The genetic epidemiology of extended-spectrum beta-lactamase producing *Escherichia coli* within the Manawatū region

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Abstract

Hospital and community infections caused by antibiotic resistant bacteria are difficult to treat, can become severe, and increase mortality rates. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) are increasing in New Zealand and commonly associated with multi-drug resistance (MDR) in urinary tract infections (UTIs). However, in New Zealand, there is limited information about antibiotic resistance and ESBL-producing *E. coli* found in the environment.

This study aimed to characterise the antibiotic resistance phenotypes and genotypes of ESBL-producing *E. coli* within the Manawatū River and MidCentral District. Human clinical ESBL-producing Enterobacterales isolates were sourced from Medlab Central (August 2019 to March 2020 and June 2020 to January 2021), and environmental isolates were sourced from six locations along the Manawatū River over two sampling periods (August 2019 to March 2020 and July 2020 to January 2021).

There were a total of 311 clinical and 86 environmental ESBL-producing *E. coli* collected from Medlab Central and the along the Manawatū River respectively. The environmental samples from along the Manawatū River consisted of water, sediment, stormwater and treated effluent. Whole genome sequencing was conducted on 189 of the clinical isolates, and on 45 of the environmental isolates. Sequencing results revealed both the clinical and environmental isolates have a variety of ESBL coding genes and other antibiotic resistance genes. The ESBL gene $bla_{\text{CTX-M-27}}$ was most prevalent amongst clinical isolates and $bla_{\text{CTX-M-15}}$ for environmental isolates. Additionally treated effluent and the point at which it flows into the Manawatū River is a source of these antibiotic resistant *E. coli*, which also had a high incidence of multi-drug resistance (MDR). Sequence type (ST) 131 was the dominant sequence type recorded for both the clinical and environmental isolates. Whole genome sequence analysis of these isolates suggested that there was sharing of the same strain between humans and the Manawatū River.

The results from this study provide insights into ESBL-producing *E. coli* within the Manawatū River and MidCentral District. Knowing the genetic relatedness of ESBL producing *E. coli* and other associated antibiotic resistance will assist in understanding different transmission pathways relating to humans, animals, and the environment.

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Declaration

There were 16 isolates included in the whole genome sequencing analysis that had previously been sequenced by Dr Sara Burgess prior to the candidate starting. The remainder of the work in this thesis was conducted by the candidate with guidance from the supervisors.

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Abbreviations

AST	Antibiotic susceptibility testing
bp	Base pairs
BPAC	Best Practice Advocacy Centre
BPW	Buffered peptone water
CTX	Cefotaxime
FOX	Cefoxitin
CAZ	Ceftazidime
C	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
cfu	Colony-forming units
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
ECB	*E. coli* broth
CHRO	ESBL chromogenic agar
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended-spectrum beta-lactamase
FNU	Formazin Nephelometric Unit
GTR	General time-reversible
GM	Gentamicin
g	G-force
g	Grams
GC	Guanine-cytosine
HGT	Horizontal gene transfer
ESR	Institute of Environmental Science and Research
MCC	MacConkey agar
MCTX	MacConkey supplemented with (1 mg/ml) cefotaxime
MCAZ	MacConkey supplemented with (1 mg/ml) ceftazidime
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
µg	Microgram
µl	Microliter
ml	Millilitres
mg	Milligram

mm	Millimetres
MIC	Minimum inhibitory concentration
min	Minutes
MDR	Multi drug resistance
MLST	Multi-locus sequence typing
ng	Nanograms
nM	Nanomolar
NCBI	National Centre for Biotechnology Information
NGS	Next generation sequencing
NI	Nitrofurantoin
NeSI	New Zealand eScience Infrastructure
ONT	Oxford Nanopore Technologies
PE	Paired-end
PCR	Polymerase chain reaction
rpm	Revolutions per minute
RNA	Ribonucleic acid
ST	Sequence type
SNP	Single nucleotide polymorphism
SMRT	Single-molecule real-time sequencing
S	Streptomycin
T	Tetracycline
TS	Trimethoprim/sulfamethoxazole
UTI	Urinary tract infection
V	Volts
wgMLST	Whole genome multi-locus sequence typing
WGS	Whole genome sequencing

1 Introduction

Antibiotic resistance is a growing global problem because first choice antibiotics are no longer effective at treating common antimicrobial infections. This means that the infections are difficult to treat and, in some instances, become severe and life threatening. A key contributor to this rising problem is the extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*. These ESBL enzymes convey resistance to beta-lactam antibiotics, specifically toward third generation cephalosporins. The main variants of ESBL enzymes include CTX-M, TEM, and SHV although there are other types such as VEB and GES. Resistance to third generation cephalosporins is indicative of ESBL production (1). This type of antibiotic resistant bacteria is often associated with urinary tract infections (UTIs) and makes treatment of this common infection increasingly difficult. Multi-drug resistance (MDR) is another factor often associated with ESBL-producing *Enterobacteriaceae* complicating treatment even further. MDR is defined as resistance shown to an antibiotic in three or more classes (2). Antibiotic resistant and multi-drug resistant bacteria make common infections difficult to treat which can lead to infections worsening and even death.

Several pathways facilitate antibiotic resistance spread, including faecal-oral, food and water sources. Humans play a large part in the increase of antibiotic resistant bacteria, but it is also important to consider how animals and the natural environment contribute to and are affected by this resistance. Humans and animals can have antibiotic resistant bacteria, simply, from being treated with antibiotics, but can also be exposed to them through ingesting contaminated food and water (from the environment) (3). Factors within the environment can also assist with antibiotic resistance spread. These can include climate change, fertilizers, and heavy metals (4). Therefore, it is important to look at this transmission cycle and how each aspect is closely connected between humans, animals, and the environment. This is considered by taking a One Health approach.

This review focuses on ESBL-producing *Enterobacteriaceae* (particularly *E. coli*), UTIs and their treatment within humans, and the current research conducted in New Zealand. This review highlights the important information about understanding ESBL resistant bacteria and why a One Health perspective is needed when looking at antibiotic resistance.

1.1 *Enterobacteriaceae* and *E .coli*

The *Enterobacteriaceae* family are gram-negative bacteria that are found in mammalian intestinal tracts. They can be opportunistic pathogens and can cause a range of infections such as pneumonia, meningitis, diarrhoea, and UTIs (5, 6). Community-acquired infections are infections acquired outside of a hospital or detected within 48 hours of admittance to a hospital (7, 8). However, a UTI that is acquired through hospitalisation is considered a nosocomial infection (9, 10). *Enterobacteriaceae* commonly detected in UTIs are *E. coli* (11, 12).

E. coli can be classified into phylogroups. There are currently up to 14 phylogroups for *E. coli*, A, B1, B2-1, B2-2, C, D1, D2, D3, E1, E2, F, G, Shig 1, and Shig 2 (13). Additionally, phylogroup H has recently been discovered (14). Phylogroup assignment is determined traditionally determined by polymerase chain reaction (PCR). Clermont typing uses deoxyribonucleic acid (DNA) markers with specific allele amplification to determine the phylogroups (15). More recently multi-locus sequence typing (MLST) and whole genome sequencing (WGS) allow for phylogroup assignment (15). This is particularly relevant concerning *E. coli*, as certain sequence types (ST) and phylogroups are associated with different *E. coli* strains, hosts or pathogenicity (6). Commensal *E. coli* are associated with the phylogroup A and B2 for humans, whereas *E. coli* from animal sources is associated with group A and B1 (13, 16, 17). More specifically for animals, phylogroups A and B1 have been linked to vertebrates in general, whereas phylogroups B2 and D have been associated with endothermic vertebrates (18). However, poultry and meat products are associated with group G (13). Extraintestinal pathogenic *E. coli* which are disease causing in humans are associated with groups G, B2, and D, whereas *E. coli* serogroup O157 is associated with phylogroup E2 (13). Uropathogenic *E. coli* are specifically associated with B2 (19). The *E. coli* sequence type (ST) 131, which is extraintestinal pathogenic and belongs to phylogroup B2-1 and is commonly associated with ESBL production (13, 20).

1.2 Beta-lactamase resistance mechanisms

1.2.1 Resistance and naming systems

Antibiotics are split into antibiotic classes based on chemical structure and mechanism of action. Classes of antibiotics include antibiotics that target the bacterial cell wall (for example, beta-lactams), inhibit protein synthesis (for example, aminoglycosides, macrolides and tetracyclines), inhibit DNA replication (quinolones) and inhibit folic acid metabolism (sulfonamides and trimethoprim) (21). As the name ESBL-producing *Enterobacteriaceae* implies, this group of bacteria is resistant to antibiotics that contain beta-lactam rings. Beta-lactam antibiotics are a class of antibiotics that contain antibiotics with a beta-lactam ring in the chemical structure. Enzymes encoded by sets of ESBL genes are responsible for hydrolysing the beta-lactam ring and rendering the antibiotic ineffective (22). Antibiotics that have a beta-lactam ring in the chemical structure can be further divided into antibiotic groups such as penicillins, cephalosporins, and monobactams. Therefore, ESBLs can be defined as beta-lactamases that allow resistance to penicillins, first and third generation cephalosporins as well as monobactams, but are not resistant to cephamycins (1, 23). However, there are beta-lactamase inhibitors, such as the serine type beta-lactamase inhibitors called sulbactam, clavulanate, and tazobactam (24) that can be combined with antibiotics (penicillins) to treat ESBL infections.

Two main classification systems are used to classify beta-lactamases, these are the Ambler and Bush-Jacoby-Medeiros classification systems. The Ambler system uses active sites to classify beta-lactamases into one of four classes, A, B, C, or D. The active site for classes A, C, and D contains serine, whereas for class B it is metallo-beta-lactamase (25). This is in comparison to

the functional classification system structure used in the Bush-Jacoby-Medeiros system. This classification system uses similarities of substrate hydrolysis and effects of the inhibitors on the beta-lactamases. There are three main groups, one to three accompanied by subgroups A to D (26, 27).

1.2.2 Beta-lactamases

Many different beta-lactamase enzymes have been discovered over time. The first group of beta-lactam enzymes described were the penicillinases, but ESBL includes a broader resistance spectrum including first and third generation cephalosporins, and monobactams (Table 1.1). The most commonly found ESBL genes are *bla*$_{SHV}$, *bla*$_{TEM}$ (not the parent type), and *bla*$_{CTX-M}$ (Table 1.1) (12, 28). These genes are usually plasmid encoded making it very easy to transfer resistance from one bacterial cell to another (28). The *bla*$_{TEM}$ and *bla*$_{SHV}$ genes were detected before the *bla*$_{CTX-M}$ gene. The SHV enzyme is thought to have originated from a chromosomally encoded gene in *Klebsiella* species (29). Origins of the TEM enzymes are not fully clear, but early documentation of them have suggested that they were plasmid encoded (30, 31). It is thought that the *bla*$_{CTX-M}$ gene was originally chromosomally encoded in *Kluyvera* sp. but then became plasmid borne due to horizontal gene transfer (HGT) (32, 33).

The enzymes are further split into variants, these can indicate the spectrum of resistance that is conveyed. Parent enzyme types: TEM-1, TEM-2, SHV-1, and SHV-11 convey resistance to narrow/standard spectrum beta-lactam antibiotics, such as the first and second generation cephalosporins (Table 1.1). There are many variants of the SHV and TEM types that occur due to point mutations in the parent enzyme active site and allow extended-spectrum beta-lactamase resistance to oxyimino-cephalosporins, which are third generation cephalosporins (30). CTX-M enzymes are ESBLs and have a preference for hydrolysing cefotaxime and ceftazidime, which are third generation cephalosporins and as of 2021 there were over 240 CTX-M variants (34).

1.2.3 AmpC

AmpC enzymes are another type of enzyme allowing resistance to beta-lactam antibiotics. These enzymes confer resistance to third generation cephalosporins (via hydrolysis of the beta-lactam ring) and the previously mentioned inhibitors, but not fourth generation cephalosporins (24, 35). *Enterobacteriaceae*, such as *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp, chromosomal *ampC* is inducible or de-repressed with exposure to inducing substrates, however, this is not the same for *E. coli* (36). For *E. coli* chromosomal mutations in the promotor or attenuator regions of the *ampC* gene, result in hyper-production of AmpC and therefore resistance (37). *E. coli* can also have plasmid mediated *ampC* genes such as *bla*$_{CMY}$ and *bla*$_{DHA}$ due to acquisition of a plasmid with the gene encoded on it. Plasmid-mediated *ampC* is thought to have been derived from the chromosomal gene from other *Enterobacteriaceae* species, the gene can be expressed constitutively or is inducible when exposed to beta-lactam antibiotics (36).

1.2.4 Carbapenemases

Many different beta-lactamases such as the OXA, KPC and NDM, also convey resistance to carbapenem antibiotics, which are considered a last resort treatment (but are increasingly commonly used) for antibiotic resistant bacteria (38). OXA variant types differ in the level of resistance against third generation cephalosporins and carbapenems, some of which are also not inhibited by clavulanic acid (30). *Klebsiella pneumoniae* (*K. pneumoniae*) carbapenemases (KPC) and New Delhi metallo-beta-lactamase (NDM) are further examples of enzymes that provide resistance against carbapenem antibiotics, as summarised and expanded on in Table 1 (39, 40). Unfortunately, both ESBL and carbapenemase resistance are usually contributing factors toward MDR seen in bacteria (41). The way that beta-lactamase enzymes have changed and evolved to the use of new antibiotics, could demonstrate future trends, as seen with emerging carbapenem resistance.

Table 1.1: Summary of enzyme types and their respective antibiotic resistance mechanism.

Enzyme	Ambler class	Bush-Jacoby–Medeiros classification	Type of Enzymes	Spectrum of antibiotic resistance
Narrow spectrum beta-lactamase	A (serine)	2b	Parent types: TEM-1, 2 and 13, SHV-1, and 11	Penicillins, first and second generation cephalosporins
ESBL	A (serine)	2be, 2br, 2f	TEM excluding parent types e.g., TEM-3 SHV excluding parent types e.g., SHV-2 CTX-M	Penicillins, third generation cephalosporins and monobactams
AmpC	C (serine)	1	AmpC CMY-2	Penicillins, third generation cephalosporins, cephamycins, and monobactams
Carbapenemases	A (serine)	2f	KPC, GES, SME1	Penicillins, cephalosporins, and carbapenems
	B (metallo-beta-lactamases (Zn^{2+}))	3	NDM, VIM, IMP, IND	Penicillins, cephalosporins, cephamycins, and carbapenems
Oxacillinases	D (serine)	2d	e.g. OXA-1	Penicillins
			e.g. OXA-14	Penicillins and cephalosporins
			e.g. OXA-24/40, OXA-23, OXA-48	Penicillins, cephalosporins, carbapenems

Adapted from (23, 42, 43). Additional references used (44, 45)

1.3 ESBL associated infections

1.3.1 UTIs

Gram-negative bacteria are the leading cause of UTIs. A UTI involves bacterial invasion of the lower or both lower and upper urinary tracts (7, 46). Cystitis is a term used to describe an infection in the lower urinary tract (46). For the more severe progression of the infection, it can spread to the kidneys, which is then an upper UTI and the patient is then regarded as having pyelonephritis (7). The invading bacteria usually originate from the gut but can also be acquired in hospital settings (8). A UTI that is acquired through hospitalisation is usually due to a catheter and, therefore, classified as a nosocomial infection (9, 10). A nosocomial infection is also defined as being an infection occurring 48 hours after patient admission and the infection not being present at the time of admission (8). This is compared to a UTI that has been acquired in the community (7).

The occurrence of UTIs differs between men and women and across age groups (47). There is a higher prevalence of UTIs in women with additional risk factors such as pregnancy, sexual activity, and age contributing to the frequency of infection, with older females (over 65) having twice the rate of UTI incidence than the remainder of the female population (48, 49). However, this is expected, given UTIs are more common in the older population regardless of gender (47).

The bacteria that cause UTIs include, *E. coli*, *K. pneumoniae*, and *Proteus mirabilis* (50). However, differences can be observed between species prevalence isolated from hospital versus community UTIs. *E. coli* remains the most common in both settings but is slightly less frequently observed in hospital samples (51). *Klebsiella* species, *Enterococcus faecalis, Proteus mirabilis, and Pseudomonas aeruginosa* have also been isolated from both community and hospital samples, and although rare *Pseudomonas aeruginosa* and *Enterococcus faecalis* have been detected in hospital settings (51).

1.3.2 Treatment of ESBL infection

Treatment of UTIs depends on the state of the infection, meaning whether it is an uncomplicated or complicated infection. In New Zealand, the Best Practice Advocacy Centre (BPAC) has an antibiotic choice for common infections guide that suggests the antibiotics to be used for UTI treatment (52). First line antibiotics recommended for the treatment of cystitis and pyelonephritis are nitrofurantoin and trimethoprim/sulfamethoxazole. The antibiotic cefalexin is reserved for use in treatment only if the organisms are known to be resistant to first line options and are susceptible to this option (52).

Treatment options for UTIs associated with ESBL-producing bacteria need to be carefully considered (Table 1.2). Current options have been reviewed and split into three main categories based on treatment options (53). These three groups are, severe infections/high-risk, non-severe intermediate risk source (e.g., catheter), and non-severe low-risk UTI. However, a low-risk infection that presents as uncomplicated has the potential to progress and become more severe

(54). It was concluded that carbapenems were the recommended option for severe high-risk infections, but not necessarily all ESBL associated infections, if there was another option (53). This is because carbapenems are still largely considered the best treatment to combat ESBL-producing bacteria and are shown to be more effective than antibiotics with third generation cephalosporin inhibitor combinations (55, 56). However, due to the emerging resistance to carbapenems alternative options are being considered including cefepime, which is a fourth-generation cephalosporin. As an alternative to carbapenem use, authors have cautioned that cefepime would only be appropriate for non-severe low-risk infections, such as UTIs, in addition to the correct dosage used and awareness that there is susceptibility to this antibiotic (56). Other studies recommend the oral treatment options of pivmecillinam (a penicillin), fosfomycin, and nitrofurantoin, as high rates of sensitivity have been shown towards these antibiotics (57).

The treatment of hospital versus community acquired UTI needs to be considered carefully. This is due to hospital acquired infections being associated with higher rates of resistance to many antibiotics. However, there was little difference in resistance rates against nitrofurantoin in hospital and community acquired infections (51). This results in nitrofurantoin being recommended as the treatment for uncomplicated cystitis for both hospital and community acquired infections (51). Treatment of UTIs and ESBL associated UTIs seem to be case dependent with several different factors dictating the treatment prescribed, such as age and severity of infection. Bacteria can also have antibiotic resistance to other classes of second option antibiotics, limiting treatment options meaning that antibiotics with more severe side effects are used.

Table 1.2: Treatment options for UTIs.

Severity of infection	Risk	Treatment	Conditions
Cystitis (lower UTI) – non severe in adults	Low-risk	First choice: nitrofurantoin Alternative: trimethoprim Alternative: cefalexin Alternatives: pivmecillinam, fosfomycin	Prescribe treatment of a longer course for pregnant women and males. Prescribe treatment of a longer course for pregnant women and males. Prescribe when organisms are known to be resistant to first line options and are susceptible.
Cystitis (lower UTI) – non severe in children	Low-risk	First choice: trimethoprim/sulfamethoxazole Alternative: amoxicillin clavulanate Alternative: cefalexin	Based on the weight of the child. Based on the weight of the child and for children older than one month.
Pyelonephritis (upper UTI) – mild pyelonephritis	Low to medium risk	First choice: trimethoprim/sulfamethoxazole Alternatives: amoxicillin clavulanate or Cefalexin	Longer course than if treating cystitis
Severe infections (for example, urosepsis)	High-risk	Carbapenems: e.g imipenem or meropenem	

References: (52-54, 56)

1.4 Detection of ESBLs

1.4.1 Phenotypic detection methods

Phenotypic detection of potential ESBLs can occur by a few different processes. To first establish if the sample or isolate could be ESBL-producing, screening takes place to determine if there is resistance to extended-spectrum antibiotics. This is followed by a confirmation test to confirm ESBL production. This is common practice in a hospital setting, samples are screened first and labelled presumptive ESBL producing bacteria, before being confirmed ESBL producing. Pre-screening the sample on selective media, such as, ESBL chromogenic agar is favoured (58). This is because there are specific substrates in the media that allow the identification of ESBL-producing *Enterobacteriaceae* based on colony colour, however, these substrates are not disclosed. Other pre-screening methods can include media with an added antibiotic. Cefotaxime is a regular choice to be added to MacConkey agar as a selective agent (59). Cefotaxime is chosen because it is a third generation cephalosporin, and is preferred over ceftazidime because the CTX-M enzyme has greater hydrolysing effects against it (25). On occasion, *E. coli* chromogenic agar has had antibiotics added to it, as a double-layered screening method (58). Utilising this method means that the *E. coli* selected from the agar plate are known to be resistant to cefotaxime, increasing the chance of the bacteria being ESBL positive. Taxonomic identification of selected isolates can then occur using matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF MS). Additional phenotypic species characterisation includes gram staining, oxidate and fermentation tests (60). VITEK-2 is also used for taxonomic identification (12, 22, 61). The VITEK-2 system allows for bacterial identification and rapid antibiotic susceptibility testing. Organism identification uses a fluorometric technique where biochemical reactions occur on an identification card. This card is inoculated with an unknown organism, incubated and then the optical signal is generated, which is compared to a reference database to identify the species (62). Antibiotic susceptibility is reported using an antibiotic susceptibility test card by measuring turbidity, to determine the minimum inhibitory concentration (MIC) (63). Once the MIC is known it is compared to a bacterium with a reference MIC to the tested antibiotics.

Disc-diffusion tests

Identification can be followed by the Kirby-Bauer disc diffusion tests. These tests use antibiotic discs to determine whether a bacterium shows resistance to a certain antibiotic or not. This can be used as another initial screening for ESBL production (12). Results from the disc diffusion tests can be interpreted following guidelines from the Clinical and Laboratory Standards Institute (CLSI) or the open access option from the European Committee on Antibiotic Susceptibility Testing (EUCAST) (64, 65). The ESBL screening test is followed by a confirmatory ESBL test also outlined by CLSI or EUCAST (64, 65) and is referred to as the combined disc diffusion test or confirmatory ESBL test. Both confirmatory methods use third generation cephalosporins, as these indicate the presence of ESBL genes. There is also the option for an additional test when other

beta-lactamases mask the presence of ESBLs. This can occur when there is high expression of AmpC, making the inhibitors in the antibiotics ineffective. The extra test, outlined by EUCAST, uses cefepime as it is not usually hydrolysed by the AmpC enzymes (65).

Using only phenotypic detection methods will not allow for differentiation between specific genes and the resulting enzyme production, such as parent versus the variant of the TEM and SHV enzymes (1). Therefore, it is equally important that genotypic identification methods are used.

1.4.2 Genotypic detection methods

Polymerase chain reaction (PCR)

Genotypic detection methods can provide greater detail than phenotypic results. A common method used is PCR to determine the presence of ESBL-producing genes. Primers used are sequence specific for ESBL resistance genes, such as *bla*$_{SHV}$, *bla*$_{TEM,}$ and *bla*$_{CTX-M}$ (12, 22). However, as previously mentioned some TEM and SHV variants are narrow-spectrum beta-lactamase enzymes (parent types) and not considered ESBLs, sequencing is needed to determine the specific variant. The *bla*$_{CTX-M}$ primer sets can further be broken down into more groupings allowing more specific identification of the gene present (66). This is based on amino acid differences of the sublineage groups CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and KLUC (66, 67). These CTX-M groups can be run as singleplex or combined for a multiplex PCR run. Gel electrophoresis is used to differentiate the amplified PCR products and in some cases, additional sequencing of the products occurs to determine the sublineage group of a specific beta-lactamase gene (68). The *ampC* gene location (chromosomal versus plasmid) can also be tested by PCR. Primers matching the attenuator or promoter region are used for chromosomal detection (66). A multiplex PCR can be used to distinguish plasmid mediated *ampC* genes based on six groups (69). Pérez-Pérez and Hanson (69) defines these six groups as ACC, CIT, DHA, ECB, FOX, and MOX.

Whole genome sequencing

WGS using second and sometimes third generation sequencing is used for determining the resistance type, virulence factors, plasmid types, and epidemiological relationships between bacterial strains. Second generation sequencing technologies use sequencers that have become more automated and have high throughput capabilities. Illumina sequencing is frequently used which involves sequencing reactions with complementary adapter sequences, which are attached to each end of a fragment of DNA. The adapter is complementary to a sequence on the flow cell, tethering the DNA. Bridge amplification then occurs where the adapter on the “free” end of the DNA fragment ligates to the matching sequence on the flow cell. Many rounds of amplification occur, creating clusters of the fragments, allowing forward and reverse read pairs to be made (70).

The method mentioned above is considered short read sequencing as the read fragments produced are 100-300 base pairs (bp). Short-read sequencing has a relatively low error rate, particularly Illumina with 0.1% in paired end reads (71). This low error rates allows for accurate genome assemblies. However, assemblies are usually fragmented due to the short read length.

Many bioinformatic tools can be used for short read sequencing analysis which include, MLST, antimicrobial resistance gene identifiers as well as a virulome analysis. MLST uses variation in defined housekeeping genes to define different sequence types. To determine whether the bacteria are genetically related, sequences can be compared with tools such as Snippy, which looks at single nucleotide polymorphisms (SNPs) (72). Tools such as Plasmidfinder and Resfinder can be utilised to provide more information about the presence of plasmids and resistance genes, based on the assembled draft genome (73, 74). The tools stated above are only a few of what is incorporated into the Nullarbor2 pipeline. The Nullarbor2 pipeline incorporates trimming of adapters, contig assembly, and annotation of the draft genome, which allows a large amount of sequencing information to be made available with one command. WGS is needed for understanding the genetic relatedness of these antibiotic resistant bacteria through the analysis of the core genome, SNPs, resistant genes, and MLST which all tools contribute evidence to indicate how antibiotic resistance spreads.

Long read sequencing

Detection of resistance genes via short read sequencing does not always lead to the location (chromosomal or plasmid) of the resistance gene to be determined, this is due to multiple contigs (75). Long read technologies, such as PacBio and Oxford Nanopore Technologies (ONT), enable complete sequencing of bacterial genomes.

PacBio utilises single molecule real time (SMRT) sequencing, where each molecule is processed one at a time. The DNA polymerase is bound to the well with the DNA molecule. Each of the four phospholinked nucleotides are in a single solution within the well, with each nucleotide having a different fluorescent label (76). As the DNA polymerase incorporates bases into the chain the fluorophore emits light and the reaction at that point is imaged, allowing base identification and therefore the DNA sequence is revealed.

ONT uses a flow cell with active protein pores in a membrane. Adapters and motor proteins are attached to the double-stranded DNA during library preparation (77). Within the flow cell device, an electrically resistant polymer membrane holds the pore, which is all supported by a microscaffold. The set voltage is passed across the membrane, sending an ionic current through the pore. As the DNA sequence passes through the pore, with the assistance of the motor proteins, a change of current is observed, indicating the base sequence (78).

The benefit of long read sequencing is that plasmids that carry antibiotic resistance genes and areas of repeats (e.g., insertion sequences or transposable elements) can be sequenced to completion, however, long read sequencing is more expensive. Achieving this information and

when used in combination with short read sequencing, gives clarity to the overall sequence of the chromosome and any plasmids that may be present (79, 80).

1.5 Transmission and spread of ESBLs within a One Health paradigm

The spread of ESBLs is important to understand as it will help establish mitigation and control measures. Selection pressure drives mutation events and the further mobilisation of resistance genes allowing the spread of antibiotic resistance (81). The two main pathways for the spread of resistance are HGT and vertical transmission. HGT of ESBL genes, located on plasmids, can happen three different ways: conjugation, transformation, and transduction (82). Vertical transmission is the direct descent of genes to daughter cells. Resistance spread is facilitated by poor hygiene, improper sewage disposal, heavy metals, sanitiser use, and the misuse and overuse of antibiotics. These factors are mainly caused by humans but contribute to downstream effects for other humans, animals, and the environment. Therefore, it is important to look at the transmission and spread from a One Health perspective.

1.5.1 The role of the natural environment

The presence of antibiotic resistance in the environment allows for further spread to humans and animals. Antibiotic resistance had been found in environments without anthropogenic inputs. These environments are pristine with minimal human impact and include locations such as permafrost, caves, and soil (83-85). However, humans are one of the main contributors of antibiotic resistance and ESBLs found in the environment. This is through household and hospital waste disposal. The most reported being through wastewater. Hospitals and health care facilities are generally seen as a hotspot for ESBL presence, especially in wastewater (58, 86, 87). *Enterobacteriaceae* frequently detected in wastewater are *E. coli* and *K. pneumoniae*, both of which are commonly associated with UTIs (60, 88). Concerningly, studies suggest that antibiotic resistant bacteria are not completely removed during wastewater treatment (89). The wastewater discharge into bodies of water is seen as a route for antibiotic resistance spread to the environment (90). This could allow for possible antibiotic resistance exchanges between clinical and environmental strains.

Humans can encounter antibiotic resistant bacteria in the wider environment through several pathways. ESBL-producing *E. coli* has been detected in recreational water samples, including freshwater locations (91). Analysis of coastal water has been used for estimating the frequency and occurrence of antibiotic resistant *E. coli* and the potential for humans to encounter them (3). It was determined that the type of water sport plays a role in the risk of exposure and ingestion of water (3).

Once antibiotic resistance bacteria are in the environment there are ways in which it is becoming easier for them to survive. There has been evidence to suggest through multivariate-adjusted

models that increasing temperatures can lead to an increase in antibiotic resistance (92). Another study modelled that an increase in temperature (until reaching 30°C) could also increase the odds that a UTI suffering patient is hospitalised (93). It is suggested by the authors that changes in human behavior when temperatures reach above 30°C is what reduces the odds of UTI hospitalisations.

Additional impacts on the environment such as pollution are influencing the surrounding bacteria. Higher levels of pollutants such as zinc, lead, and ammonia (NH_3-N) has been linked to a higher abundance of efflux pumps in bacteria (94). This means that antibiotics will be more efficiently pumped out of the bacteria having less of an effect. Bacteria having exposure to heavy metals such as zinc, copper, cadmium, and mercury could lead to co-selection of antibiotic resistance genes (95, 96). Soil and water samples have been analysed for this and it was found that higher resistance to trace elements and antibiotics were linked to sample sites where trace element levels were higher (95). This demonstrates the possibility of co-selection as the resistance mechanisms are usually found clustered on the same plasmid so will be acquired at the same time.

1.5.2 Humans

Human to human transfer is one of the main ways the ESBL-producing bacteria can spread through the community (97). This is supported by a study from the Netherlands which created models demonstrating that human to human transmission within and across households accounted for most of the community acquired ESBLs, compared to food, companion animals and environmental contact (98). Studies have also suggested that patients having recently had an ESBL-producing *Enterobacteriaceae* associated infection can be a source of household transmission (99).

Ingestion of antibiotic resistant bacteria through food is also a risk factor. A study comparing ESBL-producing *E. coli* from poultry and chicken meat to human clinical samples in the Netherlands (100) found that 35% of the human samples contained ESBL genes that were genetically similar to those found in the poultry isolates, therefore, the study suggested poultry to human transmission via the food chain (100). A systematic review that included the aforementioned study as well as 33 other peer-reviewed publications concluded that poultry is a suspect source for extraintestinal infections in humans associated with expanded-spectrum cephalosporin resistant *E. coli* (101). However, this review also states few studies show a link between food-prefacing animals and infections in humans, with those reviews acknowledging the limitations potentially affect the conclusions (101). Studies have also isolated ESBL-producing *E. coli* from vegetables (salad vegetables), raw milk, egg and meat (102, 103). Overall, it is hard to determine the exact transmission pathway between food and humans. This is further supported by another study that compares ESBL-producing *E. coli* from livestock farms and retail meat to *E. coli* from bloodstream infections in humans in the United Kingdom (104). There were few similarities in the antibiotic resistant profiles between the livestock and meat isolates compared

to humans. However, there was evidence of genetically related isolates between the same animal species across different farms (104). It concluded that there was little human infection originating from livestock (104).

ESBL-producing *Enterobacteriaceae* have been detected in salad vegetables and wastewater used for irrigation in Burkina Faso, West Africa (60). Higher numbers of ESBL-producing *Enterobacteriaceae* were detected in the wastewater, however, there was still ESBL-producing *Enterobacteriaceae* found in the produce sold at the nearby markets (60). This is not the single occasion that ESBL producers have been found associated with vegetables. From the Enterobacterales isolated, low numbers (5/856, 0.58%) have been found from vegetables sourced from supermarkets and farmers' markets in Romania (105).

1.5.3 Animals

Since animals have been domesticated there is potential for disease or infection to pass between animals and humans. It is this close contact with companion and domesticated animals that pose the greatest risk of transmission between the human and animal. Studies looking at household animals such as pets and dogs have found evidence for co-carriage between humans and pets (97, 106). Petting zoos have been identified as a potential risk factor for ESBL transmission from animals to humans (107). ESBLs were found on the animals' body surface and in the faeces of petting zoo animals that have direct contact with humans. Findings identified three main species of bacteria: *Enterobacter cloacae, E. coli,* and *Citrobacter freundii* which carry ESBL, AmpC, or multiple antibiotic resistance genes resulting in some of the *E. coli* being multi-drug resistant.

Farm animals can be considered a reservoir of ESBL-producing *Enterobacteriaceae*. ESBL-producing *Enterobacteriaceae* have been detected mainly in faecal samples (108, 109). A study from Chile looked at small scale farms, taking a larger range of farm animals including horses, goats, sheep, chickens, pigs, cows, and dogs into consideration. It was determined that dogs had the highest numbers of ESBL-producing *E. coli* detected in faecal samples (110). However, this was for small scale farms and the case is different for intensively farmed animals. There are also studies that focused on food animals with faecal samples taken at slaughterhouses. One study from South Korea reported that chickens (94.1%) had the highest numbers of ESBL-producing *E. coli* detected, followed by pigs (69.5%) and cattle (7.0%) (111). This was in agreement with a Swiss study, which found that chicken faecal samples (63.4%) had the highest percentage of ESBL-producing *Enterobacteriaceae* detected, followed by porcine (15.3%), bovine (13.7%), then ovine (8.6%) sources (112).

The presence of ESBLs in wildlife has also been studied (108, 109). Areas where humans, domesticated animals and wild animals' habitats overlap have been a particular focus as this could be a potential transmission pathway (110). Due to the wild animals not being treated with antibiotics, environmental contamination, from either humans or other animals, must be considered. This is also demonstrated with studies looking at wild boar in Europe (108, 113). Both studies found ESBL-producing *E. coli* from wild boars and theorised the source of the resistance

to be originating from human activity. Overall, the results outlined above show the potential opportunities for ESBL spread amongst humans and animals. It also demonstrates the impact that humans are having on the environment, which is in turn is affecting the animals within it.

1.6 Current and future research in New Zealand

1.6.1 Human based studies

ESBL-producing *Enterobacteriaceae* are known to be present in New Zealand, of which resistant rates are being monitored by the Institute of Environmental Science and Research (ESR) two-yearly surveys (114). Surveillance is important as identifying resistance patterns will allow for future treatment and management options. The 2016 ESR survey found 521 ESBL-producing *Enterobacteriaceae* human clinical isolates, with 386/521 (74.1%) being *E. coli* making it the most predominant species detected (114, 115). Findings of this survey saw the percentage of ESBL-producing *E. coli*, increasing from 50.2% in 2007 to 74.1% in 2016. This increase is not just limited to *E. coli* but is observed for all ESBL-producing *Enterobacteriaceae* clinical isolates collected for regular surveys since 2007. Data from this survey also shows that the majority of isolates were linked to the community, rather than hospital cases. Community or hospitalised status was classified based on whether the person was hospitalised at the time of isolate collection or had been in hospital within the last three months. ESR surveys provide a wider view of community prevalence across the country.

Other studies utilising ESR collected data delve deeper, further breaking down the data by district health board, or by ethnicity (114, 116). From the same report, samples showed the most common ESBL enzyme type in New Zealand is CTX-M, with the most frequent sequence type for *E. coli* being ST131.

A study comparing ESR isolates with additional ESBL-producing *E. coli* isolates collected from Otago was conducted. The additional isolates were collected from urine samples taken in 2015 and CTX-M was the most common ESBL enzyme type detected with ST131 the most common sequence type (117). Another study analysed third generation cephalosporin resistant *E. coli* from blood stream infections in Australia, New Zealand, and Singapore (118). It was determined that the source of the blood stream infection was caused by the urinary tract 69.6% of the time. Overall, there was a clear relationship between ST131 and the presence of $bla_{\text{CTX-M}}$ gene presence across all countries.

1.6.2 Animal studies

Multiple New Zealand studies have shown domesticated and companion animals are an important source of ESBL- and AmpC-producing *E. coli* (97, 119). Isolates from veterinary diagnostic labs showed that 60 out of 115 isolates from companion animals were ESBL- or AmpC-producing *Enterobacteriaceae*, 36 of these being *E. coli* (119). Sequence types detected were ST12, ST131, and ST648. Recent studies have expanded on these findings, comparing the carriage between

humans and household pets (97). In five out of eleven households ESBL-producing *E. coli* strains were shared between people within the same household and similarly in two out of eleven households' strains were shared between the dog and the owner. However as stated by the authors the study was unable to determine if transmission did occur between the humans and dogs, or whether the dogs picked up the bacteria from an external source like raw meat or the environment. Alternatively the humans and dogs gained the strain from the same source (97). A previous study found canine urine samples (from 2005 to 2012) (120). harboured ESBL-producing *E. coli* (1104/3135, 35.2% of isolates). The percentage of *E. coli* that were resistant to amoxicillin-clavulanic acid, cephalothin was seen to increase by 4.2% and 11.2% respectively over the 2005 to 2012 period, along with those isolates resistant to enrofloxacin (resistance increased by 1.5%) (120).

Livestock has also been identified as a potential reservoir for ESBL-producing *Enterobacteriaceae* in New Zealand. A study conducted in the Manawatū recently investigated the frequency of ESBL-producing *E. coli* on dairy farms (121). Three ESBL-producing *E. coli* strains, from dairy cattle on one farm, were identified as ST405, which is also associated with human blood and urinary infections. It was concluded that there was a low numbers of ESBL-producing *E. coli* on pasture-based farms. Another study has looked at AmpC-producing *E. coli* from dairy farms in 2017 (122). Cattle faeces were collected across 26 different farms. There were AmpC-producing *E. coli* isolates from 7/26 farms. There were no ESBL-producing isolates reported (122).

There have also been studies to detect antibiotic resistance in poultry (123). Carcass-rinse samples collected from July to December 2006 were assessed for antibiotic resistance by disc diffusion testing. The *E. coli* tested were susceptible to all the antibiotics tested, including no resistance shown to extended-spectrum cephalosporins (123). Another study included samples from young calves, pigs, poultry and fresh produce in New Zealand (124). The samples were rinsates of swabs of carcasses. No *E. coli* isolates tested were determined to be ESBL or AmpC-producing (124).

1.6.3 Environmental studies

Environmental research surrounding the diversity and spread, of ESBL-producing *Enterobacteriaceae* throughout New Zealand has been reported on, but not in as great depth as animal or human studies. There have been some studies that have broadly looked at antibiotic resistance in biofilms from freshwater sources (125, 126). There were many different resistance genes detected in the biofilms, some of which were for vancomycin, a human only antibiotic (125, 126). However, both papers concluded that agricultural runoff could be contributing the resistance seen (125, 126). A study conducted in Canterbury New Zealand reports that ESBL-producing bacteria were detected in urban and agricultural streams (127). However genotypic analysis was not carried out, so gene presence is unknown.

Further research on freshwater has been conducted that looked at isolates sourced from rivers in the Midcentral District Health Board of New Zealand, samples being collected from the Manawatū and Oroua River in 2019 (128). ESBL or AmpC-producing *E. coli* was present in eight samples, from the Manawatū River (128). All the isolates that were ESBL positive were also multi-drug resistant from disc diffusion tests (128). *E. coli* sourced from New Zealand waterways has also been reported with the presence of $bla_{\text{CTX-M}}$ being detected in water isolates (129, 130). One isolate from the Manawatū River, that was sequenced using ONT, the gene $bla_{\text{CTX-M-24}}$ was chromosomally encoded (130).

To provide the whole picture, future research could include the relationship between ESBL-producing *Enterobacteriaceae* found in humans in a hospital or community setting and those found from animals and the environment. This would allow a full One Health perspective.

1.7 Conclusion

ESBL-producing *Enterobacteriaceae* are a growing problem, not only for New Zealand, but also worldwide. ESBL-producing *E. coli* are a known problem in the hospital and community setting. The correct treatment is important to prevent the overuse of antibiotics that would drive antibiotic resistance to develop in bacteria. Therefore, the detection of ESBL genes and investigation by genome sequencing can provide information about the genetic relatedness and allows resistance spread to be monitored. A One Health approach is important to visualise the full transmission cycle. Knowing that animals carry ESBL-producing *E. coli* and like humans are a contributor to the presence in the environment allows for investigation into sources and the spread of the resistant bacteria to be pinpointed. However, it is not fully known what effect ESBL-producing bacteria in the environment have on food sources for both human and animals. This is an important area to research because the extent of ESBL-producing bacteria in the environment is unknown in New Zealand. Having the full picture will allow preventative and regulatory measures to be put in place to reduce rising resistance rates.

1.8 Objectives of the study

The purpose of this study was to compare the genetic relatedness of clinical and environmental ESBL-producing *E. coli* collected within the Manawatū River and MidCentral District of New Zealand. Whole genome sequencing will provide further understanding of how the *E. coli* isolates are genetically similar.

There are two objectives of this study:

(i) Characterise the phenotypic and genotypic antibiotic resistance profiles of ESBL-producing *E. coli* collected from clinical and environmental sources.

(ii) Assess the genetic relatedness of the ESBL-producing *E. coli* using whole genome sequencing.

2 Materials and Methods

2.1 Sample collection and processing

Environmental samples from the Manawatū River, stormwater, and treated effluent were collected over a 14 month period from August 2019 to March 2020 and July 2020 to January 2021 (excluding October 2019).

Two water and one sediment sample were collected from locations A (upstream), B, D, and F (downstream) along the Manawatū River (Figure 2.1). A stormwater sample was collected from the Centennial Drive site (site C) when water was running from the drain. Near the Tip Road site (site E), a sample was collected from the treat effluent outflow. Sample site A was originally located along State Highway Three, Manawatū Gorge (-40.305855, 175.771632), and samples from August 2019 to March 2020 were collected from this location. However, due to the construction of the Manawatū Gorge bridge, sample site A was moved to the location shown in Figure 2.1 (-40.305311, 175.758704).

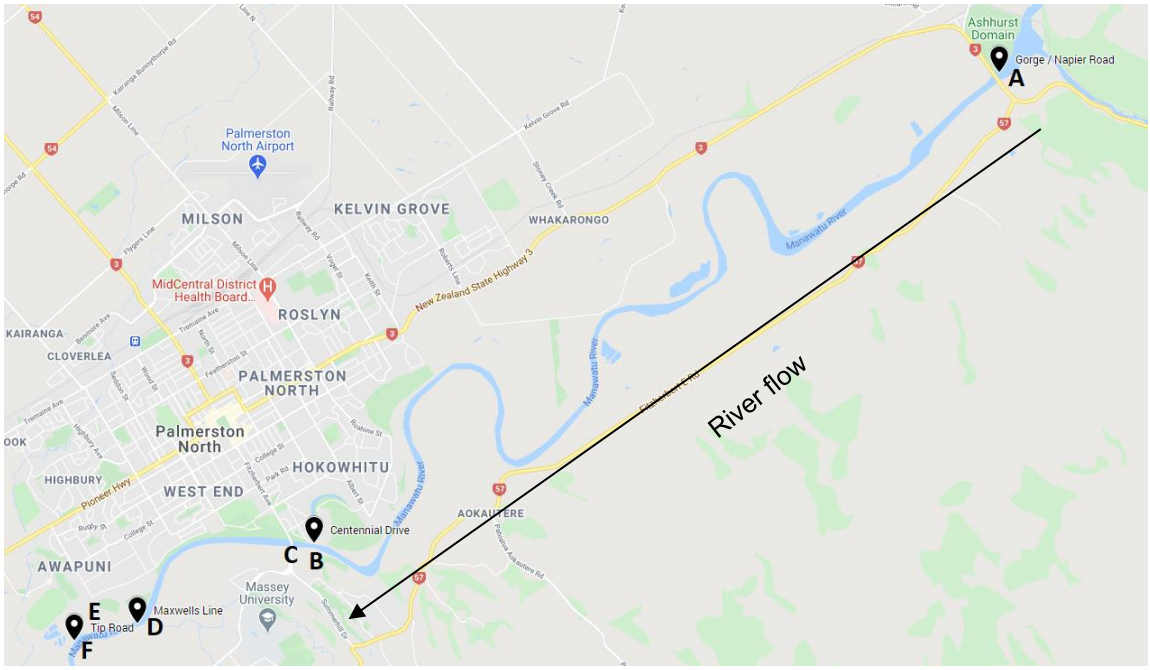

Figure 2.1: Sample collection sites along the Manawatū River. Location A = Gorge /Napier Road (-40.305311, 175.758704); B = Centennial Drive Manawatū river (-40.373511, 175.628272); C = Centennial Drive stormwater drain (-40.373294, 175.626352); D = Maxwells Line (-40.385139, 175.594736); E = Tip Road treated effluent outflow (-40.387237, 175.582637); and F = Tip Road Manawatū river samples (-40.387557, 175.5826448). Image produced using Google Maps.

Samples were prepared for enrichment by aseptically filtering 100 ml of each water sample with a vacuum filtration system using mixed cellulose ester filters (0.45 µm, Millipore, Germany). This

was done in duplicate, one filter per enrichment broth. The pieces of filter paper were then placed aseptically into 20 ml sterile universal bottles. Sediment (1 g) was aseptically weighed and placed in sterile 20 ml universal bottles. Additionally, 1 ml of treated effluent was transferred to a 20 ml sterile universal bottle. Samples were enriched by adding 10 ml of buffered peptone water (BPW; BD Difco™, Becton Dickinson, New Jersey, USA) or *E. coli* broth (ECB; Oxoid Ltd, Hants, UK) to each universal bottle. Additionally, 1 ml of treated effluent sample was pipetted into a 20 ml universal bottle with 9ml BPW and/or ECB. All universal bottles were vortexed and incubated overnight at 35°C. The next day, 2 ml from each bottle was transferred into a 2 ml Eppendorf tube, which was centrifuged for 5 min at 6000 *g*. The supernatant was discarded, and the remaining pellet was re-suspended in 1 ml of 15% (w/v) glycerol broth, then frozen at -80°C. Each sample was allocated a unique identifying number with the prefix "SB" followed by the allocated sample number. Additionally, 1 ml of treated effluent was plated onto ESBL chromogenic agar plates (CHROMagar™ ESBL, Fort Richard Laboratories, Auckland, New Zealand), in duplicate. After incubation at 35°C for 16 to 24 hours pink and blue colonies were counted with select isolates being purified (Section 2.4).

2.2 Ethics statement

This project has been evaluated by peer review and judged to be low risk (ethics notification number: 4000021252). Consequently, it has not been reviewed by one of Massey University's Human Ethics Committees. The researcher(s) named in this document are responsible for the ethical conduct of this research.

2.3 Clinical isolate collection

Presumptive ESBL-producing *Enterobacteriaceae* clinical UTI isolates were sourced weekly from MedLab Central, from August 2019 to March 2020 and June 2020 to January 2021, and had been grown on blood agar plates. MedLab Central phenotypically selected isolates with cystine-lactose-electrolyte-deficient or chromogenic agar and/or MALDI-TOF identification. The isolates MedLab Central collected are then screened for potential ESBL production with a VITEK system, followed by confirmation disc diffusion (MAST Group, Merseyside, UK, sets D68C (AmpC and ESBL Detection Set) and D63C (Cefepime ESBL ID Disc Set)). It was unknown if isolates received for this study were only presumed or confirmed ESBL-producing by MedLab Central. Therefore, all received were considered "presumptive". Upon reception of presumptive ESBL-producing clinical isolates from MedLab Central (Palmerston North), two single colonies were selected and streaked onto fresh Columbia horse blood agar (Fort Richard Laboratories, Auckland, New Zealand), which were incubated overnight for 16-24 hours at 35°C. The two purified isolates were labelled with unique identifying labels prefixed with "EH" followed by either an 'a' or 'b'. The purified isolates were suspended in 15% glycerol broth and frozen in cryovials at -80°C. The 'a' isolates were identified using MALDI-TOF MS. The 'b' isolates were stored as backup cultures if the 'a' isolate was impure.

2.4 Environmental isolate collection

Enterobacteriaceae were isolated from the enriched environmental samples by streaking the frozen enrichments onto four types of selective agar (97). Chromogenic agar contains ingredients that will indicate potential ESBL production and the species of bacteria. MacConkey agar with and without antibiotics added was used as the non-commercial option and for added selection opportunities for lactose metabolising bacteria. These included MacConkey with lactose and crystal violet (Fort Richard Laboratories, Auckland, New Zealand), MacConkey (BD Difco™, Becton Dickinson, New Jersey, USA) supplemented with cefotaxime (final concentration in agar was 1 µg/ml) (prepared in-house), MacConkey (BD Difco™, Becton Dickinson, New Jersey, USA) supplemented with ceftazidime (final concentration in agar was 1 µg/ml) (prepared in house), and ESBL chromogenic agar. The MacConkey agar (with and without added antibiotics) used below was made in-house by following the manufacturers' instructions and sterilisation requirements. The antibiotics were prepared as stock solutions of 10 mg/ml from powder bases of cefotaxime sodium salt (Sigma-Aldrich, Missouri, USA) and ceftazidime (Sigma-Aldrich, Missouri, USA), 50 mg of the powder was dissolved in 5 ml sterile MiliQ water. The solutions were filter sterilised with a 0.22 µM syringe filter (Millipore, Germany) and then diluted to 1mg/ml and stored at -80°C. The antibiotic agar was made by adding 250 µl of the specified antibiotic (working stock concentration of 1 mg/ml) to 250 ml of molten agar. Each sample was streaked onto the four selective agars and incubated for 16 to 24 hours at 35°C. Two suspected *Enterobacteriaceae* single colonies were selected from each agar type and checked for purity on Columbia horse blood agar plates (Fort Richard Laboratories, Auckland, New Zealand). Pure cultures were frozen in cryovials in 15% (w/v) glycerol broth at -80°C.

2.5 MALDI–TOF MS identification of isolates

Identification of isolates to the species level was carried out using a Bruker Microflex MALDI-TOF MS instrument (Bruker, Bremen, Germany) with a database provided by Bruker (revision C, March 2019) (131). The isolates were prepared by streaking for single colonies on Columbia horse blood agar plates (Fort Richard Laboratories, Auckland, New Zealand) from frozen pure cultures and incubated overnight at 35°C for 20 hours. Single colonies were selected to be smeared onto the target spaces of the MSP 96 target polished steel BC plate (Bruker, Bremen, Germany) in duplicate using sterile toothpicks. In a fume hood, 1 µl of 70% formic acid was added to each target spot and allowed to dry. The HCCA (α-Cyano-4-hydroxycinnamic acid) proportioned matrix for MALDI-TOF MS (Bruker, Bremen, Germany) was rehydrated with a solution consisting of 50% acetonitrile, 47.5% water and 2.5% trifluoroacetic acid (Sigma-Aldrich, Missouri, USA). Once dry, 1 µl of the rehydrated HCCA proportioned matrix for MALDI-TOF MS mixture was added on top of the formic acid dried spots and allowed to dry. The run was started using Bruker Daltonics MALDI Biotyper Compass (v4.1.100) and monitored using the software Bruker Daltonics

flexControl (v3.4). A score reported as greater than two indicated a confident species identification.

2.6 Antimicrobial susceptibility testing

2.6.1 Screening panel for environmental isolates

The environmental *E. coli* isolates, selected from either of the two antibiotic agars and the ESBL chromogenic agar, were screened for antibiotic resistance, against a panel of ten antibiotics from seven different classes (MAST Group, Merseyside, UK), including two third generation cephalosporins (Table 2.1). The clinical isolates were presumptive ESBL producers, therefore they were not screened using the ten antibiotics. From the frozen pure cultures, environmental isolates were streaked onto Columbia horse blood agar plates (Fort Richard Laboratories, Auckland, New Zealand) and incubated overnight at 35°C for 20 hours. To ensure the purity of the culture, a single colony was sub-cultured onto Columbia horse blood agar plates and incubated at 35°C overnight. From the sub-cultured growth, three single colonies were dabbed lightly using a sterile cotton bud and transferred to 2 ml of sterile saline solution, with the inoculated mixture being equivalent to a 0.5 McFarland standard. A new sterile cotton bud was used to inoculate the Muller Hinton agar plates (Fort Richard Laboratories, Auckland, New Zealand) from the saline solution, streaking in three different directions to create a lawn. The antibiotic discs were placed, the Muller Hinton agar plates which were then incubated (following CLSI guidelines) at 35°C for 16 to 18 hours. The recommended control of *E. coli* ATCC® 25922 was used and prepared in the same manner as other isolates (64). The disc diffusion zones were read using electronic callipers. Listed in Table 1 are the antibiotic disc concentrations, the zone diameters, and respective guidelines that were used to interpret the readings. Isolates that were resistant to either ceftazidime or cefotaxime, were subject to an ESBL double disc diffusion confirmation test following CLSI guidelines, as outlined below (64).

2.6.2 ESBL confirmation

Clinical and environmental *E. coli* isolates were subjected to a confirmation ESBL double disc diffusion test. This utilises third generation cephalosporins with and without an inhibitory agent. Those used were, ceftazidime (30 µg), ceftazidime-clavulanate (30/10 µg), cefotaxime (30 µg), and cefotaxime-clavulanate (30/10 µg) (MAST Group, Merseyside, UK), and the resistance status was determined following CLSI guidelines (64). The colonies were streaked into a lawn as previously stated and antibiotic discs were placed onto the lawn. The plates were incubated at 35°C for 16 to 18 hours. The recommended CLSI controls were used and prepared in the same manner as above, these were *E. coli* ATCC® 25922 as the negative control and *K. pneumoniae* ATCC® 700603 as the positive control (64). Results were read using electronic callipers. The full workflow of the above processes is displayed in Figure 2.2.

Clinical and environmental isolates were selected for Illumina sequencing from those that were ESBL positive from the disc diffusion test. Additionally, the antibiotic resistant profiles from the screening panel of ten antibiotics (in combination with ESBL positive status) were considered when the selection of the environmental isolates for sequencing occurred.

Table 2.1: Breakpoints of antibiotics used according to CLSI or EUCAST guidelines (64, 65).

Antibiotic	Abbreviation	Resistant (mm)	Intermediate (mm)	Sensitive (mm)	Guidelines used
Ciprofloxacin (5 µg)	CIP	≤21	22-25	≥26	CLSI
Chloramphenicol (30 µg)	C	≤12	13-17	≥18	CLSI
Gentamicin (10 µg)	GM	≤12	13-14	≥15	CLSI
Tetracycline (30 µg)	T	≤11	12-14	≥15	CLSI
Trimethoprim/ sulfamethoxazole (1.25/23.75 µg)	T/S	≤10	11-15	≥16	CLSI
Streptomycin (10 µg)	S	≤11	12-14	≥15	CLSI
Nitrofurantoin (100 µg)	NI	<11	-	≥11	EUCAST
Cefoxitin (30 µg)	FOX	≤14	15-17	≥18	CLSI
Ceftazidime (30 µg)	CAZ	≤17	18-20	≥21	CLSI
Cefotaxime (30 µg)	CTX	≤22	23-25	≥26	CLSI

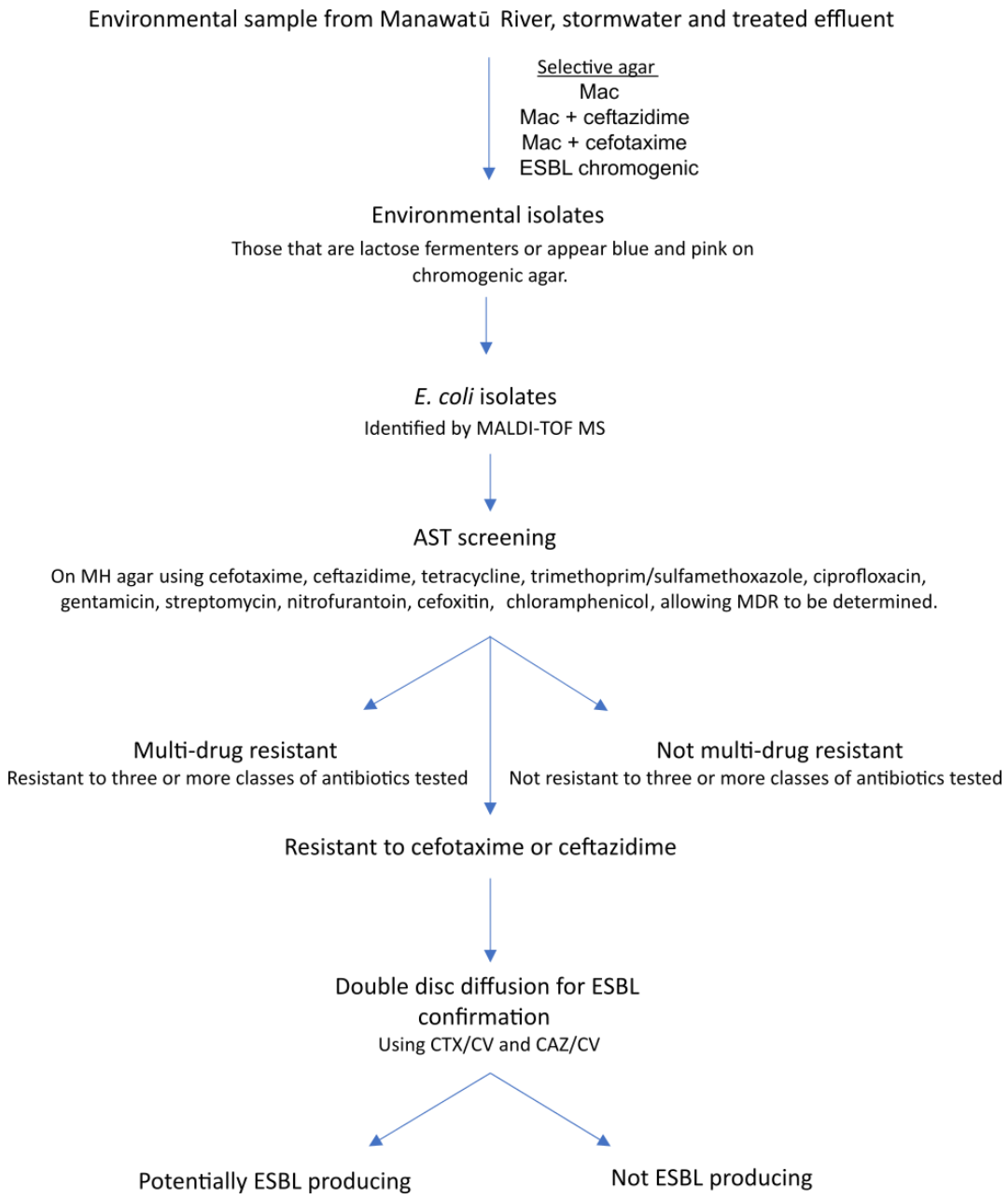

Figure 2.2: Full workflow from sample collection to isolate selections, identification, and AST.

2.7 DNA Extractions

E. coli isolates were streaked onto Columbia horse blood agar plates from frozen and incubated overnight at 35°C for 20 hours. A single colony was sub-cultured onto fresh Columbia horse blood agar plate (Fort Richard Laboratories, Auckland, New Zealand) and were grown at 35°C for at least 18 hours. A single colony was selected from the sub-culture and transferred using a sterile 1 µl loop into 5 ml of Luria-Bertani broth (made with deionised water, NaCl Scharlau, Yeast extract (BD Difco™, Becton Dickinson, New Jersey, USA) and Tryptone (BD Difco™, Becton Dickinson, New Jersey, USA)) in a 50 ml falcon tube. Liquid cultures were incubated overnight on an orbital shaker at 180 rpm for 14 to 16 hours at 35°C.

The Promega genomic DNA extractions kit (Promega, Wisconsin, USA) was used as per the protocol guidelines for “isolating DNA from gram-negative bacteria”, with minor amendments. From the overnight culture, 1 ml was pipetted into a 2 ml microcentrifuge tube, then spun for 2 min at 13,000 – 16,000 *g*. The supernatant was discarded, and the pellet was resuspended in 600 µl nuclei lysis solution. The lysis was completed by incubating the solution at 80°C for 5 min, shaking at 500 rpm, then cooled to 18°C on a Provocell Micro Incubator (Provocell™, ESCO, Singapore). To the cell lysate, 3 µl of RNase solution was added and incubated at 37°C, shaking at 1200 rpm for 1 hour on a Provocell Micro Incubator. To the cell lysate and RNase solution, 200 µl of protein precipitation solution was added and the tube was vortexed for 5 seconds, before promptly being incubated on ice for 5 min. The solution was centrifuged for 10 min at 13,000 – 16,000 *g*. The supernatant was transferred to a new microcentrifuge tube and then centrifuged at 13,000 *g* for 3 min. The supernatant was transferred to another new microcentrifuge tube. Ice cold isopropanol was added in a 1:1 ratio to the supernatant, the tube was inverted at least 7 times twice over with a 1 min incubation interval between inversion sets. The solution was centrifuged for 2 min at 13,000 – 16,000 *g*. The supernatant was decanted, and the pellet was washed with 600 µl of 70% molecular grade absolute ethanol. The solution was centrifuged for 2 min at 13,000 – 16,000 *g*, and the supernatant was decanted. The resulting pellet was dried and resuspend in 50 µl of 10 µM tris.HCl pH 8.0 (Sigma-Aldrich, Missouri, USA). Once resuspended the microcentrifuge tube was placed on a Provocell Micro Incubator at 65°C for one hour. DNA extractions were stored at 4°C until quality control checks were conducted. Post quality control DNA extractions were stored in Eppendorf tubes and were placed in freezer boxes at -20°C.

2.8 DNA extractions quality checks

2.8.1 Nanodrop

The Nanodrop microvolume spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific, Massachusetts, USA) was used to check the approximate DNA concentration, and if there was any protein or ribonucleic acid (RNA) contaminating the DNA extractions. The RNA A_{260}/A_{280} ratio value of greater than 2 indicated RNA contamination (1.8-2.0 was deemed a pure DNA sample) and the A_{260}/A_{230} value of less than 2.0 indicated presence of other contaminants (for example, phenol or guinadine) The spectrometer was cleaned with sterile Milli Q water and then blanked with 2 µl 10 µM tris.HCL pH 8.0. A volume of 2 µl was used for the DNA extractions.

2.8.2 Agarose gel electrophoresis

DNA extraction purity and quality were checked via agarose gel electrophoresis. A 1% agarose gel made with 100 ml 1x Tris-acetate-EDTA (TAE) buffer added to 1 g of Bioline molecular grade agarose powder (Meridian Bioscience®, Ohio, USA). This was microwaved to dissolve the powder into the buffer and 5 µl RedSafe (iNtRON Biotechnology, Inc., Korea) was added per 100 ml of gel. The gel was run with 5 µl of 1 kb+ DNA ladder (Invitrogen, Thermo Fisher Scientific,

Massachusetts, USA) and 2 µl of λ DNA/Hind III high molecular weight ladder (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA) loaded into each gel. Additionally, 1 µl loading dye (25 mg bromophenol blue, 4 ml glycerol, 10ml 1xTAE) per 2 µl of each DNA extraction (2 µl of each neat DNA extraction as well as 2 µl of a 1/10 dilution) was loaded into the agarose gel lanes. The agarose gel was run at 80 V until the bands had reached three-quarters of the way down the gel slab in a gel tank filled with 1x TAE buffer. Agarose gel pictures were taken using a GelDoc XR$^+$ (BioRad, California, USA).

2.8.3 Qubit DNA concentration measurement

Extractions were then diluted to a 1/10 concentration using 2 µl of DNA into 18 µl of nuclease free water. The concentrations of the 1/10 diluted DNA extractions were measured using the Qubit dsDNA HS (High Sensitivity) Assay Kit (Introven, Thermo Fisher Scientific, Massachusetts, USA) on a Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA). The samples were prepared according to the manufacturers' instructions. DNA concentrations were reported in ng/µl.

2.9 Illumina library preparation, pooling, and sequencing

2.9.1 Library preparation

Illumina libraries were prepared before submission to the Massey Genome Service (Massey University, Palmerston North, 4410, New Zealand) for sequencing. The library preparation kit used was the Illumina Nextera XT (Illumina Inc, California, USA), and the protocol guidelines used were an adaptation of the Nextera XT protocol.

The library preparation consisted of four steps, DNA tagmentation, barcoding, amplification and clean-up. The manufacturer's protocol was followed, with adaptations to the tagmentation and amplification protocols as described below. The tagmentation protocol of 55°C for 12 min was run using a SensoQuest labcycler (SensoQuest, Germany). Upon program completion, 5 µl of NT (Neutralise Tagment Buffer) was added to stop the enzymatic process. There was 5 µl of each index providing a unique barcode sequence to each isolate. To this 15 µl of NPM (Nextera PCR Master Mix) was added to each tube. The amplification program was run on a SensoQuest labcycler and consisted of 72°C for 3 min, 95°C for 30 sec followed by 12 cycles of 95°C for 10 sec, 55°C for 30 sec and 72°C for 30 sec, followed by 72°C for 5 min. The clean-up process used 40 µl of AmPure XP beads (Beckman Coulter, California, USA). The DNA library was stored in a LoBind Eppendorf tube (Eppendorf, Germany) at -20°C.

2.9.2 Library quality control

Each library preparation was diluted to a concentration of 1-2 µg/ml for LabChip analysis (PerkinElmer LabChip® GX Touch HT Nucleic Acid Analyser, Massachusetts, USA) at the Massey Genome Service (Massey University, Palmerston North,4410, New Zealand). This was

to check that the average fragment size fell within the required 400 – 1000 bp window. The concentration of each library preparation was measured using the Qubit (as described in section 2.8.3).

2.9.3 Library pooling and whole genome sequencing

To determine the volume of each library required for pooling equation 2.1 was used with the value 1.52 being the conversion factor for using 1000 bp (as 1 ng of 1000 bp equals 1.52 nM). The desired volume per library to pool was determined to be 4.57 µl. The libraries were normalised and pooled in equimolar ratios, to calculate the volume of library to add (µl) and amount of library to add (ng) were calculated using equations 2.2, and 2.3, respectively. The pooled total was diluted with PCR grade water (equation 2.4), making a total volume of 1 ml. The final concentration of the pooled library was measured using the Qubit (as described in section 2.8.3) to ensure it was within the expected range.

Equations used:

A = average molecular size (bp)

B = library concentration (ng/ µl)

C = library concentration (nM)

D = volume of library to add (µl)

E = amount of library to add (ng)

F = volume of buffer to add (µl)

(Equation 2.1)

$$\left(\frac{1000}{A}\right) \times 1.52 \times B = C$$

(Equation 2.2)

$$\frac{12\ (nM)}{(C \times 4.57\ \mu l)} = D$$

(Equation 2.3)

$$D \times B = E$$

(Equation 2.4)

$$4.57\ \mu l - D = F$$

Whole genome sequencing (WGS) was performed by Novogene (Novogene, Singapore) using one lane of Illumina HiSeq™ X sequencing (2 x 150 base paired end) resulting in 110G of raw data.

2.10 Whole genome sequencing analysis

2.10.1 FastQC

FastQC v0.11.9 was used for quality assessment, on the raw sequenced files and files post adapter removal, of sequencing quality, guanine-cytosine (GC) content, and adapter content (132). Individual reports were produced for each isolate. The purpose of conducting FastQC post adapter removal was to check that the adapter content was no longer detected in the trimmed reads.

2.10.2 Nullarbor Two

The software Nullarbor2 (v2.0.20191013) was used as the main analysis tool for the Illumina sequence data. Nullarbor2 conducted *de novo* assembly and included several other dependencies, to combine processing and visualisation into one final report (133). Default parameters were used for the Nullarbor2 pipeline. The tool trimmomatic v0.39 (134) was used to trim adapter sequences from the paired end sequence data. Parameters specified in trimmomatic included bases to be cut off at the lead and tail of the strands if the bases had a quality score of ten or below. The *MINLEN* is set at 30, meaning that the read will be dropped if it is less than 30 bp. SKESA (strategic k-mer extension for scrupulous assemblies, v2.4.0) was the *de novo* assembler utilised to construct the contigs (135). The reference option for MLST identification used was specified as *E. coli*. This software is a component of the Nullarbor2 pipeline and is used to classify the isolate and determine the sequence type (136). This was constructed by the developers of the PubMLST website (https://pubmlst.org/) (137). *Escherichia coli* ST131 strain EC968 was used as the reference (GenBank accession HG941718.1)) (138). The command line code was run using the New Zealand eScience Infrastructure (NeSI) supercomputer. The command used to run Nullarbor2 was as follows:

```
nullarbor.pl --name nullarbor_massey03217 --mlst ecoli --ref
ecoliST131.fasta --trim ON --taxoner centrifuge --input Input_all.txt
--outdir nullarbor_all_results

nice make all -j 4 -l 54 -C nullarbor_all_results 2>&1 | tee -a
nullarbor_all_results/nullarbor.log
```

A full list of software versions included in the Nullarbor2 pipeline can be found in Appendix 4.

2.10.3 Quast

Quast (v5.0.2) is a genome evaluation tool (139). The input files are genome assemblies, and it makes a report giving a statistics report on every assembly included. This was run with the *E. coli* ST131 reference genome GenBank number HG941718.1, with default parameters. The output file of particular interest was the main report that contained the assembly length, coding

sequence, and the GC content. This step was important as the Nullarbor2 output contained the GC percentage for the reads, but not the assemblies. There was no reads or assemblies discarded because of this process.

2.10.4 Fast-GeP

For whole genome MLST (wgMLST) the software Fast Genome-Profiler (Fast-GeP, v1.0.2) was used (140). This includes the tools BLAST+, DIAMOND aligner, and SplitsTree. BLAST+ or DIAMOND can be chosen as the aligner and, SplitsTree is used to compare alignments generating a phylogenetic tree to visualise the relationships (141, 142). Fast-GeP conducts a genome-by-genome approach utilising the database BLASTX to identify the alleles called during comparison of the genomes. Orthologues are aligned to the reference genomes' amino acid sequences, further measures are also taken by the program to account for orthologous genes to be found. For this project, default parameters were used. BLAST+ was the selected aligner with the inclusion of a reference genome allowing allele profiles to be made using an *ad hoc* wgMLST scheme. The reference genome used was *E. coli* EC968 (GenBank accession HG941718.1) in GenBank format (138). The output Fast-GeP tree file in *.nex* format was then imported into a desktop version of SplitsTrees (v5.3.0) and a Neighbour-Joining tree was constructed. The final tree was exported as a *.newick* file.

2.10.5 Snippy

Snippy (v4.6.0) is another tool included in the Nullarbor2 pipeline but was also utilised as a standalone tool (72). The main function of this software is to find SNPs and indels (insertions or deletions of bases) between the reference genome used and the supplied sequence reads. This also includes the function where a core SNP alignment and a phylogenetic tree is produced for the final Nullarbor2 report.

Snippy-multi was used independently from the Nullarbor2 pipeline to create the core SNP phylogenies for additional phylogenetic trees. Assembled contigs, of isolate EH0395a, were used as the reference genome for the SNP analysis of the ST131 isolates. Additionally, Illumina reads of known ST131 clades A, B and C were downloaded from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home), to allow isolates from this project to be delegated into one of the three clades. These were sourced from a study conducted in Queensland Australia, where isolates from blood stream infections were whole genome sequenced (118). The project number for these reads is PRJNA398288 with the specific run accession numbers for clade A, B and C being, MER-56 (SRR5936479), MER-53 (SRR5936492) and MER-25 (SRR5936501) respectively. Snippy-multi was run independently for only the environmental isolates as well. The assembled contigs, for isolate SB0391f1, were used as the internal reference for the SNP analysis of the environmental isolates, also creating a core-SNP phylogenetic tree.

FastTree (v2.1.11) was used for tree construction for Snippy-multi alignment results. The GTR (general time-reversible) substitution model was applied. Default parameters for both programs were used. Either of the file types *.newick* or *.tree* was uploaded to the interactive Tree Of Life (iTOL, v6.5.7) for annotation and visualisation of the phylogenetic trees (143).

2.10.6 Gubbins

Gubbins (Genealogies Unbiased By recomBinations in Nucleotide Sequences, v2.3.1) was used to remove recombination in the sequences and improve phylogenetic networks in the Snippy analysis of ST131 and the environmental isolates (144). The methods assume that mutations occur randomly throughout the genome and areas of increased substitutions are potential regions of recombination.

Gubbins was run with the default parameters for the ST131 isolates. The `--filter-percentage` flag was set at 30 for the environmental isolates to allow taxa with up to 30% of gaps to be included in the analysis. This parameter was changed for the processing of the environmental isolates due to some being excluded with the default value of 25%.

2.10.7 Centre for Genomic Epidemiology (CGE) tools

The CGE offers many individual tools publicly available through the web to use for sequence data analysis (https://www.genomicepidemiology.org/). FimTyper (v1.0) with default parameters was used for the isolates EH0318a, EH0378a, EH0389a, and EH0391a, to determine the *fimH* type (145). This was to assist in clade clarification of these ST131 isolates.

PointFinder (v3.0) was used to analyse point mutations in the sequences of the ST131 isolates (141, 146, 147). I was specifically interested in mutations of the *gyrA* and *parC* genes which confers antibiotic resistance to nalidixic acid and ciprofloxacin as well as *ampC* chromosomal mutations. All version numbers used for the analysis tools are presented in Appendix 4.

2.11 Statistical analysis

2.11.1 Phenotypic data analysis

Phenotypic results were stored in a Microsoft Access database (Microsoft 365, v16.0.1) and analysed using the software R (v4.1.2, R computing group, Auckland, New Zealand). Packages used for construction of graphs include ggplot2 (v3.3.5), lubridate (v1.8.0) and ComplexUpset (v1.3.1). The code used to construct the figures is available in Appendix 1 and package versions in Appendix 4.

2.11.2 Isolate selection for DNA sequencing

Isolate selection for sequencing was conducted using tools in R. The environmental isolates were selected based on different antibiotic resistance profiles, sample collection time, and sample type. A difference matrix was first constructed allowing the creation of a dendrogram, using base R commands, to visualise the clustering of isolates based on the resistance profiles. A distance matrix was first created using the *hclust()* command with the method set to *ward.d2* which minimises total within cluster variance. It was then plotted and then transformed into *.newick* format to be further visualised. There were 41 environmental isolates selected for sequencing. Therefore, due to the Illumina sequencing flow cell lane compacity there was space available for 178 clinical isolates to be sequenced. Collection dates of the selected environmental isolates were used to select clinical isolates that arrived during the same time period. The clinical isolates were then randomly selected without replacement across the months using an R base command and tidyverse package (v1.3.1) was used to combine columns of multiple tabular files into a single dataset. An additional 16 isolates (12 clinical and four environmental) were also included in whole genome sequencing analysis that had previously been sequenced by Dr Sara Burgess.

The code used is in Appendix 1, isolate selection options are presented in Appendix 2 and 3, and the full list of version numbers for both R code and genomic tools used in this study is detailed in Appendix 4.

2.11.3 Sequence type display

The software R (v4.1.2) was used to visualise the change in sequence types over time. This used packages ggplot2 (v3.3.5), ggalluvial (v0.12.3), and easyalluvial (0.3.0). The code for constructing this plot can be found in Appendix 1 and package versions in Appendix 4.

3 Results – Species identification and antibiotic susceptibility testing

3.1 Collection of clinical isolates

A total of 415 clinical isolates were collected across the sampling period. There were no isolates collected (due to the New Zealand Government-imposed COVID-19 restrictions) from April 2020 to May 2020. Isolates came as pure cultures, from a range of sources, the majority (395/415, 95.2%) being from urine. Other isolates came from catheter urine (14/415, 3.4%), aspirates (1/415, 0.2%), bag urine (1/415, 0.2%), wound swab (1/415, 0.2%), fluid in culture bottle (1/415, 0.2%), and blood (2/415, 0.5%). From the clinical isolates received, 359/415 (86.5%) were identified as being *E. coli* via MALDI-TOF MS (section 2.5).

Table 3.1: Clinical isolates species identified.

Species	Count
Citrobacter sp.	9
Escherichia coli	359
Enterobacter spp.	15
Other *Klebsiella* spp.	8
Klebsiella pneumoniae	17
Morganella morganii	1
Other species	3
Proteus spp.	3
Total	415

3.2 Collection of environmental samples and isolates

Manawatū River, stormwater and treated effluent samples were collected from four separate locations along the Manawatū River over a total of 14 months (August 2019 to March 2020 and July 2020 to January 2021), resulting in a total of 174 enriched samples. As above, due to the New Zealand Government-imposed COVID-19 restrictions, there was no sampling from April 2020 to June 2020. A total of 918 isolates were sourced (Table 3.2) from the 174 environmental enrichments, with 418/918 (45.5%) being identified as *E. coli* via MALDI-TOF MS (section 2.5).

Table 3.2: Identification of the environmental isolates.

	Media				
Bacterial species	MCC[a]	MCTX	MCAZ	CHRO	Total
Aeromonas spp.	33	27	19	32	111
Citrobacter spp.	38	27	34	23	122
Enterobacter spp.	17	7	10	24	58
Escherichia coli	259	41	64	54	418
Hafnia alvei	0	0	16	0	16
Klebsiella oxytoca	13	2	1	8	24
Klebsiella pneumoniae	18	8	9	6	41
Other *Klebsiella* spp.	3	0	0	0	3
Kluyvera spp.	7	0	0	1	8
Rahnella aquatilis	1	14	0	2	17
Raoultella spp.	18	1	3	0	22
Serratia spp.	1	1	0	55	57
Other species	7	3	3	2	15
Not identified	3	0	0	3	6
Total	418	131	159	210	918

[a]MCC indicates MacConkey agar, MCTX indicates MacConkey supplemented with (1 µg/ml) cefotaxime, MCAZ indicates MacConkey supplemented with (1 µg/ml) ceftazidime, and CHRO indicates ESBL chromogenic agar. “Other species” indicates other bacterial species where there were less than 6 isolates identified per species. “Not identified” represents isolates that were frozen but were not identified using MALDI-TOF MS.

3.3 Treated effluent colony counts

Table 3.3 shows the colony counts of putative ESBL-producing Enterobacterales from the treated effluent with corresponding Manawatū River metadata recorded at the time of collection.

Table 3.3: Summary of colony counts and metadata.

Month	Average (cfu/ml)	Rainfall (mm)	Rainfall Cumulative 5 days (mm)	Turbidity (FNU)	River Level (mm)
2019					
Aug	n/a[a]	0	12.6	577.3	3653
Sept	26	0	0	1.86	597
Oct	n/c	n/c	n/c	n/c	n/c
Nov	5	5.5	18.5	4.43	785
Dec	5	0	3.5	-0.2	303
2020					
Jan	3	0	0.5	0.1	225
Feb	0	0	4	-0.16	221
Mar	0	0	6	-0.35	203
Apr	n/c[b]	n/c	n/c	n/c	n/c
May	n/c	n/c	n/c	n/c	n/c
Jun	n/c	n/c	n/c	n/c	n/c
Jul	39	0	1.5	87.93	1582
Aug	3	0	4.5	1.48	454
Sept	96	0.5	28.5	12.82	1087
Oct	41	0	4	2.35	600
Nov	82	0.5	1.5	2.8	712
Dec	19	0.5	35	31.27	1443
2021					
Jan	1	0	0	2.68	426

[a] n/a represents the months where no colony count data was available.
[b] n/c represents months where no samples were collected.

Figure 3.1 displays the cumulative rainfall records overlayed with the average putative ESBL-producing colony counts (cfu/ml) recorded. The colony counts appeared to have peaked in September and November 2020 and the cumulative rainfall peaked in September, November, and December 2020. When samples were not collected, turbidity levels and rainfall levels were not included, and these months are left blank in Figure 3.1.

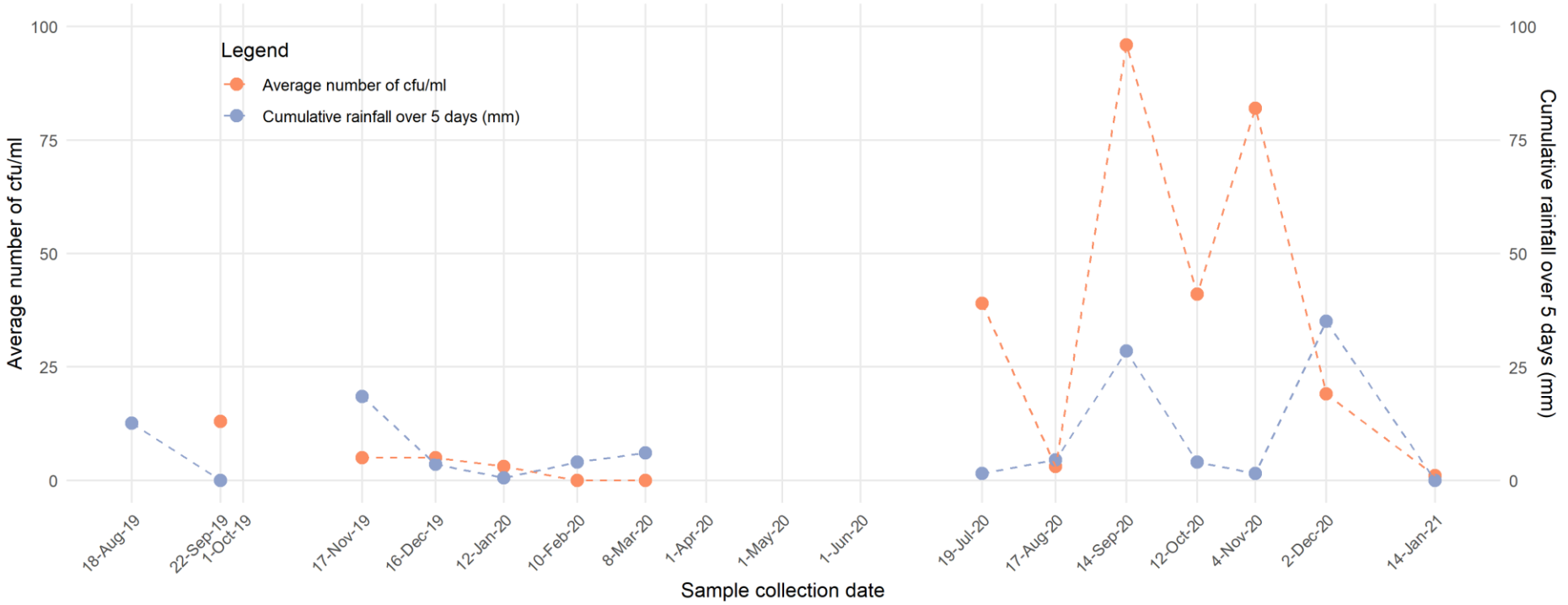

Figure 3.1: Overlay plot of average putative ESBL-producing Enterobacterales colonies per 1 ml treated effluent with the cumulative rainfall over time. The missing data over the April to June 2020 period is due to the New Zealand COVID-19 community outbreak.

3.4 Observed antibiotic resistance of presumptive third generation cephalosporin resistant environmental *E. coli* isolates

After removal of four duplicates, 155/418 (37.1%) *E. coli* isolated from antibiotic containing agar or ESBL chromogenic agar were eligible for antibiotic resistance screening (Figure 3.2). The 155 *E. coli* isolates tested for antibiotic resistance were isolated from 43 samples. A full flow chart of the process including the resulting number of isolates at each step is shown in Figure 3.2.

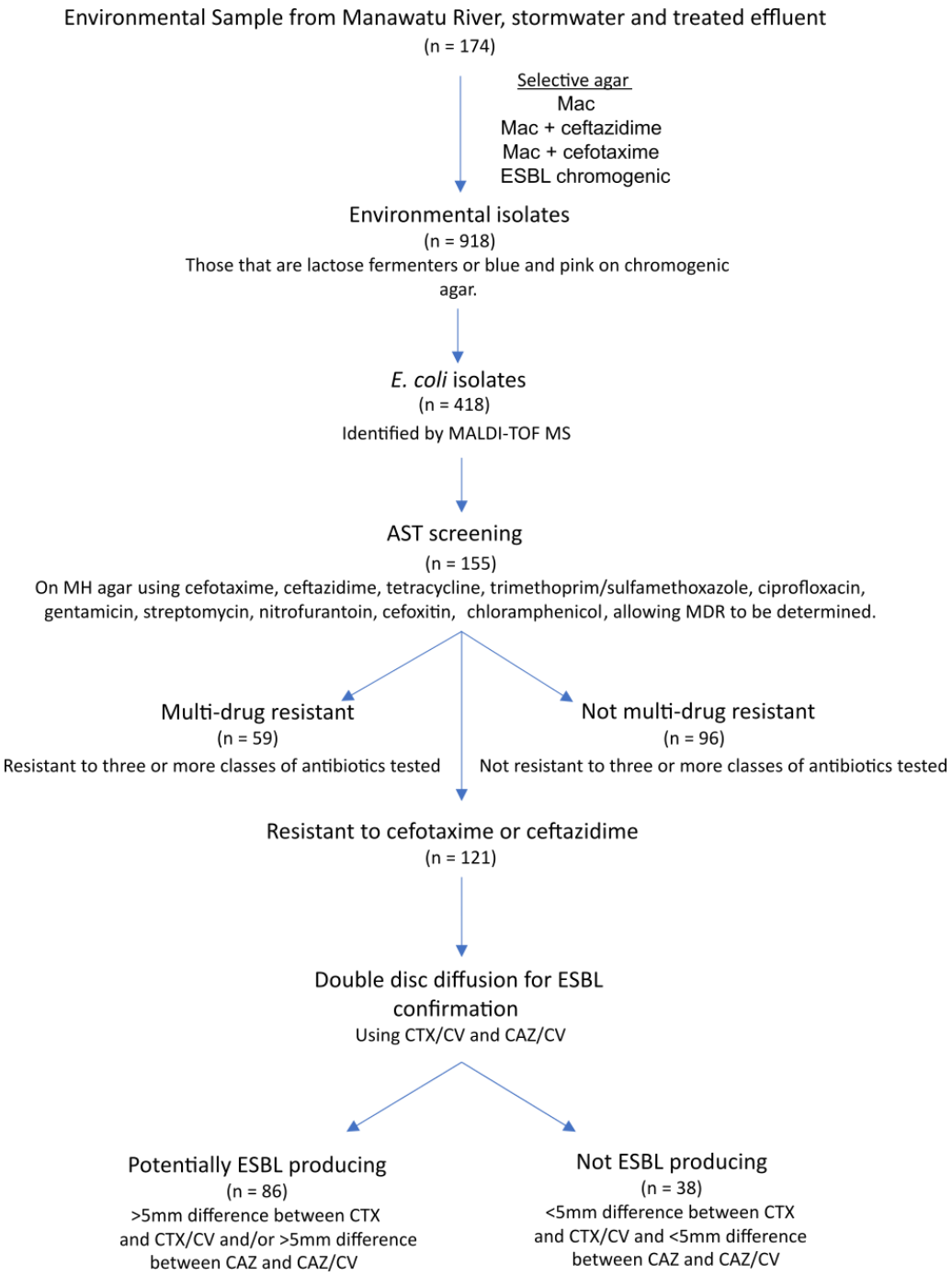

Figure 3.2: Flowchart of the environmental isolate selection process.

The 155 *E. coli* were screened with a panel of ten antibiotics, covering seven different classes, and the results are shown in Table 3.4. Of the 155 isolates 135 (87.1%) showed antibiotic resistance to one or more of the antibiotics tested. Of the total 155 *E. coli* tested there were 59/155 (38.1%) deemed multi-drug resistant (resistant to three or more antibiotics from different classes) (2) and 121/155 (78.1%) were resistant to either ceftazidime or cefotaxime.

Nitrofurantoin and trimethoprim/sulfamethoxazole are first line antibiotic choices for the treatment of UTIs in New Zealand (52). There were a total of 3/155 (1.9%) *E. coli* isolates resistant to nitrofurantoin and 46/155 (29.7%) resistant to trimethoprim/sulfamethoxazole. All three of the isolates resistant to nitrofurantoin were isolated from treated effluent, whereas resistance to trimethoprim/sulfamethoxazole was observed for isolates from both water, sediment and treated effluent.

Table 3.4: The number of presumptive third generation cephalosporin resistant *E. coli* isolates that were resistant to the ten antibiotics screened.

	Sample site								
	A		B		C	E	F		Total
Antibiotic	Water	Sediment	Water	Sediment	Stormwater	Treated effluent	Water	Sediment	Number of isolates
Cefotaxime (CTX)	0/4 (0%)	1/4 (25.0%)	3/11 (27.3%)	4/10 (40.0%)	12/13 (92.3%)	19/25 (76.0%)	72/79 (91.1%)	7/7 (100%)	118/155 (76.1%)
Ceftazidime (CAZ)	0/4 (0%)	2/4 (50%)	3/11 (27.3%)	4/10 (40.0%)	10/13 (76.9%)	13/25 (52.0&%)	46/79 (58.2%)	6/7 (85.7%)	84/155 (54.2%)
Trimethoprim/ sulfamethoxazole (TS)	0/4 (0%)	1/4 (25.0%)	3/11 (27.3%)	0/10 (0%)	0/13 (0%)	6/25 (24.0%)	35/79 (44.3%)	1/7 (14.3%)	46/155 (29.7%)
Gentamicin (GM)	0/4 (0%)	0/4 (0%)	0/11 (0%)	0/10 (0%)	6/13 (46.2%)	3/25 (12.0%)	8/79 (10.1%)	0/7 (0%)	17/155 (11.0%)
Tetracycline (T)	0/4 (0%)	0/4 (0%)	0/11 (0%)	0/10 (0%)	3/13 (23.1%)	7/25 (28.0%)	35/79 (44.3%)	1/7 (14.3%)	46/155 (29.7%)
Ciprofloxacin (CIP)	1/4 (25.0%)	0/4 (0%)	3/11 (27.3%)	1/10 (10.0%)	6/13 (46.2%)	9/25 (36.0%)	24/79 (2.5%)	2/7 (28.6%)	46/155 (29.7%)
Nitrofurantoin (NI)	0/4 (0%)	0/4 (0%)	0/11 (0%)	0/10 (0%)	0/13 (0%)	3/25 (12.0%)	0/79 (0%)	0/7 (0%)	3/155 (1.9%)
Streptomycin (S)	0/4 (0%)	0/4 (0%)	2/11 (18.2%)	0/10 (0%)	3/13 (23.1%)	5/25 (20.0%)	34/79 (43.0%)	5/7 (71.4%)	49/155 (31.6%)
Cefoxitin (FOX)	0/4 (0%)	4/4 (100%)	0/11 (0%)	5/10 (50.0%)	0/13 (0%)	13/25 (52.0%)	21/79 (26.6%)	5/7 (71.4%)	48/155 (31.0%)
Chloramphenicol (C)	0/4 (0%)	0/4 (0%)	0/11 (0%)	0/10 (0%)	0/13 (0%)	3/25 (12.0%)	4/79 (5.1%)	0/7 (0%)	7/155 (3.2%)
Multi-drug resistant	0/4 (0%)	0/4 (0%)	3/11 (27.3%)	0/10 (0%)	8/13 (61.5%)	10/25 (40.0%)	36/79 (45.6%)	2/7 (28.6%)	59/155 (38.0%)
ESBL positive	0/4 (0%)	0/4 (0%)	3/11 (27.3%)	0/10 (0%)	12/13 (92.3%)	14/25 (56.0%)	55/79 (69.6%)	2/7 (28.6%)	86/155 (55.5%)

Figure 3.3 shows the antibiotic resistant profiles of 135 *E. coli* isolates, visualised using an UpSet plot. Most isolates eligible for antibiotic screening were from site F, where treated effluent flowed into the Manawatū River. Site F isolates had the most diverse range of antibiotic resistance profiles amongst all isolates tested.

Resistance to the combination of ceftazidime (CAZ), cefotaxime (CTX) and cefoxitin (FOX) was the most observed profile amongst the isolates (21/135,15.6%). The second most observed resistance profile was the combination of ceftazidime (CAZ) and cefotaxime (CTX) (12/135, 8.9%). From this plot, it can also be seen that there were isolates resistant to streptomycin (S) and not gentamicin (GM) or *vice versa*. All three isolates that were resistant to nitrofurantoin (NI) were also resistant to ceftazidime (CAZ) and cefotaxime (CTX). Recorded zone diameters for the environmental *E. coli* antibiotic resistance disc diffusion tests can be found in Appendix 7.

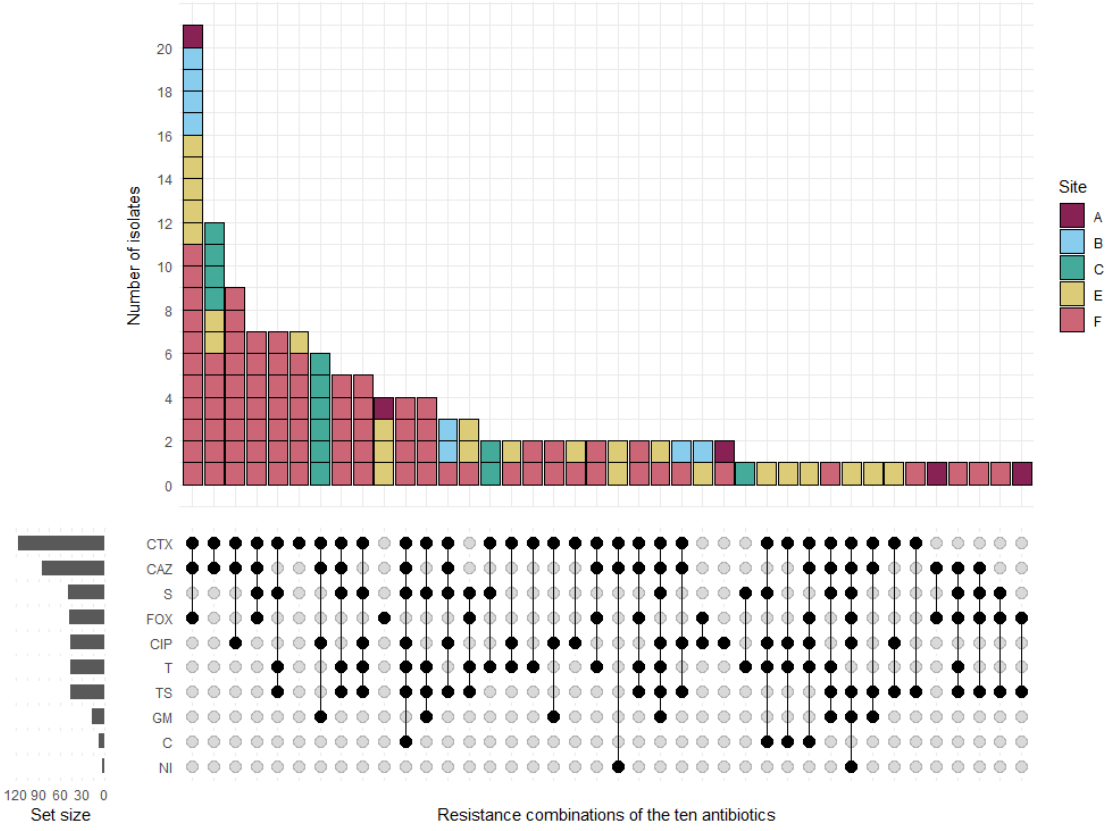

Figure 3.3: Upset plot of environmental isolates antibiotic resistance profiles in relation to collection site along the Manawatū River. (Key to antibiotic abbreviations: CTX = Cefotaxime, CAZ = Ceftazidime, TS = Trimethoprim/sulfamethoxazole, GM = Gentamicin, T = Tetracycline, CIP = Ciprofloxacin, NI = Nitrofurantoin, S = Streptomycin, FOX = Cefoxitin, C = Chloramphenicol). There were no *E. coli* isolates from sample site D that were isolated from the antibiotic agar or ESBL chromogenic agar.

In total, 311/359 (86.6%) clinical *E. coli* isolates and 86/121 (71.1%) environmental *E. coli* isolates were confirmed to be ESBL-producing. Recorded zone diameters for the clinical and environmental *E. coli* ESBL confirmation tests can be found in Appendix 5 and 7 respectively.

3.5 Sample level prevalence of antibiotic resistant, multi-drug resistant and ESBL-producing *E. coli*

E. coli was isolated from 146 of the 174 samples collected over the sampling period. Of the samples from the Manawatū River itself (sample sites A, B, D and F) sample site F had the highest number of ESBL-producing *E. coli* positive samples (17/40, 42.5%) (downstream of the treated effluent outflow) (Table 3.5). However, samples at sites C (stormwater) and E (treated effluent) had the highest numbers of ESBL-producing *E. coli*, with 50.0% of all samples positive for ESBL-producing *E. coli*. Every sample site (except site D) had at least one sample that was positive for putative third generation cephalosporin resistant *E. coli* that were resistant to at least one antibiotic tested. Multi-drug resistant *E. coli* was detected in samples sites B (urban setting upstream of the treated effluent outflow), C (urban stormwater drain, upstream of the treated effluent outflow), E (treated effluent), and F (downstream of the treated effluent outflow).

Table 3.5: The number of samples positive for antibiotic resistant *E. coli*.

Sample site	**A**			**B**			**C**	**D**			**E**	**F**		
	Sediment	Water	Total	Sediment	Water	Total	Storm water	Sediment	Water	Total	Treated effluent	Sediment	Water	Total
ESBL	0/12 (0.0%)	0/28 (0.0%)	0/40 (0.0%)	0/13 (0.0%)	1/28 (3.6%)	1/41 (2.4%)	3/6 (50.0%)	0/11 (0.0%)	0/22 (0.0%)	0/33 (0.0%)	7/14 (50.0%)	1/12 (8.3%)	16/28 (57.1%)	17/40 (42.5%)
Antibiotic resistant [a]	2/12 (16.7%)	1/28 (3.6%)	3/40 (7.5%)	2/13 (15.4%)	1/28 (3.6%)	3/41 (7.3%)	3/6 (50.0%)	0/11 (0.0%)	0/22 (0.0%)	0/33 (0.0%)	7/14 (50.0%)	2/12 (16.7%)	18/28 (64.3%)	20/40 (50.0%)
Multi-drug resistant	0/12 (0.0%)	0/28 (0.0%)	0/40 (0.0%)	0/13 (0.0%)	1/28 (3.6%)	1/41 (2.4%)	2/6 (33.3%)	0/11 (0.0%)	0/22 (0.0%)	0/33 (0.0%)	6/14 (42.9%)	1/12 (8.3%)	12/28 (42.9%)	13/40 (32.5%)

[a] Resistant to at least one of the ten antibiotics used to screen the samples.

4 Results - Genomic analysis

4.1 Overall genome characteristics

In total 234 isolates were whole genome sequenced, 189/234 (80.8%) were clinical isolates, and 45/234 (19.2%) were environmental isolates. All isolates were identified as *E. coli* with the smallest genome size being 4,537,231 bp and the largest as 5,455,391 bp. Contig numbers ranged between the smallest being 61 (EH0388a) and the largest number being 468 (EH0095a) for an isolate. The GC content across the isolates ranged from 50.37% to 51.12%. A full summary table containing isolate information can be found in Appendix 8.

Table 4.1 demonstrates the number of isolates with each resistance gene grouped by class of antibiotic. The most common type of ESBL gene across both the clinical and environmental *E. coli* isolates was $bla_{\text{CTX-M-15}}$ (107/234, 45.7%) followed by $bla_{\text{CTX-M-27}}$ (94/234, 40.2%). The ESBL gene $bla_{\text{CTX-M-15}}$ was detected in a higher proportion in the environmental isolates (28/45, 62.2%) compared with the clinical isolates (79/189, 41.8%), which harboured a higher proportion of $bla_{\text{CTX-M-27}}$ (86/189, 45.5%). Isolate EH0394a tested positive for ESBL production but did not have an ESBL $bla_{\text{CTX-M}}$ gene present. However, EH0394a does have the gene $bla_{\text{SHV-12}}$, which can provide ESBL production and resistance to third generation cephalosporins (29, 148).

There were 11 isolates with plasmid associated *ampC* genes, 9/11(81.8%) clinical and 2/11 (18.2%) environmental isolates. A full table of all the resistance genes with associated counts and proportions can be found in Appendix 9.

Table 4.1: Antibiotic resistance genes detected in clinical and environmental *E. coli*, grouped by antibiotic class.

Antibiotic class and genes	Clinical isolate totals per gene	Clinical overall resistance per antibiotic class	Environmental isolate totals per gene	Environmental overall resistance per antibiotic class	All isolate totals per gene	All overall resistance per antibiotic class
Aminoglycosides						
aac	78/189 (41.3%)	140/189 (74.15)	6/45 (13.3%)	22/45 (48.9%)	91/234 (38.9%)	162/234 (69.2%)
aad	110/189 (52.9%)		4/45 (8.9%)		125/234 (53.4%)	
ant	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
aph	89/189 (47.1%)		13/45 (28.9%)		102/234 (4.26%)	
Beta-lactam substrates						
Narrow spectrum beta-lactamase						
bla$_{\text{TEM-1}}$	78/189 (41.3%)	78/189 (41.3%)	9/45 (20.0%)	9/45 (20.0%)	87/234 (37.2%)	87/234 (37.2%)
ESBL						
bla$_{\text{CTX-M-1}}$	4/189 (2.1%)	189/189 (100%)	0 (0%)	45/45 (100%)	4/234 (1.7%)	234/234 (100%)
bla$_{\text{CTX-M-121}}$	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{\text{CTX-M-14}}$	17/189 (9%)		7/45 (15.6%)		24/234 (10.3%)	
bla$_{\text{CTX-M-15}}$	79/189 (41.8%)		28/45 (62.2%)		107/234 (45.7%)	
bla$_{\text{CTX-M-27}}$	86/189 (45.5%)		8/45 (17.8%)		94/234 (40.2%)	
bla$_{\text{CTX-M-3}}$	2/189 (1.1%)		0 (0%)		2/234 (0.9%)	
bla$_{\text{CTX-M-55}}$	1/189 (0.5%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{\text{SHV-12}}$	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
bla$_{\text{TEM-235}}$	5/189 (2.6%)		0 (0%)		5/234 (2.1%)	
bla$_{\text{TEM-30}}$	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
AmpC						
bla$_{\text{CMY-138}}$	0 (0%)	9/189 (4.8%)	1/45 (2.2%)	2/45 (4.4%)	1/234 (0.4%)	11/234 (4.7%)
bla$_{\text{CMY-2}}$	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{\text{DHA-1}}$	9/189 (4.8%)		0 (0%)		9/234 (3.8%)	

Oxacillinases						
bla$_{OXA-1}$	22/189 (11.6%)	28/189 (14.8%)	5/45 (11.1%)	6/45 (13.3%)	27/234 (11.5%)	34/234 (14.5%)
bla$_{OXA-10}$	6/189 (3.2%)		1/45 (2.2%)		7/234 (3.0%)	
Chloramphenicol						
cat	16/189 (8.5%)	19/189 (10.1%)	1/45 (2.2%)	4/45 (8.9%)	17/234 (7.3%)	23/234 (9.8%)
cml	6/189 (3.2%)		3/45 (6.7%)		9/234 (3.8%)	
Trimethoprim/sulfamethoxazole						
dfrA	133/189 (70.4%)	133/189 (70.4%)	20/45 (44.4%)	20/45 (44.4%)	153/234 (65.4%)	153/234 (65.4%)
sul1	122/189 (64.6%)	144/189 (76.25)	13/45 (28.9%)	18/45 (40%)	135/234 (57.7%)	162/234 (69.2%)
sul2	101/189 (53.4%)		15/45 (33.3%)		116/234 (49.6%)	
Quinolones						
qep	1/189 (0.5%)	23/189 (12.2%)	0 (0%)	18/45 (40%)	1/234 (0.4%)	41/234 (17.5%)
qnr	22/189 (11.6%)		18/45 (40%)		40/234 (17.1%)	
Tetracycline						
tet(A)	98/189 (51.9%)	122/189 (64.6%)	14/45 (31.1%)	16/45 (35.6%)	112/234 (47.9%)	128/234 (54.7%)
tet(B)	20/189 (10.6%)		3/45 (6.7%)		23/234 (9.8%)	
tet(D)	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
Macrolides						
ere	1/189 (0.5%)	119/189 (63%)	0 (0%)	14/45 (31.1%)	1/234 (0.4%)	133/234 (56.8%)
erm	8/189 (4.2%)		1/45 (2.2%)		9/234 (3.8%)	
mph	118/189 (62.4%)		13/45 (28.9%)		131/234 (56.0%)	
msr	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
Fosfomycin						
fosA	0 (0%)	0 (0%)	2/45 (4.4%)	2/45 (4.4%)	2/234 (0.9%)	2/234 (0.9%)

4.2 Genetic diversity of ESBL-producing *E. coli*

To determine genetic relationships between the isolates, whole genome MLST (wgMLST) was performed. From the unrooted phylogenetic tree, displayed in Figure 4.1, there is distinct clustering present by sequence type (ST). The wgMLST tree (Figure 4.2) displays the full distribution of sequence types across all isolates.

The bar chart around the perimeter of the phylogenetic tree in Figure 4.2 provides the total number of resistance genes present for each given isolate. The genomes did not group by resistance gene type or sample origin. The environmental isolates (prefixed with “SB”) were distributed throughout the phylogenetic tree, demonstrating the diversity of these isolates.

There was evidence of clonal sharing of genes between clinical and environmental isolates. This was determined by the pairwise difference being less than or equal to ten loci shared between the isolates, potentially indicating a transmission event (149, 150).

Approximately half of the isolates were typed as ST131 (107/234, 45.7%), with the next most common types being ST1193 (18/234, 7.6%), ST69 (17/234, 7.3%), ST38 (14/234, 6.0%), ST648 (13/234, 5.6%), and ST988 (12/234, 5.1%). There were 21/234 (9.0%) isolates that were singleton sequence types, 16/24 were clinical and 8/24 of these were environmental isolates.

The observed sequence types were plotted over time in Figure 4.3. ST131 was dominant across all the collection months. Singleton sequence types were found across the whole sampling period. There appeared to be no clear trends for any specific sequence type to any specific time of the year.

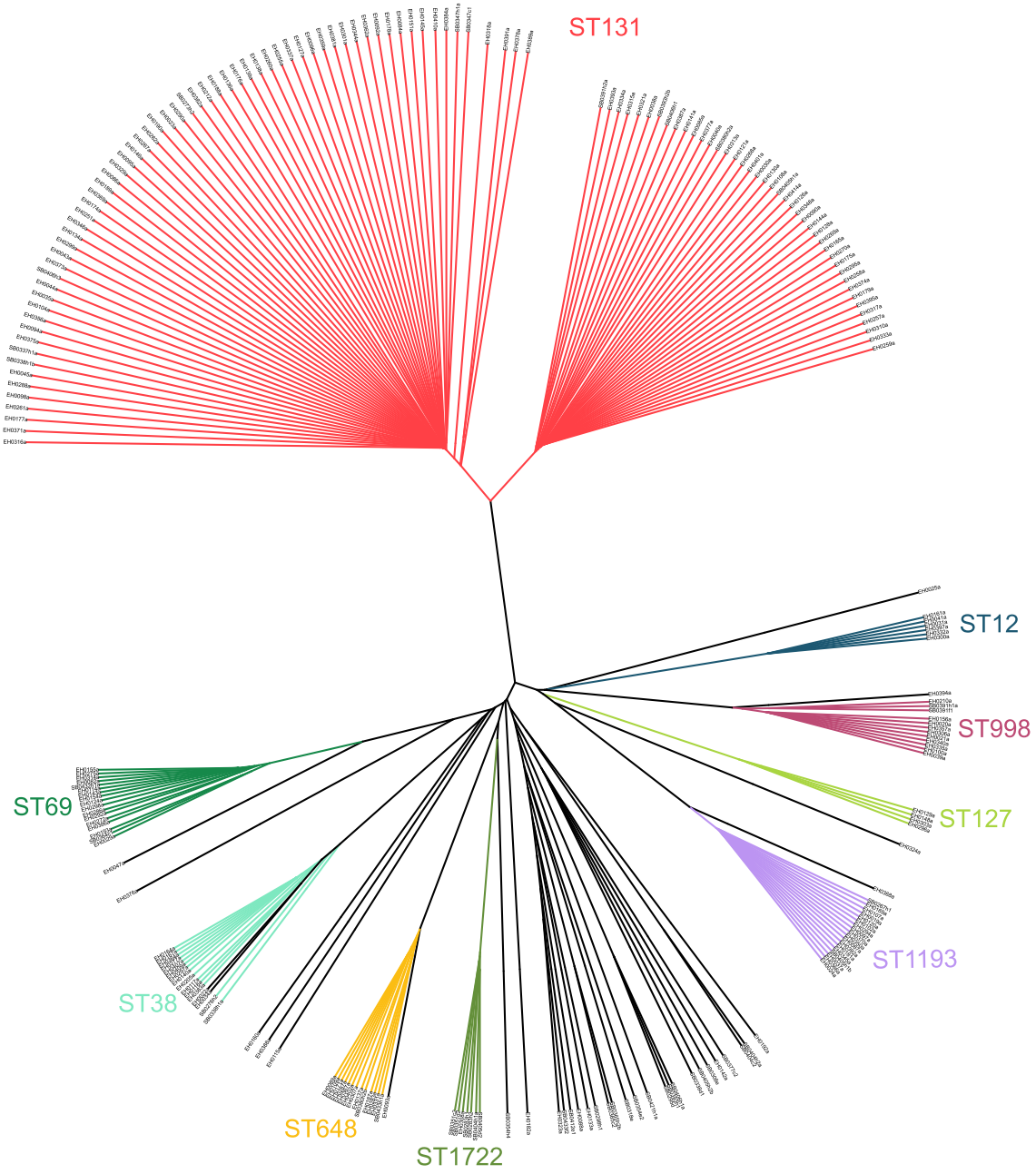

Figure 4.1: An unrooted wgMLST tree of 234 genomic sequences with 2331 loci shared. The tree was produced using Fast-GeP, constructed with SplitsTree using the neighbour-joining model and visualised in iTOL.

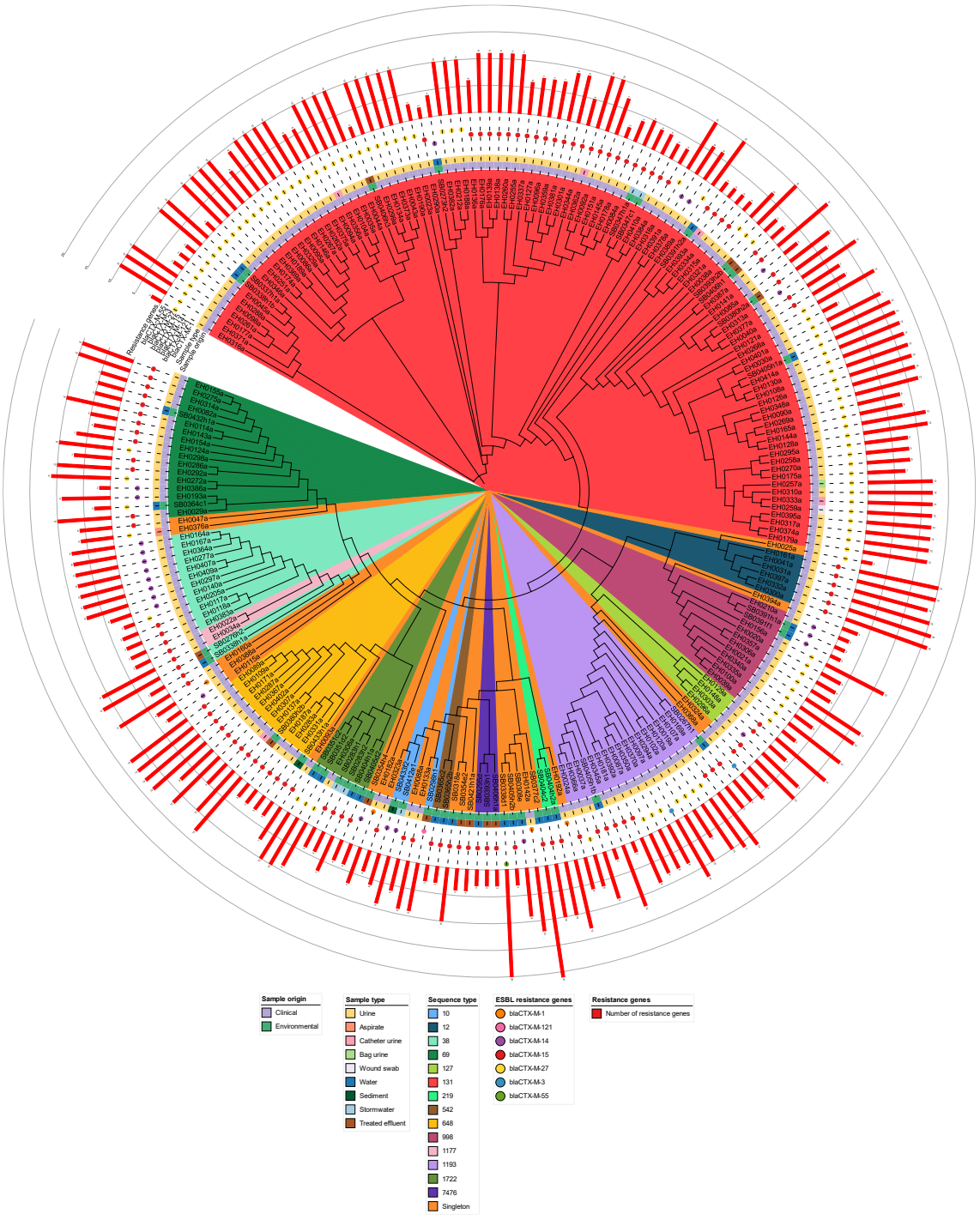

Figure 4.2: A wgMLST tree of 234 genomic sequences with 2331 loci shared. The tree was produced using Fast-GeP, constructed with SplitsTree using the neighbour-joining model and visualised in iTOL.

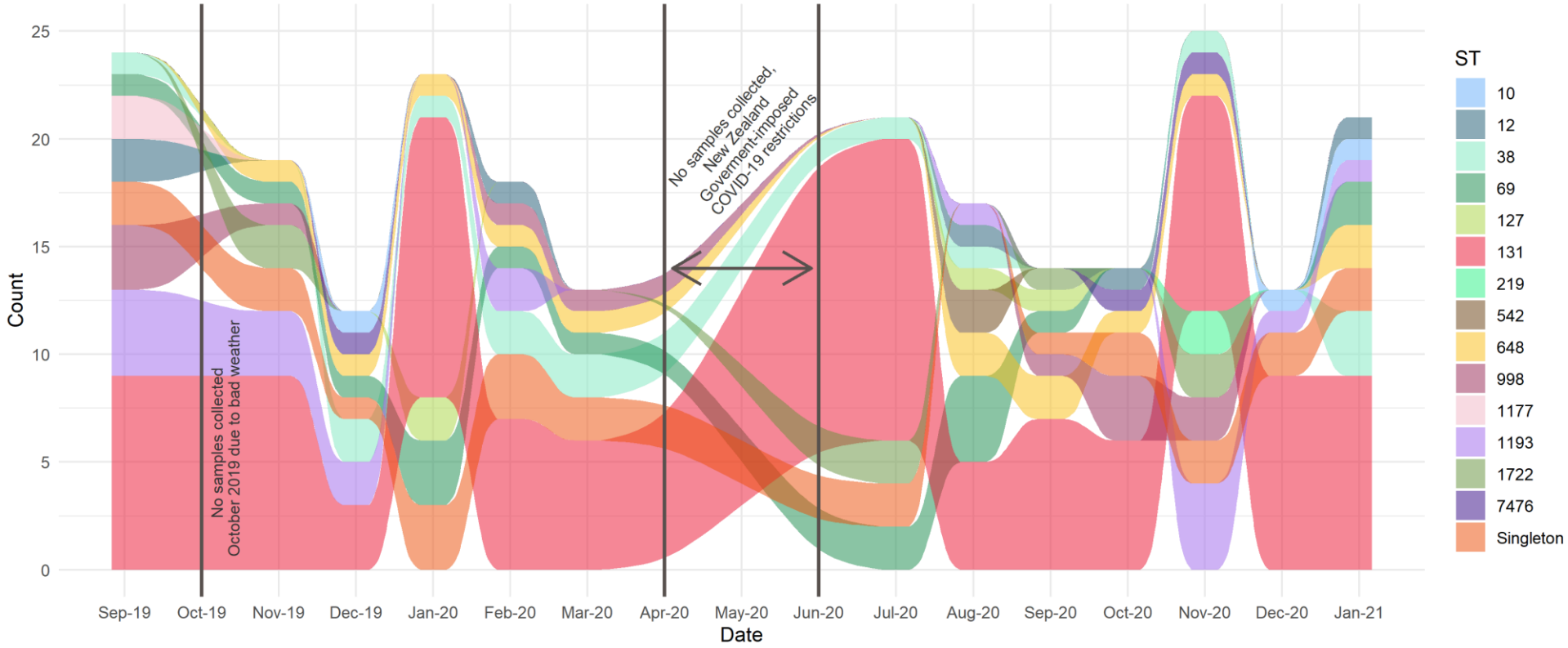

Figure 4.3: Number of sequence types over time from clinical and environmental isolates sequenced.

4.3 SNP phylogeny of the environmental isolates

The genetic relationship between the environmental isolates showed there was distinct clustering by sequence type (Figure 4.4). ST131 (11/45, 24.4%) was the most common sequence type detected, followed by ST1722 (6/45, 13.3%). There were also three isolates of ST7476, this sequence type being unique to the environmental isolates in this study. ST7476 was detected in treated effluent, water and sediment over the months of September, October, and November of 2020, showing persistence over time. ST131 was also detected sporadically throughout the sampling period, but there was no clear seasonal trend. ST131 isolates were detected in river water, stormwater, and treated sewage, but not sediment. ST10 isolates were detected in treated effluent, sediment and stormwater from collection sites E, F and C respectively.

The most predominant ESBL gene across the isolates was *bla*$_{\text{CTX-M-15}}$ (28/45, 62.2%), there were no isolates with the gene *bla*$_{\text{CTX-M-1}}$ or *bla*$_{\text{CTX-M-3}}$. There was variation of the resistome profile amongst the environmental ESBL-producing *E. coli* isolates. The ST131 isolates did not present any resistance genes against chloramphenicol, or quinolones. There were two isolates (SB406h1 and SB0377c2) that each had *bla*$_{\text{CMY}}$, plasmid mediated AmpC. The sequence types for the isolates are ST131 and ST2079 respectively.

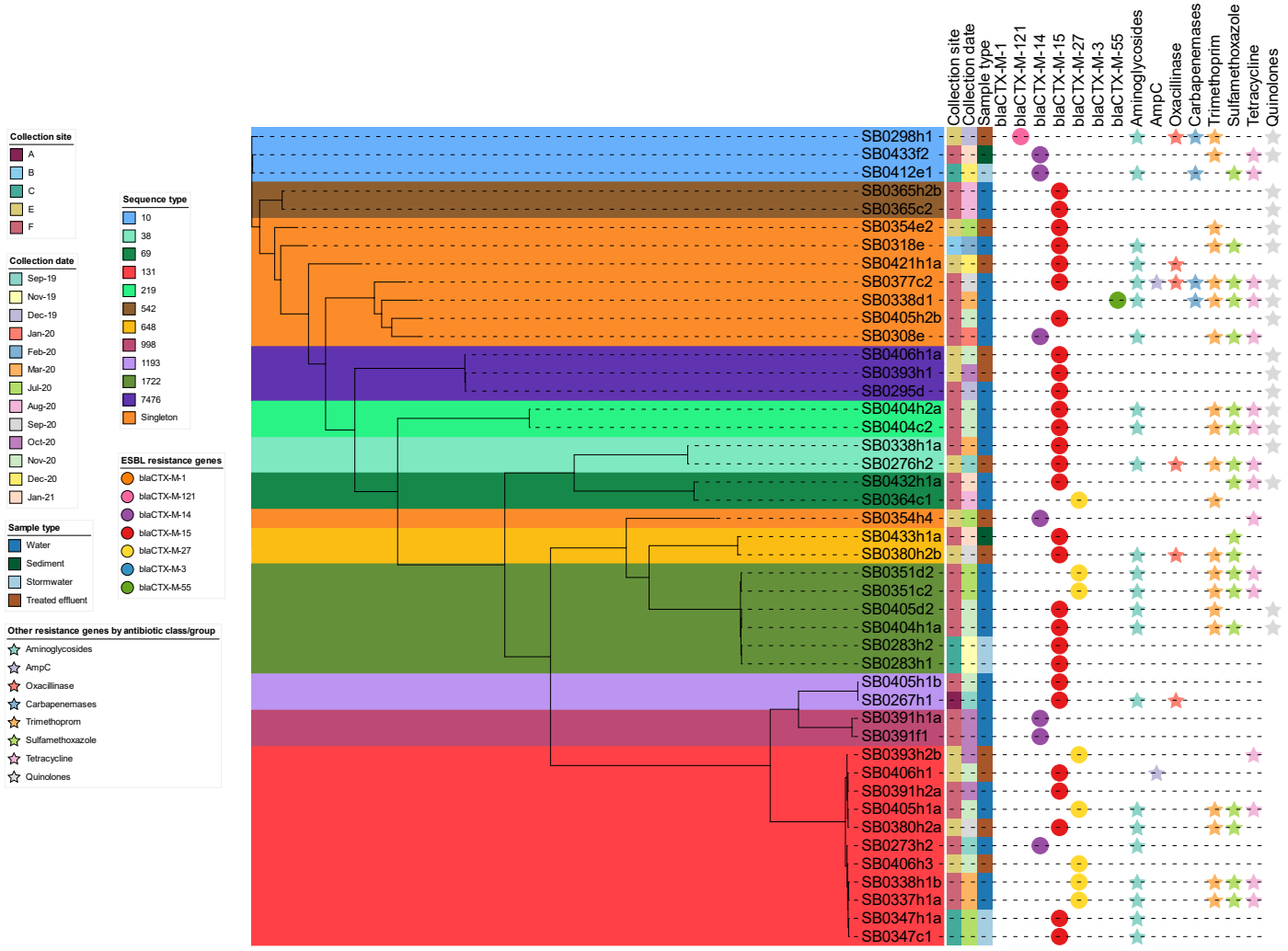

Figure 4.4: Core SNP phylogeny of 23,642 SNPs for the environmental isolates produced using Snippy with SB0391f1 used as the reference genome. The tree was constructed with FastTree using maximum-likelihood GTR model and visualised in iTOL.

4.4 SNP phylogeny of the ST131 isolates

Isolates that were ST131 were delegated into clades by using reference genomes of known clade types. The unrooted tree in Figure 4.5 was split into three clades A, B and C. The largest group was classified as clade C, containing 60/107 (56.1%) isolates, followed by clade A, 43/107 (40.2%) isolates and clade B 4/107 (3.7%) isolates.

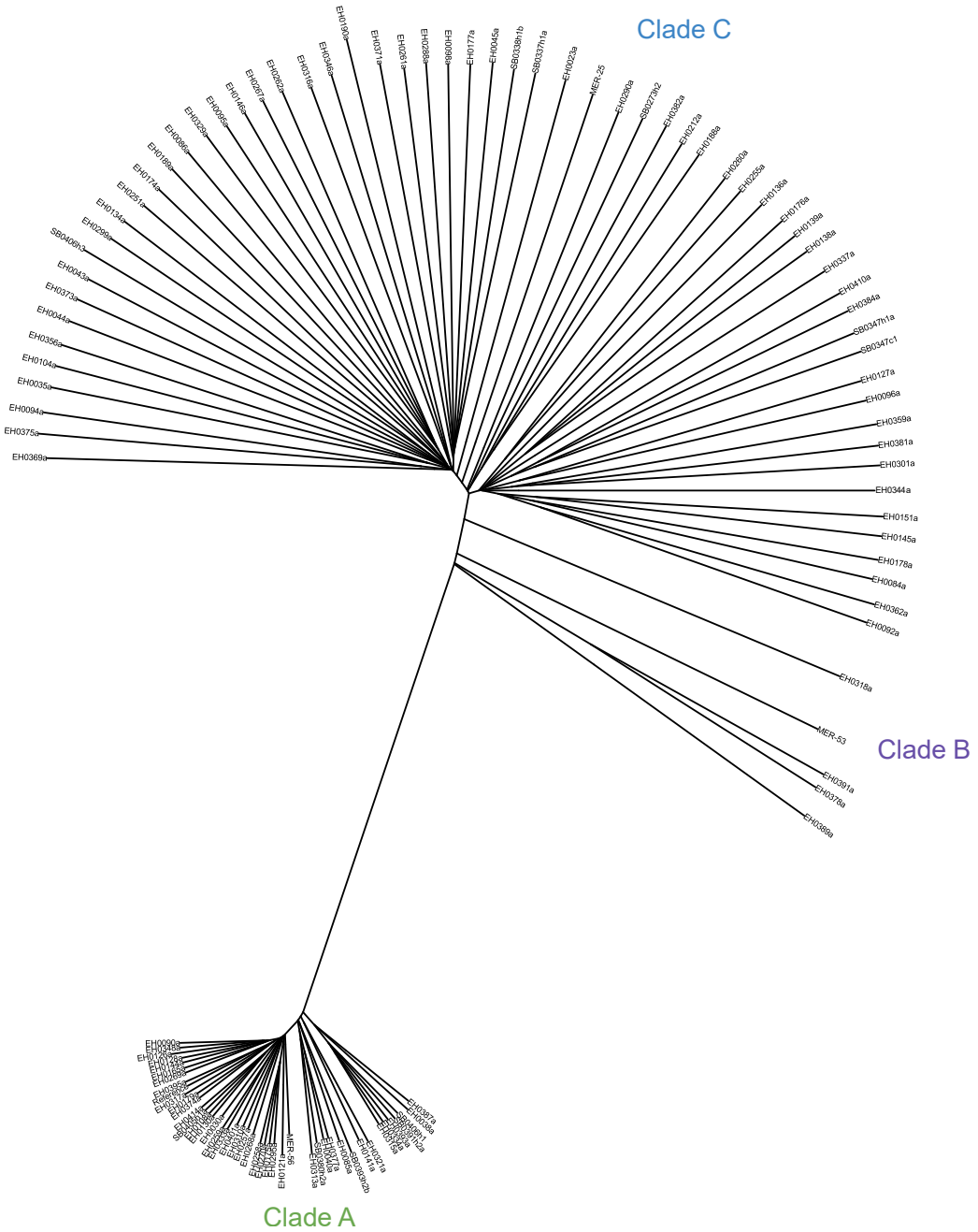

Figure 4.5: Unrooted core SNP phylogeny of 2254 SNPs for the ST131 isolates, comprising clades A, B, and C. A core SNP alignment was produced using Snippy with EH0395a used as the reference genome. The tree was constructed with FastTree using the maximum-likelihood GTR model and visualised in iTOL. The genomes used as references for clades A, B and C were MER-56 (SRR5936479), MER-53 (SRR5936492), and MER-25 (SRR5936501) respectively from the study conducted by Harris *et al.* (118).

Figure 4.6 displays the genetic relationships of the ST131 isolates across clade A, B and C, showing that there is clear clustering by clade but not collection date (column 4). The environmental isolates (predominantly isolated from water and treated effluent) were distributed throughout the phylogenetic tree. The predominant ESBL gene variant was *bla*$_{\text{CTX-M-27}}$ (68/107, 63.6%), followed by *bla*$_{\text{CTX-M-15}}$ (34/107, 31.8%) and *bla*$_{\text{CTX-M-14}}$ (5/107. 4.7%).

Next, I investigated whether there were mutations in chromosomal genes that were associated with resistance in the ST131 isolates. The genes of main interest were *parC* and *gyrA* as point mutations in these genes confer resistance to nalidixic acid and ciprofloxacin (151). Two mutations in the *gyrA* gene were needed to qualify those isolates as having clinically important resistance and 66/107 (61.7%) had these mutations (151). There were 67/107 (62.6%) with a mutation in the *parC* gene. Additionally, I investigated if there were mutations in the promoter region of the chromosomal *ampC* gene, as this would convey resistance to second and third generation cephalosporins (23). There were no mutations detected in the promoter region of the *ampC* gene. Virulence genes were included as a heat map and were selected based on what is considered of importance in uropathogenic infections (152-155). Following the criteria stated by Johnson *et al.* (155) and Spurbeck *et al.* (154), two of the four genes, *chuA*, *fyuA*, *vat*, and *yfcV*, were needed to deem the isolate uropathogenic. All ST131 isolates had the virulence genes *chyA* and *fyuA*, therefore deeming all ST131 isolates uropathogenic.

There were three ESBL-producing *E. coli* ST131 isolates seen to indicate near clonal relatedness, by having seven SNPs different between the clinical isolate EH0177a and both the environmental isolates SB0337h1a and SB0338h1b (from the core SNP phylogeny of 234 isolates). The clinical isolate (EH0177a) was collected on the 24th of February 2020 and the environmental isolates (SB0337h1a and SB0338h1b, both water isolates from site F) were collected on the 8th of March 2020.

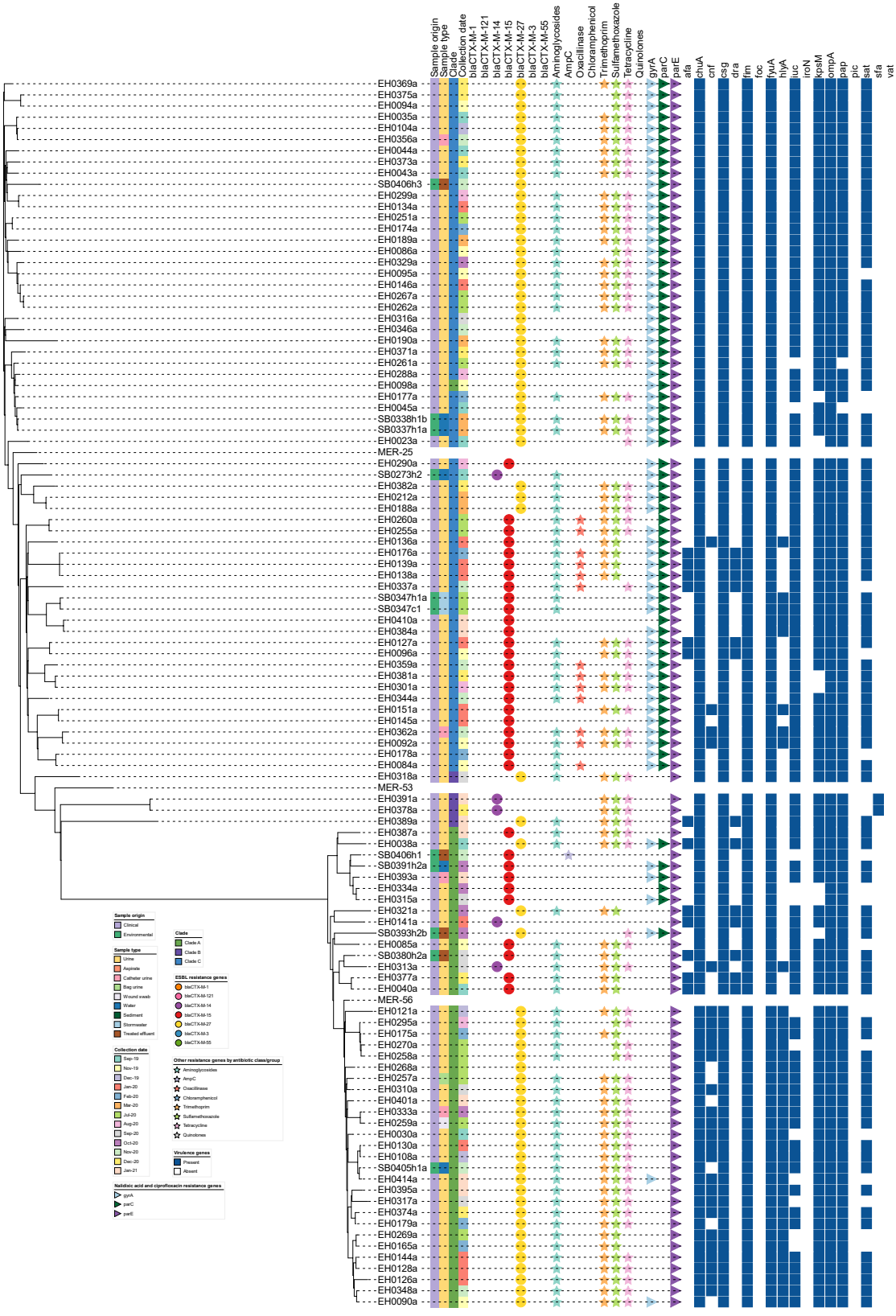

Figure 4.6: Core SNP phylogeny of ST131 isolates, clade A, B and C. The Tree was produced using Snippy with EH0395a used as the reference genome. The phylogenetic tree was constructed with FastTree using the maximum-likelihood GTR model and visualised in iTOL. The genomes used as references for clades A, B and C were MER-56 (SRR5936479), MER-53 (SRR5936492), and MER-25 (SRR5936501) respectively from the study conducted by Harris *et al.* (118).

5 Discussion

5.1 Summary of findings

The study aimed to compare the genetic relationship of ESBL-producing *E. coli* from the Manawatū River and MidCentral District. There were two hypotheses of this study, firstly, that treated effluent was a source of antibiotic resistant *E. coli*, and secondly that antibiotic resistant *E. coli* from the Manawatū River were genetically related to human clinical isolates. To address these aims and hypotheses, whole genome sequencing was carried out on ESBL-producing *E. coli* and these ESBL-producing *E. coli* were characterised using various comparative genomic tools. This allowed isolates from humans to be assessed as potential sources of ESBL-producing *E. coli* in the environment.

The analyses determined that treated effluent and the point it flows into the Manawatū River is a source of antibiotic resistance, and amongst the antibiotic resistant *E. coli* there was a high incidence of MDR (resistance shown to antibiotics from three different classes). It was also observed that the clinical and environmental ESBL-producing *E. coli* from both the environment and humans contained the same resistance genes, and ST131 was the dominant sequence type. There was limited evidence of the environmental and clinical isolates being genetically related, and further sampling would be needed to determine the frequency of transmission events.

5.2 Antibiotic resistance detected in waterways

The antibiotic resistant *E. coli* isolated from the Manawatū River displayed a variety of different resistance phenotypes and genotypes, highlighting the potential impact of discharging treated effluent into the river. Multi-drug resistant and ESBL-producing *E. coli* were isolated from four (B – within Palmerston North City; C – the stormwater drain; E – treated effluent; and F – downstream of the treated effluent) of the six sample sites. Sample sites A and D had no multi-drug resistant or ESBL-producing *E. coli* isolated. Sample site A was upstream of Palmerston North City which could explain this finding, however, sample site D is in Palmerston North City, so further sampling of this site might be beneficial to clarify this finding.

The samples from sites C (stormwater) and E (treated effluent) had the highest numbers of ESBL-producing *E. coli*, 3/6 (50%) and 7/14 (50%) respectively. Sample site F (downstream of the treated effluent outflow) had the greatest number of samples tested for ESBL-producing *E. coli* as there were more samples of water and sediment collected over the sampling period, with 42.5% of samples being positive for ESBL-producing *E. coli*. This is not surprising as it is known that wastewater treatment is not completely effective at removing antibiotic resistant bacteria (156-158) and a previous study by Fagerström *et al.* (90) also isolated *E. coli* from the immediate river where treated effluent was discharged. As stated by Pattis *et al.* (159) there is little information available regarding antibiotic bacteria in wastewater treatment plants in New Zealand

and is an area that needs further research. However, there has been a previous study conducted in New Zealand that detected the *bla*$_{CTX-M}$ gene in sediment samples from rivers in Canterbury (160).

E. coli isolates from the water at site F showed resistance to 9/10 antibiotics (except nitrofurantoin) used in the screening panel. A study conducted in Tunisia also took samples downstream of the wastewater treatment plant and found *E. coli* resistant to nalidixic acid, ciprofloxacin, sulphonamide, trimethoprim/sulfamethoxazole, tetracycline, gentamicin, and chloramphenicol were present (59).

The methods used in my study selected for third generation cephalosporin resistant *E. coli*; therefore, as expected the ESBL-producing *E. coli* were present in greater numbers compared to other antibiotic resistance phenotypes. It is not thus surprising that the highest numbers of antibiotic resistance were observed for cefotaxime and ceftazidime. My findings agree with other studies where there was a greater frequency of ESBL-producing *E. coli* resistant to cefotaxime and to a lesser extent ceftazidime (58, 91, 161). These previous studies also screened samples on selective antibiotic containing media to give a greater chance of ESBL-producing *E. coli* being isolated.

Resistance to nitrofurantoin (3/155,1.9% of *E. coli* isolates) was only observed in isolates from the site E (treated effluent) samples, whereas trimethoprim/sulfamethoxazole resistance (46/155, 29.7% of *E. coli* isolates) was observed in isolates from the samples from sites A, B, E and F, which included water, sediment and treated effluent sources. Both nitrofurantoin and trimethoprim/sulfamethoxazole are first line treatments of UTIs for humans in New Zealand (52); thus, antibiotic resistance to these antibiotics in environmental isolates is concerning as these antibiotics are of human clinical importance.

Previous studies have also isolated *E. coli* from wastewater that are resistant to a variety of antibiotics including nitrofurantoin and trimethoprim/sulfamethoxazole (58, 91). Studies from Canada and Norway have also reported trimethoprim/sulfamethoxazole resistant *E. coli* in treated effluent (89, 91). A study looking at all stages of the wastewater treatment process in Tunisia supports the finding that resistance in *E. coli* to trimethoprim/sulfamethoxazole is present in the incoming raw sewage and persists throughout the treatment process (59). Nitrofurantoin resistant *E. coli* have been isolated from treated effluent in South Africa (12/223, 5.4% of *E. coli* isolates) and Norway (6/91, 7.0% of *E. coli* isolates) (91, 162). The study by Adefisoye and Okoh (162) did not culture the *E. coli* isolates on an antibiotic-containing agar so were not presumptive ESBL producers, however, Jørgensen *et al.* (91) screened *E. coli* using chromogenic agar before antibiotic susceptibility testing and therefore were presumptively ESBL-producing, similar to the isolates tested in my study.

There were many similarities and differences between the phenotypic (disc diffusion tests) and genotypic results (sequencing data) of the environmental isolates. All environmental *E. coli* isolates that were confirmed as ESBL-producing by confirmation ESBL disc diffusion tests had an ESBL gene. However, there were some discrepancies where resistance was seen in disc

diffusion tests, but a resistance gene was not seen in the sequencing results or *vice versa*. Trimethoprim/sulfamethoxazole resistant *E. coli* isolates (17/45, 37.8%) had a *dfr* and/or a *sul* gene present. However, there were six isolates that had either a *dfr* and/or a *sul* present in sequencing results but did not present with trimethoprim/sulfamethoxazole resistance in disc diffusion tests.

The screening panel of ten antibiotics used to test antibiotic resistance of the environmental isolates, included two aminoglycosides, gentamicin and streptomycin. Some isolates showed resistance to one but not both of these aminoglycoside antibiotics. Whole genome sequencing revealed that some isolates harboured different aminoglycoside resistance enzymes. Acetylation (ACC) enzyme types are mostly associated with gentamicin resistance, however, adenylylation (ANT) and aminoglycoside phosphotransferase (APH) enzymes have many different genes some of which provide resistance to gentamicin and others to streptomycin (163). For example, the *ant(2")-I* gene provides resistance to gentamicin, tobramycin, dibekacin, and sisomicin, whereas *ant(6)-I* provides resistance to streptomycin (163). One environmental isolate (SB0377c) had resistance to only streptomycin from the disc diffusion tests, and had the resistance gene *aph(6)-Id,* supporting the observed resistance. However, SB0377c2 also had the resistance gene *ant(2")-Ia* which would provide resistance to gentamicin, but gentamicin resistance was not seen phenotypically. There were additionally two *E. coli* isolates that showed resistance to cefoxitin in disc diffusion tests but did not have an AmpC associated resistance gene or chromosomal mutation. An AmpC confirmation disc diffusion test would clarify the phenotypic result. The difference seen between phenotypic and genotypic results highlights the importance of confirmation tests, followed by genome sequencing. Additional and deeper investigation of sequencing results could reveal mutation or changes to the resistance gene that could be affecting the resulting active site of the enzyme, and therefore the phenotypic presentation. Further investigation is required in this area to fully understand why these differences have occurred as it has important clinical reporting implications.

5.2.1 Antibiotic resistance genes present in *E. coli* from the Manawatū River

In New Zealand, there is little information about antibiotic resistance genes from *E. coli* isolates found in the environment. Therefore, this study was conducted in part to fill this knowledge gap. The most common ESBL resistance gene was *bla*$_{\text{CTX-M-15}}$ and resistance genes for the antibiotic classes aminoglycosides, beta-lactams (these include gene encoding for AmpC, and oxacillinase enzymes), chloramphenicol, fosfomycin, macrolides, quinolones, tetracycline, and trimethoprim/sulfamethoxazole were detected in varying numbers for the environmental isolates.

It has previously been established that there is antibiotic resistant *E. coli* present in New Zealand freshwater environments (127, 164). However, to my knowledge, only three studies have undertaken whole genome sequencing on 19 isolates sourced from the New Zealand water and urban environment before this study (129, 130, 165). Across these 19 sequenced isolates ten isolates harboured CTX-M type enzymes, including the variants, *bla*$_{\text{CTX-M-15}}$ (5/19, 26.3%), *bla*$_{\text{CTX-}}$

$_{M\text{-}14}$ (3/19, 15.8%), *bla*$_{CTX\text{-}M\text{-}24}$ (1/19, 5.3%), and *bla*$_{CTX\text{-}M\text{-}3}$ (1/19, 5.3%). Additional resistance genes were identified that conferred resistance to aminoglycosides, chloramphenicol, macrolides, tetracycline, and trimethoprim/sulfamethoxazole (129, 130). Therefore, it had been previously established that antibiotic resistance is present in the New Zealand environment, and the results of this study support this finding. To my understanding, this study is the first in New Zealand that has taken a longitudinal approach to investigate the prevalence of ESBL-producing *E. coli* in the environment. Findings suggest that effluent is the main source of ESBL-producing *E. coli* and that there is a variety of ESBL-producing encoding genes detected over time, rather than the same genes persisting.

5.3 Distribution of antibiotic resistance genes in the clinical *E. coli* isolates

It is widely known that ST131, harbouring the CTX-M-15 ESBL type, is the dominant ST found worldwide (20). In my study the most frequently detected ESBL gene types from the clinical isolates were *bla*$_{CTX\text{-}M\text{-}27}$ (86/189, 45.5%), and *bla*$_{CTX\text{-}M\text{-}15}$ (79/189, 41.8%), with ST131 being the dominant sequence type recorded. A New Zealand survey of human clinical *Enterobacteriaceae* isolates carried out in 2016 found that the ESBL types CTX-M-15 (211/394, 53.6%) and CTX-M-27 (88/394, 22.3%) were predominant across isolates from the *Enterobacteriaceae* family (114). Results from Hapuarachchi *et al.* (117) supported this finding with types CTX-M-27 (18/65, 27.7%), CTX-M-14 (17/65, 26.1%), and CTX-M-15 (14/65, 21.5%) being the most common amongst the urinary ESBL-producing *E. coli* isolates collected from the Otago region.

In my study the clinical isolates were not screened in the same manner as the environmental isolates as they were received as presumptive ESBL producers from a clinical diagnostics laboratory. From the sequencing results, isolate EH0394a exhibited an ESBL phenotype, however, whole genome sequencing revealed that it did not contain a *bla*$_{CTX\text{-}M}$ gene, but instead contained the *bla*$_{SHV\text{-}12}$ gene. As SHV-12 has evolved from the narrow-spectrum parent type, this gene allows for ESBL production and therefore accounts for the resistance demonstrated in the ESBL confirmation disc diffusion test (23, 166).

5.4 Association of sequence types and collection source

MLST determined that the majority of the ESBL *E. coli* isolates in this study were ST131. It is well established that ST131 is associated with human disease particularly as an extraintestinal pathogen, and is a major cause of bloodstream and UTIs (20, 167-170). *E. coli* wastewater isolates have also been recorded with this sequence type (89, 91, 171). ST131 is known to be associated with human disease, therefore, this is another factor linking the clinical and environmental isolates, given that ST131 was the most frequent sequence type detected in this study. This indicates that humans potentially influence the presence of ST131 *E. coli* detected in the environment.

The other frequent STs detected in the environmental isolates for this study were ST1722, ST10, and ST7476. A previous study conducted in Sweden determined that ST131, ST38, and ST10 are present throughout treated effluent and where it flows into the river (90). The same study detected ST1722, but in the samples collected from the wastewater treatment plant (90). Another study conducted in Norway showed that ST131 and ST38 were common in urine and wastewater, whereas ST10 was present in the water samples and was only detected in one urine sample (91). Jørgensen *et al.* (91) acknowledged that ST10 had previously been described in humans and animals from other countries, but the prevalence in Norway would need to be investigated (172). This could be due to a low sample size of clinical isolates collected in that study. There were only three ST10 isolates detected in this study, and these were from environmental samples, no clinical ST10 isolates were detected. ST7476 was only found in the environmental isolates from treated effluent (site E) and water (site F). To my knowledge there is little evidence of this ST being reported, however, EnteroBase (https://enterobase.warwick.ac.uk/, accessed May 2022 (173, 174)) had three records for *E. coli* ST476, two of which were isolated from humans in the Netherlands and no metadata was available for the third isolate (172, 175). ST1722, as stated above, has been isolated from wastewater, but has also been detected in humans, and livestock according to EnteroBase (https://enterobase.warwick.ac.uk/, accessed May 2022 (173, 174)). My study detected ST1722 in both humans (1/189, 0.5%) and environmental (6/45, 13.3%) isolates which appears consistent with other studies examined (130, 176-178).

The frequency of ST131 clinical ESBL-positive *E. coli* isolates in this study was 96/189 (50.8%). The next most frequent STs observed were ST1193 (16/189, 8.5%) and ST69 (15/189, 7.9%). Previous studies assessing clinical ESBL-producing *E. coli* isolates found ST131 was also frequently reported, demonstrating the global dominance of this ST (169, 179, 180). However, there are a variety of other STs commonly detected with the prevalence seemingly varying across the countries of origin, these include ST73, ST38, ST69, ST10, ST127, ST95, and ST405 (90, 91, 169, 180-182). ST1193 is also an important global multi-drug resistant clone as reported by Pitout *et al.* (183). In their review, it stated that ESBL-producing *E. coli* ST1193 have been found in China, Germany, Thailand, and Bangladesh, with the authors concluding that it could be the next high risk ST like ST131 (183). ST69, ST73, and ST95 are common in UTIs and bloodstream infections, but do not harbour as many resistance determinants as ST131 (184, 185). Additionally, ST405, ST410, ST167, and ST648 are commonly associated with MDR (181, 186-188).

Other common UTI associated STs were also detected throughout the clinical ESBL-producing *E. coli* isolates of my study, these included ST38 (12/189, 6.3%), ST648 (11/189, 5.8%), ST127 (4/189, 2.1%), ST405 (1/189, 0.5%) and ST73 (1/189, 0.5%). However, these STs were not limited to the clinical isolates, ST1193, ST69, ST38, and ST648 all had two environmental ESBL-producing *E. coli* recorded for each of the specified STs.

In New Zealand ST131 remains the most prominent ST found in clinical specimens as reported by the two-yearly ESR surveys (177/293, 60.1%, (114)) and in a study by Hapuarachchi *et al.* (117) (27/65, 41.5%). Other STs such as ST38 (20/293, 6.8%) and ST1193 (20/293, 6.8%) were

also detected during ESR's survey (114). The study by Hapuarachchi *et al.* (117) also found ST38 (7/65, 10.8%) to be common among ESBL-producing *E. coli* from urine isolates within the region of Otago. There many STs associated with companion animals with some key ones including ST131, ST38, ST68, ST405, ST617 and ST648 but these do not account for the vast majority as reviewed by Salgado-Caxito *et al.* (189). The spread of STs detected in one species is demonstrated by ST1193, ST4553, ST746, ST2541 and ST10 being found in dogs alone by Toombs-Ruane *et al.* (97). There are also a variety of STs associated with dairy farms, such as ST648, ST58, ST410 and ST10 (121). The few examples above illustrate the variability of STs detected in different animal groups. Further sampling, including animals' sources, would be beneficial for determining differences in source prevalence of persistent *E. coli* sequence types in New Zealand.

5.5 Potential transmission of antibiotic resistant *E. coli*

5.5.1 Drivers of antibiotic resistance

The overuse and misuse of antibiotics is the main driver for the spread of bacteria harbouring antibiotic resistance genes, which can then spread from human and animal populations to the environment. My study looked at the antibiotic resistance phenotypes, genotypes, and prevalence of ESBL-producing *E. coli* from both human clinical and environmental sources. The antibiotic resistance profiles of the environmental and clinical ESBL-producing *E. coli* isolates of this study will indicate the potential of certain antibiotics having reduced efficacy for treating infections.

In New Zealand, a wide variety of antibiotics are used in both animals and humans, which could be a driver of MDR development in bacteria. Although it was not the focus of my study the use of antibiotics should also be considered to determine why multi-drug resistant and ESBL-producing *E. coli* are common in the community and environment. Information is available about the sales of antibiotics for the agricultural and veterinary sectors, which focuses on antibiotics that are critical for human health as well as the usage of antibiotics for humans in New Zealand (190-192). The use of antibiotics in animals should also be considered when determining potential drivers of antibiotic resistant *E. coli* prevalence in the environment. Overall antibiotic active ingredient sales of antibiotics (a proxy for use data as the data for actual usage in animals is not available) for use in animals decreased by 10.8% from 2018 to 2019, but human consumption of antibiotics remained constant over the 2011 to 2014 period (190-192). Penicillins and clavulanic acid (29.3%) had the second highest sales for animal consumption in 2019 (190). Cephalosporins were mainly used to treat cattle, however, it was reported that sales of third and fourth generation cephalosporins had decreased in 2019 due to instructions from the primary sector to limit the use of these antibiotic compounds. Data from Williamson *et al.* (192) reports that in humans the penicillin group (both narrow and broad-spectrum) had the highest consumption. There is information about cephalosporins and other beta-lactams, but this does not include third generation cephalosporins (192). Without information about human consumption rates of third

generation cephalosporins, it cannot be determined if consumption of these antibiotics is a driver for the spread of ESBL-producing *E. coli* in hospital and community settings.

5.5.2 The source and transmission of antibiotic resistance

It is important to determine the source of antibiotic resistance because mitigation strategies can be implemented to prevent further antibiotic resistance spread throughout the community and into the environment. In this study both the treated effluent and Manawatū River samples taken downstream of the effluent outlet showed high numbers of antibiotic resistant *E. coli*. This supports my hypothesis that humans are contributing to the antibiotic resistance observed in the Manawatū River. However, further sampling of animal populations in urban and rural areas is needed to see if any ESBL-producing *E. coli* animal isolates are related to environmental ESBL-producing *E. coli* isolates, particularly those upstream (sample sites A, B, C, and D) of sample sites E (treated effluent) and F (river sample downstream of the treated effluent), because the antibiotic resistance could be of animal origin. It also needs to be acknowledged that it is not just human waste that may be going into treated effluent, therefore it would be important to compare animal sourced isolates to those collected from treated effluent.

To determine the genetic relatedness and potential transmission events from humans to the Manawatū River, this study considered the date of sample collection, wgMLST allele differences and core SNP phylogeny. To potentially indicate a direct transmission event the clinical isolate and environmental sample had to be collected within a short time from one another and have less than or equal to ten allele differences or SNPs between the isolates (149, 150).

A notable event of potential transmission occurring is evidenced by the human clinical isolate EH0177a, and Manawatū River isolates SB0337h1a, and SB0338h1b. The clinical isolate was collected on the 24th of February 2020 and the environmental isolates were collected on the 8th of March 2020, hence there was 14 days difference between these dates. All three isolates were ST131 and there were seven SNPs difference (over 204,120 SNPs from the core SNP phylogeny of the 234 *E. coli* isolates) between the clinical isolate EH0177a and both the environmental isolates SB0337h1a and SB0338h1b cultured from a Manawatū River sample, downstream of the effluent outlet. To my knowledge there are only two previous studies that compared clinical and environmental isolates and have shown similar results to this study (using different methods), indicating that isolates found from water sources could be linked to human sources. A study by Fagerström *et al.* (90) compared ESBL-producing *E. coli* isolates from urine infections to those sourced from environmental waters and found that some isolates had less than ten allele differences using a core genome MLST approach (90). Although Fagerström *et al.* (90) acknowledged that the isolates could have originated from other sources, considering the genetic similarity of the ESBL coding genes the authors concluded that the ESBL-producing *E. coli* in the environment were likely due to humans (90).

A study conducted in Germany found that ST949 *E. coli* isolates collected from swimming and bathing sites were potentially related to human clinical ST949 isolates from New Zealand and

Sweden(193). This was determined by comparing the ST949 water isolates to human ST949 clinical isolates sourced from EnteroBase which originated from New Zealand and Sweden. Falgenhauer *et al.* (193) concluded that ST949 isolates sourced from the water site in their study were related to human clinical *E. coli* isolates. The authors acknowledged that the epidemiological link between the water isolates from German and the human clinical *E. coli* isolates from New Zealand and Sweden is not clear.

5.6 Limitations

The aim of this study was to compare the genetic relatedness of clinical and environmental isolates within the Manawatū River and MidCentral District. Therefore, it was important to match the collection date of both the environmental isolates and clinical isolates as closely as possible. However, only one potential transmission event was identified through whole genome sequence analysis of the clinical and environmental isolates. This may be due to the frequency of environmental sampling. The environmental samples were a single “grab sample” at a one-time point, once a month. In future, more frequent sampling, for example, every week, or over a day at different time points, would potentially allow for a greater variety of isolates to be collected and further comparisons to be made about antibiotic resistance spread between isolates sourced from humans and the Manawatū River. Additionally, there were no *E. coli* counts recorded from the environmental samples, so no correlations could be made between antibiotic resistance and total *E. coli* numbers. Further sampling throughout the wastewater treatment process would also be beneficial to see how many antibiotic resistant bacteria persist through treatment compared to the incoming raw sewage.

The study was also limited as the clinical isolates were not screened for resistance to the same ten antibiotics used for the environmental isolates. Therefore, nitrofurantoin resistance frequency is unknown amongst the clinical ESBL-producing *E. coli*. The clinical Enterobacterales isolates were received as presumptive ESBLs from a clinical diagnostics laboratory, but only 311/359 (86.6%) of the *E. coli* were confirmed to be ESBL-positive. Additionally, AmpC prevalence amongst both the clinical and environmental isolates was not tested. The lower number of ESBL-positive isolates was expected as the antibiotic cefepime was not included in the double disc confirmation assay. Cefepime would be beneficial to include in the double disc diffusion tests as this would allow testing for ESBL production in the presence of AmpC enzymes. This is important to consider because it is known that the presence of AmpC enzymes can mask the results of the ESBL confirmation test (24). This occurs due to AmpC enzymes breaking down the inhibitor clavulanic acid, resulting in a reduced zone size in the disc diffusion test and appearing negative for ESBL production. However, it is important that diagnostic laboratories account for this situation as it would affect the reporting of antibiotic resistant bacterial presence in the hospital or community setting and the true prevalence of ESBL-producing bacteria will be unknown.

The geographic area was limited to a small region of the Manawatū region of New Zealand. Therefore, this study did not provide information on the prevalence of ESBL-producing *E. coli* for the whole of New Zealand. Unfortunately, the original location of sample site A (-40.305855, 175.771632) became inaccessible and had to be moved closer to the Ashhurst Domain (-40.305311, 175.758704). Therefore, the geographic area was also limited predominantly to the urban area of Palmerston North, apart from sample site A (upstream of Palmerston North City). More widespread sampling sites, including more rural sampling sites would be beneficial to investigate other potential reservoirs, such as animals both domesticated and wild.

The study was also limited by the timeframe. Unfortunately, due to New Zealand being in Government-imposed COVID-19 restrictions, there were no environmental samples collected over the April to June period of 2020. This was the only time that these months fell into the sampling timeframe. This is over the autumn/winter months in New Zealand and higher levels of rainfall would have been expected. There were some spikes in the colony counts from the treated effluent collected in other months, when there were higher levels of turbidity or rainfall, but this was not always the case. There are no studies to my knowledge that specifically look at the levels of ESBL-producing *E. coli* in relation to rainfall and turbidity in water environments in New Zealand. However, there have been studies that have found fluctuations in antibiotic resistant bacteria and phylogroups dependent on season (90, 194). Future studies could benefit from a sampling period over two years, this would allow more trends over time to be seen clearly.

The experimental design was limited to Illumina short read sequencing data analysis for ease of use and to minimise the cost of sequencing. Although short read sequencing is beneficial for genomic confirmation of the resistance profile, long read sequencing would allow for the exact genomic location of the resistance genes, thereby determining whether resistance is chromosomal, or plasmid encoded (71).

5.7 Future research

From literature searches conducted throughout this research, there are no studies to my knowledge that take a One Health approach considering the transmission of ESBL-producing *E. coli* in New Zealand. Therefore, a larger study that looks at how animals, humans and the environment link together would be beneficial.

The next step for this project would be to collect another round of samples from the Manawatū River. An appropriate duration for new samples to be collected would be over two years, allowing isolates and metadata from the winter months to be captured to determine if any weather-related events impact the number of ESBL-producing *E. coli* collected.

Further sampling of rivers in rural areas as well as farm and wild animals would allow for animals as contributors to antibiotic resistance in the environment to be assessed. Additional sampling would also provide an opportunity to look at the prevalence of antibiotic resistant bacteria, isolated

from humans and the environment, over time. Coastal waters, as well as campsites in New Zealand, would also be points to look at sampling, as these areas have seasons of increased human prevalence, followed by little human presence during the colder months of the year. This would allow for an opportunity to compare locations of high human activity, followed by relative dormancy.

Immediate work for this project could include sequencing the remaining ESBL-producing isolates, both clinical and environmental, that did not get sequenced at the time of this study, which would allow for more information and genetic comparisons to be made. Long read sequencing, using ONT for example would allow a more in-depth analysis of the sequences to determine where the ESBL-producing genes are located, indicating what type of transmission event occurred.

6 Conclusion

In conclusion, this study found that antibiotic resistant bacteria, particularly ESBL-producing *E. coli* are present in water, sediment, stormwater, and treated effluent samples collected along the Manawatū River. It was shown that treated while effluent is a source of antibiotic resistant *E. coli*, these resistant bacteria were also present in the Manawatū River upstream of the treated effluent outflow. Further investigation is required through the sampling of rural areas as well as animals to confirm that human activity is the main source of antibiotic resistance in the Manawatū River. There was limited evidence for the sharing of genetically related ESBL-producing *E. coli* between clinical and environmental sources. The study was limited by the number of isolates collected and therefore sequenced. More frequent sampling would provide a clearer picture of the genetic relatedness between environmental and human ESBL-producing *E. coli* isolates. The results collected from this study provide new insights into antibiotic resistance specifically ESBL-producing *E. coli* present in the Manawatū River and MidCentral District of New Zealand. This is a public health concern as humans and animals can be exposed to these antibiotic resistant bacteria providing the potential for further antibiotic resistance spread and therefore limited treatment options for disease.

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Appendices

Appendix 1 – R code used for isolate selction and figure generation

A1.1 Clinical isolate selection for sequencing

```
############ Clinical isolate selection for sequencing ################

# selection of isolates from all available

Total <- read.csv("~/Clinical/Total.csv")

Tot<-Total[c(1:291), c(1:2)]
index_Tot<-sample(1:nrow(Total), size = 178, replace = FALSE)
S_from_total<-Tot[index_Tot, ]

write.csv(S_from_total, "~/Clinical/Clinical_selection/Subset_from_total.csv
")

# selection of the isolates from the same months as environmental samples wer
e collected

Months <- read.csv("~/Clinical_selection_all/Months.csv")
sample_months<-Months[c(1:236), c(1:2)]
index_sample_months<-sample(1:nrow(sample_months), size = 178, replace = FALS
E)
S_only_sample_month<-sample_months[index_sample_months, ]

write.csv(S_only_sample_month, "~/Clinical/Clinical_selection/Subset_only_sam
ple_month.csv")

# compiling different time frames to determine which one is best for isolate
selection
# libraries
library("tidyverse")
library("fs")

# setwd
setwd("~/Clinical/Clinical_selection")
data_dir <- getwd()

# load source data
baseData <- read_csv("../Total.csv", col_names = TRUE, na = c("", "NA"))
baseData <- as_tibble(baseData) %>% mutate(sampleDate = str_c(Isolate, DatePr
ocessed, sep = "_"))
head(baseData)

# load in files
csv_files <- fs::dir_ls(data_dir, regexp = "\\.csv$")
csv_files
df <- csv_files %>% map_dfr(read_csv, .id = "source") %>% as_tibble() %>% sel
ect(-DateProcessed)
head(df)
df = df %>% mutate(file = str_remove(source, "C:/Users/holly/Documents/Clinic
al/Clinical_selection/")) %>%
  mutate(file = str_remove(file, ".csv"))
```

```
# compiling all csv files into one
baseData2 <- baseData %>%  full_join(df, by = "Isolate") %>% select(-source)
final_data <- spread(baseData2, file, Isolate) %>% arrange(sampleDate)
colnames(final_data)[ncol(final_data)] <- "notSelected"
write_tsv(final_data, "sortedByCategories.txt")
```

A1.2 Enviromental dendrogram for selection of isolates for sequencing

```
############ Environmental isolate selection for sequencing ##############
#creating the dendrogram to select environmental isolates for sequencing.

# data
SB <- read.csv("~/SB_Final.csv")
str(SB)
data2<-SB[, -c(1,1)]

# making and plotting dendrogram
dd<-dist(scale(data2), method = "euclidean")
hc_SB<-hclust(dd, method = "ward.D2")
plot(hc)
plot(hc, labels = SB$EnvIsolateID, hang = -1, cex = 0.7, xlab = "Isolate", ma
in = "Sequencing selection")

write(hc2Newick(hc_SB),file="~/hc_SB.newick")
```

A1.3 Figure 3.1 overlay plot code

```
############ Figure 3.1 ##############
### Overlayed metadata 1ml colony counts ###
# libraries
library("ggplot2")
library("lubridate")

Plate_counts <- read.csv("C:/Users/holly/Downloads/1ml_plate_counts.csv")

# making the dates factors
str(Plate_counts)
Plate_counts$Month.. <- factor(Plate_counts$Month.., levels=c("18-Aug-19", "2
2-Sep-19", "1-Oct-19", "17-Nov-19", "16-Dec-19", "12-Jan-20", "10-Feb-20", "8
-Mar-20", "1-Apr-20", "1-May-20", "1-Jun-20", "19-Jul-20", "17-Aug-20", "14-S
ep-20", "12-Oct-20", "4-Nov-20", "2-Dec-20", "14-Jan-21"))

# making the intervals for the x-axis true to time
dmy("18-Aug-19", "22-Sep-19", "1-Oct-19", "17-Nov-19", "16-Dec-19", "12-Jan-2
0", "10-Feb-20", "8-Mar-20", "1-Apr-20", "1-May-20", "1-Jun-20", "19-Jul-20",
"17-Aug-20", "14-Sep-20", "12-Oct-20", "4-Nov-20", "2-Dec-20", "14-Jan-21")
# This sets the labels of the timepoints and keeps them true to time
Plate_counts$Month..<- dmy("18-Aug-19", "22-Sep-19", "1-Oct-19", "17-Nov-19",
"16-Dec-19", "12-Jan-20", "10-Feb-20", "8-Mar-20", "1-Apr-20", "1-May-20", "1
-Jun-20", "19-Jul-20", "17-Aug-20", "14-Sep-20", "12-Oct-20", "4-Nov-20", "2-
Dec-20", "14-Jan-21")

# doubled y-axis plot
```

```
ggplot(data = Plate_counts, aes(Month.., Average..CFU.ml.., group = 1))+
  geom_point(aes(y = Average..CFU.ml.., color = "Average number of cfu/ml"),
size = 3) +
  geom_point(aes(y = Rainfall.Cumulative.5.days..mm..., color = "Cumulative r
ainfall over 5 days (mm)"), size = 3) +
  geom_line(aes(y = Average..CFU.ml.., color = "Average number of cfu/ml"), l
inetype = "dashed", size = 0.5) +
  geom_line(aes(y = Rainfall.Cumulative.5.days..mm..., color = "Cumulative ra
infall over 5 days (mm)"), linetype = "dashed", size = 0.5) +
  theme_minimal()+
  theme(panel.grid.minor = element_blank())+
  scale_color_manual(values=c("#fc8d62", "#8da0cb", "#66c2a5"))+
  scale_x_continuous(breaks=Plate_counts$Month.., labels=c("18-Aug-19", "22-S
ep-19", "1-Oct-19", "17-Nov-19", "16-Dec-19", "12-Jan-20", "10-Feb-20", "8-Ma
r-20", "1-Apr-20", "1-May-20", "1-Jun-20", "19-Jul-20", "17-Aug-20", "14-Sep-
20", "12-Oct-20", "4-Nov-20", "2-Dec-20", "14-Jan-21"))  +
  scale_y_continuous(
    name = ("Average number of cfu/ml"),
    sec.axis = sec_axis(~ . * 1 , name = "Cumulative rainfall over 5 days (m
m)"),
    limits = c(0, 100))+
  labs(x= "Sample collection date", y="counts", colour= "Legend") +
  labs(caption="Sample collection date") +
  theme(plot.caption = element_text(hjust=0.5, vjust = -12,size=rel(1.5))) +
  theme(
    legend.position = c(.10, .95),
    legend.justification = c("left", "top"),
    legend.box.just = "right",
    legend.margin = margin(6, 6, 6, 6)
  ) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))

# Saving the plot in a wider format
ggsave("Figure_3.1.png", width=12)
```

A1.4 Figure 3.3 upset plot code

```
############ Figure 3.3 #############
# libraries
library(ggplot2)
library(RColorBrewer)
library(ComplexUpset)


# reading in the data
FULL <- read.csv("C:/Users/holly/Downloads/FULL (1).csv")
View(Full) #checking that the correct data is loaded
str(Full) # structure of the variables, seeing that there is the binary 0,1 m
atrix

# specifying names used for the antibiotic labels
antibiotics <- c("CTX", "CAZ", "TS",  "GM","T", "CIP", "NI", "S", "FOX", "C")
names(antibiotics) <- antibiotics

# constructing the plot with ggplot2

upset(
```

```
  FULL,
  antibiotics,
  name = "Resistance combinations of the ten antibiotics",
  base_annotations=list(
    'Intersection size'=intersection_size(
      counts=FALSE,
      mapping=aes(fill=Site)
    )
    + geom_bar(stat="identity",colour="black")
    + ylab('Number of isolates')
    + labs(fill = "Site") +
      scale_y_continuous(breaks = c(0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20))
  ),
  width_ratio=0.1,
  stripes='white'
) & scale_fill_manual(values = c("#882255","#88ccee", "#44aa99", "#DDCC77", "
#cc6677"))

ggsave("Figure_3.3.png", width=10)

#export as 1000 x 813 or similar (updates automatically)
```

A1.5 Figure 4.3 alluvial plot code

```
############ Figure 4.3 #############
# libraries
library(ggplot2)
library(gplots)
install.packages("devtools")
devtools::install_github("erblast/easyalluvial")
library("alluvial")
library(ggalluvial)
require(easyalluvial)

# read in the data
ST_by_date_28Apr22 <- read.csv("C:/Users/holly/Downloads/ST_by_date_28Apr22.c
sv")

# formatting data
ST_by_date_28Apr22$Date<- as.factor(ST_by_date_28Apr22$Date, levels=c("Sep-19
", "Nov-19", "Dec-19", "Jan-20", "Feb-20", "Mar-20", "Jul-20", "Aug-20", "Sep
-20", "Oct-20", "Nov-20", "Dec-20", "Jan-21"))
is_alluvia_form(as.data.frame(ST_by_date_28Apr22), axes = 1, silent = TRUE)
ST_by_date_28Apr22$ST<- factor(ST_by_date_28Apr22$ST, levels=c("10", "12", "3
8","69", "127", "131", "219","542","648","998","1177","1193","1722","7476","S
ingleton"))
# run again
ST_by_date_28Apr22$Date<- factor(ST_by_date_28Apr22$Date, levels=c("Sep-19",
"Oct-19","Nov-19", "Dec-19", "Jan-20", "Feb-20", "Mar-20", "Apr-20", "May-20
", "Jun-20","Jul-20", "Aug-20", "Sep-20", "Oct-20", "Nov-20", "Dec-20", "Jan-
21"))

# plot
ggplot(data = ST_by_date_28Apr22,
       aes(x = Date, stratum = Count, alluvium = ST,
           y = Count, label = Count)) +
  geom_alluvium(aes(fill = ST)) +
```

```
  theme_minimal()+
  scale_x_discrete(limits = c("Sep-19", "Oct-19","Nov-19", "Dec-19", "Jan-20
", "Feb-20", "Mar-20", "Apr-20", "May-20", "Jun-20","Jul-20", "Aug-20", "Sep-
20", "Oct-20", "Nov-20", "Dec-20", "Jan-21"), expand = c(.05, .05)) +
  scale_fill_manual(values = c("#68affc", "#1c5872", "#7ee8c0", "#16894a", "#
a9d541", "#ec102f", "#2af385", "#683d0d", "#fbbd13", "#972554", "#f4b6c7", "#
9f66ed", "#64903a", "#340785","#ed4b04"), na.value = NA) +
  scale_color_manual(values = c("#68affc", "#1c5872", "#7ee8c0", "#16894a", "
#a9d541", "#ec102f", "#2af385", "#683d0d", "#fbbd13", "#972554", "#f4b6c7", "
#9f66ed", "#64903a", "#340785","#ed4b04")) +
  geom_vline(xintercept = 8, size=1, colour = "#57504d") +
  geom_vline(xintercept = 10, size=1, colour = "#57504d") +
  annotate(
    "text", x = 9.0, y = 20, angle = 50, family = "Poppins", size = 3, color
= "gray20", lineheight = .9,
    label = "No samples collected, \n New Zealand \n Government-imposed \n CO
VID-19 restrictions ") +
  geom_vline(xintercept = 2, size=1, colour = "#57504d") +
  annotate(
    "text", x = 2.3, y = 6.0, angle = 90, family = "Poppins", size = 3, color
= "gray20", lineheight = .9,
    label = "No samples collected \n October 2019 due to bad weather") +
  geom_segment(mapping=aes(x=8.1, y=14, xend=9.9, yend=14), arrow=arrow(ends=
'both'), size=0.8, color="#57504d")


ggsave("Figure_4.3.png", width=12)
```

Appendix 2

List of ESBL producing *E. coli* clinical isolates available to select for sequencing, previously sequenced isolates had been removed from the list. Columns display when the isolate was received in relation to when the environmental sample was collected.

Key

- One_week_after = clinical isolate received within one week after environmental sample collection date
- One_week_before = clinical isolate received within one week before the environmental sample collection date
- Subset_from_total = 178 randomly sampled without replacement from the total number of clinical ESBL producing *E. coli* isolates available for sequencing
- Subset_only_sample_month = 178 randomly sampled without replacement from the clinical ESBL producing *E. coli* isolates available for sequencing that were received within the month that an environmental sample was collected.
- Two_weeks_after = clinical isolate received within two weeks after environmental sample collection date
- Two_weeks_before = clinical isolate received within two weeks before the environmental sample collection date
- Not Selected = isolates that were not sampled or within the date range selected

Date Processed	Isolate ID	One_week_after	One_week_before	Subset_from_total	Subset_only_sample_month	Two_weeks_after	Two_weeks_before	Not Selected
5-Aug-19	EH0002a	NA	NA	YES	NA	NA	NA	NA
5-Aug-19	EH0003a	NA	NA	NA	NA	NA	NA	YES
5-Aug-19	EH0006a	NA	NA	YES	NA	NA	NA	NA
5-Aug-19	EH0007a	NA	NA	YES	NA	NA	NA	NA
13-Aug-19	EH0009a	NA	NA	NA	NA	NA	NA	YES
13-Aug-19	EH0010a	NA	NA	YES	NA	NA	NA	NA
21-Aug-19	EH0012a	NA	NA	NA	NA	NA	NA	YES
21-Aug-19	EH0013a	NA	NA	YES	NA	NA	NA	NA
21-Aug-19	EH0014a	NA	NA	YES	NA	NA	NA	NA
27-Aug-19	EH0016a	NA	NA	YES	NA	NA	NA	NA
27-Aug-19	EH0018a	NA	NA	YES	NA	NA	NA	NA
2-Sep-19	EH0019a	NA	NA	NA	YES	NA	NA	NA
2-Sep-19	EH0020a	NA	NA	YES	YES	NA	NA	NA

2-Sep-19	EH0022a	NA	NA	NA	YES	NA	NA	NA
9-Sep-19	EH0024a	NA	NA	YES	YES	NA	YES	NA
9-Sep-19	EH0025a	NA	NA	YES	YES	NA	YES	NA
9-Sep-19	EH0026a	NA	NA	YES	NA	NA	YES	NA
9-Sep-19	EH0027a	NA	NA	YES	NA	NA	YES	NA
19-Sep-19	EH0029a	NA	YES	YES	YES	NA	YES	NA
19-Sep-19	EH0031a	NA	YES	YES	YES	NA	YES	NA
19-Sep-19	EH0033a	NA	YES	NA	NA	NA	YES	NA
19-Sep-19	EH0034a	NA	YES	YES	YES	NA	YES	NA
19-Sep-19	EH0035a	NA	YES	NA	YES	NA	YES	NA
19-Sep-19	EH0036a	NA	YES	YES	NA	NA	YES	NA
19-Sep-19	EH0037a	NA	YES	YES	YES	NA	YES	NA
23-Sep-19	EH0038a	YES	YES	YES	YES	YES	YES	NA
23-Sep-19	EH0040a	YES	YES	NA	YES	YES	YES	NA
23-Sep-19	EH0041a	YES	YES	NA	YES	YES	YES	NA
23-Sep-19	EH0043a	YES	YES	YES	YES	YES	YES	NA
30-Sep-19	EH0044a	NA	NA	YES	YES	YES	NA	NA
30-Sep-19	EH0046a	NA	NA	YES	NA	YES	NA	NA
30-Sep-19	EH0047a	NA	NA	NA	YES	YES	NA	NA
30-Sep-19	EH0049a	NA	NA	NA	NA	YES	NA	NA
8-Oct-19	EH0052a	NA	NA	YES	NA	NA	NA	NA
8-Oct-19	EH0053a	NA	NA	NA	NA	NA	NA	YES
8-Oct-19	EH0054a	NA	NA	YES	NA	NA	NA	NA
8-Oct-19	EH0055a	NA	NA	NA	NA	NA	NA	YES
8-Oct-19	EH0056a	NA	NA	YES	NA	NA	NA	NA
8-Oct-19	EH0057a	NA	NA	YES	NA	NA	NA	NA
14-Oct-19	EH0059a	NA	NA	NA	NA	NA	NA	YES
14-Oct-19	EH0060a	NA	NA	NA	NA	NA	NA	YES
14-Oct-19	EH0062a	NA	NA	YES	NA	NA	NA	NA
14-Oct-19	EH0065a	NA	NA	YES	NA	NA	NA	NA
14-Oct-19	EH0066a	NA	NA	YES	NA	NA	NA	NA
21-Oct-19	EH0069a	NA	NA	YES	NA	NA	NA	NA
21-Oct-19	EH0070a	NA	NA	NA	NA	NA	NA	YES
21-Oct-19	EH0072a	NA	NA	YES	NA	NA	NA	NA
21-Oct-19	EH0073a	NA	NA	YES	NA	NA	NA	NA
21-Oct-19	EH0074a	NA	NA	NA	NA	NA	NA	YES
21-Oct-19	EH0075a	NA	NA	NA	NA	NA	NA	YES
21-Oct-19	EH0076a	NA	NA	YES	NA	NA	NA	NA
21-Oct-19	EH0077a	NA	NA	NA	NA	NA	NA	YES
29-Oct-19	EH0079a	NA	NA	NA	NA	NA	NA	YES
29-Oct-19	EH0080a	NA	NA	YES	NA	NA	NA	NA
29-Oct-19	EH0081a	NA	NA	NA	NA	NA	NA	YES
4-Nov-19	EH0083a	NA	NA	YES	NA	NA	YES	NA

4-Nov-19	EH0084a	NA	NA	YES	YES	NA	YES	NA
4-Nov-19	EH0085a	NA	NA	YES	YES	NA	YES	NA
4-Nov-19	EH0086a	NA	NA	NA	YES	NA	YES	NA
12-Nov-19	EH0088a	NA	YES	YES	YES	NA	YES	NA
12-Nov-19	EH0089a	NA	YES	YES	YES	NA	YES	NA
12-Nov-19	EH0090a	NA	YES	NA	YES	NA	YES	NA
19-Nov-19	EH0093a	YES	NA	YES	YES	YES	NA	NA
19-Nov-19	EH0094a	YES	NA	NA	YES	YES	NA	NA
19-Nov-19	EH0095a	YES	NA	YES	YES	YES	NA	NA
19-Nov-19	EH0096a	YES	NA	YES	YES	YES	NA	NA
19-Nov-19	EH0097a	YES	NA	NA	YES	YES	NA	NA
25-Nov-19	EH0100a	NA	NA	NA	YES	YES	NA	NA
25-Nov-19	EH0102a	NA	NA	YES	YES	YES	NA	NA
2-Dec-19	EH0105a	NA	NA	YES	NA	NA	NA	NA
2-Dec-19	EH0107a	NA	NA	NA	YES	NA	NA	NA
11-Dec-19	EH0109a	NA	YES	NA	YES	NA	YES	NA
11-Dec-19	EH0111a	NA	YES	NA	NA	NA	YES	NA
11-Dec-19	EH0112a	NA	YES	YES	NA	NA	YES	NA
11-Dec-19	EH0113a	NA	YES	NA	NA	NA	YES	NA
11-Dec-19	EH0114a	NA	YES	YES	YES	NA	YES	NA
16-Dec-19	EH0115a	YES	YES	YES	YES	YES	YES	NA
16-Dec-19	EH0116a	YES	YES	NA	NA	YES	YES	NA
16-Dec-19	EH0118a	YES	YES	YES	YES	YES	YES	NA
16-Dec-19	EH0119a	YES	YES	YES	NA	YES	YES	NA
16-Dec-19	EH0120a	YES	YES	YES	YES	YES	YES	NA
16-Dec-19	EH0121a	YES	YES	YES	YES	YES	YES	NA
16-Dec-19	EH0123a	YES	YES	YES	NA	YES	YES	NA
9-Jan-20	EH0124a	NA	YES	NA	YES	NA	YES	NA
9-Jan-20	EH0126a	NA	YES	YES	YES	NA	YES	NA
9-Jan-20	EH0127a	NA	YES	YES	YES	NA	YES	NA
9-Jan-20	EH0128a	NA	YES	YES	YES	NA	YES	NA
9-Jan-20	EH0129a	NA	YES	YES	YES	NA	YES	NA
9-Jan-20	EH0130a	NA	YES	NA	YES	NA	YES	NA
13-Jan-20	EH0133a	YES	YES	YES	YES	YES	YES	NA
13-Jan-20	EH0134a	YES	YES	NA	YES	YES	YES	NA
13-Jan-20	EH0135a	YES	YES	NA	NA	YES	YES	NA
22-Jan-20	EH0136a	NA	NA	YES	YES	YES	NA	NA
22-Jan-20	EH0137a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0138a	NA	NA	YES	YES	YES	NA	NA
22-Jan-20	EH0139a	NA	NA	YES	YES	YES	NA	NA
22-Jan-20	EH0140a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0141a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0142a	NA	NA	NA	YES	YES	NA	NA

22-Jan-20	EH0143a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0144a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0145a	NA	NA	YES	YES	YES	NA	NA
22-Jan-20	EH0146a	NA	NA	YES	YES	YES	NA	NA
22-Jan-20	EH0147a	NA	NA	NA	NA	YES	NA	NA
22-Jan-20	EH0148a	NA	NA	NA	YES	YES	NA	NA
22-Jan-20	EH0149a	NA	NA	NA	NA	YES	NA	NA
28-Jan-20	EH0151a	NA	NA	YES	YES	NA	YES	NA
28-Jan-20	EH0153a	NA	NA	NA	NA	NA	YES	NA
28-Jan-20	EH0154a	NA	NA	NA	YES	NA	YES	NA
2-Feb-20	EH0155a	NA	NA	YES	YES	NA	YES	NA
2-Feb-20	EH0156a	NA	NA	NA	YES	NA	YES	NA
2-Feb-20	EH0160a	NA	NA	YES	YES	NA	YES	NA
2-Feb-20	EH0161a	NA	NA	YES	YES	NA	YES	NA
2-Feb-20	EH0162a	NA	NA	YES	NA	NA	YES	NA
2-Feb-20	EH0163a	NA	NA	YES	NA	NA	YES	NA
2-Feb-20	EH0164a	NA	NA	NA	YES	NA	YES	NA
2-Feb-20	EH0165a	NA	NA	YES	YES	NA	YES	NA
2-Feb-20	EH0167a	NA	NA	YES	YES	NA	YES	NA
2-Feb-20	EH0168a	NA	NA	YES	NA	NA	YES	NA
2-Feb-20	EH0169a	NA	NA	YES	YES	NA	YES	NA
18-Feb-20	EH0171a	NA	NA	NA	YES	YES	NA	NA
18-Feb-20	EH0172a	NA	NA	YES	NA	YES	NA	NA
18-Feb-20	EH0174a	NA	NA	YES	YES	YES	NA	NA
18-Feb-20	EH0175a	NA	NA	YES	YES	YES	NA	NA
24-Feb-20	EH0176a	NA	NA	YES	YES	NA	YES	NA
24-Feb-20	EH0177a	NA	NA	YES	YES	NA	YES	NA
24-Feb-20	EH0178a	NA	NA	NA	YES	NA	YES	NA
24-Feb-20	EH0179a	NA	NA	NA	YES	NA	YES	NA
24-Feb-20	EH0180a	NA	NA	NA	NA	NA	YES	NA
24-Feb-20	EH0181a	NA	NA	YES	YES	NA	YES	NA
24-Feb-20	EH0182a	NA	NA	NA	YES	NA	YES	NA
24-Feb-20	EH0184a	NA	NA	YES	NA	NA	YES	NA
2-Mar-20	EH0187a	NA	YES	NA	YES	NA	YES	NA
2-Mar-20	EH0188a	NA	YES	NA	YES	NA	YES	NA
2-Mar-20	EH0189a	NA	YES	YES	YES	NA	YES	NA
2-Mar-20	EH0190a	NA	YES	YES	YES	NA	YES	NA
2-Mar-20	EH0191a	NA	YES	NA	NA	NA	YES	NA
2-Mar-20	EH0192a	NA	YES	YES	YES	NA	YES	NA
9-Mar-20	EH0193a	YES	NA	NA	YES	YES	NA	NA
16-Mar-20	EH0205a	NA	NA	YES	YES	YES	NA	NA
16-Mar-20	EH0206a	NA	NA	YES	NA	YES	NA	NA
16-Mar-20	EH0208a	NA	NA	YES	NA	YES	NA	NA

16-Mar-20	EH0210a	NA	NA	NA	YES	YES	NA	NA
16-Mar-20	EH0212a	NA	NA	YES	YES	YES	NA	NA
17-Jun-20	EH0213a	NA	NA	NA	NA	NA	NA	YES
17-Jun-20	EH0214a	NA	NA	YES	NA	NA	NA	NA
17-Jun-20	EH0215a	NA	NA	NA	NA	NA	NA	YES
17-Jun-20	EH0216a	NA	NA	YES	NA	NA	NA	NA
17-Jun-20	EH0217a	NA	NA	YES	NA	NA	NA	NA
17-Jun-20	EH0220a	NA	NA	YES	NA	NA	NA	NA
17-Jun-20	EH0224a	NA	NA	NA	NA	NA	NA	YES
17-Jun-20	EH0229a	NA	NA	YES	NA	NA	NA	NA
17-Jun-20	EH0230a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0231a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0232a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0233a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0235a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0237a	NA	NA	YES	NA	NA	NA	NA
26-Jun-20	EH0238a	NA	NA	NA	NA	NA	NA	YES
26-Jun-20	EH0239a	NA	NA	NA	NA	NA	NA	YES
26-Jun-20	EH0240a	NA	NA	YES	NA	NA	NA	NA
29-Jun-20	EH0241a	NA	NA	YES	NA	NA	NA	NA
29-Jun-20	EH0242a	NA	NA	YES	NA	NA	NA	NA
29-Jun-20	EH0243a	NA	NA	NA	NA	NA	NA	YES
29-Jun-20	EH0244a	NA	NA	YES	NA	NA	NA	NA
29-Jun-20	EH0248a	NA	NA	YES	NA	NA	NA	NA
6-Jul-20	EH0251a	NA	NA	YES	YES	NA	YES	NA
6-Jul-20	EH0254a	NA	NA	YES	NA	NA	YES	NA
6-Jul-20	EH0255a	NA	NA	YES	YES	NA	YES	NA
6-Jul-20	EH0256a	NA	NA	NA	NA	NA	YES	NA
21-Jul-20	EH0257a	YES	NA	YES	YES	YES	NA	NA
21-Jul-20	EH0258a	YES	NA	NA	YES	YES	NA	NA
21-Jul-20	EH0259a	YES	NA	YES	YES	YES	NA	NA
21-Jul-20	EH0260a	YES	NA	NA	YES	YES	NA	NA
21-Jul-20	EH0261a	YES	NA	YES	YES	YES	NA	NA
21-Jul-20	EH0262a	YES	NA	NA	YES	YES	NA	NA
21-Jul-20	EH0263a	YES	NA	YES	NA	YES	NA	NA
21-Jul-20	EH0264a	YES	NA	YES	NA	YES	NA	NA
21-Jul-20	EH0265a	YES	NA	YES	NA	YES	NA	NA
21-Jul-20	EH0267a	YES	NA	YES	YES	YES	NA	NA
21-Jul-20	EH0268a	YES	NA	NA	YES	YES	NA	NA
21-Jul-20	EH0269a	YES	NA	YES	YES	YES	NA	NA
21-Jul-20	EH0270a	YES	NA	NA	YES	YES	NA	NA
21-Jul-20	EH0271a	YES	NA	YES	NA	YES	NA	NA
21-Jul-20	EH0272a	YES	NA	YES	YES	YES	NA	NA

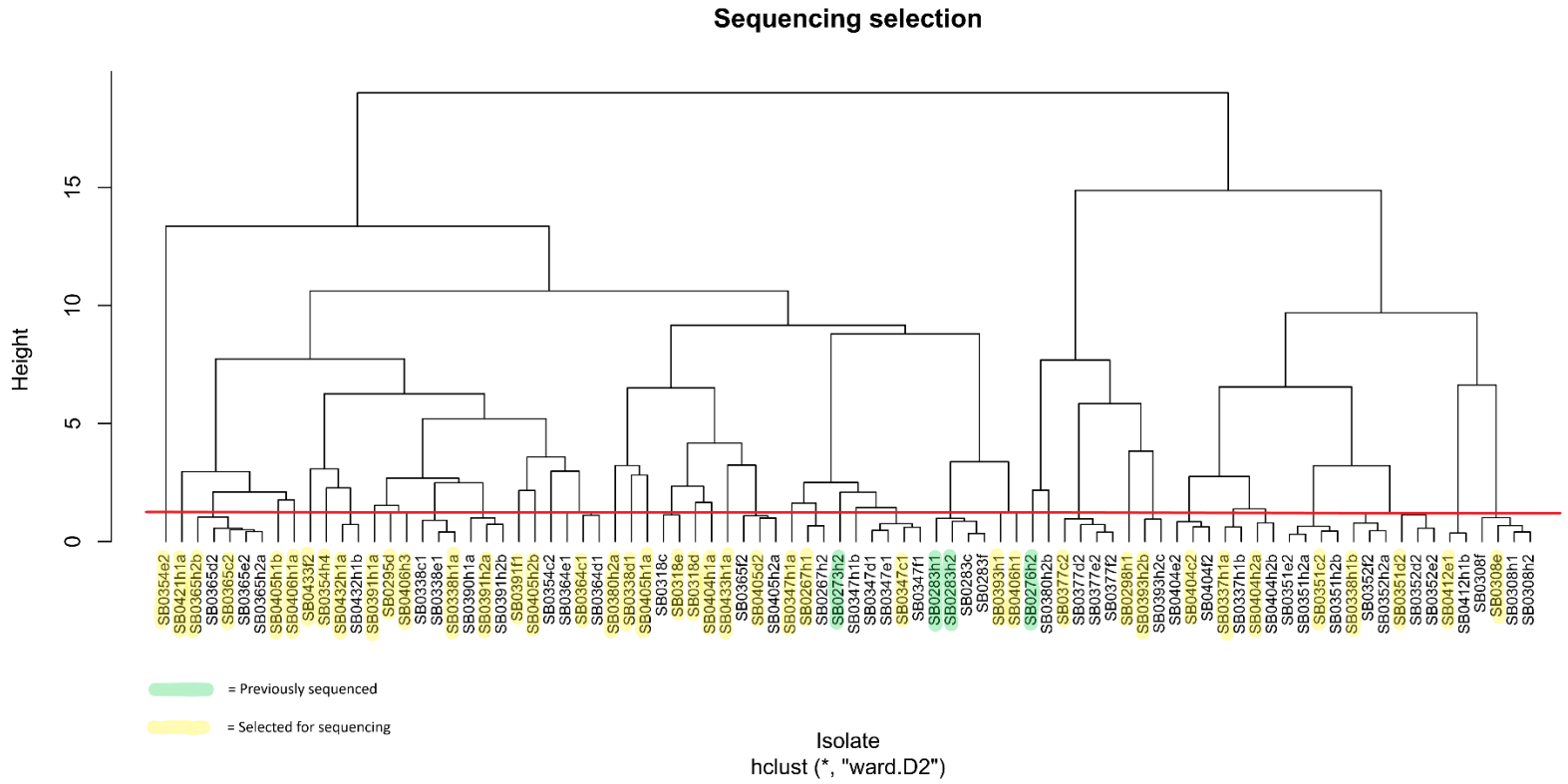
21-Jul-20	EH0275a	YES	NA	YES	YES	YES	NA	NA
28-Jul-20	EH0277a	NA	NA	YES	YES	YES	NA	NA
3-Aug-20	EH0279a	NA	NA	YES	NA	NA	NA	NA
3-Aug-20	EH0280a	NA	NA	YES	NA	NA	NA	NA
3-Aug-20	EH0282a	NA	NA	YES	NA	NA	NA	NA
31-Aug-20	EH0283a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0284a	NA	NA	YES	NA	NA	NA	NA
31-Aug-20	EH0285a	NA	NA	NA	NA	NA	NA	YES
31-Aug-20	EH0286a	NA	NA	YES	YES	NA	NA	NA
31-Aug-20	EH0287a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0288a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0290a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0292a	NA	NA	YES	YES	NA	NA	NA
31-Aug-20	EH0293a	NA	NA	YES	NA	NA	NA	NA
31-Aug-20	EH0294a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0295a	NA	NA	YES	YES	NA	NA	NA
31-Aug-20	EH0296a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0297a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0298a	NA	NA	YES	YES	NA	NA	NA
31-Aug-20	EH0299a	NA	NA	YES	YES	NA	NA	NA
31-Aug-20	EH0300a	NA	NA	NA	YES	NA	NA	NA
31-Aug-20	EH0301a	NA	NA	NA	YES	NA	NA	NA
8-Sep-20	EH0303a	NA	YES	YES	YES	NA	YES	NA
8-Sep-20	EH0304a	NA	YES	YES	NA	NA	YES	NA
8-Sep-20	EH0306a	NA	YES	NA	YES	NA	YES	NA
8-Sep-20	EH0307a	NA	YES	YES	YES	NA	YES	NA
8-Sep-20	EH0308a	NA	YES	YES	YES	NA	YES	NA
14-Sep-20	EH0310a	YES	YES	YES	YES	YES	YES	NA
28-Sep-20	EH0313a	NA	NA	YES	YES	NA	NA	NA
28-Sep-20	EH0314a	NA	NA	NA	YES	NA	NA	NA
28-Sep-20	EH0315a	NA	NA	YES	YES	NA	NA	NA
28-Sep-20	EH0316a	NA	NA	YES	YES	NA	NA	NA
28-Sep-20	EH0317a	NA	NA	NA	YES	NA	NA	NA
28-Sep-20	EH0318a	NA	NA	YES	YES	NA	NA	NA
28-Sep-20	EH0319a	NA	NA	YES	NA	NA	NA	NA
14-Oct-20	EH0321a	YES	NA	YES	YES	YES	NA	NA
14-Oct-20	EH0322a	YES	NA	NA	NA	YES	NA	NA
14-Oct-20	EH0323a	YES	NA	YES	YES	YES	NA	NA
14-Oct-20	EH0324a	YES	NA	YES	YES	YES	NA	NA
20-Oct-20	EH0327a	NA	NA	NA	NA	YES	NA	NA
20-Oct-20	EH0328a	NA	NA	NA	NA	YES	NA	NA
20-Oct-20	EH0329a	NA	NA	NA	YES	YES	NA	NA
20-Oct-20	EH0330a	NA	NA	YES	NA	YES	NA	NA

20-Oct-20	EH0331a	NA	NA	YES	YES	YES	NA	NA
20-Oct-20	EH0332a	NA	NA	NA	YES	YES	NA	NA
28-Oct-20	EH0333a	NA	NA	NA	YES	NA	YES	NA
28-Oct-20	EH0334a	NA	NA	NA	YES	NA	YES	NA
28-Oct-20	EH0335a	NA	NA	YES	YES	NA	YES	NA
28-Oct-20	EH0337a	NA	NA	YES	YES	NA	YES	NA
2-Nov-20	EH0339a	NA	YES	NA	NA	NA	YES	NA
2-Nov-20	EH0340a	NA	YES	NA	YES	NA	YES	NA
2-Nov-20	EH0344a	NA	YES	YES	YES	NA	YES	NA
2-Nov-20	EH0345a	NA	YES	YES	YES	NA	YES	NA
9-Nov-20	EH0346a	YES	NA	NA	YES	YES	NA	NA
9-Nov-20	EH0347a	YES	NA	YES	NA	YES	NA	NA
9-Nov-20	EH0348a	YES	NA	NA	YES	YES	NA	NA
9-Nov-20	EH0350a	YES	NA	YES	YES	YES	NA	NA
9-Nov-20	EH0353a	YES	NA	NA	NA	YES	NA	NA
16-Nov-20	EH0354a	NA	NA	YES	NA	YES	NA	NA
23-Nov-20	EH0356a	NA	NA	YES	YES	NA	YES	NA
23-Nov-20	EH0357a	NA	NA	YES	YES	NA	YES	NA
30-Nov-20	EH0358a	NA	YES	NA	NA	NA	YES	NA
30-Nov-20	EH0359a	NA	YES	NA	YES	NA	YES	NA
30-Nov-20	EH0361a	NA	YES	NA	NA	NA	YES	NA
30-Nov-20	EH0362a	NA	YES	YES	YES	NA	YES	NA
30-Nov-20	EH0363a	NA	YES	NA	YES	NA	YES	NA
30-Nov-20	EH0364a	NA	YES	NA	YES	NA	YES	NA
30-Nov-20	EH0365a	NA	YES	NA	NA	NA	YES	NA
30-Nov-20	EH0366a	NA	YES	YES	YES	NA	YES	NA
30-Nov-20	EH0367a	NA	YES	YES	YES	NA	YES	NA
30-Nov-20	EH0368a	NA	YES	YES	YES	NA	YES	NA
30-Nov-20	EH0369a	NA	YES	YES	YES	NA	YES	NA
7-Dec-20	EH0370a	YES	NA	NA	YES	YES	NA	NA
7-Dec-20	EH0371a	YES	NA	YES	YES	YES	NA	NA
7-Dec-20	EH0373a	YES	NA	NA	YES	YES	NA	NA
7-Dec-20	EH0374a	YES	NA	YES	YES	YES	NA	NA
7-Dec-20	EH0375a	YES	NA	YES	YES	YES	NA	NA
7-Dec-20	EH0376a	YES	NA	YES	YES	YES	NA	NA
14-Dec-20	EH0377a	NA	NA	NA	YES	YES	NA	NA
14-Dec-20	EH0378a	NA	NA	NA	YES	YES	NA	NA
14-Dec-20	EH0379a	NA	NA	NA	NA	YES	NA	NA
14-Dec-20	EH0381a	NA	NA	YES	YES	YES	NA	NA
14-Dec-20	EH0382a	NA	NA	YES	YES	YES	NA	NA
11-Jan-21	EH0383a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0384a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0386a	NA	YES	NA	YES	NA	YES	NA

11-Jan-21	EH0387a	NA	YES	NA	YES	NA	YES	NA
11-Jan-21	EH0388a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0389a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0391a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0392a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0393a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0394a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0395a	NA	YES	YES	YES	NA	YES	NA
11-Jan-21	EH0396a	NA	YES	YES	NA	NA	YES	NA
11-Jan-21	EH0397a	NA	YES	NA	YES	NA	YES	NA
12-Jan-21	EH0398a	NA	YES	YES	NA	NA	YES	NA
12-Jan-21	EH0401a	NA	YES	NA	YES	NA	YES	NA
12-Jan-21	EH0402a	NA	YES	NA	YES	NA	YES	NA
12-Jan-21	EH0404a	NA	YES	YES	NA	NA	YES	NA
12-Jan-21	EH0406a	NA	YES	YES	NA	NA	YES	NA
12-Jan-21	EH0407a	NA	YES	NA	YES	NA	YES	NA
12-Jan-21	EH0409a	NA	YES	YES	YES	NA	YES	NA
18-Jan-21	EH0410a	YES	NA	NA	YES	YES	NA	NA
18-Jan-21	EH0413a	YES	NA	YES	NA	YES	NA	NA
18-Jan-21	EH0414a	YES	NA	YES	YES	YES	NA	NA

Appendix 3

Dendrogram of the environmental ESBL producing *E. coli* isolates available for selection for sequencing. The dendrogram was constructed based of antibiotic resistance profiles recorded from the disc diffusion tests which are displayed in Appendix 5. The red line on the dendrogram was used to assist me in picking an isolate from each cluster.



Appendix 4

The version numbers used for any R code and the software used for genomic analysis of the isolates.

Software	Version	Software	Version
R software	4.1.2	**Newick-Utils**	1.6
R library "tidyverse"	1.3.1	**Nullarbor 2**	2.0.20191013
R library "ggplot2"	3.3.5	**Prokka**	1.14.6
R library "lubridate"	1.8.0	**Quast**	5.0.2
R library "ggalluvial"	0.12.3	**ResFinder**	4.0
R library "easyalluvial"	0.3.0	**Roary**	3.13.0
R library "alluvial"	0.1-2	**SAMtools**	1.12
R library "fs"	1.5.0	**SKESA**	2.4.0
R library "ComplexUpset"	1.3.1	**Shovill**	1.1.0
R library "gplots"	3.1.1	**Snippy**	4.6.0
R library "RColorBrewer"	1.1-2	**SPAdes**	3.14.1
R library "devtools"	2.4.2	**SplitTree**	5.3.0
BiocManager	1.30.16	**Trimmomatic**	0.39
Abricate	1.0.1	**seqret**	(unable to determine version)
BWA MEM	0.7.17-r1188	**seqtk**	1.3-r106
Centrifuge	1.0.4	**snp-dists**	0.8.2
Fast-GeP	1.0.2	**Pointfinder**	3.0
FastQC	0.11.9	**Pointfinder database**	March 2022
FastTree	2.1.10 Double precision (No SSE3) and 2.1.11	**VFDB**	March 2021
FimTyper	1.0	**MALDI-TOF**	
FreeBayes	1.3.5	**flexcontrol**	3.4
Gubbins	2.3.1	**Biotyper compass**	4.1.100
IQtree	2.1.4-beta COVID-edition for Linux 64-bit built Jun 24 2021	**Database**	Revision C March 2019
iTol	6.5.7		
Kraken	1.1.1		
LabChip GX Reviewer	5.3.2115.0		
MLST	2.19.0		
MegaHit	1.2.9		

Appendix 5

The table below outlines *E. coli* isolates collected from Medlab Central that tested positive for ESBL production. Zone diameters from the double-disc diffusion test are included, -1 is indicative of the positive result.

Clinical IsolateID	Date Processed	Sample Type	ESBL_CTXz1	ESBL_CTXz2	ESBL_CTXdiff	ESBL_CTX	ESBL_CAZz1	ESBL_CAZz2	ESBL_CAZdiff	ESBL_CAZ
EH0001a	5-Aug-19	Urine	10.6	31.0	20.3	-1	18.9	27.6	8.7	-1
EH0002a	5-Aug-19	Urine	8.4	29.7	21.3	-1	16.0	26.3	10.3	-1
EH0003a	5-Aug-19	Urine	17.6	28.5	10.9	-1	26.3	27.1	0.8	
EH0006a	5-Aug-19	Urine	8.6	24.4	15.8	-1	20.3	24.1	3.8	
EH0007a	5-Aug-19	Urine	15.4	30.7	15.2	-1	20.8	27.8	7.0	-1
EH0008a	13-Aug-19	Urine	17.5	30.2	12.7	-1	19.0	27.2	8.2	-1
EH0009a	13-Aug-19	Urine	10.1	30.6	20.6	-1	17.1	27.8	10.7	-1
EH0010a	13-Aug-19	Urine	19.6	30.1	10.5	-1	27.7	27.6	-0.1	
EH0011a	21-Aug-19	Urine	17.2	30.0	12.8	-1	25.5	28.6	3.1	
EH0012a	21-Aug-19	Urine	10.5	28.9	18.4	-1	14.4	26.4	12.0	-1
EH0013a	21-Aug-19	Urine	17.1	26.5	9.4	-1	19.9	19.4	-0.4	
EH0014a	21-Aug-19	Urine	12.4	31.3	18.9	-1	18.8	28.2	9.4	-1
EH0015a	27-Aug-19	Urine	7.8	30.0	22.2	-1	16.3	28.0	11.7	-1
EH0016a	27-Aug-19	Urine	11.6	31.7	20.1	-1	20.4	28.1	7.6	-1
EH0018a	27-Aug-19	Urine	12.0	30.7	18.7	-1	20.5	26.9	6.4	-1

EH0019a	2-Sep-19	Urine	6.2	24.4	18.2	-1	19.4	27.6	8.3	-1
EH0020a	2-Sep-19	Urine	6.0	24.3	18.3	-1	13.9	27.7	13.8	-1
EH0021a	2-Sep-19	Urine	8.8	31.7	22.9	-1	17.5	28.0	10.6	-1
EH0022a	2-Sep-19	Urine	6.0	26.1	20.1	-1	6.1	23.8	17.8	-1
EH0023a	9-Sep-19	Urine	12.8	31.3	18.5	-1	19.5	28.6	9.1	-1
EH0024a	9-Sep-19	Urine	7.6	31.5	23.9	-1	15.1	29.6	14.6	-1
EH0025a	9-Sep-19	Urine	15.7	33.9	18.2	-1	22.5	28.5	6.0	-1
EH0026a	9-Sep-19	Urine	12.6	31.3	18.7	-1	19.7	28.2	8.5	-1
EH0027a	9-Sep-19	Urine	14.0	27.9	13.9	-1	25.6	28.1	2.5	
EH0029a	19-Sep-19	Urine	10.2	24.1	13.9	-1	15.8	26.0	10.1	-1
EH0030a	19-Sep-19	Urine	9.1	28.7	19.6	-1	18.3	27.4	9.1	-1
EH0031a	19-Sep-19	Urine	6.0	28.6	22.6	-1	14.0	22.7	8.7	-1
EH0033a	19-Sep-19	Urine	13.6	30.8	17.2	-1	19.5	30.2	10.7	-1
EH0034a	19-Sep-19	Urine	6.0	25.1	19.1	-1	8.5	23.2	14.6	-1
EH0035a	19-Sep-19	Urine	14.1	29.8	15.7	-1	19.7	26.2	6.6	-1
EH0036a	19-Sep-19	Urine	7.1	29.0	21.9	-1	14.8	25.6	10.8	-1
EH0037a	19-Sep-19	Urine	7.3	31.0	23.7	-1	17.4	28.4	11.0	-1
EH0038a	23-Sep-19	Urine	12.9	31.4	18.4	-1	20.4	27.0	6.6	-1
EH0039a	23-Sep-19	Urine	10.3	30.4	20.1	-1	17.0	27.6	10.6	-1
EH0040a	23-Sep-19	Urine	6.9	30.3	23.3	-1	14.8	27.0	12.2	-1
EH0041a	23-Sep-19	Urine	7.2	27.2	20.0	-1	15.5	28.3	12.8	-1
EH0043a	23-Sep-19	Urine	14.1	32.7	18.7	-1	18.9	26.7	7.9	-1
EH0044a	30-Sep-19	Urine	14.9	30.1	15.2	-1	20.0	27.6	7.7	-1
EH0045a	30-Sep-19	Urine	15.7	33.0	17.4	-1	21.0	28.4	7.4	-1
EH0046a	30-Sep-19	Catheter urine	13.5	28.9	15.5	-1	18.5	24.9	6.4	-1
EH0047a	30-Sep-19	Urine	12.0	30.0	18.1	-1	17.7	29.2	11.5	-1
EH0049a	30-Sep-19	Urine	8.2	28.5	20.2	-1	13.0	25.6	12.6	-1

EH0050a	8-Oct-19	Urine	14.5	31.4	16.9	-1	20.7	28.3	7.6	-1
EH0052a	8-Oct-19	Urine	19.3	27.7	8.3	-1	19.9	20.5	0.7	
EH0053a	8-Oct-19	Urine	6.0	28.5	22.5	-1	11.8	25.5	13.7	-1
EH0054a	8-Oct-19	Urine	6.8	29.8	22.9	-1	16.7	26.9	10.3	-1
EH0055a	8-Oct-19	Urine	10.9	30.7	19.8	-1	19.5	27.2	7.7	-1
EH0056a	8-Oct-19	Urine	6.3	26.7	20.4	-1	18.5	24.2	5.7	-1
EH0057a	8-Oct-19	Urine	8.4	23.6	15.2	-1	15.7	22.5	6.8	-1
EH0058a	14-Oct-19	Urine	15.0	31.4	16.4	-1	23.4	29.8	6.4	-1
EH0059a	14-Oct-19	Urine	11.6	25.9	14.2	-1	18.8	24.7	5.9	-1
EH0060a	14-Oct-19	Urine	12.8	26.6	13.8	-1	20.8	26.5	5.7	-1
EH0062a	14-Oct-19	Urine	8.9	34.6	25.7	-1	13.4	30.2	16.8	-1
EH0065a	14-Oct-19	Urine	12.1	22.5	10.4	-1	17.2	16.9	-0.2	
EH0066a	14-Oct-19	Urine	6.0	20.3	14.3	-1	12.5	17.4	4.9	
EH0068a	21-Oct-19	Catheter urine	9.1	28.8	19.7	-1	16.3	27.0	10.6	-1
EH0069a	21-Oct-19	Urine	6.2	21.8	15.7	-1	15.5	23.3	7.8	-1
EH0070a	21-Oct-19	Urine	6.0	19.1	13.1	-1	11.4	20.1	8.7	-1
EH0072a	21-Oct-19	Urine	6.0	18.3	12.3	-1	8.6	19.1	10.5	-1
EH0073a	21-Oct-19	Urine	22.8	38.0	15.3	-1	29.7	36.6	6.9	-1
EH0074a	21-Oct-19	Urine	13.1	26.7	13.6	-1	21.3	25.9	4.7	
EH0075a	21-Oct-19	Urine	10.6	24.4	13.7	-1	18.8	25.6	6.7	-1
EH0076a	21-Oct-19	Urine	13.2	21.3	8.0	-1	23.2	22.6	-0.6	
EH0077a	21-Oct-19	Urine	6.1	23.0	16.9	-1	17.0	23.3	6.3	-1
EH0078a	29-Oct-19	Urine	6.6	26.5	19.9	-1	11.3	27.3	16.0	-1
EH0079a	29-Oct-19	Urine	15.4	26.0	10.6	-1	26.0	27.3	1.3	
EH0080a	29-Oct-19	Urine	15.3	27.1	11.9	-1	19.6	25.9	6.3	-1
EH0081a	29-Oct-19	Urine	12.3	24.2	11.9	-1	15.4	15.0	-0.3	
EH0082a	4-Nov-19	Urine	14.2	30.7	16.5	-1	22.1	29.4	7.3	-1

EH0083a	4-Nov-19	Urine	6.0	23.8	17.8	-1	13.8	23.4	9.6	-1
EH0084a	4-Nov-19	Urine	10.4	24.9	14.6	-1	16.7	24.4	7.7	-1
EH0085a	4-Nov-19	Urine	8.9	22.3	13.3	-1	15.9	22.4	6.5	-1
EH0086a	4-Nov-19	Urine	12.3	24.3	12.0	-1	18.3	22.6	4.4	
EH0087a	12-Nov-19	Urine	12.3	28.9	16.6	-1	20.4	29.5	9.1	-1
EH0088a	12-Nov-19	Urine	6.6	27.1	20.5	-1	8.4	23.1	14.6	-1
EH0089a	12-Nov-19	Urine	6.0	26.7	20.7	-1	11.3	23.7	12.5	-1
EH0090a	12-Nov-19	Urine	9.8	31.0	21.2	-1	16.8	26.7	9.9	-1
EH0092a	19-Nov-19	Urine	6.0	26.5	20.5	-1	6.2	23.5	17.3	-1
EH0093a	19-Nov-19	Urine	11.4	28.0	16.7	-1	19.1	23.9	4.8	-1
EH0094a	19-Nov-19	Urine	13.7	30.9	17.2	-1	19.7	28.2	8.5	-1
EH0095a	19-Nov-19	Urine	13.9	31.8	18.0	-1	21.3	29.3	8.0	-1
EH0096a	19-Nov-19	Urine	16.8	34.9	18.1	-1	24.6	30.1	5.5	-1
EH0097a	19-Nov-19	Urine	6.1	26.1	20.0	-1	19.0	23.8	4.8	
EH0098a	25-Nov-19	Urine	13.7	31.7	18.0	-1	20.5	28.4	8.0	-1
EH0100a	25-Nov-19	Urine	13.2	32.1	18.9	-1	20.9	30.2	9.4	-1
EH0102a	25-Nov-19	Urine	9.6	31.8	22.2	-1	18.9	29.9	11.0	-1
EH0104a	2-Dec-19	Urine	12.7	29.9	17.2	-1	19.4	27.0	7.7	-1
EH0105a	2-Dec-19	Urine	14.6	30.4	15.8	-1	22.0	26.7	4.7	-1
EH0107a	2-Dec-19	Urine	6.0	27.8	21.8	-1	12.8	26.1	13.3	-1
EH0108a	11-Dec-19	Urine	9.8	31.7	21.9	-1	18.4	28.4	10.0	-1
EH0109a	11-Dec-19	Urine	6.7	23.3	16.6	-1	13.3	26.0	12.7	-1
EH0111a	11-Dec-19	Urine	11.4	28.8	17.4	-1	20.0	29.6	9.6	-1
EH0112a	11-Dec-19	Urine	8.5	28.1	19.6	-1	14.9	27.7	12.9	-1
EH0113a	11-Dec-19	Urine	6.6	24.8	18.2	-1	11.8	25.8	14.0	-1
EH0114a	11-Dec-19	Urine	14.5	24.6	10.1	-1	16.7	18.9	2.3	
EH0115a	16-Dec-19	Urine	18.2	28.1	9.9	-1	26.8	31.0	4.1	

EH0116a	16-Dec-19	Urine	13.2	31.2	18.0	-1	19.9	28.2	8.2	-1
EH0117a	16-Dec-19	Urine	8.8	32.3	23.5	-1	16.5	29.9	13.4	-1
EH0118a	16-Dec-19	Urine	10.4	28.0	17.6	-1	15.3	21.9	6.6	-1
EH0119a	16-Dec-19	Urine	6.6	20.6	14.0	-1	7.6	17.2	9.6	-1
EH0120a	16-Dec-19	Urine	10.6	27.0	16.5	-1	15.9	21.9	6.0	-1
EH0121a	16-Dec-19	Urine	10.5	26.4	15.9	-1	16.6	20.9	4.3	
EH0123a	16-Dec-19	Urine	9.8	19.5	9.7	-1	20.2	20.0	-0.2	
EH0124a	9-Jan-20	Urine	10.1	22.5	12.4	-1	17.2	22.7	5.6	-1
EH0126a	9-Jan-20	Urine	10.5	27.5	17.0	-1	18.6	21.3	2.7	
EH0127a	9-Jan-20	Urine	16.9	30.9	14.1	-1	23.5	25.2	1.7	
EH0128a	9-Jan-20	Urine	10.9	27.6	16.7	-1	18.0	22.1	4.2	
EH0129a	9-Jan-20	Urine	7.6	21.3	13.7	-1	16.3	20.0	3.7	
EH0130a	9-Jan-20	Urine	13.1	21.6	8.6	-1	17.8	20.5	2.8	
EH0133a	13-Jan-20	Urine	9.9	22.5	12.6	-1	21.3	22.9	1.6	
EH0134a	13-Jan-20	Urine	13.5	30.7	17.2	-1	20.0	23.6	3.6	
EH0135a	13-Jan-20	Urine	6.6	23.3	16.7	-1	8.1	16.9	8.8	-1
EH0136a	22-Jan-20	Urine	6.6	19.7	13.1	-1	10.8	19.2	8.4	-1
EH0137a	22-Jan-20	Catheter urine	6.6	17.8	11.2	-1	6.9	19.8	12.9	-1
EH0138a	22-Jan-20	Urine	6.6	21.2	14.6	-1	9.6	17.6	8.0	-1
EH0139a	22-Jan-20	Urine	6.6	20.0	13.4	-1	9.1	21.3	12.3	-1
EH0140a	22-Jan-20	Urine	12.0	20.7	8.7	-1	25.0	23.7	-1.3	
EH0141a	22-Jan-20	Urine	12.1	20.0	7.9	-1	24.6	23.7	-0.9	
EH0142a	22-Jan-20	Urine	10.7	18.8	8.1	-1	23.0	20.2	-2.8	
EH0143a	22-Jan-20	Urine	13.2	28.2	15.0	-1	18.3	26.4	8.1	-1
EH0144a	22-Jan-20	Urine	10.6	24.8	14.2	-1	18.7	20.3	1.5	
EH0145a	22-Jan-20	Urine	10.1	23.3	13.2	-1	15.0	23.6	8.6	-1
EH0146a	22-Jan-20	Urine	15.4	26.0	10.6	-1	24.0	27.0	3.0	

EH0147a	22-Jan-20	Urine	13.0	22.6	9.6	-1	23.9	20.8	-3.0	
EH0148a	22-Jan-20	Urine	8.2	22.2	14.0	-1	15.9	21.0	5.1	-1
EH0149a	22-Jan-20	Urine	6.6	18.4	11.8	-1	7.5	19.7	12.3	-1
EH0151a	28-Jan-20	Urine	8.4	23.6	15.2	-1	15.1	24.8	9.7	-1
EH0153a	28-Jan-20	Urine	7.3	20.8	13.5	-1	12.8	21.6	8.9	-1
EH0154a	28-Jan-20	Urine	13.0	28.4	15.4	-1	19.5	25.0	5.5	-1
EH0155a	2-Feb-20	Urine	15.4	25.0	9.6	-1	17.6	20.1	2.5	
EH0156a	2-Feb-20	Urine	9.9	29.8	19.9	-1	18.0	27.2	9.2	-1
EH0160a	2-Feb-20	Urine	6.0	20.0	14.0	-1	10.6	17.5	6.9	-1
EH0161a	2-Feb-20	Urine	9.9	24.7	14.8	-1	18.0	20.6	2.7	
EH0162a	2-Feb-20	Urine	22.2	36.6	14.4	-1	33.8	39.4	5.6	-1
EH0163a	2-Feb-20	Urine	9.3	24.3	15.0	-1	15.4	20.5	5.1	-1
EH0164a	2-Feb-20	Urine	17.7	25.2	7.5	-1	26.0	23.4	-2.6	0
EH0165a	2-Feb-20	Urine	6.0	17.1	11.1	-1	7.6	13.7	6.0	-1
EH0167a	2-Feb-20	Urine	14.7	22.5	7.8	-1	23.1	22.3	-0.9	
EH0168a	2-Feb-20	Urine	12.2	25.5	13.3	-1	18.3	23.6	5.3	-1
EH0169a	2-Feb-20	Urine	8.6	22.4	13.8	-1	12.7	18.9	6.2	-1
EH0171a	18-Feb-20	Urine	6.6	24.6	18.0	-1	11.6	26.5	14.9	-1
EH0172a	18-Feb-20	Urine	12.2	28.7	16.6	-1	19.1	29.8	10.7	-1
EH0174a	18-Feb-20	Urine	13.4	29.5	16.1	-1	19.2	29.3	10.2	-1
EH0175a	18-Feb-20	Urine	9.7	28.7	19.0	-1	15.7	28.0	12.3	-1
EH0176a	24-Feb-20	Urine	6.0	18.1	12.1	-1	6.4	16.7	10.4	-1
EH0177a	24-Feb-20	Urine	11.6	24.7	13.2	-1	19.1	24.1	4.9	
EH0178a	24-Feb-20	Urine	10.9	34.1	23.2	-1	18.8	27.6	8.8	-1
EH0179a	24-Feb-20	Urine	9.8	32.5	22.7	-1	19.0	25.5	6.5	-1
EH0180a	24-Feb-20	Urine	10.4	34.0	23.7	-1	17.1	29.7	12.6	-1
EH0181a	24-Feb-20	Urine	15.0	30.8	15.8	-1	21.3	26.4	5.1	-1

EH0182a	24-Feb-20	Urine	13.3	27.5	14.2	-1	23.2	27.9	4.7	
EH0184a	24-Feb-20	Urine	8.1	31.5	23.4	-1	13.8	24.9	11.1	-1
EH0187a	2-Mar-20	Urine	6.7	27.9	21.2	-1	14.4	22.9	8.5	-1
EH0188a	2-Mar-20	Urine	11.9	34.4	22.5	-1	19.1	25.3	6.2	-1
EH0189a	2-Mar-20	Urine	12.9	27.7	14.8	-1	20.2	23.0	2.8	
EH0190a	2-Mar-20	Urine	12.8	33.5	20.7	-1	18.9	29.1	10.1	-1
EH0191a	2-Mar-20	Urine	14.4	28.8	14.5	-1	23.5	25.5	2.0	
EH0192a	2-Mar-20	Urine	10.0	22.8	12.9	-1	20.6	18.9	-1.7	
EH0193a	9-Mar-20	Urine	14.6	26.4	11.8	-1	23.8	27.1	3.3	
EH0205a	16-Mar-20	Urine	9.2	26.0	16.8	-1	18.6	21.4	2.8	
EH0206a	16-Mar-20	Urine	12.9	28.3	15.5	-1	18.4	22.8	4.4	
EH0208a	16-Mar-20	Urine	11.0	26.6	15.6	-1	20.3	23.1	2.8	
EH0210a	16-Mar-20	Urine	8.7	25.1	16.4	-1	19.5	23.3	3.9	
EH0212a	16-Mar-20	Urine	11.7	32.1	20.4	-1	17.9	28.2	10.3	-1
EH0213a	17-Jun-20	Urine	12.5	39.4	26.9	-1	19.4	27.4	8.0	-1
EH0214a	17-Jun-20	Urine	13.1	31.9	18.8	-1	19.4	26.4	7.0	-1
EH0215a	17-Jun-20	Urine	6.6	27.8	21.2	-1	11.7	24.4	12.7	-1
EH0216a	17-Jun-20	Urine	8.0	33.0	25.1	-1	15.2	30.2	15.0	-1
EH0217a	17-Jun-20	Urine	14.6	25.8	11.2	-1	18.1	17.8	-0.2	
EH0220a	17-Jun-20	Urine	13.6	32.3	18.7	-1	21.0	28.8	7.8	-1
EH0224a	17-Jun-20	Urine	11.5	20.6	9.1	-1	20.1	31.9	11.8	-1
EH0229a	17-Jun-20	Urine	11.3	33.7	22.4	-1	18.8	28.4	9.7	-1
EH0230a	17-Jun-20	Urine	10.4	29.4	19.0	-1	16.9	28.7	11.8	-1
EH0231a	26-Jun-20	Urine	11.7	29.2	17.6	-1	17.7	26.2	8.6	-1
EH0232a	26-Jun-20	Urine	15.3	31.0	15.7	-1	24.1	29.9	5.8	-1
EH0233a	26-Jun-20	Fluid in blood culture bottle	13.3	33.4	20.1	-1	20.9	30.7	9.8	-1
EH0235a	26-Jun-20	Urine	13.1	32.4	19.3	-1	22.2	30.2	8.0	-1

EH0237a	26-Jun-20	Urine	12.6	30.6	18.0	-1	20.0	27.3	7.3	-1
EH0238a	26-Jun-20	Urine	11.2	32.6	21.4	-1	20.5	29.6	9.1	-1
EH0239a	26-Jun-20	Urine	18.4	29.7	11.2	-1	26.3	28.5	2.2	
EH0240a	26-Jun-20	Urine	14.9	34.8	19.9	-1	24.2	32.0	7.8	-1
EH0241a	29-Jun-20	Urine	10.2	29.7	19.5	-1	15.2	29.3	14.1	-1
EH0242a	29-Jun-20	Urine	17.1	30.1	13.0	-1	27.3	27.2	-0.1	
EH0243a	29-Jun-20	Urine	12.0	30.2	18.3	-1	18.9	26.3	7.4	-1
EH0244a	29-Jun-20	Urine	15.0	37.6	22.6	-1	17.9	37.2	19.2	-1
EH0248a	29-Jun-20	Urine	6.7	21.0	14.3	-1	13.6	22.5	8.9	-1
EH0251a	6-Jul-20	Urine	11.8	23.8	12.0	-1	17.9	23.6	5.7	-1
EH0254a	6-Jul-20	Urine	6.3	18.5	12.2	-1	9.6	19.6	9.9	-1
EH0255a	6-Jul-20	Urine	7.7	20.5	12.8	-1	11.0	19.0	8.1	-1
EH0256a	6-Jul-20	Urine	12.0	24.2	12.2	-1	18.7	24.8	6.1	-1
EH0257a	21-Jul-20	Bag urine	10.1	27.0	16.9	-1	20.2	24.1	3.8	
EH0258a	21-Jul-20	Urine	8.0	26.9	19.0	-1	15.8	24.1	8.3	-1
EH0259a	21-Jul-20	Wound swab	11.1	26.6	15.5	-1	17.5	25.1	7.6	-1
EH0260a	21-Jul-20	Urine	6.2	23.0	16.8	-1	9.2	20.0	10.8	-1
EH0261a	21-Jul-20	Urine	13.5	25.5	12.0	-1	20.9	24.2	3.4	
EH0262a	21-Jul-20	Urine	17.6	27.5	9.9	-1	26.8	28.7	1.9	
EH0263a	21-Jul-20	Urine	8.1	20.2	12.1	-1	16.0	21.3	5.2	-1
EH0264a	21-Jul-20	Urine	16.5	27.2	10.6	-1	27.5	27.8	0.3	
EH0265a	21-Jul-20	Urine	12.0	24.4	12.4	-1	17.8	23.7	5.9	-1
EH0267a	21-Jul-20	Urine	16.4	27.8	11.4	-1	26.3	28.3	2.0	
EH0268a	21-Jul-20	Urine	11.6	23.9	12.3	-1	19.2	23.0	3.8	
EH0269a	21-Jul-20	Urine	6.6	21.6	15.1	-1	16.2	20.8	4.6	
EH0270a	21-Jul-20	Urine	9.9	23.8	13.9	-1	16.8	22.2	5.4	-1
EH0271a	21-Jul-20	Urine	17.0	24.6	7.6	-1	27.0	25.9	-1.0	

EH0272a	21-Jul-20	Urine	13.0	25.7	12.7	-1	21.2	24.6	3.4	
EH0275a	21-Jul-20	Urine	14.9	22.9	8.0	-1	28.4	27.8	-0.5	
EH0277a	28-Jul-20	Urine	15.8	23.6	7.8	-1	21.2	22.7	1.5	
EH0279a	3-Aug-20	Urine	10.0	24.9	14.9	-1	19.5	24.2	4.8	
EH0280a	3-Aug-20	Catheter urine	6.9	22.1	15.2	-1	13.8	18.1	4.2	
EH0282a	3-Aug-20	Urine	9.0	23.8	14.8	-1	15.3	23.8	8.6	-1
EH0283a	31-Aug-20	Urine	6.6	26.8	20.2	-1	13.1	24.9	11.8	-1
EH0284a	31-Aug-20	Urine	8.0	29.2	21.3	-1	15.0	27.7	12.8	-1
EH0285a	31-Aug-20	Urine	6.6	25.9	19.3	-1	10.0	22.3	12.4	-1
EH0286a	31-Aug-20	Urine	6.7	27.9	21.3	-1	13.0	27.4	14.4	-1
EH0287a	31-Aug-20	Urine	6.6	20.9	14.3	-1	9.5	24.5	15.0	-1
EH0288a	31-Aug-20	Urine	12.3	20.8	8.5	-1	18.9	26.0	7.1	-1
EH0290a	31-Aug-20	Urine	10.6	31.8	21.2	-1	16.1	28.2	12.1	-1
EH0292a	31-Aug-20	Urine	7.0	27.9	20.9	-1	13.2	28.4	15.2	-1
EH0293a	31-Aug-20	Urine	10.1	18.0	7.9	-1	12.5	6.6	-5.9	
EH0294a	31-Aug-20	Urine	9.2	33.4	24.2	-1	20.9	29.6	8.8	-1
EH0295a	31-Aug-20	Urine	9.8	30.0	20.2	-1	19.2	27.8	8.5	-1
EH0296a	31-Aug-20	Urine	7.9	30.8	22.8	-1	15.7	28.6	12.9	-1
EH0297a	31-Aug-20	Urine	20.9	30.4	9.6	-1	28.3	30.4	2.0	
EH0298a	31-Aug-20	Urine	10.5	30.4	19.9	-1	19.0	27.1	8.1	-1
EH0299a	31-Aug-20	Urine	12.0	32.3	20.3	-1	19.8	27.6	7.8	-1
EH0300a	31-Aug-20	Urine	6.8	28.9	22.1	-1	14.9	24.3	9.4	-1
EH0301a	31-Aug-20	Urine	10.2	31.6	21.4	-1	16.7	28.3	11.6	-1
EH0303a	8-Sep-20	Urine	25.5	32.5	7.1	-1	32.2	32.2	0.0	
EH0304a	8-Sep-20	Urine	10.8	30.5	19.8	-1	16.3	27.2	10.8	-1
EH0306a	8-Sep-20	Urine	6.0	29.2	23.2	-1	14.8	26.6	11.7	-1
EH0307a	8-Sep-20	Urine	6.0	28.3	22.3	-1	12.8	25.8	12.9	-1

EH0308a	8-Sep-20	Urine	10.9	19.4	8.5	-1	12.9	6.0	-6.9	
EH0310a	14-Sep-20	Urine	8.0	28.6	20.6	-1	16.8	27.0	10.2	-1
EH0313a	28-Sep-20	Urine	12.7	29.9	17.2	-1	23.1	26.6	3.5	
EH0314a	28-Sep-20	Urine	15.2	31.1	15.9	-1	22.1	27.2	5.1	-1
EH0315a	28-Sep-20	Urine	6.0	29.0	23.0	-1	11.0	26.7	15.7	-1
EH0316a	28-Sep-20	Urine	13.5	31.5	18.0	-1	19.8	27.2	7.4	-1
EH0317a	28-Sep-20	Urine	9.5	32.7	23.2	-1	18.8	29.4	10.6	-1
EH0318a	28-Sep-20	Urine	12.2	30.3	18.1	-1	14.0	27.8	13.8	-1
EH0319a	28-Sep-20	Urine	8.8	29.1	20.3	-1	14.0	27.8	13.8	-1
EH0321a	14-Oct-20	Urine	9.0	32.5	23.5	-1	19.7	29.8	10.2	-1
EH0322a	14-Oct-20	Urine	13.1	31.1	18.0	-1	19.0	26.9	7.8	-1
EH0323a	14-Oct-20	Urine	13.9	30.2	16.3	-1	20.2	28.6	8.4	-1
EH0324a	14-Oct-20	Urine	11.0	32.5	21.5	-1	20.2	30.0	9.8	-1
EH0327a	20-Oct-20	Urine	14.6	32.7	18.2	-1	20.8	29.1	8.3	-1
EH0328a	20-Oct-20	Urine	11.1	30.1	19.1	-1	18.5	28.8	10.3	-1
EH0329a	20-Oct-20	Urine	13.5	31.9	18.4	-1	21.1	28.1	7.0	-1
EH0330a	20-Oct-20	Catheter urine	12.4	32.6	20.3	-1	20.6	30.3	9.7	-1
EH0331a	20-Oct-20	Urine	6.0	28.8	22.8	-1	12.6	25.0	12.4	-1
EH0332a	20-Oct-20	Urine	8.7	28.1	19.4	-1	15.3	23.2	7.9	-1
EH0333a	28-Oct-20	Catheter urine	11.3	30.0	18.7	-1	18.2	25.9	7.7	-1
EH0334a	28-Oct-20	Urine	7.0	28.4	21.3	-1	12.5	25.4	13.0	-1
EH0335a	28-Oct-20	Urine	13.2	30.6	17.4	-1	21.2	27.3	6.1	-1
EH0337a	28-Oct-20	Urine	10.8	30.5	19.7	-1	15.7	26.2	10.5	-1
EH0339a	2-Nov-20	Urine	6.4	30.2	23.9	-1	12.9	26.8	13.9	-1
EH0340a	2-Nov-20	Urine	9.3	31.5	22.2	-1	18.3	28.9	10.7	-1
EH0344a	2-Nov-20	Urine	10.2	33.5	23.3	-1	14.7	30.3	15.6	-1
EH0345a	2-Nov-20	Urine	6.0	27.5	21.5	-1	14.1	24.9	10.8	-1

EH0346a	9-Nov-20	Urine	14.1	31.7	17.7	-1	22.9	29.5	6.7	-1
EH0347a	9-Nov-20	Urine	6.0	26.2	20.2	-1	12.0	25.3	13.4	-1
EH0348a	9-Nov-20	Urine	9.3	26.5	17.2	-1	15.6	27.3	11.7	-1
EH0350a	9-Nov-20	Urine	12.4	31.4	19.0	-1	20.4	28.5	8.0	-1
EH0353a	9-Nov-20	Urine	10.0	29.2	19.1	-1	17.3	25.3	8.0	-1
EH0354a	16-Nov-20	Urine	9.1	29.3	20.2	-1	15.9	25.9	10.1	-1
EH0356a	23-Nov-20	Catheter urine	12.8	31.1	18.3	-1	18.4	28.7	10.3	-1
EH0357a	23-Nov-20	Urine	6.6	25.0	18.4	-1	16.2	27.0	10.8	-1
EH0358a	30-Nov-20	Urine	14.3	30.7	16.4	-1	21.6	29.1	7.5	-1
EH0359a	30-Nov-20	Urine	9.9	29.2	19.3	-1	13.9	25.3	11.4	-1
EH0361a	30-Nov-20	Urine	10.9	30.1	19.1	-1	18.5	27.8	9.3	-1
EH0362a	30-Nov-20	Catheter urine	8.2	30.8	22.6	-1	14.6	27.1	12.5	-1
EH0363a	30-Nov-20	Urine	21.3	34.3	13.0	-1	33.5	33.2	-0.3	
EH0364a	30-Nov-20	Urine	6.8	24.4	17.6	-1	19.5	23.9	4.4	
EH0365a	30-Nov-20	Catheter urine	12.7	30.2	17.5	-1	20.1	27.1	7.0	-1
EH0366a	30-Nov-20	Urine	12.5	31.9	19.4	-1	22.0	29.6	7.7	-1
EH0367a	30-Nov-20	Urine	6.6	27.2	20.6	-1	8.2	25.2	17.0	-1
EH0368a	30-Nov-20	Urine	13.7	30.5	16.8	-1	24.9	28.8	3.9	
EH0369a	30-Nov-20	Urine	10.9	32.7	21.8	-1	17.4	28.8	11.5	-1
EH0370a	7-Dec-20	Urine	6.6	33.7	27.1	-1	7.2	27.5	20.3	-1
EH0371a	7-Dec-20	Urine	11.4	29.8	18.4	-1	15.3	24.1	8.8	-1
EH0373a	7-Dec-20	Urine	15.1	32.8	17.7	-1	24.0	30.1	6.2	-1
EH0374a	7-Dec-20	Urine	6.6	30.2	23.6	-1	15.8	27.1	11.2	-1
EH0375a	7-Dec-20	Urine	13.3	32.2	18.9	-1	18.9	28.5	9.6	-1
EH0376a	7-Dec-20	Aspirate	6.6	32.3	25.7	-1	15.4	28.2	12.8	-1
EH0377a	14-Dec-20	Urine	6.6	29.7	23.1	-1	11.9	28.4	16.6	-1
EH0378a	14-Dec-20	Urine	12.1	27.8	15.7	-1	25.5	29.5	4.0	

EH0379a	14-Dec-20	Urine	14.4	31.4	17.0	-1	21.2	29.4	8.2	-1
EH0381a	14-Dec-20	Urine	9.7	29.8	20.1	-1	16.6	27.3	10.7	-1
EH0382a	14-Dec-20	Urine	9.2	28.6	19.4	-1	15.0	28.5	13.5	-1
EH0383a	11-Jan-21	Urine	7.3	30.5	23.2	-1	15.0	26.1	11.1	-1
EH0384a	11-Jan-21	Urine	9.4	28.1	18.7	-1	15.7	25.4	9.7	-1
EH0386a	11-Jan-21	Urine	16.2	30.7	14.5	-1	26.3	28.6	2.3	
EH0387a	11-Jan-21	Urine	6.9	29.0	22.1	-1	15.6	26.8	11.2	-1
EH0388a	11-Jan-21	Urine	9.0	27.3	18.2	-1	18.4	25.4	7.0	-1
EH0389a	11-Jan-21	Urine	7.4	29.4	22.0	-1	17.3	27.3	10.0	-1
EH0391a	11-Jan-21	Urine	13.5	27.9	14.4	-1	24.2	26.3	2.2	
EH0392a	11-Jan-21	Urine	6.6	30.4	23.8	-1	12.5	27.2	14.7	-1
EH0393a	11-Jan-21	Catheter urine	6.6	30.3	23.7	-1	12.0	27.2	15.2	-1
EH0394a	11-Jan-21	Urine	21.1	35.6	14.4	-1	13.7	27.3	13.7	-1
EH0395a	11-Jan-21	Urine	9.1	27.0	17.9	-1	19.4	25.1	5.7	-1
EH0396a	11-Jan-21	Urine	6.6	26.7	20.1	-1	15.4	24.0	8.7	-1
EH0397a	11-Jan-21	Urine	7.9	29.6	21.7	-1	15.8	27.0	11.1	-1
EH0398a	12-Jan-21	Urine	11.7	32.3	20.6	-1	20.8	28.9	8.0	-1
EH0401a	12-Jan-21	Urine	10.3	31.1	20.9	-1	19.3	29.0	9.7	-1
EH0402a	12-Jan-21	Blood culture	6.6	28.6	22.0	-1	10.8	25.4	14.5	-1
EH0404a	12-Jan-21	Urine	15.5	32.6	17.2	-1	20.3	28.6	8.3	-1
EH0406a	12-Jan-21	Urine	15.6	33.0	17.4	-1	24.5	30.2	5.8	-1
EH0407a	12-Jan-21	Urine	15.0	27.8	12.8	-1	22.6	25.4	2.7	
EH0409a	12-Jan-21	Urine	14.8	28.2	13.4	-1	22.4	25.4	3.0	
EH0410a	18-Jan-21	Urine	6.6	29.0	22.4	-1	6.6	26.3	19.7	-1
EH0413a	18-Jan-21	Urine	18.9	30.8	11.9	-1	28.1	28.7	0.5	
EH0414a	18-Jan-21	Urine	9.3	30.3	21.0	-1	16.7	26.3	9.6	-1

Appendix 6

Table of the breakpoint diameters for the 155 environmental presumptive ESBL producing *E. coli* tested against ten antibiotics.

Date Collected	Street Address	Sample Type	Env SampleID	Env IsolateID	CTX diff	CAZ diff	TS diff	GM diff	T diff	CIP diff	NI diff	S diff	FOX diff	C diff
19-Aug-19	Napier Road	Water	SB0260	SB0260c	24.3	18.7	29.3	23.0	18.4	33.6	16.5	17.0	15.3	20.7
19-Aug-19	Napier Road	Water	SB0260	SB0260d	24.0	19.7	28.1	23.4	18.6	17.1	16.9	16.7	15.7	19.3
19-Aug-19	Napier Road	Water	SB0261	SB0261c	24.6	19.9	27.1	22.9	22.2	36.8	17.4	18.0	15.3	22.6
19-Aug-19	Napier Road	Water	SB0261	SB0261d	24.4	20.8	28.5	21.0	21.6	34.4	15.1	17.3	15.4	23.4
19-Aug-19	Centennial Drive	Water	SB0262	SB0262c	24.7	19.5	28.3	23.6	18.1	35.5	16.4	17.1	15.5	20.4
19-Aug-19	Centennial Drive	Water	SB0262	SB0262d	24.1	19.0	29.6	22.5	18.8	33.4	15.1	17.7	15.4	21.5
19-Aug-19	Centennial Drive	Water	SB0263	SB0263c	24.9	20.9	28.7	24.2	20.5	34.6	17.0	17.3	15.6	22.7
19-Aug-19	Centennial Drive	Water	SB0263	SB0263d	23.3	18.8	26.4	23.6	20.2	33.2	17.6	17.3	15.4	22.0
19-Aug-19	Tip Rd	Water	SB0264	SB0264c	24.0	19.9	28.6	24.5	18.8	34.9	14.8	18.7	16.8	24.1
19-Aug-19	Tip Rd	Water	SB0264	SB0264d	25.2	20.3	30.0	25.1	21.3	32.1	21.2	17.7	16.4	24.6
19-Aug-19	Tip Rd	Water	SB0264	SB0264e	23.1	18.2	6.6	22.5	6.6	27.1	16.0	6.6	12.2	25.2
19-Aug-19	Tip Rd	Water	SB0264	SB0264f	24.2	19.4	6.6	23.9	7.5	38.5	15.8	6.6	14.4	24.0
23-Sep-19	Napier Road	Water	SB0267	SB0267h1	8.7	17.8	27.8	7.7	21.2	6.6	17.1	15.6	23.6	25.4
23-Sep-19	Napier Road	Water	SB0267	SB0267h2	9.5	17.8	30.6	8.9	22.0	6.6	17.3	16.6	24.9	24.8
23-Sep-19	Tip Rd	Water	SB0273	SB0273h2	8.0	21.7	26.0	6.6	21.4	6.6	18.3	15.4	20.2	22.1
23-Sep-19	Tip Rd	Treated effluent	SB0276	SB0276h2	6.6	8.8	6.6	6.6	6.6	6.6	11.4	6.6	15.0	20.8
17-Nov-19	Centennial Drive	Storm Water	SB0283	SB0283c	6.6	11.4	24.5	23.4	16.2	35.9	12.9	15.5	18.8	21.6
17-Nov-19	Centennial Drive	Storm Water	SB0283	SB0283f	6.6	10.8	26.1	23.9	16.4	36.1	12.6	15.6	18.1	21.3

17-Nov-19	Centennial Drive	Storm Water	SB0283	SB0283h1	6.6	10.3	27.4	22.7	18.3	35.9	12.1	15.8	20.3	22.4
17-Nov-19	Centennial Drive	Storm Water	SB0283	SB0283h2	6.6	10.7	27.1	23.0	18.2	34.9	14.3	16.8	17.9	23.2
17-Nov-19	Tip Rd	Sediment	SB0286	SB0286c	15.8	12.3	23.5	24.5	28.6	22.6	16.1	10.6	7.1	24.2
17-Nov-19	Tip Rd	Sediment	SB0286	SB0286d	16.5	12.9	25.8	24.7	19.5	24.5	18.1	10.2	7.1	24.8
17-Nov-19	Tip Rd	Sediment	SB0286	SB0286e	15.4	12.6	24.0	26.3	18.2	23.5	17.1	11.0	7.9	23.6
17-Nov-19	Tip Rd	Sediment	SB0286	SB0286h1	13.4	10.5	21.2	23.0	16.3	22.6	17.9	9.9	6.6	22.7
17-Nov-19	Tip Rd	Sediment	SB0286	SB0286h2	14.4	11.0	22.1	22.1	17.9	22.0	17.0	9.2	6.6	23.1
16-Dec-19	Tip Rd	Water	SB0295	SB0295d	9.3	16.8	26.0	21.6	18.9	21.0	21.1	16.3	22.7	23.3
16-Dec-19	Tip Rd	Water	SB0296	SB0296c	18.0	13.0	15.5	21.4	6.6	24.7	16.8	16.5	6.6	18.1
16-Dec-19	Tip Rd	Water	SB0296	SB0296d	18.2	14.0	15.7	21.7	6.6	23.9	18.7	17.0	6.6	21.3
16-Dec-19	Tip Rd	Water	SB0296	SB0296e	10.9	7.1	6.6	21.8	6.6	21.7	18.4	13.2	6.6	20.8
16-Dec-19	Tip Rd	Water	SB0296	SB0296f	10.1	7.6	6.6	21.9	6.6	24.7	20.6	12.6	7.6	19.5
16-Dec-19	Tip Rd	Treated effluent	SB0298	SB0298h1	11.3	22.2	14.6	22.9	6.6	6.6	13.8	8.6	20.6	6.6
13-Jan-20	Tip Rd	Water	SB0308	SB0308e	15.1	25.8	6.6	7.8	6.6	23.3	19.6	9.4	21.8	20.9
13-Jan-20	Tip Rd	Water	SB0308	SB0308f	16.6	27.2	6.6	7.6	6.6	23.5	21.6	8.7	23.0	20.0
13-Jan-20	Tip Rd	Water	SB0308	SB0308h1	15.6	24.6	6.6	8.0	6.6	20.0	20.1	10.7	21.5	18.9
13-Jan-20	Tip Rd	Water	SB0308	SB0308h2	15.3	24.9	6.6	8.1	6.6	21.6	21.0	9.1	21.7	18.8
09-Feb-20	Centennial Drive	Water	SB0318	SB0318c	6.6	11.7	6.6	23.0	19.5	13.9	22.3	11.7	24.9	22.3
09-Feb-20	Centennial Drive	Water	SB0318	SB0318d	6.6	11.1	6.6	22.6	17.7	12.9	15.5	10.0	23.8	22.3
09-Feb-20	Centennial Drive	Water	SB0318	SB0318e	6.6	12.2	6.6	22.5	17.7	12.2	20.0	11.1	22.2	22.1
08-Mar-20	Tip Rd	Water	SB0337	SB0337h1a	12.7	20.5	6.6	21.6	6.6	6.6	22.4	6.6	23.0	21.8
08-Mar-20	Tip Rd	Water	SB0337	SB0337h1b	12.4	19.6	6.6	21.8	6.6	6.6	21.2	6.6	21.9	23.2
08-Mar-20	Tip Rd	Water	SB0338	SB0338c1	9.3	17.5	29.0	22.2	17.6	25.6	16.4	15.4	19.2	22.4
08-Mar-20	Tip Rd	Water	SB0338	SB0338d1	6.8	15.2	6.6	8.3	6.6	6.6	21.9	6.6	18.1	18.1
08-Mar-20	Tip Rd	Water	SB0338	SB0338e1	8.9	18.2	27.2	23.4	19.2	23.6	18.4	16.2	19.9	21.6

08-Mar-20	Tip Rd	Water	SB0338	SB0338h1a	8.6	17.3	25.9	22.9	19.3	23.9	18.0	16.4	20.7	21.1
08-Mar-20	Tip Rd	Water	SB0338	SB0338h1b	9.7	17.5	6.6	20.4	6.6	6.6	20.7	6.6	22.0	22.2
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347c1	8.2	14.7	26.3	6.6	20.3	6.6	18.2	16.7	21.7	24.0
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347d1	7.8	13.9	26.2	6.6	16.6	6.6	19.3	16.9	20.9	23.8
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347e1	8.3	13.6	26.1	6.6	18.0	6.6	18.2	16.0	21.4	23.3
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347f1	9.3	15.8	27.1	6.6	18.1	6.6	19.4	16.8	21.9	24.2
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347h1a	8.5	12.7	29.9	6.6	19.5	6.6	20.2	17.9	24.6	24.1
19-Jul-20	Centennial Drive	Storm Water	SB0347	SB0347h1b	7.0	13.3	25.1	6.6	16.9	6.6	18.2	16.2	21.6	19.6
19-Jul-20	Tip Rd	Water	SB0351	SB0351c2	8.4	17.6	6.6	21.0	6.6	21.7	13.9	7.0	20.2	21.0
19-Jul-20	Tip Rd	Water	SB0351	SB0351d2	8.5	17.4	6.6	21.1	6.6	21.7	18.4	7.2	20.7	19.9
19-Jul-20	Tip Rd	Water	SB0351	SB0351e2	9.2	17.4	6.6	21.8	6.6	21.5	15.0	7.0	20.7	20.8
19-Jul-20	Tip Rd	Water	SB0351	SB0351h2a	8.3	17.2	6.6	22.3	6.6	21.6	15.3	6.7	20.6	21.2
19-Jul-20	Tip Rd	Water	SB0351	SB0351h2b	7.8	19.0	6.6	21.8	6.6	23.0	13.7	6.6	21.0	21.2
19-Jul-20	Tip Rd	Water	SB0352	SB0352d2	7.7	17.4	6.6	21.8	6.6	32.8	18.0	6.6	23.4	22.1
19-Jul-20	Tip Rd	Water	SB0352	SB0352e2	9.3	17.5	6.6	20.6	6.6	28.6	18.9	6.6	23.0	22.1
19-Jul-20	Tip Rd	Water	SB0352	SB0352f2	8.7	17.6	6.6	21.2	6.6	29.7	21.6	6.6	22.8	19.7
19-Jul-20	Tip Rd	Water	SB0352	SB0352h2a	9.4	18.2	6.6	21.9	6.6	30.5	20.2	6.6	23.0	20.1
19-Jul-20	Tip Rd	Treated effluent	SB0354	SB0354c1	13.4	9.9	26.1	22.7	14.3	32.3	15.2	16.4	6.6	20.2
19-Jul-20	Tip Rd	Treated effluent	SB0354	SB0354c2	9.9	19.3	9.1	21.2	18.7	19.2	14.5	16.4	22.7	17.2
19-Jul-20	Tip Rd	Treated effluent	SB0354	SB0354e2	12.8	20.7	15.1	23.0	19.3	2078.0	22.0	16.9	24.8	18.2
19-Jul-20	Tip Rd	Treated effluent	SB0354	SB0354h4	8.5	19.0	26.6	21.0	6.6	6.6	17.3	15.3	20.6	20.2
17-Aug-20	Tip Rd	Water	SB0364	SB0364c1	12.0	20.5	8.6	24.3	23.2	40.4	21.4	16.8	26.6	22.1
17-Aug-20	Tip Rd	Water	SB0364	SB0364d1	11.1	20.6	13.6	22.2	20.2	26.3	18.8	17.3	25.4	21.5
17-Aug-20	Tip Rd	Water	SB0364	SB0364e1	11.5	20.7	16.9	20.5	22.0	39.7	22.9	18.0	26.5	21.6

17-Aug-20	Tip Rd	Water	SB0365	SB0365c2	7.9	13.7	28.7	24.3	18.6	21.5	24.4	19.8	23.1	20.0
17-Aug-20	Tip Rd	Water	SB0365	SB0365d2	8.8	14.2	28.4	24.0	17.5	20.3	23.7	19.2	23.2	21.5
17-Aug-20	Tip Rd	Water	SB0365	SB0365e2	8.3	13.7	28.5	24.9	19.8	19.3	23.0	19.5	23.4	20.9
17-Aug-20	Tip Rd	Water	SB0365	SB0365f2	7.2	13.3	13.6	24.2	18.1	20.7	22.3	17.3	22.3	20.2
17-Aug-20	Tip Rd	Water	SB0365	SB0365h2a	7.8	14.4	30.1	24.1	20.1	22.5	24.1	19.5	23.5	21.2
17-Aug-20	Tip Rd	Water	SB0365	SB0365h2b	7.8	13.4	28.2	23.2	16.8	20.6	21.5	18.6	23.3	22.5
14-Sep-20	Napier Road	Sediment	SB0370	SB0370 f1	22.9	17.4	28.2	22.5	17.4	33.0	17.0	16.5	8.4	22.0
14-Sep-20	Napier Road	Sediment	SB0370	SB0370c1	20.9	17.0	28.6	21.8	16.2	30.9	16.9	15.9	8.9	18.3
14-Sep-20	Napier Road	Sediment	SB0370	SB0370e1	23.2	17.6	28.1	22.9	16.9	34.1	17.0	17.2	8.4	18.3
14-Sep-20	Centennial Drive	Water	SB0372	SB0372c1	25.2	22.9	31.4	22.8	20.7	38.5	15.1	18.9	15.8	24.2
14-Sep-20	Centennial Drive	Water	SB0372	SB0372d1	26.8	22.4	29.4	22.6	18.8	39.3	16.7	17.7	17.2	21.5
14-Sep-20	Centennial Drive	Water	SB0372	SB0372e1	28.2	24.9	30.9	22.2	19.6	40.4	13.7	18.4	17.6	21.6
14-Sep-20	Centennial Drive	Water	SB0372	SB0372f1	30.0	25.6	30.2	22.9	19.3	42.1	16.1	20.0	17.5	23.0
14-Sep-20	Tip Rd	Water	SB0377	SB0377c2	7.5	10.0	6.6	19.2	6.6	17.3	20.0	6.6	21.1	6.6
14-Sep-20	Tip Rd	Water	SB0377	SB0377d2	6.7	6.6	6.6	18.0	6.6	15.9	18.2	6.6	19.4	6.6
14-Sep-20	Tip Rd	Water	SB0377	SB0377e2	6.6	9.9	6.6	18.0	6.6	14.3	18.2	6.6	19.0	6.6
14-Sep-20	Tip Rd	Water	SB0377	SB0377f2	6.6	9.6	6.6	18.3	6.6	15.7	18.9	6.6	20.0	6.6
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380c2	15.2	11.4	30.0	23.2	18.3	38.1	15.7	18.2	6.6	20.4
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380d2	14.7	11.5	29.1	22.8	13.7	37.0	15.5	17.5	6.6	22.8
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380e2	15.2	11.8	30.7	22.9	16.6	35.8	15.9	19.2	6.6	19.3
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380f2	14.7	11.1	31.0	22.4	18.4	38.2	16.0	18.0	6.6	21.4
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380h2a	6.6	12.8	6.6	6.6	19.8	24.4	25.3	14.6	23.1	21.6
14-Sep-20	Tip Rd	Treated effluent	SB0380	SB0380h2b	6.6	9.2	6.6	6.6	11.7	6.6	10.2	6.6	10.5	15.0

12-Oct-20	Centennial Drive	Sediment	SB0386	SB0386c1	28.7	25.0	29.5	21.7	26.9	37.1	20.0	18.3	18.5	24.3
12-Oct-20	Centennial Drive	Sediment	SB0386	SB0386d1	28.8	22.9	27.7	21.6	24.8	36.9	19.0	19.4	18.1	22.2
12-Oct-20	Tip Rd	Water	SB0390	SB0390h1a	8.1	12.9	26.9	23.0	18.8	22.0	17.3	17.0	23.3	19.0
12-Oct-20	Tip Rd	Water	SB0391	SB0391c1	29.5	25.8	27.6	21.7	21.4	6.6	20.9	20.0	14.6	21.6
12-Oct-20	Tip Rd	Water	SB0391	SB0391c2	17.6	12.9	27.6	23.3	18.8	38.1	19.7	17.8	6.6	24.0
12-Oct-20	Tip Rd	Water	SB0391	SB0391d2	16.0	12.4	32.6	23.5	23.9	34.9	25.0	19.9	6.6	24.1
12-Oct-20	Tip Rd	Water	SB0391	SB0391e2	17.0	11.6	30.1	23.5	21.2	37.6	24.1	20.6	6.6	23.5
12-Oct-20	Tip Rd	Water	SB0391	SB0391f1	12.1	25.9	31.2	24.8	20.9	40.0	20.0	19.2	28.5	23.1
12-Oct-20	Tip Rd	Water	SB0391	SB0391f2	16.3	11.3	30.9	23.0	20.6	36.6	23.8	20.3	6.6	22.2
12-Oct-20	Tip Rd	Water	SB0391	SB0391h1a	9.1	22.8	29.1	22.8	20.3	37.9	19.9	16.6	23.6	21.2
12-Oct-20	Tip Rd	Water	SB0391	SB0391h2a	8.4	15.0	29.1	22.6	19.9	6.6	18.3	17.1	22.8	21.3
12-Oct-20	Tip Rd	Water	SB0391	SB0391h2b	8.2	14.4	29.1	23.5	20.8	6.6	20.4	17.4	24.0	20.9
12-Oct-20	Tip Rd	Treated effluent	SB0393	SB0393e1	24.8	18.3	28.3	23.7	19.3	33.4	16.7	16.5	13.9	23.6
12-Oct-20	Tip Rd	Treated effluent	SB0393	SB0393f1	23.0	20.1	27.5	22.6	20.9	30.2	15.5	16.8	13.6	21.9
12-Oct-20	Tip Rd	Treated effluent	SB0393	SB0393h1	8.4	15.0	27.6	21.4	20.1	20.8	9.4	15.8	20.9	21.0
12-Oct-20	Tip Rd	Treated effluent	SB0393	SB0393h2b	10.6	15.6	19.9	20.7	6.6	6.6	18.6	17.2	14.9	10.2
12-Oct-20	Tip Rd	Treated effluent	SB0393	SB0393h2c	8.8	17.5	22.4	21.8	6.6	6.6	20.1	17.6	15.7	11.9
04-Nov-20	Centennial Drive	Sediment	SB0399	SB0399c1	17.5	12.5	26.6	23.2	16.1	34.0	16.2	17.1	7.4	20.1
04-Nov-20	Centennial Drive	Sediment	SB0399	SB0399d1	16.8	12.0	27.9	23.2	13.1	34.2	15.0	16.8	2.7	19.3
04-Nov-20	Centennial Drive	Sediment	SB0399	SB0399e1	17.4	12.7	28.6	22.7	15.1	35.0	14.4	17.8	8.3	23.0
04-Nov-20	Centennial Drive	Sediment	SB0399	SB0399f1	14.5	12.3	25.8	22.2	16.6	31.6	15.0	16.8	7.8	20.4
04-Nov-20	Tip Rd	Water	SB0404	SB0404c2	12.8	21.3	6.6	22.6	6.6	22.0	22.8	6.6	27.2	27.3
04-Nov-20	Tip Rd	Water	SB0404	SB0404d2	23.8	16.6	6.6	22.5	6.6	21.6	23.0	6.6	6.6	22.0

04-Nov-20	Tip Rd	Water	SB0404	SB0404e2	13.7	22.6	6.6	21.3	6.6	23.6	21.2	6.6	26.3	25.5
04-Nov-20	Tip Rd	Water	SB0404	SB0404f2	14.8	20.8	6.6	22.0	6.6	23.6	22.7	6.6	27.0	27.4
04-Nov-20	Tip Rd	Water	SB0404	SB0404h1a	6.6	12.8	6.6	21.5	20.6	20.7	15.7	6.6	19.6	21.9
04-Nov-20	Tip Rd	Water	SB0404	SB0404h2a	13.1	20.8	6.6	22.5	6.6	19.3	20.4	6.8	24.5	21.4
04-Nov-20	Tip Rd	Water	SB0404	SB0404h2b	14.2	19.2	6.6	22.3	6.6	19.8	20.7	7.6	25.5	23.7
04-Nov-20	Tip Rd	Water	SB0405	SB0405c2	24.2	16.9	6.6	22.3	21.8	25.3	23.5	6.6	6.6	24.8
04-Nov-20	Tip Rd	Water	SB0405	SB0405d2	8.4	14.9	12.7	22.3	20.9	24.1	21.5	19.3	22.3	22.9
04-Nov-20	Tip Rd	Water	SB0405	SB0405h1a	10.0	9.1	6.6	6.6	6.6	24.5	23.5	6.6	24.9	20.4
04-Nov-20	Tip Rd	Water	SB0405	SB0405h1b	6.6	12.7	30.4	21.5	22.7	6.6	24.5	19.7	26.2	22.8
04-Nov-20	Tip Rd	Water	SB0405	SB0405h2a	6.7	15.4	18.2	21.1	20.2	23.0	20.8	18.7	21.0	21.3
04-Nov-20	Tip Rd	Water	SB0405	SB0405h2b	14.7	20.8	29.5	21.4	21.7	25.9	22.9	19.2	24.6	19.9
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406c1	26.6	21.8	32.4	22.7	21.5	21.0	19.0	19.8	6.9	16.1
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406c2	22.9	18.4	6.6	21.8	6.6	26.6	23.9	6.6	6.6	23.2
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406d1	27.6	21.0	32.0	22.4	21.8	22.7	20.9	22.4	6.6	17.8
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406d2	25.0	18.7	6.6	22.2	6.6	26.4	23.3	6.6	6.6	22.4
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406h1	8.8	15.5	28.1	20.7	21.1	21.0	6.6	16.2	23.1	23.4
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406h1a	9.9	16.4	33.6	23.1	24.5	25.0	26.0	23.1	23.6	21.9
04-Nov-20	Tip Rd	Treated effluent	SB0406	SB0406h3	12.7	19.6	24.8	20.7	20.2	6.6	20.8	15.2	23.9	23.8
02-Dec-20	Napier Road	Sediment	SB0409	SB0409c1	26.0	20.4	6.6	21.0	21.2	31.4	23.4	18.4	13.5	21.8
02-Dec-20	Centennial Drive	Storm Water	SB0412	SB0412e1	21.9	30.5	22.9	21.4	6.6	29.0	25.9	8.0	26.6	21.4
02-Dec-20	Centennial Drive	Storm Water	SB0412	SB0412h1a	22.5	33.6	25.1	21.1	6.6	29.3	27.1	9.3	26.9	20.0
02-Dec-20	Centennial Drive	Storm Water	SB0412	SB0412h1b	21.6	29.9	22.7	21.8	6.6	27.7	26.2	7.6	27.4	20.9
02-Dec-20	Centennial Drive	Sediment	SB0414	SB0414c1	27.6	23.9	31.2	22.4	25.6	36.0	18.2	18.9	17.3	21.3

02-Dec-20	Centennial Drive	Sediment	SB0414	SB0414d1	29.2	24.0	29.8	21.9	25.6	34.5	19.0	19.2	20.3	22.5
02-Dec-20	Maxwell Line	Water	SB0416	SB0416c1	28.8	23.5	30.6	22.4	22.7	36.3	23.6	19.1	20.6	21.0
02-Dec-20	Maxwell Line	Water	SB0416	SB0416d1	28.7	23.3	28.8	20.7	22.6	33.0	22.7	18.4	20.2	21.1
02-Dec-20	Tip Rd	Treated effluent	SB0421	SB0421h1a	6.6	6.6	34.0	27.6	23.9	34.2	25.8	20.9	23.8	26.8
14-Jan-21	Centennial Drive	Sediment	SB0427	SB0427c1	25.0	19.5	28.0	22.9	21.9	34.4	20.9	17.1	21.8	21.4
14-Jan-21	Centennial Drive	Sediment	SB0427	SB0427d1	24.0	18.1	28.6	22.5	19.4	20.8	20.5	17.6	11.8	21.4
14-Jan-21	Tip Rd	Water	SB0432	SB0432c1	16.4	11.8	30.8	21.2	22.0	41.7	21.7	20.7	7.4	23.4
14-Jan-21	Tip Rd	Water	SB0432	SB0432c2	16.8	12.1	27.1	22.6	21.4	43.9	22.3	12.5	8.3	24.2
14-Jan-21	Tip Rd	Water	SB0432	SB0432d1	16.7	10.9	30.1	20.9	20.2	40.9	21.6	18.5	7.6	22.1
14-Jan-21	Tip Rd	Water	SB0432	SB0432d2	18.3	12.0	26.0	22.1	22.0	39.6	23.4	11.6	6.9	19.9
14-Jan-21	Tip Rd	Water	SB0432	SB0432e1	15.8	12.7	30.5	21.4	19.4	41.5	20.3	20.4	7.0	22.8
14-Jan-21	Tip Rd	Water	SB0432	SB0432f1	17.7	12.2	31.3	22.3	22.0	43.8	22.5	19.2	7.5	22.7
14-Jan-21	Tip Rd	Water	SB0432	SB0432f2	14.1	11.4	25.2	23.6	21.5	39.4	21.4	13.3	7.1	21.9
14-Jan-21	Tip Rd	Water	SB0432	SB0432h1a	13.3	22.7	20.0	22.1	6.6	23.5	16.0	16.4	21.5	17.9
14-Jan-21	Tip Rd	Water	SB0432	SB0432h1b	14.0	22.1	20.4	22.5	6.6	23.3	15.0	18.4	21.5	20.0
14-Jan-21	Tip Rd	Water	SB0432	SB0432h2a	16.9	11.8	25.7	24.4	21.3	39.8	18.6	11.0	8.2	21.3
14-Jan-21	Tip Rd	Water	SB0432	SB0432h2b	17.6	12.0	26.5	23.2	22.0	41.5	24.3	11.0	8.0	23.3
14-Jan-21	Tip Rd	Sediment	SB0433	SB0433f2	17.3	26.0	24.0	22.3	6.6	10.6	21.0	18.3	23.9	21.9
14-Jan-21	Tip Rd	Sediment	SB0433	SB0433h1a	6.6	7.9	6.6	23.9	18.6	6.6	19.7	18.3	15.8	19.3

Appendix 7

Environmental *E. coli* isolates ESBL confirmation test disc diffusion breakpoints. A value of -1 indicates a positive result for ESBL production.

Env IsolateID	Env SampleID	Date Collected	ESBL_ CTXz1	ESBL_ CTXz2	ESBL_ CTXdiff	ESBL_ CTX	ESBL_ CAZz1	ESBL_ CAZz2	ESBL_ CAZdiff	ESBL_ CAZ
SB0267h1	SB0267	23-Sep-19	8.98	23.2	-14.22	-1	17.33	23.78	-6.45	-1
SB0267h2	SB0267	23-Sep-19	11.05	23.28	-12.23	-1	17.96	24.53	-6.57	-1
SB0273h2	SB0273	23-Sep-19	7.36	22.77	-15.41	-1	22.18	20.35	1.83	
SB0276h2	SB0276	23-Sep-19	6.6	24.97	-18.37	-1	9.89	20.41	-10.52	-1
SB0283c	SB0283	17-Nov-19	6.6	22.81	-16.21	-1	12.43	25.56	-13.13	-1
SB0283f	SB0283	17-Nov-19	7.97	23.37	-15.4	-1	11.8	25.44	-13.64	-1
SB0283h1	SB0283	17-Nov-19	6.6	21.07	-14.47	-1	10.06	15.63	-5.57	-1
SB0283h2	SB0283	17-Nov-19	6.6	21.67	-15.07	-1	10.53	16.69	-6.16	-1
SB0295d	SB0295	16-Dec-19	11.17	23.25	-12.08	-1	16.6	23.57	-6.97	-1
SB0298h1	SB0298	16-Dec-19	14.14	26.64	-12.5	-1	22.58	25.44	-2.86	
SB0308e	SB0308	13-Jan-20	16.87	26.94	-10.07	-1	24.11	23.29	0.82	
SB0308f	SB0308	13-Jan-20	17.97	27.46	-9.49	-1	25.34	23.94	1.4	
SB0308h1	SB0308	13-Jan-20	17.8	28.7	-10.9	-1	26.93	28.67	-1.74	
SB0308h2	SB0308	13-Jan-20	17.89	29.43	-11.54	-1	26.89	29.27	-2.38	
SB0318c	SB0318	09-Feb-20	11.73	25.31	-13.58	-1	13.46	26.53	-13.07	-1
SB0318d	SB0318	09-Feb-20	7.02	26.58	-19.56	-1	12.68	25.39	-12.71	-1
SB0318e	SB0318	09-Feb-20	8	25.4	-17.4	-1	12.3	25.82	-13.52	-1
SB0337h1a	SB0337	08-Mar-20	14.12	30.44	-16.32	-1	19.81	28.81	-9	-1
SB0337h1b	SB0337	08-Mar-20	13.25	29.22	-15.97	-1	19.37	27.43	-8.06	-1
SB0338c1	SB0338	08-Mar-20	9.56	29.03	-19.47	-1	17.66	27.09	-9.43	-1

SB0338d1	SB0338	08-Mar-20	9.81	27.43	-17.62	-1	14.44	23.45	-9.01	-1
SB0338e1	SB0338	08-Mar-20	9.25	28.28	-19.03	-1	17.18	26.93	-9.75	-1
SB0338h1a	SB0338	08-Mar-20	9.67	26.31	-16.64	-1	17.84	25.12	-7.28	-1
SB0338h1b	SB0338	08-Mar-20	13.45	29.86	-16.41	-1	20.35	28.06	-7.71	-1
SB0347c1	SB0347	19-Jul-20	9.11	24.28	-15.17	-1	13.95	28.29	-14.34	-1
SB0347d1	SB0347	19-Jul-20	8.8	26.4	-17.6	-1	14.42	21.13	-6.71	-1
SB0347e1	SB0347	19-Jul-20	9.92	25.87	-15.95	-1	14.6	21.45	-6.85	-1
SB0347f1	SB0347	19-Jul-20	8.81	23.79	-14.98	-1	15.59	19.95	-4.36	
SB0347h1a	SB0347	19-Jul-20	7.98	28.38	-20.4	-1	15.48	26.14	-10.66	-1
SB0347h1b	SB0347	19-Jul-20	8.87	25.4	-16.53	-1	15.04	19.97	-4.93	
SB0351c2	SB0351	19-Jul-20	9.13	26.84	-17.71	-1	18.27	22.3	-4.03	
SB0351d2	SB0351	19-Jul-20	8.81	25.41	-16.6	-1	17.73	20.92	-3.19	
SB0351e2	SB0351	19-Jul-20	11.39	29.22	-17.83	-1	18.84	28.34	-9.5	-1
SB0351h2a	SB0351	19-Jul-20	10.46	28.32	-17.86	-1	17.72	27.09	-9.37	-1
SB0351h2b	SB0351	19-Jul-20	9.36	27.26	-17.9	-1	18.65	21.52	-2.87	
SB0352d2	SB0352	19-Jul-20	10.08	24.99	-14.91	-1	17.83	20.16	-2.33	
SB0352e2	SB0352	19-Jul-20	9.52	23.93	-14.41	-1	17.53	28.78	-11.25	-1
SB0352f2	SB0352	19-Jul-20	10.34	27	-16.66	-1	18.25	27.97	-9.72	-1
SB0352h2a	SB0352	19-Jul-20	9.87	24.23	-14.36	-1	18.74	29.25	-10.51	-1
SB0354c2	SB0354	19-Jul-20	16.29	29.41	-13.12	-1	20.7	24.71	-4.01	
SB0354e2	SB0354	19-Jul-20	15.34	28.49	-13.15	-1	21.13	27.57	-6.44	-1
SB0354h4	SB0354	19-Jul-20	9.94	23.57	-13.63	-1	19.72	25.32	-5.6	-1
SB0364c1	SB0364	17-Aug-20	11.5	26.43	-14.93	-1	19.81	22.26	-2.45	
SB0364d1	SB0364	17-Aug-20	11.18	27.67	-16.49	-1	19.43	22.39	-2.96	
SB0364e1	SB0364	17-Aug-20	12.18	27.57	-15.39	-1	20.17	23.02	-2.85	
SB0365c2	SB0365	17-Aug-20	8.43	25.57	-17.14	-1	13.54	19.68	-6.14	-1

SB0365d2	SB0365	17-Aug-20	9.03	24.36	-15.33	-1	13.18	18.28	-5.1	-1
SB0365e2	SB0365	17-Aug-20	8.39	25.22	-16.83	-1	12.67	18.88	-6.21	-1
SB0365f2	SB0365	17-Aug-20	8.12	26.39	-18.27	-1	13.3	18.91	-5.61	-1
SB0365h2a	SB0365	17-Aug-20	8.86	25.71	-16.85	-1	13.17	19.11	-5.94	-1
SB0365h2b	SB0365	17-Aug-20	8.47	26.85	-18.38	-1	13.52	20.32	-6.8	-1
SB0377c2	SB0377	14-Sep-20	9.72	21.15	-11.43	-1	11.19	17.29	-6.1	-1
SB0377d2	SB0377	14-Sep-20	8.91	20.45	-11.54	-1	11.11	16.97	-5.86	-1
SB0377e2	SB0377	14-Sep-20	8.17	20.33	-12.16	-1	10.13	17.01	-6.88	-1
SB0377f2	SB0377	14-Sep-20	8.66	21.2	-12.54	-1	10.85	17.22	-6.37	-1
SB0380h2a	SB0380	14-Sep-20	7.33	22.25	-14.92	-1	12.06	19.29	-7.23	-1
SB0380h2b	SB0380	14-Sep-20	6.6	23.44	-16.84	-1	10.77	24.27	-13.5	-1
SB0390h1a	SB0390	12-Oct-20	8.18	23.01	-14.83	-1	14.21	27.51	-13.3	-1
SB0391f1	SB0391	12-Oct-20	11.98	18.95	-6.97	-1	24.61	22.41	2.2	
SB0391h1a	SB0391	12-Oct-20	9.75	20.83	-11.08	-1	22.14	28.94	-6.8	-1
SB0391h2a	SB0391	12-Oct-20	8.8	25.24	-16.44	-1	12.92	29.2	-16.28	-1
SB0391h2b	SB0391	12-Oct-20	8.23	24.75	-16.52	-1	14.47	26.74	-12.27	-1
SB0393h1	SB0393	12-Oct-20	8.58	27.29	-18.71	-1	14.81	26.81	-12	-1
SB0393h2b	SB0393	12-Oct-20	13.2	31.7	-18.5	-1	18.4	28.7	-10.3	-1
SB0393h2c	SB0393	12-Oct-20	13.7	34.5	-20.8	-1	19.8	28.8	-9	-1
SB0404c2	SB0404	04-Nov-20	13.67	28.89	-15.22	-1	20.45	25.61	-5.16	-1
SB0404e2	SB0404	04-Nov-20	16.33	30.41	-14.08	-1	21.92	29.63	-7.71	-1
SB0404f2	SB0404	04-Nov-20	11.82	30.07	-18.25	-1	21.2	24.28	-3.08	
SB0404h1a	SB0404	04-Nov-20	7.8	27.7	-19.9	-1	12.4	27.1	-14.7	-1
SB0404h2a	SB0404	04-Nov-20	13.85	26.17	-12.32	-1	19.14	21.75	-2.61	
SB0404h2b	SB0404	04-Nov-20	12.66	30.28	-17.62	-1	18.64	25.39	-6.75	-1
SB0405d2	SB0405	04-Nov-20	8.47	23.04	-14.57	-1	14.55	21.86	-7.31	-1

SB0405h1a	SB0405	04-Nov-20	12.9	26.6	-13.7	-1	18.6	27.2	-8.6	-1
SB0405h1b	SB0405	04-Nov-20	8.8	28.4	-19.6	-1	11.7	26.3	-14.6	-1
SB0405h2a	SB0405	04-Nov-20	7.92	22.28	-14.36	-1	12.88	21.33	-8.45	-1
SB0405h2b	SB0405	04-Nov-20	13.56	28.25	-14.69	-1	19.43	22.42	-2.99	
SB0406h1	SB0406	04-Nov-20	9.98	29.5	-19.52	-1	16.78	28.73	-11.95	-1
SB0406h1a	SB0406	04-Nov-20	9.17	22.32	-13.15	-1	12.97	17.81	-4.84	
SB0406h3	SB0406	04-Nov-20	13.68	32.15	-18.47	-1	20.23	29.32	-9.09	-1
SB0412e1	SB0412	02-Dec-20	19.05	24.79	-5.74	-1	27.76	30.35	-2.59	
SB0412h1b	SB0412	02-Dec-20	19.75	25.3	-5.55	-1	26	30.49	-4.49	
SB0421h1a	SB0421	02-Dec-20	6.6	36	-29.4	-1	6.6	27.4	-20.8	-1
SB0432h1a	SB0432	14-Jan-21	14.09	26.83	-12.74	-1	19.73	24.13	-4.4	
SB0432h1b	SB0432	14-Jan-21	12.46	26.15	-13.69	-1	20.46	25.41	-4.95	
SB0433f2	SB0433	14-Jan-21	15.75	23.3	-7.55	-1	24.53	22.98	1.55	
SB0433h1a	SB0433	14-Jan-21	6.6	19.08	-12.48	-1	7.55	16.8	-9.25	-1

Appendix 8

Summary of the 234 ESBL-producing *E. coli* that were whole genome sequenced for this project. Results are gathered from the Nullarbor and quast outputs.

Isolate ID	Collection date	Sample origin		Sample type	Species	MLST	Genome size (bp)	GC (%)	Depth	Contigs	CDS	N50
EH0019a	2-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5099444	50.66	53	147	4796	131115
EH0020a	2-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	998	4951070	50.64	55	93	4560	161716
EH0021a	2-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	998	5156495	50.55	213	82	4778	226471
EH0022a	2-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	1177	5123288	50.61	66	140	4744	83097
EH0023a	9-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5129793	50.91	210	245	4858	39217
EH0024a	9-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	1193	4946742	50.75	61	329	4606	32874
EH0025a	9-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	73	5189060	50.63	144	228	4800	65439
EH0029a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	69	5254149	50.87	57	399	4852	31944
EH0030a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5122515	50.59	213	92	4769	191227
EH0031a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	12	5230523	50.57	86	113	4886	155708
EH0034a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	1177	5141625	50.63	140	116	4767	125042
EH0035a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5200228	50.72	86	122	4878	124278
EH0037a	19-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5043172	50.63	71	136	4707	108342
EH0038a	23-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	4968846	50.71	51	115	4616	140714
EH0039a	23-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	998	5177583	50.55	202	78	4820	213140
EH0040a	23-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5078695	50.75	94	163	4739	92880
EH0041a	23-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	12	5231644	50.56	101	113	4903	152665
EH0043a	23-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5038498	50.83	73	171	4686	65443
EH0044a	30-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5147112	50.87	73	244	4812	44186

EH0045a	30-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	131	5010995	50.78	207	131	4701	103598
EH0047a	30-Sep-19	Clinical	Medlab Central	Urine	*E. coli*	394	5190988	50.53	59	163	4832	78627
EH0082a	4-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	69	5233659	50.77	263	168	4895	66721
EH0084a	4-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5010663	50.74	102	72	4722	229617
EH0085a	4-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5177497	50.73	101	114	4828	160246
EH0086a	4-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5082486	50.72	100	106	4770	140912
EH0087a	12-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5039610	50.76	191	190	4690	68905
EH0088a	12-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	617	4822356	50.77	82	133	4475	97305
EH0089a	12-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	648	5311885	50.82	49	371	4948	29648
EH0090a	12-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5016632	50.75	57	238	4655	52437
EH0092a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5294503	50.73	255	147	5018	124628
EH0093a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	8881	5062151	50.61	164	110	4688	140160
EH0094a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5053279	50.9	74	308	4742	33364
EH0095a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5076939	51.07	53	468	4733	22930
EH0096a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5093441	51.12	42	449	4781	23187
EH0097a	19-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	1193	4993352	50.6	67	95	4655	172279
EH0098a	25-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	131	5045408	50.83	244	196	4717	59110
EH0100a	25-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	998	5170358	50.58	67	162	4785	83408
EH0102a	25-Nov-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5131577	50.84	53	304	4764	38411
EH0104a	2-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	131	5172785	50.82	278	209	4843	58935
EH0107a	2-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5120192	50.83	57	277	4759	44609
EH0108a	11-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	131	5145833	50.58	261	79	4782	208215
EH0109a	11-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	648	5390465	50.58	139	102	5048	174811
EH0114a	11-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	69	5200634	50.88	53	302	4849	40998
EH0115a	16-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	349	5226224	50.53	114	80	4917	184239
EH0117a	16-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	38	5235039	50.51	245	137	4873	130089
EH0118a	16-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	38	5209778	50.55	54	269	4844	42621
EH0120a	16-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	1193	5037318	50.69	57	226	4667	49794

EH0121a	16-Dec-19	Clinical	Medlab Central	Urine	*E. coli*	131	5066943	50.73	64	226	4706	53971
EH0124a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	69	5303063	50.8	51	303	4923	36635
EH0126a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5106107	50.58	106	106	4754	228224
EH0127a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5188518	50.85	118	150	4884	138706
EH0128a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5068001	50.61	189	91	4711	191082
EH0129a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	127	5157911	50.44	121	155	4784	97256
EH0130a	9-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5136016	50.6	114	102	4771	171706
EH0133a	13-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	450	4994201	50.83	55	217	4689	52335
EH0134a	13-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5103144	50.71	127	106	4747	140533
EH0136a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5282689	50.73	70	102	5008	160252
EH0137a	22-Jan-20	Clinical	Medlab Central	Catheter urine	*E. coli*	648	5257498	50.49	66	126	4947	162274
EH0138a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5341189	50.71	115	180	5057	110454
EH0139a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5348755	50.68	120	156	5067	136868
EH0140a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	38	5207973	50.56	93	104	4819	150763
EH0141a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5015134	50.67	136	96	4627	186554
EH0142a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	448	4876729	50.86	63	95	4589	131425
EH0143a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	69	5192680	50.71	67	103	4875	103194
EH0144a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5066440	50.6	68	97	4704	147403
EH0145a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5090664	50.69	71	114	4809	128588
EH0146a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5122462	50.71	79	87	4784	191306
EH0148a	22-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	127	5158341	50.37	69	103	4778	122601
EH0151a	28-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	131	5304570	50.63	84	116	5044	141076
EH0154a	28-Jan-20	Clinical	Medlab Central	Urine	*E. coli*	69	5219841	50.72	59	149	4888	86955
EH0155a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	69	5286120	50.73	98	150	4948	94995
EH0156a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	998	5232095	50.55	113	93	4858	244195
EH0160a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	405	5396439	50.56	110	211	5029	74305
EH0161a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	12	5240176	50.55	86	79	4909	277201
EH0164a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	38	5095547	50.55	115	107	4718	139264

EH0165a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5034284	50.6	73	101	4674	188474
EH0167a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	38	5083740	50.55	71	101	4710	155568
EH0169a	2-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	1193	4927488	50.62	145	65	4589	205606
EH0171a	18-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	648	5362513	50.69	81	247	5010	55349
EH0174a	18-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5060029	50.82	75	212	4735	54278
EH0175a	18-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5098229	50.62	70	155	4731	83352
EH0176a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5355598	50.65	64	144	5079	158933
EH0177a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5046079	50.81	117	82	4755	155039
EH0178a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	4953896	50.93	79	224	4647	51936
EH0179a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	131	5069664	50.79	70	292	4708	44230
EH0181a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5110759	50.62	73	183	4793	79720
EH0182a	24-Feb-20	Clinical	Medlab Central	Urine	*E. coli*	1163	4947168	50.95	63	87	4635	146356
EH0187a	2-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	648	5271932	50.43	61	185	4967	82737
EH0188a	2-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	131	5157779	50.73	77	109	4855	134730
EH0189a	2-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	131	4936398	50.83	84	145	4577	88949
EH0190a	2-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	131	5095304	50.73	82	110	4782	116868
EH0192a	2-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	57	5083247	50.51	80	122	4690	95236
EH0193a	9-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	69	5240956	50.74	64	283	4849	42232
EH0205a	16-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	38	5210490	50.68	69	112	4868	135179
EH0210a	16-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	998	5127566	50.7	60	228	4784	47431
EH0212a	16-Mar-20	Clinical	Medlab Central	Urine	*E. coli*	131	5155868	50.74	89	118	4852	117548
EH0251a	6-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5086738	50.72	78	94	4770	136365
EH0255a	6-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5135143	50.76	79	103	4807	138707
EH0257a	21-Jul-20	Clinical	Medlab Central	Bag urine	*E. coli*	131	5140663	50.64	56	149	4765	123244
EH0258a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5063132	50.6	55	109	4691	188674
EH0259a	21-Jul-20	Clinical	Medlab Central	Wound swab	*E. coli*	131	5131203	50.58	93	92	4770	154671
EH0260a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5121612	50.81	76	140	4786	115569
EH0261a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5046534	50.84	82	137	4731	102758

EH0262a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5117274	50.73	66	125	4771	109280
EH0267a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5124879	50.72	88	96	4789	192404
EH0268a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5142099	50.63	58	227	4782	66928
EH0269a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5070127	50.63	70	103	4723	135993
EH0270a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	131	5067998	50.57	110	83	4714	235770
EH0272a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	69	5243447	50.72	51	172	4927	87028
EH0275a	21-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	69	5231357	50.84	56	222	4876	63428
EH0277a	28-Jul-20	Clinical	Medlab Central	Urine	*E. coli*	38	5145239	50.55	83	101	4770	150168
EH0283a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	648	5299902	50.43	103	133	5003	125241
EH0286a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	69	5222824	50.73	101	169	4889	94992
EH0287a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	648	5358274	50.64	39	227	4996	62933
EH0288a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	131	4984035	50.76	60	81	4660	137777
EH0290a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	131	5064780	50.81	58	202	4751	70789
EH0292a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	69	5218426	50.73	87	187	4884	78661
EH0294a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5039967	50.57	74	64	4715	205549
EH0295a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	131	5104966	50.59	82	116	4742	167314
EH0296a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	127	5286150	50.46	101	113	4975	197372
EH0297a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	38	5220913	50.43	100	113	4870	150181
EH0298a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	69	5170830	50.75	80	119	4817	139614
EH0299a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	131	5115140	50.71	77	95	4757	160252
EH0300a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	12	5375003	50.56	101	99	5070	174092
EH0301a	31-Aug-20	Clinical	Medlab Central	Urine	*E. coli*	131	5245295	50.75	119	108	4987	155182
EH0303a	8-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	127	5166092	50.39	94	96	4801	221804
EH0306a	8-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	998	5196492	50.43	109	94	4795	153274
EH0307a	8-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	648	5291705	50.56	118	161	4927	113323
EH0308a	8-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	1722	5010417	50.65	49	136	4603	96635
EH0310a	14-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	5114495	50.58	70	98	4744	154990
EH0313a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	5358591	50.7	109	123	5068	172956

EH0314a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	69	5261131	50.78	101	147	4966	88263
EH0315a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	4934858	50.75	106	71	4595	168695
EH0316a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	5115617	50.77	78	133	4789	124475
EH0317a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	5096673	50.61	84	90	4741	146918
EH0318a	28-Sep-20	Clinical	Medlab Central	Urine	*E. coli*	131	5313318	50.71	84	121	5031	170216
EH0321a	14-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	131	5234620	50.72	89	251	4944	66173
EH0323a	14-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	8025	4963592	50.78	100	163	4691	80575
EH0324a	14-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	636	5075096	50.66	86	171	4774	72163
EH0329a	20-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	131	5237913	50.72	87	163	4935	92187
EH0331a	20-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	648	5287699	50.46	103	212	4974	66056
EH0332a	20-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	12	5328489	50.6	90	103	5006	237571
EH0333a	28-Oct-20	Clinical	Medlab Central	Catheter urine	*E. coli*	131	5134679	50.58	96	86	4774	191082
EH0334a	28-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	131	4909209	50.82	89	136	4573	79049
EH0335a	28-Oct-20	Clinical	Medlab Central	Urine	*E. coli*	998	5162181	50.52	94	111	4775	120925
EH0337a	2-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	131	5203759	50.73	88	101	4923	159259
EH0340a	2-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	998	5183919	50.47	92	110	4761	162891
EH0344a	2-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	131	5254486	50.73	108	121	4960	131179
EH0345a	9-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5072796	50.81	94	203	4737	65935
EH0346a	9-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	131	5072057	50.68	114	99	4746	138707
EH0348a	9-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	131	5054039	50.62	88	125	4687	109484
EH0350a	23-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5068567	50.65	90	105	4738	135129
EH0356a	23-Nov-20	Clinical	Medlab Central	Catheter urine	*E. coli*	131	5188591	50.74	100	136	4864	103594
EH0357a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	998	5188556	50.45	82	110	4780	153274
EH0359a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	131	5291785	50.71	97	151	5029	138698
EH0362a	30-Nov-20	Clinical	Medlab Central	Catheter urine	*E. coli*	131	5288545	50.73	81	139	5016	110014
EH0364a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	38	5086956	50.58	87	154	4700	95685
EH0366a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5063365	50.6	94	124	4805	89474
EH0367a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	648	5384361	50.5	90	206	5040	67044

EH0368a	30-Nov-20	Clinical	Medlab Central	Urine	*E. coli*	404	5240443	50.74	75	231	4922	55564
EH0369a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5012669	50.82	68	126	4671	109308
EH0370a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	1193	5039274	50.66	103	112	4712	120578
EH0371a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5112345	50.75	75	107	4788	124477
EH0373a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5100385	50.73	99	151	4783	116284
EH0374a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5142881	50.56	103	116	4788	130342
EH0375a	7-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5090385	50.74	100	128	4774	123535
EH0376a	7-Dec-20	Clinical	Medlab Central	Aapirate	*E. coli*	414	5324512	50.61	97	191	5016	71064
EH0377a	14-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5089157	50.69	86	147	4747	86256
EH0378a	14-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5367910	50.62	95	126	5091	124345
EH0381a	14-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	5215615	50.84	107	211	4934	60210
EH0382a	14-Dec-20	Clinical	Medlab Central	Urine	*E. coli*	131	4994172	50.83	96	75	4682	178382
EH0383a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	38	5340908	50.56	103	245	4988	61572
EH0384a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5193338	50.7	85	128	4907	149542
EH0386a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	69	5455391	50.67	99	214	5176	49444
EH0387a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5095616	50.69	108	124	4778	157464
EH0388a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	1883	5030856	50.51	104	61	4629	215024
EH0389a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5198083	50.74	107	94	4913	190180
EH0391a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5352315	50.67	92	146	5061	84106
EH0392a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	1193	4989216	50.64	82	130	4653	109224
EH0393a	11-Jan-21	Clinical	Medlab Central	Catheter urine	*E. coli*	131	4802848	50.8	84	137	4457	84514
EH0394a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	141	4996269	50.59	109	140	4624	103881
EH0395a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5215801	50.62	132	84	4867	246144
EH0397a	11-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	12	5235662	50.55	94	95	4905	197851
EH0401a	12-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5068879	50.68	106	198	4700	80421
EH0402a	12-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	648	5363988	50.48	100	162	4996	96099
EH0407a	12-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	38	5259642	50.54	96	197	4908	82774
EH0409a	12-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	38	5254832	50.55	92	217	4900	57186

EH0410a	18-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5180456	50.73	86	181	4893	87650
EH0414a	18-Jan-21	Clinical	Medlab Central	Urine	*E. coli*	131	5115880	50.59	73	101	4752	137532
SB0267h1	22-Sep-19	Environmental	Manwatu Gorge Walk	Water	*E. coli*	1193	4966939	50.61	134	89	4619	196912
SB0273h2	22-Sep-19	Environmental	Tip Rd	Water	*E. coli*	131	5053825	50.71	235	90	4739	183662
SB0276h2	22-Sep-19	Environmental	Tip Rd	Treated effluent	*E. coli*	38	5307898	50.53	266	141	4939	104826
SB0283h1	17-Nov-19	Environmental	Hardie Street Reserve	Stormwater	*E. coli*	1722	5113088	50.64	272	131	4744	76301
SB0283h2	17-Nov-19	Environmental	Hardie Street Reserve	Stormwater	*E. coli*	1722	5110158	50.68	276	171	4744	65817
SB0295d	16-Dec-19	Environmental	Tip Rd	Water	*E. coli*	7476	4625117	50.83	78	116	4320	95405
SB0298h1	16-Dec-19	Environmental	Tip Rd	Treated effluent	*E. coli*	10	4763513	50.77	105	150	4453	75127
SB0308e	13-Jan-20	Environmental	Tip Rd	Water	*E. coli*	442	5043706	50.75	121	76	4722	155386
SB0318e	9-Feb-20	Environmental	Hardie Street Reserve	Water	*E. coli*	1584	4705444	50.89	120	223	4450	43078
SB0337h1a	8-Mar-20	Environmental	Tip Rd	Water	*E. coli*	131	5029530	50.77	86	103	4702	134824
SB0338d1	8-Mar-20	Environmental	Tip Rd	Water	*E. coli*	156	5038501	50.48	103	93	4702	143444
SB0338h1a	8-Mar-20	Environmental	Tip Rd	Water	*E. coli*	38	4906071	50.64	112	88	4510	123686
SB0338h1b	8-Mar-20	Environmental	Tip Rd	Water	*E. coli*	131	5032659	50.78	125	121	4704	115848
SB0347c1	19-Jul-20	Environmental	Hardie Street Reserve	Stormwater	*E. coli*	131	5186039	50.6	104	137	4882	126813
SB0347h1a	19-Jul-20	Environmental	Hardie Street Reserve	Stormwater	*E. coli*	131	5187526	50.58	103	100	4894	181521
SB0351c2	19-Jul-20	Environmental	Tip Rd	Water	*E. coli*	1722	5104594	50.65	111	93	4761	146773
SB0351d2	19-Jul-20	Environmental	Tip Rd	Water	*E. coli*	1722	5102772	50.65	107	68	4754	188823
SB0354e2	19-Jul-20	Environmental	Tip Rd	Treated effluent	*E. coli*	540	4622193	50.83	79	117	4361	81790
SB0354h4	19-Jul-20	Environmental	Tip Rd	Treated effluent	*E. coli*	354	5052250	50.64	121	84	4694	163573
SB0364c1	17-Aug-20	Environmental	Tip Rd	Water	*E. coli*	69	5339688	50.56	124	131	5003	112965
SB0365c2	17-Aug-20	Environmental	Tip Rd	Water	*E. coli*	542	5160656	50.71	78	239	4933	54970
SB0365h2b	17-Aug-20	Environmental	Tip Rd	Water	*E. coli*	542	5181786	50.63	115	131	4970	147017

SB0377c2	14-Sep-20	Environmental	Tip Rd	Water	*E. coli*	2079	5121352	50.74	117	105	4822	134990
SB0380h2a	14-Sep-20	Environmental	Tip Rd	Treated effluent	*E. coli*	131	4997140	50.85	84	120	4654	142847
SB0380h2b	14-Sep-20	Environmental	Tip Rd	Treated effluent	*E. coli*	648	5118040	50.58	86	145	4788	107757
SB0391f1	12-Oct-20	Environmental	Tip Rd	Water	*E. coli*	998	5197684	50.53	112	72	4874	383630
SB0391h1a	12-Oct-20	Environmental	Tip Rd	Water	*E. coli*	998	5188465	50.59	92	134	4849	122353
SB0391h2a	12-Oct-20	Environmental	Tip Rd	Water	*E. coli*	131	4834467	50.71	111	70	4474	177082
SB0393h1	12-Oct-20	Environmental	Tip Rd	Treated effluent	*E. coli*	7476	4610033	50.78	120	115	4324	92378
SB0393h2b	12-Oct-20	Environmental	Tip Rd	Treated effluent	*E. coli*	131	4858660	50.77	79	113	4497	124735
SB0404c2	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	219	5236102	50.73	122	87	4892	187092
SB0404h1a	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	1722	4802813	50.57	105	94	4400	149407
SB0404h2a	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	219	5232287	50.74	161	104	4894	135916
SB0405d2	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	1722	4787834	50.53	87	62	4376	260796
SB0405h1a	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	131	5115122	50.67	85	183	4734	86002
SB0405h1b	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	1193	5110336	50.57	92	112	4789	130213
SB0405h2b	4-Nov-20	Environmental	Tip Rd	Water	*E. coli*	1324	4823376	50.87	88	195	4527	51397
SB0406h1	4-Nov-20	Environmental	Tip Rd	Treated effluent	*E. coli*	131	5074919	50.99	88	127	4742	106499
SB0406h1a	4-Nov-20	Environmental	Tip Rd	Treated effluent	*E. coli*	7476	4537231	50.82	93	236	4223	38109
SB0406h3	4-Nov-20	Environmental	Tip Rd	Treated effluent	*E. coli*	131	5015977	50.8	96	178	4701	74372
SB0412e1	2-Dec-20	Environmental	Hardie Street Reserve	Stormwater	*E. coli*	10	5011394	51.03	80	436	4766	25855
SB0421h1a	2-Dec-20	Environmental	Tip Rd	Treated effluent	*E. coli*	635	5238835	50.71	95	233	5001	52343
SB0432h1a	14-Jan-21	Environmental	Tip Rd	Water	*E. coli*	69	5183644	50.84	102	179	4859	59360
SB0433f2	14-Jan-21	Environmental	Tip Rd	Sediment	*E. coli*	10	5020258	50.87	85	126	4746	89830
SB0433h1a	14-Jan-21	Environmental	Tip Rd	Sediment	*E. coli*	648	5287913	50.4	140	142	4987	92330

Appendix 9

Extended version of table 4.1.

Antibiotic class and genes	Clinical isolate totals per gene	Clinical overall resistance per antibiotic class	Environmental isolate totals per gene	Environmental overall resistance per antibiotic class	All isolate totals per gene	All overall resistance per antibiotic class
Aminoglycosides						
aac(3)-IId	50/189 (26.5%)	131/189 (69.3%)	6/45 (13.3%)	22/45 (48.9%)	56/234 (23.9%)	162/234 (69.2%)
aac(3)-IIe	22/189 (11.6%)		4/45 (8.9%)		26/234 (11.1%)	
aac(3)-IVa	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
aac(6')-Ib-AKT	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
aac(6')-Ib-D181Y	23/189 (12.2%)		1/9 (11.1%)		28/234 (12.0%)	
aac(6')-Ib-G	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
aadA1	8/189 (4.2%)		4/45 (8.9%)		12/234 (5.1%)	
aadA2	2/189 (1.1%)		2/45 (4.4%)		4/234 (1.7%)	
aadA5	100/189 (52.(%)		11/45 (24.4%)		111/234 (47.4%)	
ant(2'')-Ia	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
aph(3'')-Ib	82/189 (43.3%)		13/45 (28.9%)		95/234 (40.6%)	
aph(3')-Ia	6/189 (3.2%)		0 (0%)		6/234 (2.6%)	
aph(4)-Ia	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
aph(6)-Id	80/189 (42.3%)		13/45 (28.9%)		93/234 (39.7%)	
Beta-lactam substrates						
Narrow spectrum beta-lactamase						
bla_{TEM-1}	78/189 (41.3%)	78/189 (41.3%)	9/45 (20.0%)	9/45 (20.0%)	87/234 (37.2%)	87/234 (37.2%)
ESBL						

bla$_{CTX-M-1}$	4/189 (2.1%)	189/189 (100%)	0 (0%)	45/45 (100%)	4/234 (1.7%)	234/234 (100%)
bla$_{CTX-M-121}$	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{CTX-M-14}$	17/189 (9%)		7/45 (15.6%)		24/234 (10.3%)	
bla$_{CTX-M-15}$	79/189 (41.8%)		28/45 (62.2%)		107/234 (45.7%)	
bla$_{CTX-M-27}$	86/189 (45.5%)		8/45 (17.8%)		94/234 (40.2%)	
bla$_{CTX-M-3}$	2/189 (1.1%)		0 (0%)		2/234 (0.9%)	
bla$_{CTX-M-55}$	1/189 (0.5%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{SHV-12}$	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
bla$_{TEM-235}$	5/189 (2.6%)		0 (0%)		5/234 (2.1%)	
bla$_{TEM-30}$	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
AmpC						
bla$_{CMY-138}$	0 (0%)	189/189 (100%)	1/45 (2.2%)	45/45 (100%)	1/234 (0.4%)	234/234 (100%)
bla$_{CMY-2}$	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
bla$_{DHA-1}$	9/189 (4.8%)		0 (0%)		9/234 (3.8%)	
bla$_{EC}$	2/189 (1.1%)		10/45 (22.2%)		12/234 (5.1%)	
bla$_{EC-13}$	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
bla$_{EC-15}$	2/189 (1.1%)		3/45 (6.7%)		5/234 (2.1%)	
bla$_{EC-18}$	1/189 (0.5%)		4/45 (8.9%)		5/234 (2.1%)	
bla$_{EC-19}$	24/189 (12.7%)		11/45 (24.4%)		35/234 (15.0%)	
bla$_{EC-5}$	125/189 (66.1%)		13/45 (28.9%)		138/234 (59.0%)	
bla$_{EC-8}$	34/189 (18%)		4/45 (8.9%)		38/234 (16.2%)	
Oxacillinases						
bla$_{OXA-1}$	22/189 (11.6%)	28/189 (14.8%)	5/45 (11.1%)	6/45 (13.3%)	27/234 (11.5%)	34/234 (14.5%)
bla$_{OXA-10}$	6/189 (3.2%)		1/45 (2.2%)		7/234 (3.0%)	
Cloramphenicol						
catA1	13/189 (6.9%)	19/189 (10.1%)	1/45 (2.2%)	4/45 (8.9%)	14/234 (6.0%)	23/234 (9.8%)
catA2	3/189 (1.6%)		0 (0%)		3/234 (1.3%)	
catB3	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	

cmlA1	0 (0%)		2/45 (4.4%)		2/234 (0.9%)	
cmlA5	6/189 (3.2%)		1/45 (2.2%)		7/234 (3.0%)	
Trimethoprim/sulfamethoxazole						
dfrA1	3/189 (1.6%)	133/189 (70.4%)	3/45 (6.7%)	20/45 (44.4%)	6/234 (2.6%)	153/234 (65.4%)
dfrA12	2/189 (1.1%)		0 (0%)		2/234 (0.9%)	
dfrA14	11/189 (5.8%)		7/45 (15.6%)		18/234 (7.7%)	
dfrA17	107/189 (56.6%)		11/45 (24.4%)		118/234 (50.4%)	
dfrA19	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
dfrA5	6/189 (3.2%)		1/45 (2.2%)		7/234 (3.0%)	
dfrA7	5/189 (2.6%)		0 (0%)		5/234 (2.1%)	
dfrA8	1/189 (0.5%)		0 (0%)		1/234 (0.4%)	
sul1	122/189 (64.6%)	144/189 (76.25)	13/45 (28.9%)	18/45 (40%)	135/234 (57.7%)	162/234 (69.2%)
sul2	101/189 (53.4%)		15/45 (33.3%)		116/234 (49.6%)	
Quinolone						
qepA8	1/189 (0.5%)	23/189 (12.2%)	0 (0%)	18/45 (40%)	1/234 (0.4%)	41/234 (17.5%)
qnrA1	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
qnrB1	2/189 (1.1%)		0 (0%)		2/234 (0.9%)	
qnrB4	5/189 (2.6%)		0 (0%)		5/234 (2.1%)	
qnrS1	18/189 (9.5%)		17/45 (37.8%)		35/234 (15.0%)	
Tetracycline						
tet(A)	98/189 (51.9%)	122/189 (64.6%)	14/45 (31.1%)	16/45 (35.6%)	112/234 (47.9%)	128/234 (54.7%)
tet(B)	20/189 (10.6%)		3/45 (6.7%)		23/234 (9.8%)	
tet(D)	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
Macrolides						
ere(A)	1/189 (0.5%)	119/189 (63%)	0 (0%)	14/45 (31.1%)	1/234 (0.4%)	133/234 (56.8%)
erm(B)	8/189 (4.2%)		1/45 (2.2%)		9/234 (3.8%)	
mph(A)	118/189 (62.4%)		13/45 (28.9%)			
mph(E)	0 (0%)		1/45 (2.2%)		131/234 (56.0%)	

msr(E)	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
Fosfomycin						
fosA	0 (0%)	0 (0%)	2/45 (4.4%)	2/45 (4.4%)	2/234 (0.9%)	2/234 (0.9%)
Other resistance genes						
arr-2	6/189 (3.2%)		1/45 (2.2%)		7/234 (3.0%)	
floR	6/189 (3.2%)		2/45 (4.4%)		8/234 (3.4%)	
lnu(F)	0 (0%)		1/45 (2.2%)		1/234 (0.4%)	
sat2_gen	2/189 (1.1%)		2/45 (4.4%)		4/234 (1.7%)	