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

## National human *Campylobacter* Sentinel Surveillance Reference Laboratory Service Annual Report 2021

## Summary

- 392 specimens were received
- 391 clinical specimens received from 389 patients
- 1 specimen isolated from a water sample
- 204 isolates/392(52%) samples characterized:
  - 147/329 stools (45%)
  - 57/63 isolate swabs (91%) (1 with mixed infection)
- 64.7% (n=132) susceptible to all three antimicrobials tested
  - 18% (n=37) isolates were resistant to ciprofloxacin only.
  - 6.9% (n=14) were resistant to tetracycline only.
  - 0.5% (n=1) were resistant to erythromycin only.
  - 9.5% (n=19) were resistant to ciprofloxacin and tetracycline.
  - 0.5% (n=1) were resistant to ciprofloxacin and erythromycin.
- 200/204 passed WGS QC for analysis
  - 90.5 % (n=181) *C. jejuni,* 8.5% *C. coli* (n=17), 1% *C. lari* (n=2)
  - 58 STs and 23 clonal complexes were detected in this *Campylobacter* dataset.
  - ST-21 clonal complex most prevalent 26.87% (n=54)
- Some phenotypic-genotypic congruence for antibiotic sensitivity detected
- WGS identified 22 potential clusters for public health alert in 2021.

## Introduction

This is the 2021 Annual 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

From January 1st 2021 to December 31st 2021 we received:

- A total of 392 specimens, 391 clinical specimens from 389 patients, and 1 specimen from a water sample, the specimens comprised of 329 stool specimens and 63 isolate swabs (1 mixed infection).
- A total of 204 (52%) *Campylobacter* spp. bacterial isolates were recovered from submitted specimens; 147/329 (45%) from PCR positive stool specimens and 57/63 (91%) from isolate swabs.

This low culture yield from stool specimens was previously documented in our Q3 2021 report 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 consider potential reasons (see Appendix). 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 for isolates sent, it was 9 days (range 1-21).

Consequently, to improve culture positivity of samples sent to the NRL, we recommend;

- Laboratories should send (in appropriate transport media) their positive samples/isolates daily to NRL within your scheduled week for sample submission. Please do not batch positive specimens until the end of your designated week of submission prior to sending, as this incurs unnecessary delay in NRL receipt.
- Consider your stool PCR Ct threshold result, when evaluating for clinical significance.

## 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=204) for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline. 64.7% (n=132) susceptible to all three antimicrobials tested

- 18% (n=37) isolates were resistant to ciprofloxacin only
- 6.9% (n=14) were resistant to tetracycline only
- 0.5% (n=1) were resistant to erythromycin only
- 9.5% (n=19) were resistant to ciprofloxacin and tetracycline
- 0.5% (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.

**Table 1: AST results 2019-2021**

| Year | Total isolated | Susceptible n(%) | Ciprofloxacin resistance n(%) | Tetracycline resistance n(%) | Erythromycin resistance n(%) |
|---|---|---|---|---|---|
| 2021 | 204 | 132 (64.7%) | 57(27.94%) | 33(16.18%) | 2 (0.98%) |
| 2020 | 85* | 48 (56.5%) | 26 (30.59%) | 20(23.53%) | 0 |
| 2019 | 277 | 140(50.54%) | 109(39.35%) | 73(26.35%) | 2 (0.72%) |

* This dataset of sentinel surveillance was truncated due to SARS-CoV-2 monitoring

## Whole Genome Sequence *Campylobacter* characterization

All 204 *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 200/204 isolates that passed the quality criteria.

*C. jejuni* accounted for 90.5% (n=181) of isolates and *C. coli* 8.5% (n=17) *and C. lari 1% (n=2)*. There was a diversity of sequence types (ST) with 58 STs found in total – 49 STs in *C. jejuni* and 9 STs in *C. coli* and there were no STs assigned for the *C. lari* isolates.

The most prevalent STs were ST-48 (12.5%) and ST-21 (12.5%). These STs resolved into 23 clonal complexes, with ST-21 clonal complex being the most prevalent at 26.9% (n=54). A clonal complex was not assigned to 20 isolates (10%) (Table1, Figure 1). 15/17 *C. coli* isolates belonged to the ST-828 clonal complex, but a clonal complex was not assigned to 2/17 *C. coli* isolates. The ST-828 clonal complex is exclusively associated with *C. coli.*

**Table 2: Breakdown of speciation & the most prevalent STs & clonal complexes 2019-2021**

| Year | WGS* | *C. jejuni* n(%) | *C.coli* n(%) | *C.lari* n(%) | ST-21 n(%) | ST-48 n(%) | ST-21 clonal complex** n(%) |
|---|---|---|---|---|---|---|---|
| 2021 | 200 | 181 (90.5%) | 17(8.5%) | 2(1.0%) | 25(12.5%) | 25(12.5%) | 54(27.0%) |
| 2020*** | 74 | 67(90.54%) | 7(9.5%) | 0.00% | 9(12.16%) | 10(13.51%) | 27(36.5%) |
| 2019 | 257 | 223(86.77%) | 29(11.28%) | 5(1.95%) | 31(12.06%) | 26(10.12%) | 69(26.9%) |

*The number of isolates that pass the WGS QC analysis criteria
** ST-21 clonal complex (CC) was the most prevalent CC for all three years
*** This dataset of sentinel surveillance was truncated due to SARS-CoV-2 monitoring

**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-19, ST-50, ST-53, ST-262, ST-266, ST-917 ST-6175, ST-7144, ST-9993) that matches the central genotype *i.e.* ST-21 at four or more of the conventional MLST seven housekeeping gene alleles.

**Table 3: Breakdown of 58 locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection 2021 (n=200). STs with more than four representative isolates shown. * denote *C. coli* complex**

| Sequencing Type | Clonal Complex | N | % |
|---|---|---|---|
| 48 | ST-48 complex | 25 | 12.5% |
| 21 | ST-21 complex | 25 | 12.5% |
| 45 | ST-45 complex | 17 | 8.5% |
| 19 | ST-21 complex | 13 | 6.5% |
| 42 | ST-42 complex | 8 | 4.0% |
| 828 | ST-828 complex* | 7 | 3.5% |
| 50 | ST-21 complex | 7 | 3.5% |
| 206 | ST-206 complex | 6 | 3.0% |
| 137 | ST-45 complex | 5 | 2.5% |
| 61 | ST-61 complex | 5 | 2.5% |
| 257 | ST-257 complex | 5 | 2.5% |
| 9401 | Unassigned CC | 4 | 2.0% |
| < 4 | N/A | 73 | 36.50% |

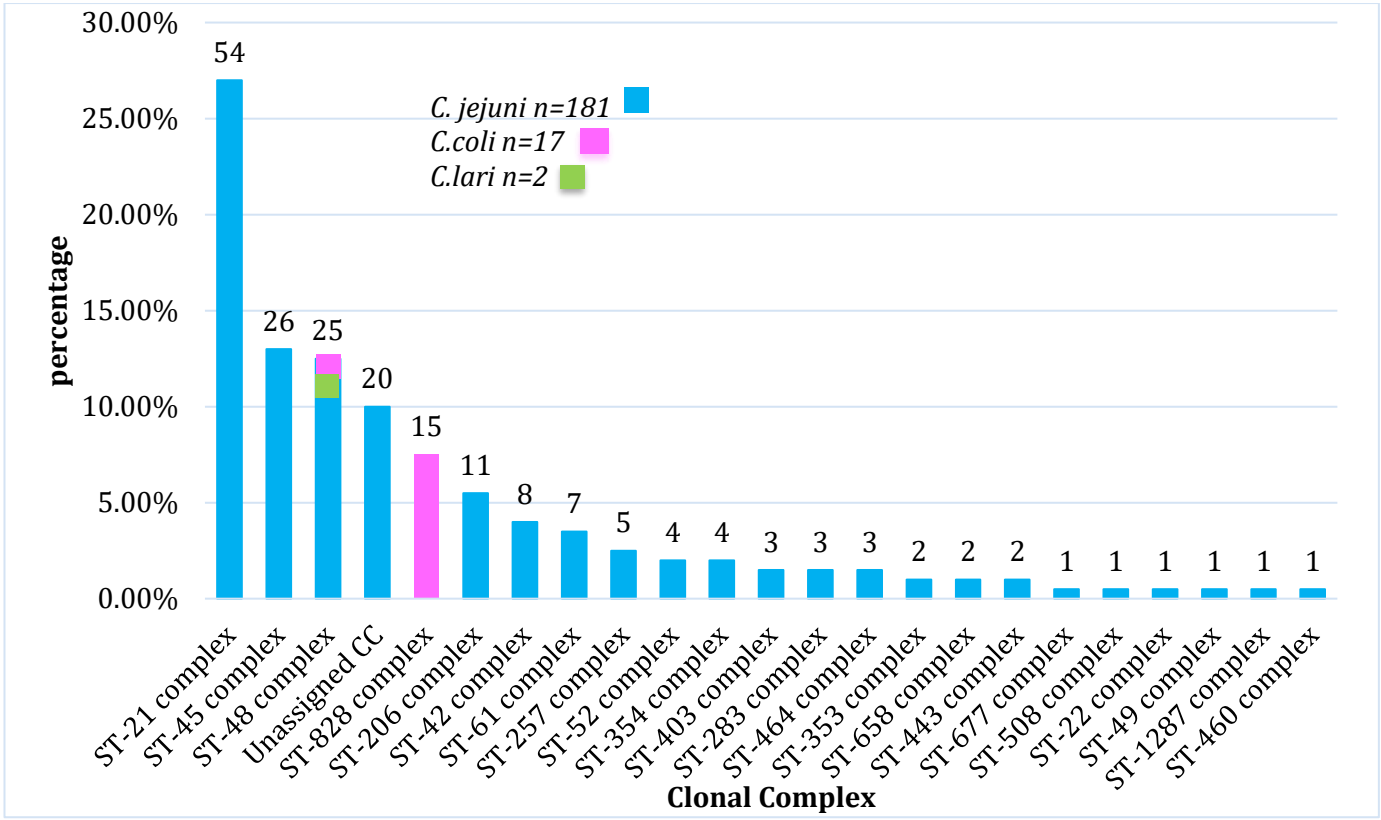


**Figure 1: 2021 *Campylobacter* spp. isolates (n=200) by clonal complex.**

## AST phenotype and genotype comparison

Of the 57 isolates with phenotypic ciprofloxacin resistance, 56 passed WGS validity criteria. Of these 56, 51 contained the *gyrA* mutation Thr86Ile/Val. Of the 147 isolates that were susceptible to ciprofloxacin, 144 passed WGS validity criteria. Of these 144, 143 did not have the *gyrA* mutation. Therefore, there was 91% and 99% sensitivity and specificity for WGS to predict ciprofloxacin sensitivity with a corresponding positive predictive value of 98% and negative predictive value of 97%. The sensitivity result is higher than that observed in 2020.

Of the 33 isolates with phenotypic tetracycline resistance, 33 passed WGS validity criteria. Of these 33, 26 harboured the gene *tetO*. Of the 171 isolates that were susceptible to tetracycline, 167 passed WGS validity criteria. Of these 168, 162 did not harbour *tetO*. Therefore, there was 79% sensitivity and 97% specificity for WGS to predict tetracycline sensitivity with a corresponding positive predictive value of 84% and negative predictive value of 96%. The sensitivity result is lower than that observed in 2020.

Two isolates were 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 result is not aligned with that observed in 2019 and 2020. 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 4: *Campylobacter* resistance associated genes and phenotype concordance amongst isolates, 2021. N=200**

| | phenotype: resistant | | phenotype: susceptible | | | | | |
|---|---|---|---|---|---|---|---|---|
| antibiotic class | genotype: resistant | genotype: susceptible | genotype: resistant | genotype: susceptible | Sensitivity (%) | Specificity (%) | Positive Predictive Value | Negative Predictive Value |
| tetracycline | 26 | 7 | 5 | 162 | 79% | 97% | 84% | 96% |
| erythromycin | 0 | 2 | 0 | 198 | 0.00% | 100% | n/a | 99% |
| ciprofloxacin | 51 | 5 | 1 | 143 | 91% | 99% | 98% | 97% |

## Virulence factors

The following is a breakdown of the virulence factors found in the *Campylobacter* isolates (n=200).The adherence and colonization associated factor genes *flaA, cadF* and *dnaJ* were found in 193/200 isolates, whereas *racR* was present in 187 isolates. *cdtA* was present in 189 isolates, *cdtB* was present in 193 isolates, while *cdtC* was present in 176 of the *C. jejuni* isolates but not present in any of the *C. coli* & *C. lari* isolates. The invasion associated *virB11* gene was found in 8 isolates (*C. jejuni*). The *iam* and *ciaB* genes were present in 193 and 166 isolates respectively (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 5: Virulence factors presence detected by WGS among *Campylobacter* isolates 2021 (N=200)**

| mechanism | gene | no. | % |
|---|---|---|---|
| Cytotoxin production | *cdtA* | 189 | 95 |
| | *cdtB* | 193 | 97 |
| | *cdtC* | 176 | 88 |
| Adherence and colonization | *flaA* | 193 | 97 |
| | *cadF* | 193 | 97 |
| | *dnaJ* | 193 | 97 |
| | *racR* | 187 | 94 |
| Invasion | *virB11* | 8 | 4 |
| | *iam* | 193 | 97 |
| | *ciaB* | 166 | 83 |

## 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 22 sets of 2021 isolates that were closely related genomically and warranted a public health alert to consider investigation for potential epidemiological links. This compared to 31 clusters in 2019 and 7 clusters in 2020 (which was truncated due to SARS-CoV-2 monitoring). These sets of clusters ranged from 2 isolates per cluster up to seven isolates per cluster in each public health alert. Three of these sets also clustered with isolates from Quarter 4 2020, which were included in the public health alerts.

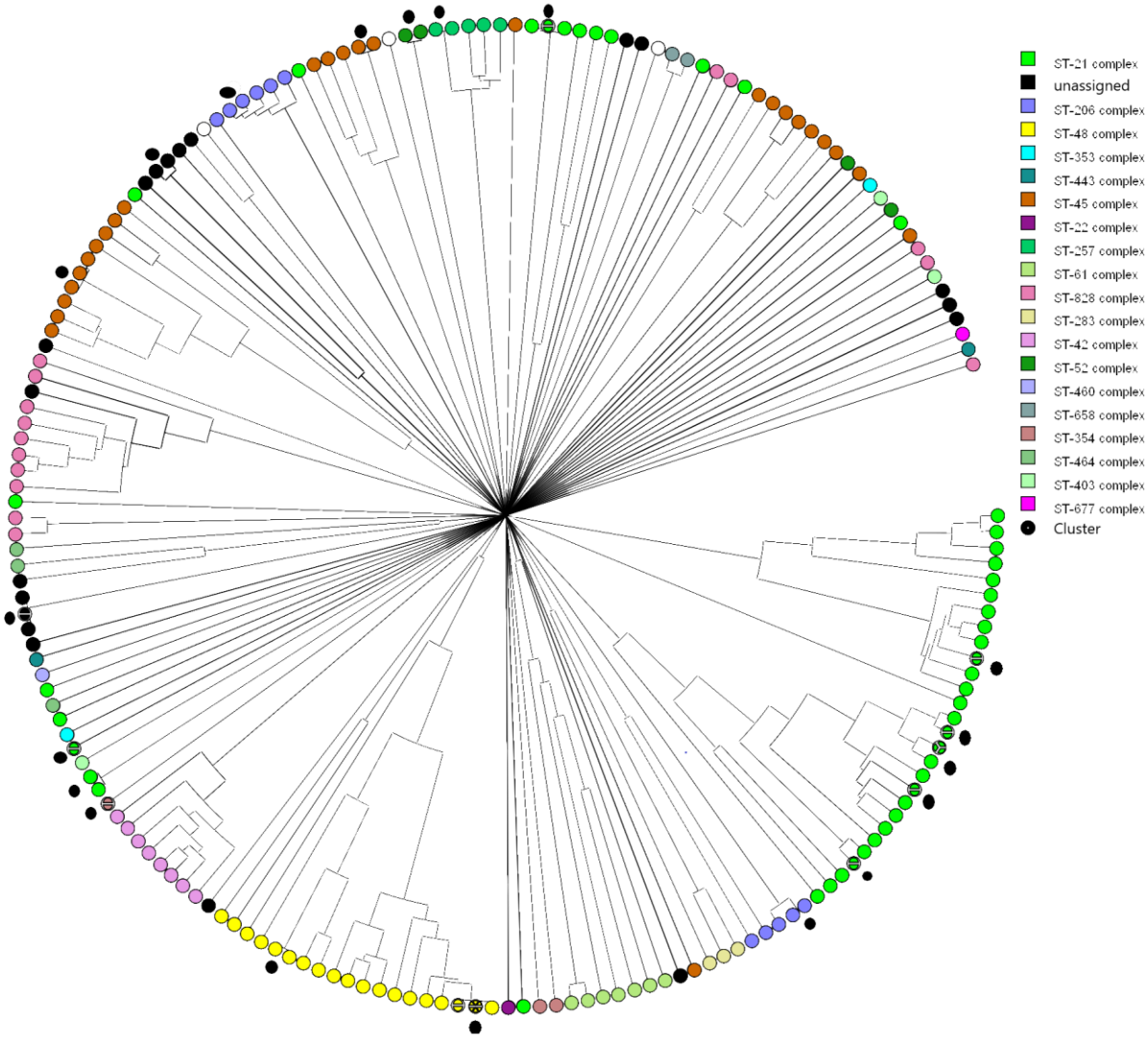


**Figure 2: UPGMA tree of cgMLST differences amongst *Campylobacter* spp. isolates (n=200) 2021. Each circle represents an isolate and they are coloured according to their clonal complex. Isolates with ≤5 cgMLST allele differences are indicated with a black circle.**

## Conclusion

This is the 2021 annual report 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 Annual 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 22 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, 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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## Appendix

### Audit Quarter 3 2021 of PCR positive *Campylobacter* stool specimens sent to the NRL

Over a number of years extensive work at the NRL determined the PCR Ct threshold for *Campylobacter* clinical significance & culture positivity. Although many manufacturer's instructions state that a Ct value of ≤36 is 'positive', continuous surveillance has shown that the NRL accredited *Campylobacter* PCR has an optimum culture positivity when the Ct value is ≤30 associated with clinical infection. Most referring laboratories use commercial PCR assays for *Campylobacter* detection, as with the NRL the thresholds for positivity will be specific to the assay utilised by the individual laboratory. The audit data presented below is an overview of referring laboratories Ct values recorded and the days taken for the positive sample to arrive at the NRL. Each laboratory should monitor their own Ct trends associated with clinical symptomatology and subsequent NRL culture positivity to allow further genomic analysis.

The low culture yield from referred PCR positive stool specimens at the NRL was remarkable in quarter 3 2021, therefore an audit was performed to review potential reasons.

In quarter 3 (n=83 referred stool specimens) it was noted:

- That the percentage of isolates cultured from senders PCR positive stools was low 30.1% (25/83) compared to previous quarters.
- That there was a higher yield of culture-positive isolates at the NRL with senders PCR positive stool specimens with low Ct values ≤30.
- On review of the specimen referral forms, 79 had senders Ct value results provided on the referral form, while n=4 of the specimens had no Ct specified.

Of the 25 culture-positive stools (by the NRL), 24 had Ct values provided on the referral form:

- The average Ct value was 28.46 (range 21-37.78).
- The average time between detection and receipt of the specimen at the NRL was 4 days (range 1-8 days).

Of the 58 culture negative specimens (by the NRL), 55 had a Ct values provided on the referral form:

- The average Ct value was 31.18 (range 23.7-40.97).
- The average time between detection and receipt of the specimen at the NRL was 5 days (range 1-8 days).

Of the 79 referred PCR-positive stools with a senders Ct value provided on the referral form:

- 40 had a Ct ≤30, of which 19 were culture positive at the NRL (47.5%).
- 58 had a Ct ≤32, of which 22 were culture positive at the NRL (37.9%).
- 39 had a Ct >30, of which 5 were culture positive at the NRL (12%).
- 21 had a Ct >32, of which 2 were culture positive at the NRL (9.5%)

Therefore we recommend not to batch specimens but to send them daily within your designated week as per schedule. The culture yield is highest on specimens referred with a Ct value of ≤30 to enable further genomic analysis.

**Table 6: Senders Ct values & days between detection & receipt at NRL for culture positive specimens**

| Culture positive stools n=24 | Senders Ct values | Length between detection & receipt in days |
|---|---|---|
| Average | 28.46 | 4 |
| Minimum | 21 | 1 |
| Maximum | 37.78 | 8 |

**Table 7: Senders Ct values & days between detection and receipt at NRL for culture negative specimens**

| Culture negative stools n=55 | Ct values | Length between detection & receipt in days |
|---|---|---|
| Average | 31.18 | 5 |
| Minimum | 23.7 | 1 |
| Maximum | 40.97 | 8 |