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

National human *Campylobacter* Sentinel Surveillance Reference laboratory service Quarter 1 Report 2019

Summary

- 24 participating laboratories
- 49 isolates:
 - 32/63 (51%) stools;
 - 17/19 (90%) isolate swabs
- 92% (n=45) *C. jejuni*; 8% (n=4) *C. coli*
- 22 STs and 11 clonal complexes
- ST-21 clonal complex most prevalent 29% (n=14)
- 49% (n=24) susceptible to all three antimicrobials tested
 - 38% (n=19) isolates were resistant to ciprofloxacin
 - 2% (n=1) were resistant to erythromycin
 - 31% (n=15) were resistant to tetracycline
- Some phenotypic-genotypic discordance for antibiotic sensitivity detected
- No clusters of public health significance detected
- Phenotypic speciation will be discontinued and replaced with quarterly genomic speciation

Introduction

This is the Quarter 1 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 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 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

A total of 14 (58%) laboratories confirm *Campylobacter* diagnosis by PCR-based methods alone using either BD-Max (n=3) or Enteric-Bio (n=11) systems. A total of 10 laboratories use culture alone. The Quarter 1 period was from February 1st to March 31st inclusive. For this period we received;

- a total of 82 specimens comprising 63 stool specimens and 19 isolate swabs
- A total of 49 bacterial isolates were recovered from submitted specimens; 32/63 (51%) from stool specimens and 17/19 (90%) 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 using the MAST ID *Campylobacter* kit.

A single *C. coli* isolate (2%) was detected using the MAST ID kit.

Antimicrobial Sensitivity Testing-phenotypic

Antimicrobial susceptibility testing (AST) by disk diffusion was performed according to EUCAST guidelines on all retrieved cultured isolates for sensitivity to the antimicrobials; ciprofloxacin, erythromycin and tetracycline.

- 49% of isolates (n=24) were susceptible to all three antimicrobials tested
- 38% (n=19) isolates were resistant to ciprofloxacin
- 2% (n=1) were resistant to erythromycin
- 31% (n=15) were resistant to tetracycline
- 22% (n=11) were resistant to two antimicrobials
 - One isolate was resistant to erythromycin and tetracycline and the remainder (n=10) were resistant to both ciprofloxacin and tetracycline

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

Whole Genome Sequence *Campylobacter* characterisation

All 49 *Campylobacter* isolates were stored and available for batch whole genome sequencing (WGS) at the end of Q1. 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.

C. jejuni accounted for 92% (n=45) of isolates with the remainder being *C. coli* (n=4). There was a diversity of sequence types (ST) with 22 STs found in total – 19 STs in *C. jejuni* and 3 in *C. coli* (Table 1). The most prevalent was ST-21 (12.5%). These STs resolved into 11 clonal complexes, with ST-21 clonal complex being the most prevalent at 29% (n=14) (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.

WGS identified 4 *C. coli* isolates with correlation of just one of these (25%) with the MAST ID biochemical *Campylobacter* speciation test. Thus the use of this biochemical test for speciation will be discontinued given this low correlation with the WGS data.

In future the phenotypic genus result without speciation will be reported contemporaneously and further genomic speciation will be reported quarterly.

Table 1: Breakdown of seven locus Sequence Type (STs) and clonal complexes found in the *Campylobacter* Sentinel collection Q1 2019 (n=49). N=number. * denotes *C. coli* clonal complex. All others were *C. jejuni*

ST	N	%	Clonal Complex
21	6	12.5	ST-21 complex
50	5	10.4	ST-21 complex
42	4	8.3	ST-42 complex
48	4	8.3	ST-48 complex
51	3	6.3	ST-443 complex
257	3	6.3	ST-257 complex
6209	3	6.3	ST-464 complex
61	2	4.2	ST-61 complex
206	2	4.2	ST-206 complex
354	2	4.2	ST-354 complex
827	2	4.2	ST-828 complex*
2079	2	4.2	ST-48 complex
122	2	4.2	ST-206 complex
45	1	2.1	ST-45 complex
432	1	2.1	ST-61 complex
475	1	2.1	ST-48 complex
806	1	2.1	ST-21 complex
825	1	2.1	ST-828 complex*
2191	1	2.1	ST-206 complex
6175	1	2.1	ST-21 complex
6543	1	2.1	ST-828 complex*
Unass	1	2.1	ST-21 complex

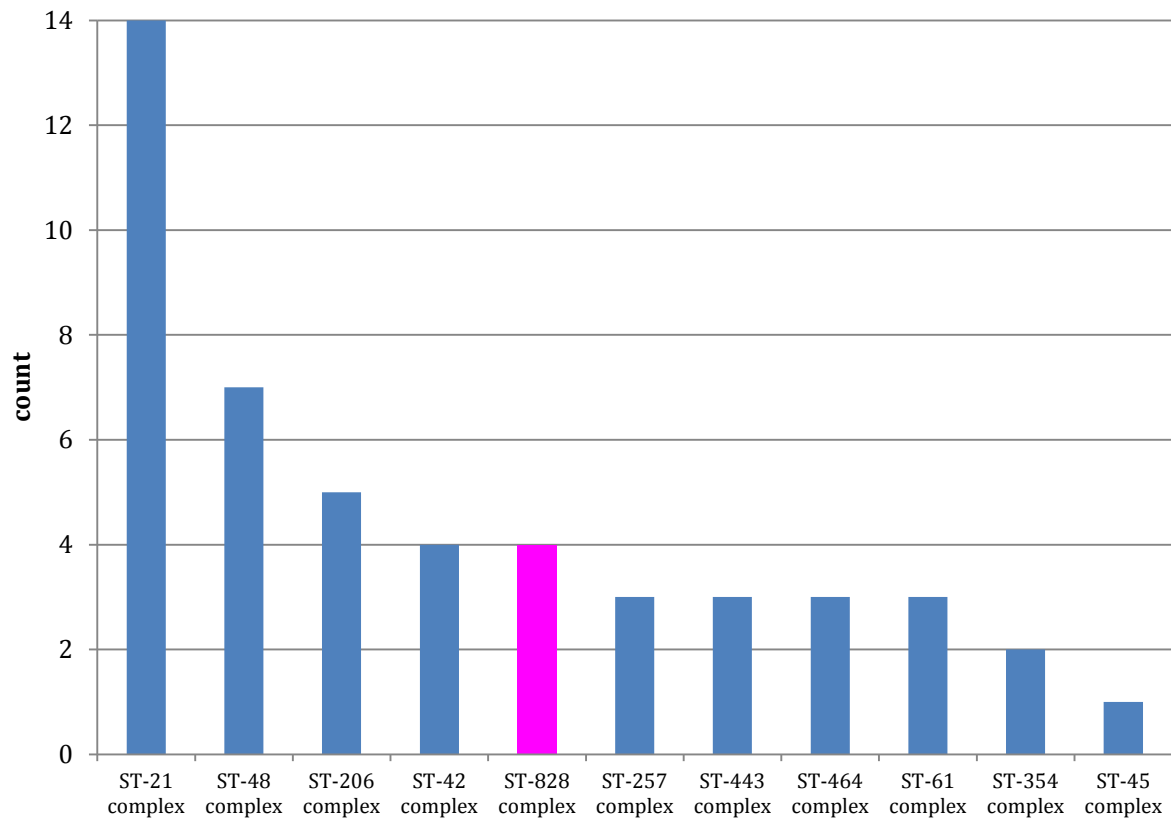


Figure 1: Q1, 2019 *Campylobacter* spp. isolates (n=49) by clonal complex. Blue indicates *C. jejuni* clonal complexes, pink indicates the *C. coli* ST-828 clonal complex.

AST phenotype and genotype comparison

A total of 39% (19/49) of isolates displayed phenotypic resistance to ciprofloxacin (Table 2). 63% (12/19) of these contained the amino acid change T86I in the *gyrA* gene known to be associated with quinolone resistance. 7/49 (14%) were phenotypically resistant to ciprofloxacin, but did not contain the *gyrA* mutation. This may indicate that other resistance mechanisms were responsible for resistance, for example, the multidrug resistance efflux pump genes *cmeA*, *cmeB*, *cmeC* were present in all 49 isolates. Another 7/49 (14%) had the *gyrA* mutation, but no phenotypic ciprofloxacin resistance evident. Thus the *gyrA* gene may have been 'switched' off.

Of the isolates with tetracycline phenotypic resistance 31% (n=15/49), all contained the *tetO* gene.

The single isolate phenotypically resistant to erythromycin did not contain mutations in the 23S rRNA gene or possess the *ermB* gene, both of which are associated with macrolide resistance. Thus erythromycin resistance may have been mediated by another mechanism *e.g.* efflux pumps as mentioned previously. These two genes mediating macrolide resistance were not detected in any of the *Campylobacter* spp. isolates.

Discrepancies between genotype and phenotype in terms of antimicrobial resistance invites caution in relying solely on genetic determinants for prediction of antibiotic sensitivity.

Table 2: *Campylobacter* resistance associated genes and phenotype correlation amongst isolates, Q1 2019. N=49

resistance	mechanism	phenotypic resistance n=49 (%)	genotype - phenotype correlation (%)	gene absent but resistant phenotype (%)	gene present but susceptible phenotype (%)
tetracycline	<i>tetO</i>	15 (31)	15 (100)	0 (0)	0 (0)
ciprofloxacin	<i>gyrA</i> Thr86Ile	19 (39)	12 (63)	7 (14)	7 (14)
erythromycin	<i>ermB/23S rRNA A2059G</i>	1	0 (0)	0 (0)	0 (0)

Virulence factors

There were a number of virulence factors found in all of the *Campylobacter* isolates (Table 3). These were the adherence and colonization associated factor genes *flaA*, *cadF*, *dnaJ* and *racR* and the invasion associated gene *iam*. The cytotoxin genes *cdtA*, *cdtB* and *cdtC* were found in all *C. jejuni* isolates but not in the four *C. coli* isolates. The invasion associated *virB11* gene was found in just two *C. jejuni* isolates, while another invasion associated gene *ciaB* was not found in any *Campylobacter* isolates.

Table 3: Virulence factors presence detected by WGS among *Campylobacter* isolates. Q1 2019 (N=49)

mechanism	gene	N	%
Cytotoxin production	<i>cdtA</i>	45	92
	<i>cdtB</i>	45	92
	<i>cdtC</i>	45	92
Adherence and colonization	<i>flaA</i>	49	100
	<i>cadF</i>	49	100
	<i>dnaJ</i>	49	100
	<i>racR</i>	49	100
Invasion	<i>virB11</i>	2	4
	<i>iam</i>	49	100
	<i>ciaB</i>	0	0

Cluster analysis

Isolate genomes were compared for relatedness by comparison at 3529 genes using whole genome MLST (wgMLST) Figure 2. The fewest differences were 43 alleles. A difference of 20 alleles or less is suggested as an alert threshold to consider cluster investigation for *Campylobacter* (Cody *et al*, 2013). Thus, using this criterion on this dataset, no clinical *Campylobacter* clusters of potential public health significance were detected in Ireland in Q1 2019. Genotypes were spread throughout the country with no apparent geographic clustering by HSE region (Figure 3).

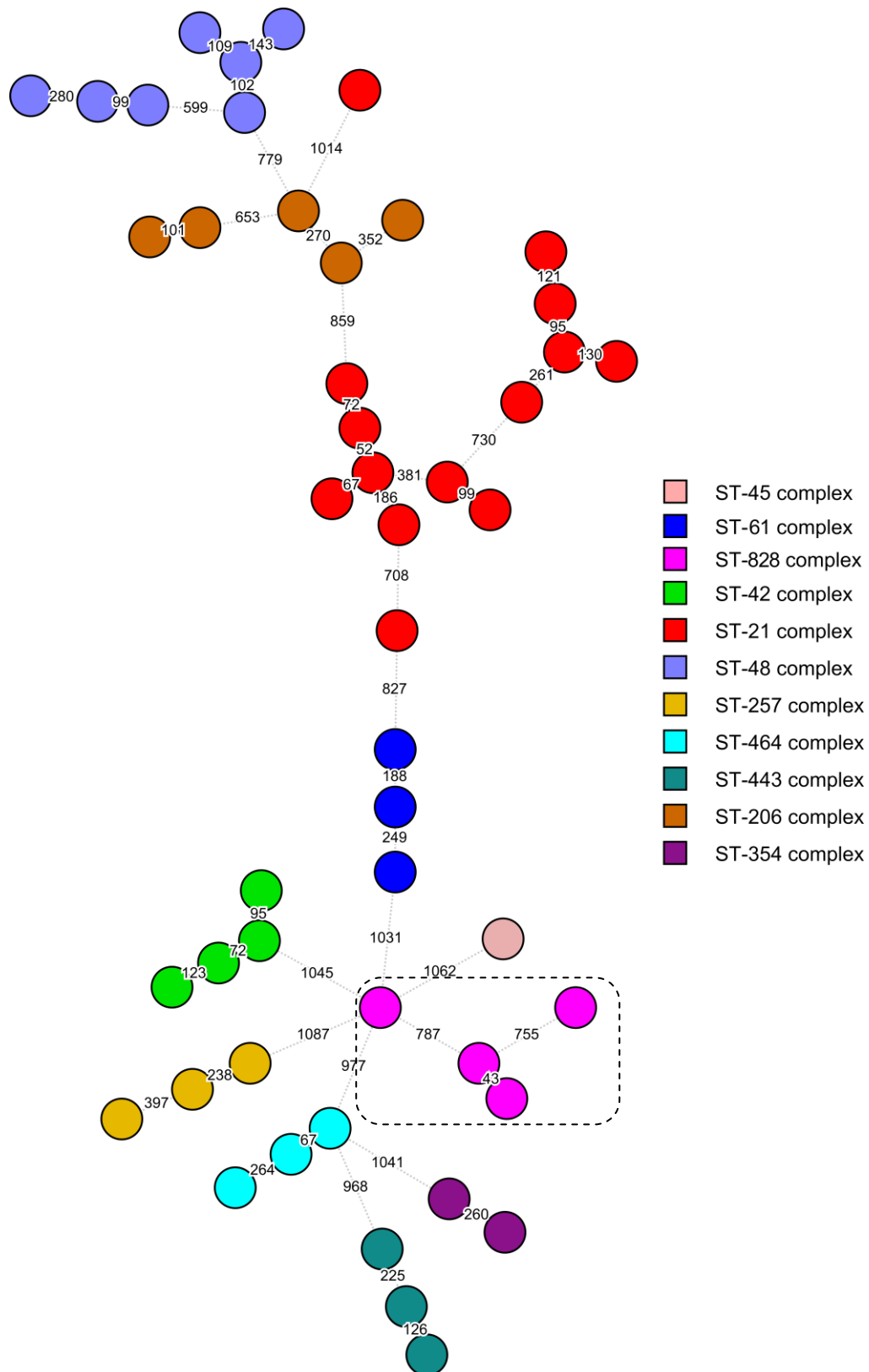


Figure 2: Minimum-spanning tree of wgMLST differences amongst *Campylobacter* spp. isolates (n=49) Q1 2019. Each circle represents an isolate and they are coloured according to their clonal complex. Lines between circles indicate number of allele differences between connected circles/isolates. *C. coli* isolates are ST828 complex (cerise pink and in the box).

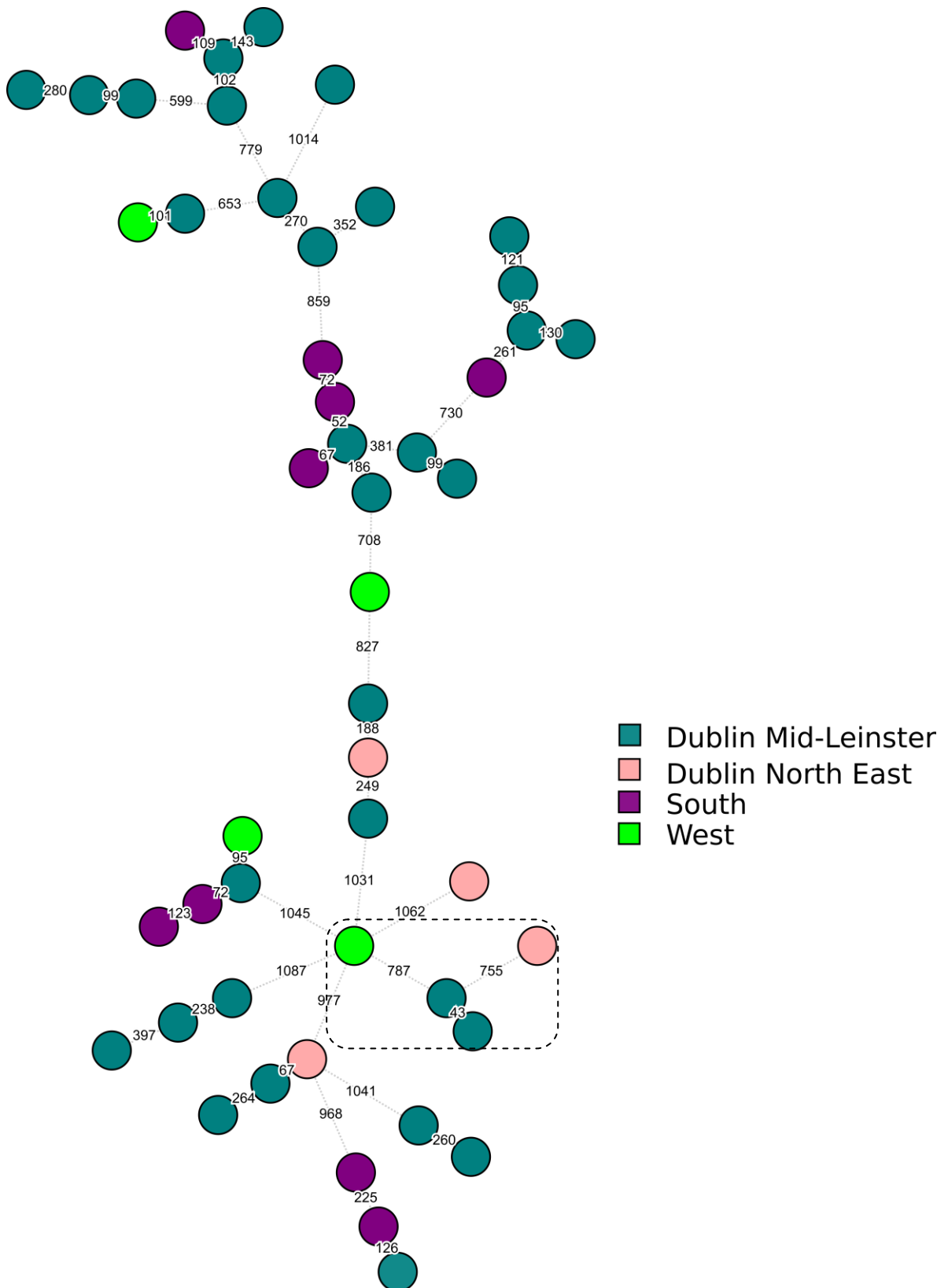


Figure 3: Minimum-spanning tree of wgMLST differences amongst *Campylobacter* spp. isolates (n=49) Q1 2019. Each circle represents an isolate and they are coloured according to their HSE region. Lines between circles indicate number of allele differences between connected circles/isolates. *C. coli* are marked in the box.