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

National human Campylobacter Sentinel Surveillance Reference Laboratory Service Quarter 2 Report 2021

Summary

- 143 clinical specimens received from 143 patients
- 89 isolates/143 (62%) samples isolated and characterized:
  - 68/121 stools (56.20%)
  - 21/22 isolate swabs (95.45%)
- 62.92% (n=56) susceptible to all three antimicrobials tested
  - 22.47% (n=20) isolates were resistant to ciprofloxacin only.
  - 6.74% (n=6) were resistant to tetracycline only.
  - 1.12 % (n=1) were resistant to Erythromycin only.
  - 6.74 % (n=6) were resistant to two antimicrobials (ciprofloxacin and tetracycline)
- 81/89 passed WGS QC for analysis
  - 90.12% (n=73) *C. jejuni,* 8.64 % *C. coli* (n=7), 1.23 % *C. lari* (n=1)
  - 27 STs and 15 clonal complexes were detected in this *Campylobacter* dataset.
  - ST-21 clonal complex most prevalent 24.69% (n=20)
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected
- WGS identified Quarter 2 2021 8 potential clusters for public health alert.

Introduction

This is the 2021 Quarter 2 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 2021 schedule began on January 14th 2021 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 2021. 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 2 period was from 01/04/2021to 30/06/2021. We received:

- A total of 143 specimens from 143 patients comprising 121 stool specimens and 22 isolate swabs.
- A total of 89 *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 68/121 (56.20%) from PCR positive stool specimens and 21/22 (95.45%) from isolate swabs.

Speciation

Campylobacter spp. were confirmed by culture immediately on receipt 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 v 11.0 2021 guidelines on all retrieved cultured isolates (n=89) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline. 62.92% (n=56) susceptible to all three antimicrobials tested

- 22.47 % (n=20) isolates were resistant to ciprofloxacin only
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- 6.74 % (n=6) were resistant to two antimicrobials (ciprofloxacin and tetracycline)

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 89 *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 81/89 isolates that passed the quality criteria. The remaining 8 will be repeated and reported at a later date.

C. jejuni accounted for 90.12 % (n=73) of isolates and *C. coli* 8.64% (n=7) *and C. lari 1.23 % (n=1)*. There was a diversity of sequence types (ST) with 27 STs found in total – 23 STs in *C. jejuni* and 4 STs in *C. coli* and an unassigned ST in *1 C. lari isolate*.

The most prevalent STs were ST-45 (14.81%), ST-19 (9.88%) and ST-21(9.88%). These STs resolved into 15 clonal complexes, with ST-21 clonal complex being the most prevalent at 24.69% (n=20). A clonal complex was not assigned to 6 isolates (7.41 %)(Table1, Figure 1). 7/7 *C. coli* (8.64%) 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 clonal complex includes STs (e.g. ST-21, ST-19, ST-50, ST-262, 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 the 27 locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection Quarter 2 2021 (n=81). STs with less than four representative isolates not shown.

ST	Clonal Complex	N	%
45	ST-45 complex	12	14.81%
19	ST-21 complex	8	9.88%
21	ST-21 complex	8	9.88%
48	ST-48 complex	7	8.64%
42	ST-42 complex	6	7.41%
137	ST-45 complex	4	4.94%
827	ST-828 complex	4	4.94%
257	ST-257 complex	4	4.94%
<4		28	34.57%

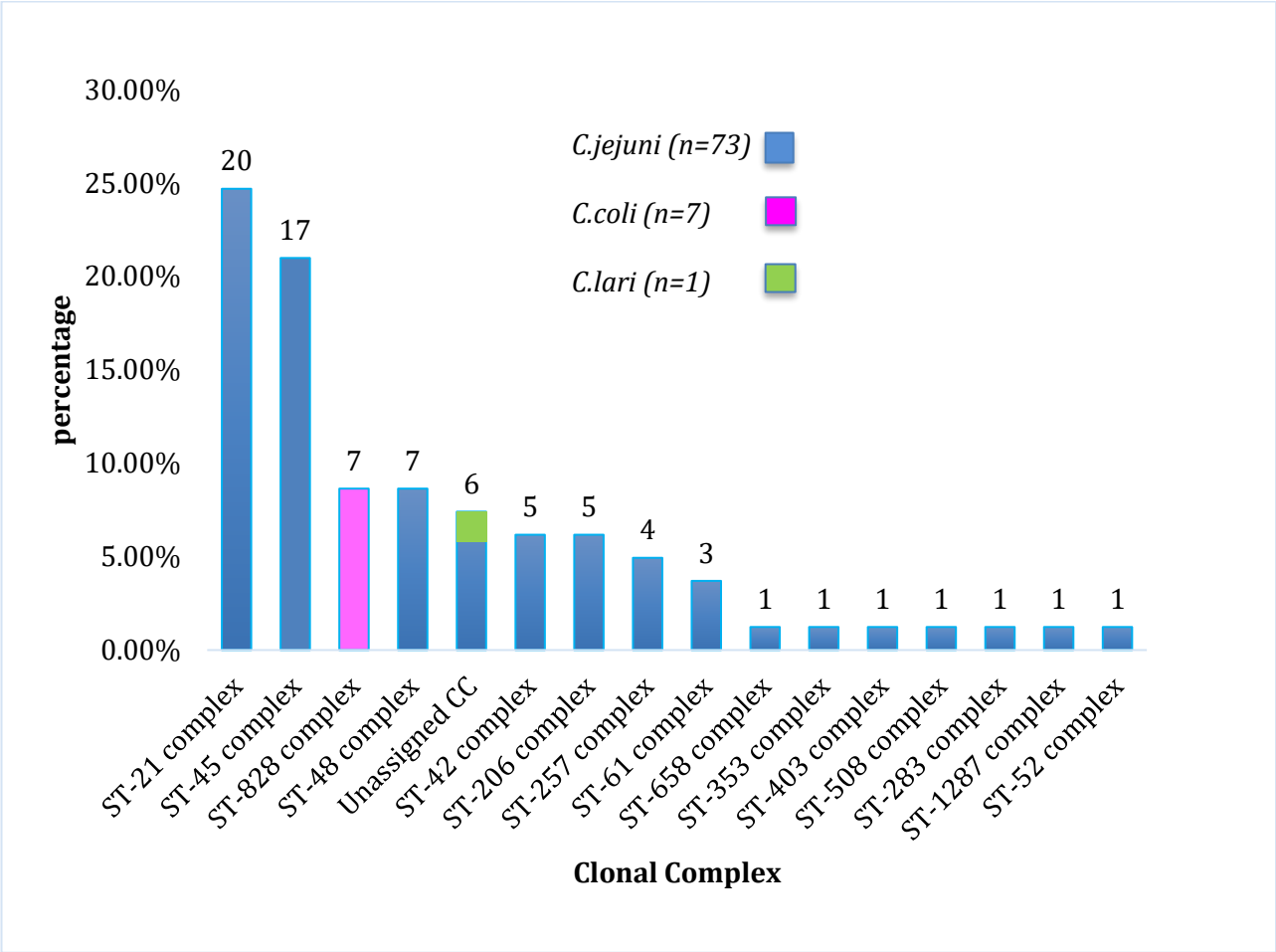

Figure 1: Quarter 2 2021 *Campylobacter* spp. isolates (n=81) by clonal complex

AST phenotype and genotype comparison

Of the 26 isolates with phenotypic ciprofloxacin resistance, 24 passed WGS validity criteria. Of these 24, 20 contained the *gyrA* mutation *Thr86Ile/Val*. However in the other 4 isolates, other resistance mechanisms maybe responsible for mediating ciprofloxacin phenotypic resistance. Of the 63 isolates that were susceptible to ciprofloxacin, 57 passed WGS validity criteria. All 57 did not have the *gyrA* mutation. Therefore, there was 83.3% and 100% sensitivity and specificity for WGS to predict ciprofloxacin AMR with a corresponding positive predictive value of 100% and negative predictive value of 93.4%.

Of the 12 isolates with phenotypic tetracycline resistance, 10 passed WGS validity criteria. 5 of the 10 harboured the gene *tetO,* which indicates that other resistance mechanisms maybe responsible for mediating tetracycline phenotypic resistance. Of the 77 isolates that were susceptible to tetracycline, 71 passed WGS validity criteria. 70 of 71 did not harbour *tetO*. Therefore, there was 50.00% sensitivity and 98.6% specificity for WGS to predict tetracycline AMR with a corresponding positive predictive value of 83.3% and negative predictive value of 93.3%.

Of the 81 isolates that passed the WGS validity criteria 1/81 isolates were found to have phenotypic erythromycin resistance. Of these 81 isolates, the *23S rRNA* gene/mutation and *ermB* gene associated with mediating macrolide resistance were not detected in any of the Campylobacter spp. isolates. This may indicate that other resistant mechanisms were responsible for mediating macrolide phenotypic resistance.

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 2 2021 N=81

	phenotype: resistant		phenotype: susceptible					
antibiotic class	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible	Sensitivity (%)	Specificity (%)	Positive Predictive Value	Negative Predictive Value
tetracycline	5	5	1	70	50.0%	98.6%	83.3%	93.3%
erythromycin	0	1	0	80	0.0%	100.0%	0.0%	98.8%
ciprofloxacin	20	4	0	57	83.3%	100.0%	100.0%	93.4%

Virulence factors

There were a number of virulence factors found in all of the campylobacter isolates. The adherence and colonization associated genes *cadF, racR & dnaJ* were found in 75/81 Campylobacter isolates, with *flaA* present in 76/81 isolates. *cdtA* was present in 73 isolates, *cdtB* was present in 74 isolates, while *cdtC* was present in 67 of the *C. jejuni* isolates and present in one of the *C. coli* isolates. The invasion associated genes, *virB11* gene was found in 1 isolate *(C. jejuni).* The *iam* and *ciaB* genes were present in 75 and 47 isolates respectively (see 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* 2021 (N=81)

mechanism	gene	no.	%
Cytotoxin production	*cdtA*	73	90.1
	cdtB	74	91.4
	cdtC	68	84.0
Adherence and colonization	*flaA*	76	93.8
	cadF	75	92.6
	dnaJ	75	92.6
	racR	75	92.6
Invasion	*virB11*	1	1.2
	iam	75	92.6
	ciaB	47	58.0

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 8 sets of Quarter 2 2021 isolates that were closely related genomically and may have warranted public health alerts to consider investigation for potential epidemiological links. However due to the recent cyber-attack the WGS cluster analysis was delayed, thus rendering these clusters historic. However they will be monitored along with future clusters.

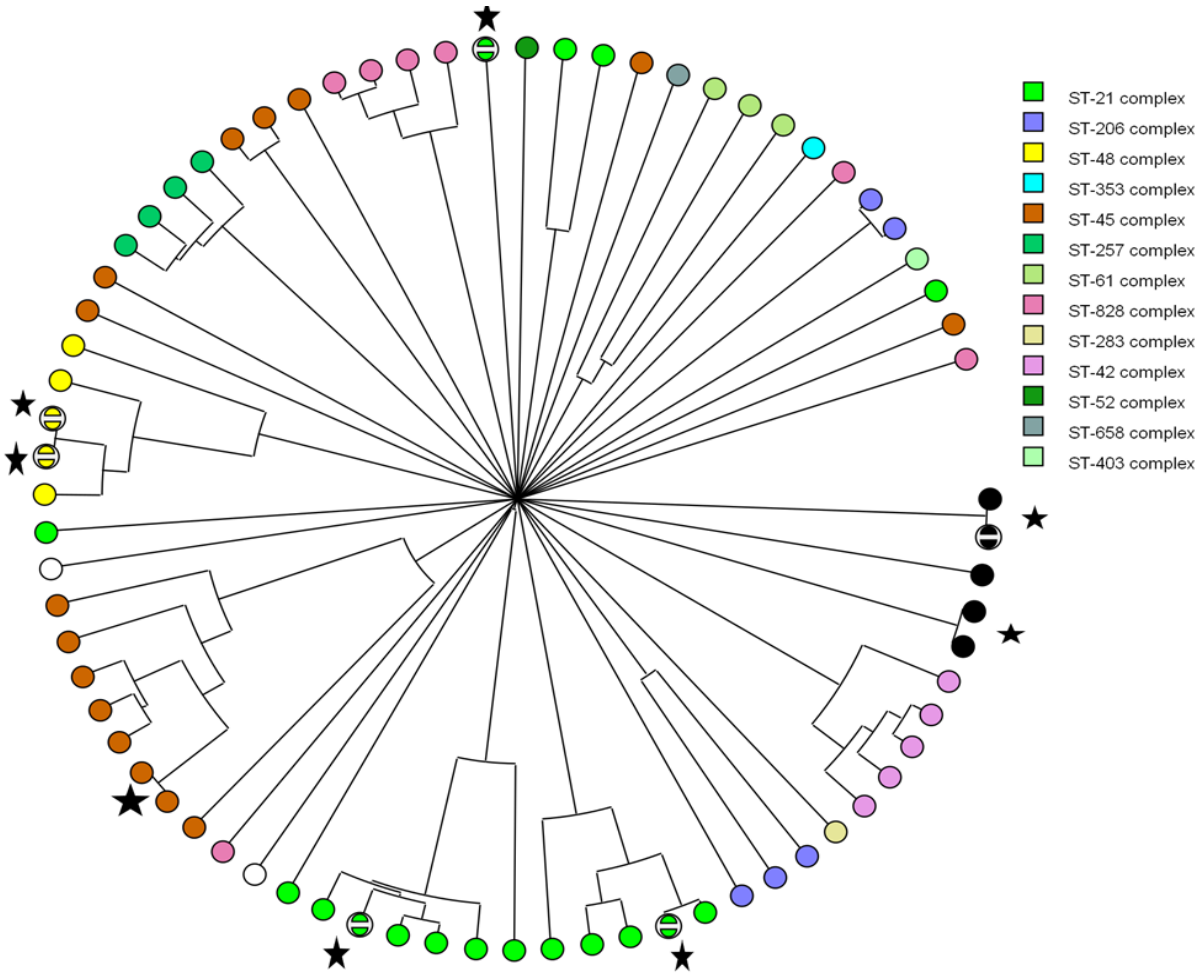

Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=81) Quarter 2 2021. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with black pentagon. Nodes in black are unassigned clonal complexes.

Conclusion

This is the Quarter 2 2021 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 2 2021 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 8 Quarter 2 2021 historic 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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