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

National human Campylobacter Sentinel Surveillance Reference Laboratory Service Quarter 3 Report 2021

Summary

- 99 specimens were received
- 98 clinical specimens received from 98 patients
- 1 specimen isolated from a water sample
- 38 isolates/99 specimens isolated and characterized:
  - 25/83 stools (30.1%)
  - 13/16 isolate swabs (81.3%)
- 60.52% (n=23) susceptible to all three antimicrobials tested
  - 15.79% (n=6) isolates were resistant to ciprofloxacin only.
  - 2.63% (n=1) were resistant to tetracycline only.
  - 18.42% (n=7) were resistant to ciprofloxacin and tetracycline
  - 2.63% (n=1) were resistant to ciprofloxacin and erythromycin
- 37/38 passed WGS QC for analysis
  - 94.59% (n=35) *C. jejuni,* 5.41% *C. coli* (n=2).
  - 19 STs and 12 clonal complexes were detected in this *Campylobacter* dataset.
  - ST-21 clonal complex most prevalent 43.24% (n=16)
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected
- Quarter 3 2021 WGS identified 5 potential clusters for public health alert.

Introduction

This is the 2021 Quarter 3 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 1st, 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 3 period was from 01/07/2021 to 30/09/2021. We received:

- A total of 99 specimens, 98 clinical specimens from 98 patients, and 1 specimen from a water sample, the specimens comprised of 83 stool specimens and 16 isolate swabs.
- A total of 38 *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 25/83 (30.1%) from PCR positive stool specimens and 13/16(81.3%) from isolate swabs. This was an unusually low culture positive yield compared to previous periods. See the consequential audit results in the final 'Conclusion Section' of this report.

Speciation

Campylobacter spp. were confirmed by culture contemporaneously, once receipted 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 guidelines on all retrieved cultured isolates (n=38) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin, and tetracycline. 60.52 % (n=23) susceptible to all three antimicrobials tested

- 15.79 % (n=6) isolates were resistant to ciprofloxacin only
- 2.63 % (n=1) were resistant to tetracycline only
- 18.42% (n=7) were resistant to ciprofloxacin and tetracycline
- 2.63% (n=1) were resistant to ciprofloxacin and erythromycin

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 38 *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 37/38 isolates that passed the quality criteria.

C. jejuni accounted for 94.59% (n=35) of isolates and *C. coli* 5.41% (n=2).There was a diversity of sequence types (ST) with 19 STs found in total – 18 STs in *C. jejuni* and 1 ST in *C. coli.*

The most prevalent STs were ST-21 (21.62%) and ST-50 (10.81%). These STs resolved into 12 clonal complexes, with ST-21 clonal complex being the most prevalent at 43.24% (n=16). A clonal complex was not assigned to 5 isolates (13.51%) (Table1, Figure 1). 1/2 *C. coli* isolates belonged to the ST-828 clonal complex, but a clonal complex was not assigned to 1/2 *C. coli* isolates. 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. ST-21, ST-19, ST-50, ST-262, ST-6175) that match the central genotype *i.e.* ST-21 at four or more of the conventional MLST seven housekeeping gene alleles.

Table 1: Breakdown of 19 locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection Quarter 3 2021 (n=37). STs with more than four representative isolates are shown.

ST	Clonal Complex	N	%
21	ST-21 complex	8	21.62%
50	ST-21 complex	4	10.81%
< 4	NA	25	67.57%

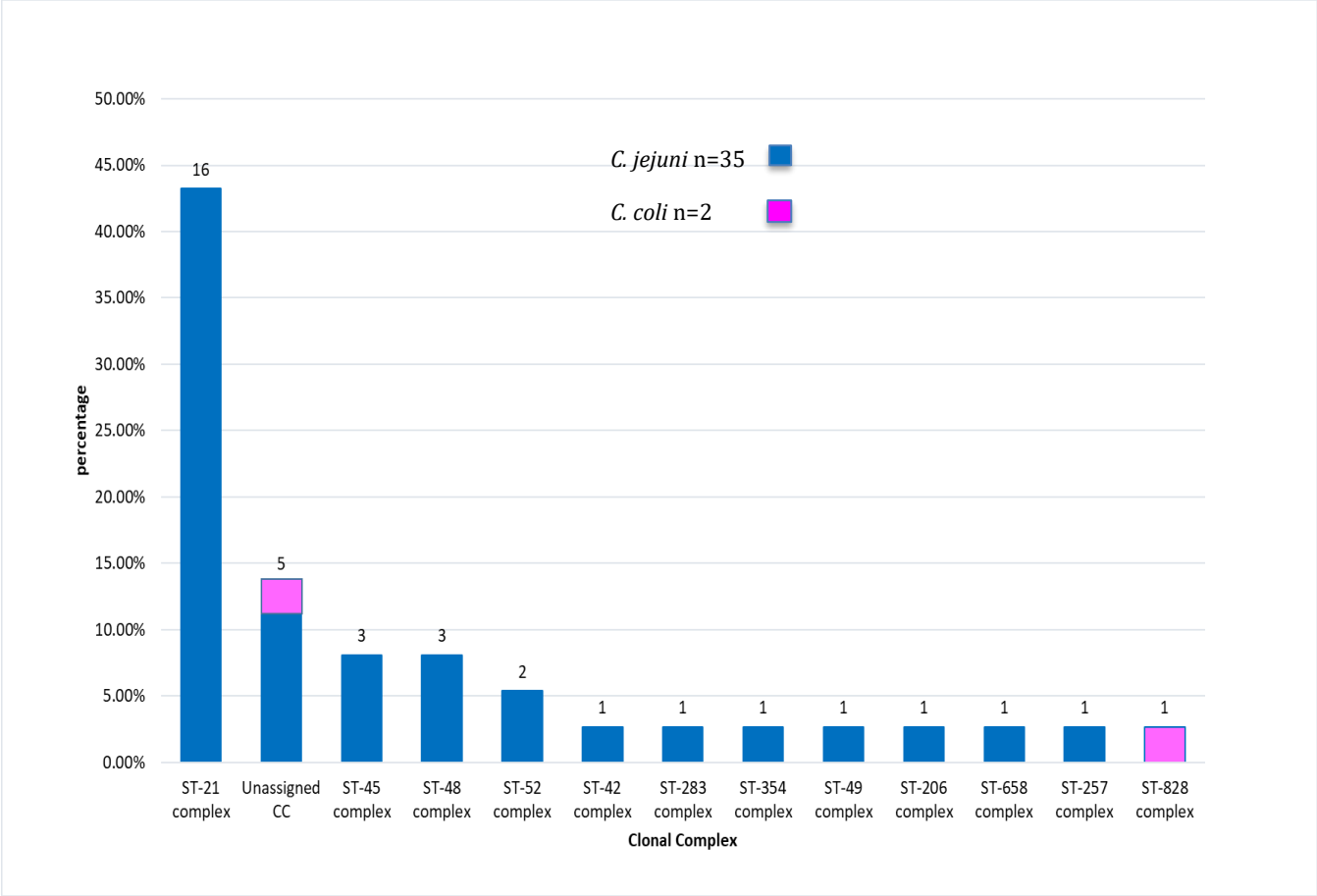

Figure 1: Quarter 3 2021 *Campylobacter* spp. isolates (n=37) by clonal complex.

AST phenotype and genotype comparison

Of the 14 isolates with phenotypic ciprofloxacin resistance, 14 passed WGS validity criteria. Of these 14, 12 contained the *gyrA* mutation Thr86Ile/Val. However, in the other two isolates, other resistance mechanisms may be responsible for mediating ciprofloxacin phenotypic resistance. Of the 24 isolates that were phenotypically susceptible to ciprofloxacin, 23 passed WGS validity criteria. Of these 23, 23 did not have the *gyrA* mutation. Therefore, there was 85.7% and 100% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 100% and a negative predictive value of 92%.

Of the 8 isolates with phenotypic tetracycline resistance, 8 passed WGS validity criteria. Of these 8, 6 harboured the gene *tetO*, which indicates that other resistance mechanisms may be responsible for tetracycline resistance. Of the 30 isolates that were phenotypically susceptible to tetracycline, 29 passed WGS validity criteria. Of these 29, 27 did not harbour *tetO*. Therefore, there was 75.0% sensitivity and 93.1% specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 75.0% and negative predictive value of 96.1%.

1 isolate was 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 may indicate that other resistant mechanisms were responsible for mediating macrolide phenotypic resistance.

Please note that the relatively low number of samples received in Quarter 3 2021 may falsely represent the apparent low sensitivity of WGS to detect the *gyrA*, *tetO, 23rRNA,* and *ermB* mutations in phenotypic resistant isolates.

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

Table 2: *Campylobacter* resistance-associated genes and phenotype concordance amongst isolates, Quarter 3 2021. N=37

	phenotype: resistant		phenotype: susceptible					
antibiotic class	**genotype: resistant**	**genotype: susceptible**	**genotype: resistant**	**genotype: susceptible**	**Sensitivity (%)**	**Specificity (%)**	**Positive Predictive Value**	**Negative Predictive Value**
tetracycline	6	2	2	27	75.0%	93.1%	75.0%	93.1%
erythromycin	0	1	0	36	0.0%	100.0%	0.0%	97.3%
ciprofloxacin	12	2	0	23	85.7%	100.0%	100.0%	92.0%

Virulence factors

There were a number of virulence factors found in all of the *Campylobacter* isolates (n=37) including the adherence and colonization associated factor genes *cadF, dnaj & flaA,* however, *racR* was present in 29/37 isolates. *cdtA* and *cdtB* were present in all 37 isolates, while *cdtC* was present in all of the 35 *C. jejuni* isolates but not present in any of the 2 *C. coli* isolates. The invasion-associated virB11 gene was found in 6 isolates *(C. jejuni).* The *iam* gene and *ciaB* were present in all 37 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 Quarter 3 2021 (N=37)

mechanism	gene	N	%
Cytotoxin production	*cdtA*	37	100.0%
	cdtB	37	100.0%
	cdtC	35	94.6%
Adherence and colonization	*flaA*	37	100.0%
	cadF	37	100.0%
	dnaJ	37	100.0%
	racR	29	78.4%
Invasion	*virB11*	6	16.2%
	iam	37	100.0%
	ciaB	37	100.0%

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 5 sets of Quarter 3 2021 isolates that were closely related genomically and warranted a public health alert to consider investigation for potential epidemiological links.

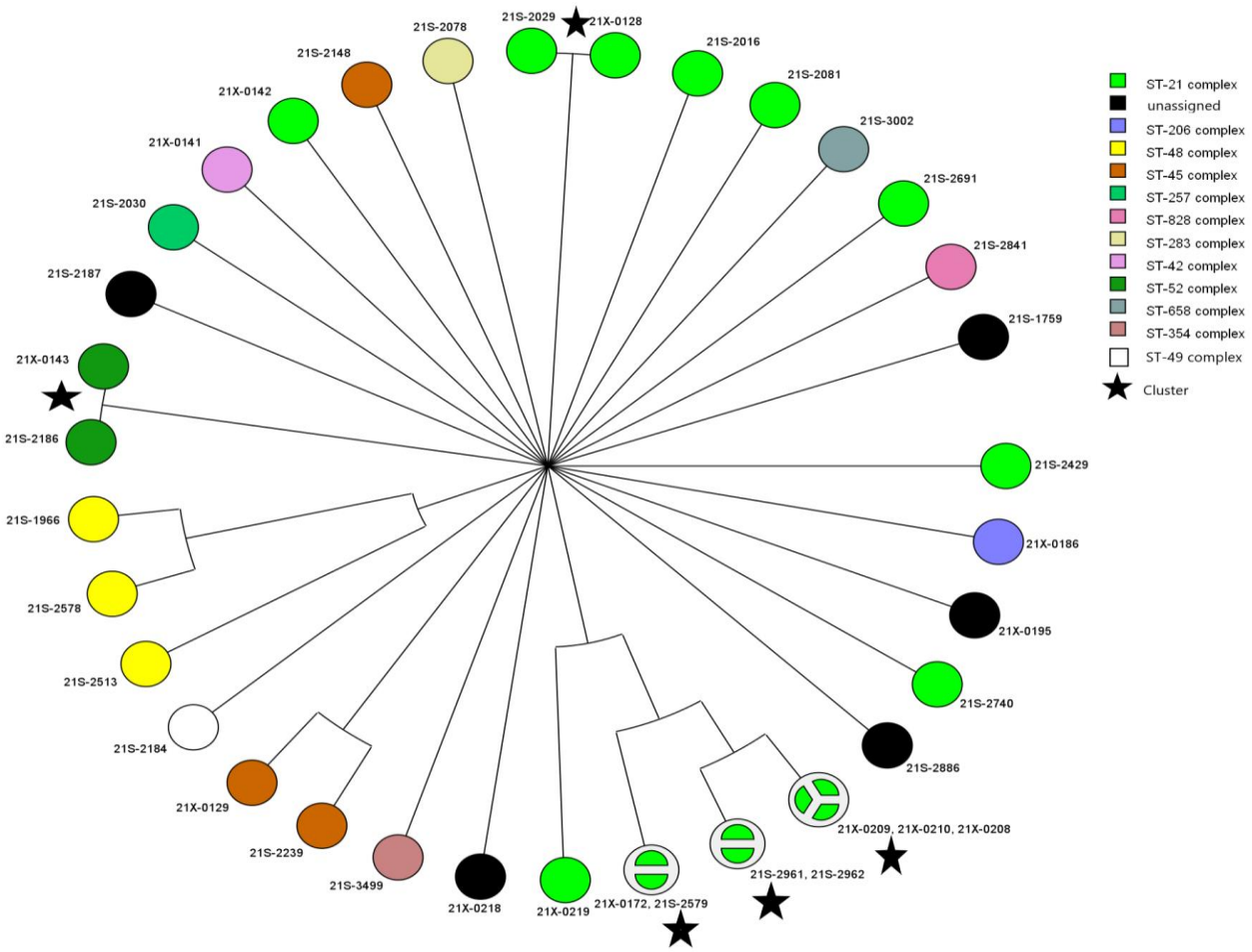

Figure 2: UPGMA tree of cgMLST differences amongst Campylobacter spp. isolates (n=37) Quarter 3 2021. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with black pentagon.

Conclusion

This is the Quarter 3 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.

The low culture yield from stool specimens was remarkable compared to previous quarters, therefore an audit was performed (reviewing senders stool Ct values recorded and time of sampling/positivity to receipt at the NRL compared to NRL culture yield) to review potential reasons. The audit found for culture positivity at the NRL, the mean Ct stool value of the sender laboratories was 29 (range 21-38) and the mean time of stool sample receipt at the NRL associated with culture positivity was 4 days (range 1-8 days) and 9 days (range 1-21) for isolates sent. Consequently we recommend;

- Laboratories to send positive samples/isolates daily to NRL within your scheduled week for submission and not to delay by batching specimens.

- Consider your stool Ct result threshold when evaluating for clinical significance.

On the basis of these Quarter 3 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 5 Quarter 3 2021 exclusive 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, 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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