 possible clusters *i.e.* pairs/groups of isolates with 15 or fewer wgMLST allelic differences. These clusters warrant a public health alert to consider investigation for potential epidemiological links.

Figure 2: UPGMA tree of wgMLST differences amongst *Campylobacter* spp. isolates (n=83) Q2 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 are indicated with an *. Isolates with 1 to 7 allele differences are indicated with square brackets.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