A STUDY OF COLONISATION OF THE HUMAN GASTROINTESTINAL TRACT BY MULTIDRUG RESISTANT *ESCHERICHIA COLI*

by

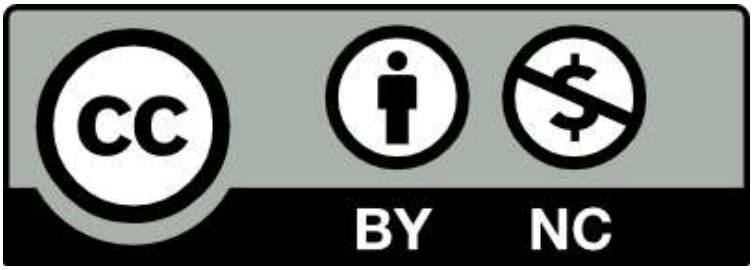
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Abstract

Escherichia coli is a commensal organism recognised as a globally important pathogen as it can cause severe invasive disease, including meningitis and bacteraemia. *E. coli* is a known carrier of antimicrobial resistance (AMR) and is a major contributor to the global spread of AMR. Mobile genetic elements (MGEs), including plasmids and insertion sequence (IS) elements, are reported to play a crucial role in the spread of AMR and in the creation of multidrug resistant (MDR) organisms. The occurrence of MDR organisms, including MDR *E. coli,* is a cause of significant concern as it restricts treatment options, and increases patient morbidity and mortality. International travel and hospital admissions often result in increased colonisation with MDR organisms, and both are known to contribute to the global spread of AMR.

Colonisation with drug resistant organisms is a potential route of AMR spread which has not been previously characterised during a traveller stay and there is limited information on colonisation in a hospital inpatient stay. Historical studies of clinical isolates have previously been used to demonstrate the impact of the discovery and increased use of antimicrobials on the rise of AMR, particularly in the last few decades. Previous historical studies have focussed on adult populations which are more likely to have had exposure to a wide range of antimicrobials over longer periods of time. This thesis is a study of *E. coli* in three different settings. It looks at MDR *E. coli* colonising healthy travellers to Laos and UK intensive care unit patients, and *E. coli* causing infection in Dutch neonates. The overall aim of this thesis was to understand the dynamics of colonisation with MDR organisms and the dynamics of MDR infection

in the absence of antibiotic selection. Colonisation and infection with MDR *E. coli* both in the absence of antibiotic selection can improve understanding of AMR in general with no bias to a resistance driven by a specific antibiotic.

Strain level sampling methods in combination with short and long read whole genome sequencing (WGS) were used to explore *E. coli* from these three different settings. Bioinformatic analysis highlighted key sequence types (STs), AMR gene and plasmid profiles in the three datasets. These techniques and daily sampling helped to characterise the dynamic nature of *E. coli* colonisation in travellers to Laos, showing the high level of MDR *E. coli* present and the rapid acquisition and loss of STs during their traveller stay. Detailed characterisation of the plasmids carried by these MDR *E. coli* highlighted the diversity of plasmids and the diversity of AMR gene settings within these AMR plasmids. Study of *E. coli* in ICU patients, demonstrated the presence of persistent colonisation during a hospital stay, and the lack of transmission, AMR carriage and ST diversity, widely differing from the Laos traveller dataset. Strain level analysis revealed circulating strains in the traveller population and lack of circulating strains in the ICU population. The clinical infection *E. coli* isolated from neonates with meningitis or bloodstream infection (BSI), demonstrated that over 47-years resistance carriage remained surprisingly low but there were interesting changes to the composition of the ST population causing invasive disease, including the loss of a predominant ST, ST567, which had not been previously widely reported.

Overall, this study revealed the different dynamics of the three *E. coli* populations investigated. Strain level sampling in these relevant settings showed the three very different antimicrobial resistance profiles and different ST populations in the absence of any antibiotic selection.

For my family

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Acronyms

AMR	Antimicrobial resistance
BBB	Blood brain barrier
BSI	Bloodstream infection
CPE	Carbapenemase-producing *Enterobacteriaceae*
CRE	Carbapenem-resistant *Enterobacteriaceae*
CSF	Cerebrospinal fluid
CTAB	Cetyl trimethylammonium bromide
DAEC	Diffusely adherent *E. coli*
DNA	Deoxyribonucleic acid
EAEC	Enteroaggregative *E. coli*
ECDC	European Centre for Disease Prevention and Control
EHEC	Enterohaemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended spectrum beta-lactamase
ESPAUR	English Surveillance Programme for Antimicrobial Utilisation and Resistance
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestinal pathogenic *E. coli*
FQR	Fluroquinolone
GLASS	Global Antimicrobial Resistance Surveillance System

HGT	Horizontal gene transfer
ICU	Intensive Care Unit
IR	Inverted repeat
IS	Insertion sequences
LB	Lysogeny broth
MDR	Multidrug resistant
MGE	Mobile genetic element
MITE	Miniature inverted repeat transposable elements
MLST	Multilocus sequence typing
MNEC	Meningitis associated *E. coli*
NEB	New England BioLabs Inc.
NICU	Neonatal ICU
NRLBM	Netherlands Reference Laboratory for Bacterial Meningitis
ONT	Oxford Nanopore Technologies
QEHB	Queen Elizabeth Hospital Birmingham
SEDRIC	Surveillance and Epidemiology of Drug-resistant Infections Consortium
SNP	Single nucleotide polymorphism
ST	Sequence type
TSD	Target site duplication
UHB	University Hospitals Birmingham NHS Foundation Trust
UKHSA	UK Health Security Agency
UoB	University of Birmingham
UPEC	Uropathogenic *E. coli*

UTI	Urinary tract infection
VF	Virulence factor
WGS	Whole genome sequencing
WHO	World Health Organisation
XDR	Extensively drug resistant

1. Introduction

1.1 Antimicrobial Resistance – The Global Problem

The spread of antimicrobial resistance (AMR) is a global problem which is acknowledged internationally and is a current focus of the World Health Organisation (WHO) (1). Antimicrobial resistance has a broad definition. It encompasses a wide range of organisms, including parasites, viruses and fungi well as bacteria, which evolve traits to survive antimicrobial therapy (2). AMR knows no boundaries and has been a recognised cause of concern for years (3) as it affects the treatment of an increasing number of diseases and is reducing the number of drugs available to treat infections effectively. The development of new drug resistance mechanisms can be driven by the overuse of antimicrobials and make treatment options more limited. The carriage of drug resistance by an organism affects the ability to prevent and treat infections, leading to poorer patient outcome, with increased morbidity and mortality (4). Medical procedures (including, chemotherapy, organ transplants and major surgery) become more high risk with increased risk of complicated and persistent infections causing longer hospital stays and increased hospital expense (2).

The spread of these antimicrobial-resistant organisms across the globe is driven by increased travel and migration, so antimicrobial-resistant organisms are becoming more prevalent, and spread is becoming more difficult to control (5). Climate change and increased pollution could also be contributing to the rise in AMR, with changing climates allowing different organisms to be successful(6). WHO are coordinating global action by supporting global surveillance programs and by investing in research and

development of new antimicrobial therapies, in order to, raise awareness of AMR, combat the spread and increase treatment options (1).

1.2 AMR – The Current Situation

The total global burden of AMR is hard to estimate but known to be high and increasing in areas where data is available (7-9). Some infections are now becoming so resistant to known drug therapies that they are almost untreatable. A recent global study by Murray et al (7) demonstrated the great impact that bacterial AMR had on deaths and disability-adjusted life-years, from a range of pathogens and diverse pathogen-drug class combinations. AMR pathogens currently causing concern on a global level include: the high level of Carbapenemase-producing *Enterobacteriaceae* (CPE) in Europe (10); antiviral resistance in HIV (11) ; artemisinin-based combination therapy resistant *Plasmodium Falciparum* (12) ; multi drug resistant (MDR) and extensively drug Mycobacterium tuberculosis (XDR) (13) as well as colistin resistance in *Enterobacteriaceae* and *Acinetobacter baumannii* (14).

The global action plan developed by WHO has identified the importance of surveillance and research to strengthen knowledge and provide evidence to establish incidence, prevalence, pathogen type and geographical patterns of AMR (1). This information allows local, national, and regional authorities to provide guidance on treatment and to monitor the success of any interventions. The WHO initiative, Global Antimicrobial Resistance Surveillance System (GLASS), is a programme that helps standardise the sharing, analysis and collection of global AMR resistance data in order to help local and national action (15). The need for improved surveillance methods and improved laboratory infrastructure at a global level has been reinforced by current research on

the global burden of AMR (7) and will be critical to help combat the burden of AMR and improve patient care.

European surveillance is carried out by The European Centre for Disease Prevention and Control (ECDC) which publishes an annual surveillance report that notes significant trends in AMR observed across a group of 29 countries in Europe. Third generation cephalosporin resistance in *Escherichia coli* was reported by ECDC as the largest AMR burden between 2016-2020, and from 2020-2021 a significant increase of *Acinetobacter* species was deemed the most concerning rise in AMR (16). Between 2015-2021 there were moderate changes to mean resistance percentages of antimicrobial group combinations detected in most Gram-negative species reported by ECDC but overall they remained at a high level (17). It was also common for an isolate to have resistance to multiple antimicrobial groups showing that multi-drug resistance is a significant problem and public health risk in Europe (17).

In the UK there is national level surveillance, carried out by the UK Health Security Agency (UKHSA) which provides an annual report, the English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) report that monitors AMR trends and antimicrobial prescription. The ESPAUR report stated in 2021 in England there had been an increase by 2.2% in severe antibiotic resistant infections since 2020 (18). *E. coli* was a critical pathogen, mirroring ECDC findings, with it being the organism to cause the highest incidence of bloodstream infection (BSI) in the UK in 2021 (18). Charities, including the Wellcome Trust, also support initiatives such as the Surveillance and Epidemiology of Drug-resistant Infections Consortium (SEDRIC) (https://sedric.org.uk/) which facilitates the collaboration of international experts who aim to improve and advance current surveillance schemes.

1.3 The Spread of AMR

Potential routes for the spread of antimicrobial resistant organisms include both person to person and zoonotic routes (19). Person to person transmission of organisms can occur through poor infection control, poor sanitation, or poor food handling practices. Zoonotic transmission of organisms can take place either directly between animal and person or from food of animal origin to person (20). AMR can therefore be expected to spread by the same routes. Agriculture is a particular problem as antimicrobials have been used to increase productivity and profit, for example, antimicrobials have been used as growth promoters (e.g., colistin), as well as to improve animal health and animal crop yield (21). The environment is also a key reservoir for AMR (22) and environmental transmission has the potential to spread AMR to humans and animals. Wastewater and river water studies in different areas of the globe highlight the high levels of AMR in the environment (23, 24). The WHO One Health initiative is an attempt to reduce these problems recognising that drug-resistant bacteria are not confined to specific geographical areas and can easily spread between animals and humans and the environment. It is widely accepted that over prescription of antibiotics in both the animal and human population has had a significant effect on the spread of AMR, with the overuse of antimicrobials driving the selection of resistant strains.

International travel and migration are also important factors in the spread of AMR at a global level (5). Migration and international travel (for both leisure and business) have increased as they have become quicker, easier, and more affordable in recent years facilitating the spread of AMR. The rapid international spread of the novel SARS-CoV-2 virus and the COVID-19 pandemic demonstrated the impact that the increased amount of international travel and trade that occurs globally has had on the spread of

infection. It was hard to contain and control the spread of the novel virus whilst effective treatment or a vaccine was developed (25).The clear parallel with the potential global spread of novel and current AMR highlights the urgent need for international cooperation and commitment to tackle local AMR issues to help minimise their global spread.

1.4 Mechanisms of AMR

Development of resistance is accelerating due to misuse and overuse of antimicrobials in both healthcare settings and the wider world. An organism can have intrinsic or acquired resistance to an antimicrobial therapy. Natural, intrinsic, resistance to antibiotics can be due to resistance genes in the bacterial chromosome e.g., those that code for efflux pumps (26). Alternatively, intrinsic resistance can occur because bacteria are missing the target of the antibiotic e.g., expression of alternative proteins, including *mec*A in methicillin-resistant *Staphylococcus aureus* (27). Acquired resistance mechanisms can cause the development of resistance to a drug for which the organism has no inherent resistance (28).

Resistant organisms do not just cause infections, they can also be found as colonising organisms (29). Common examples of resistance mechanisms seen in bacteria colonising the gut (e.g., *Escherichia coli, Klebsiella pneumoniae*) include: Extended spectrum beta-lactamase (ESBL) producing organisms, CPEs and colistin resistant organisms. It is important to understand colonisation of these organisms in patients, specifically those who are immunocompromised as there is a risk colonisation can cause infection if these organisms spread to other sites in the body e.g., urinary tract or bloodstream. There is high awareness of these resistant organisms which cause

more serious infections as they are often harder to treat and have a poorer patient outcome (4). Resistance mechanisms (e.g., ESBLs, CPEs) vary significantly, each conferring resistance to different types of drug treatment. This could potentially delay effective treatment as treatment failure of first line antimicrobial therapy may occur, and extended laboratory testing on the organism could be required to detect these resistance mechanisms prior to prescription of a suitable antimicrobial.

ESBL-producing organisms have enzymes that confer resistance to beta-lactam antibiotics including penicillin, cephalosporins and monobactams. They do not affect cephamycins or carbapenems (30). ESBLs can be split into two classes, serine-beta-lactamases (Ambler Scheme Classes A, C and D) and metallo-beta-lactamases (Ambler Scheme Class D). Ambler class A ß lactamases, serine-beta-lactamases, most major types of ESBLs, including *bla*$_{\text{CTX-M}}$*, bla*$_{\text{TEM}}$*, bla*$_{\text{SHV}}$ and *bla*$_{\text{VEB}}$ (31). Ambler class D beta lactamases, also serine-beta-lactamases, contains another important ESBL, *bla*$_{OXA}$. The ESBL groups of most interest are *bla*$_{\text{CTX-M}}$*, bla*$_{TEM}$ and *bla*$_{SHV}$ (31). TEM beta-lactamases are cephalosporin-hydrolysing mutants of *bla*$_{\text{TEM}}$ plasmid mediated penicillinases (32). SHV beta-lactamases are mainly found in *Klebsiella* species and are found on self-transmissible plasmids (33). CTX-M beta-lactamases are derived from *Kluyvera* species. There are over 170 *bla*$_{\text{CTX-M}}$ *types,* with the two most widespread being *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-15}}$ (34).

Carbapenemase-producing *Enterobacteriaceae* (CPE) produce enzymes that confer resistance to carbapenems. Carbapenems are a group of effective broad-spectrum antibiotics which are stable against beta-lactamases and ESBLs and so carbapenems are frequently used to treat more complex infections (35, 36). The production of

carbapenemases by *Enterobacteriaceae* causes the destruction of beta-lactam antibiotics including the broad spectrum carbapenems (including imipenem, meropenem, ertapenem). There are many different types of carbapenemases, the four most common being *bla*$_{KPC}$*, bla*$_{OXA-48}$*, bla*$_{NDM}$ and *bla*$_{VIM}$*.*

Hospital patients that test positive for carriage of colonising *Enterobacteriaceae* are a cause for concern, as CPEs can cause complex, hard to treat invasive infections, including pneumonia, bloodstream infections (BSIs) and meningitis (37). CPE positive hospital patients commonly carry *Klebsiella* species and *E. coli* as their carbapenemases-producing organism (38). Colonisation with CPEs is often linked with travel, hospital attendance outside of the UK and to specific hotspot hospitals in the UK (39, 40). Patients who have a history of travel or stay in a high-risk environment, are monitored for CPE colonisation (41). If detected infection control precautions are put in place to prevent the spread of CPE colonisation between patients e.g., isolation in side rooms, personal protective equipment, enhanced cleaning protocols.

Colistin resistance is also known to occur in gut colonising organisms and was first seen in China in 2011, where the plasmid mediated *mcr* gene was identified (42). Colistin is a polymyxin (Polymyxin B) so is in the main family of antibiotics still available to treat CPE infections. Polymyxins have become increasingly important with the increase of CPE infections. Few new drugs have been developed to treat MDR Gram negative bacteria making colistin especially important. There is a global effort to reduce colistin use and to save it as last resort therapy and colistin is in the reserve category of the WHO Essentials Medicines List (43). This means that colistin can only be used to treat life-threatening infections caused by MDR bacteria sensitive to colistin. The

objective of this restriction is to prevent the overuse of colistin which could cause selection for any resistant isolates present and this should help to prevent further development and spread of AMR. Colistin resistance is also spread through plasmids which are easily transferred between bacteria species and strains (42). The colistin plasmid mediated resistance *mcr* genes in 2011, code for phosphoethanolamine transferase enzymes which modify lipopolysaccharide permanently by adding a phosphoethanolamine to the colistin binding site Lipid A. This change prevents the binding of colistin causing drug resistance (44, 45). At least 10 colistin resistance genes have been identified (46). Variants of these plasmid mediated resistance genes have also been detected, and can be caused by modification to the lipopolysaccharide membrane (14).

1.5 *Escherichia coli* and AMR

E. coli is a globally important pathogen, a known carrier of AMR, and recognised as a contributor to the global spread of AMR (47). Recent research linked to hospitals and travel shows an increase in resistance found in both colonising and infectious *E. coli* isolates, often isolates in these settings are MDR (29, 48-50). In the UK community and nosocomial *E. coli* infections have historically, and are still, often treated with cephalosporins, fluoroquinolones, cotrimoxazole or a nitrofuran depending on the site of infection, resistance profile and local antibiotic guidelines (https://bnf.nice.org.uk/treatment-summaries/urinary-tract-infections/) but these antibiotics are increasingly failing to be effective.

Commensal and pathogenic *E. coli* both play important roles as reservoirs for AMR (51) . *E. coli* are classified into seven phylogroups which are associated with specific sources and epidemiological niches (52). The Clermont method is used to assign

phylogroup to *E. coli* classifying them as phylogroup A, B1, B2, C, D, E, F or *Escherichia* cryptic clades (52) (Figure 1.1). This can also be carried out *in silico* using ClermonTyping (53). Cryptic clades are thought to be environmental isolates rarely causing infection or found colonising human faecal stool samples (52, 54). They cannot be distinguished from *E. coli* isolates by standard biochemical typing methods but are all phylogenetically distinct and are possibly evolutionary lineages (54). Cryptic clade lineages are divided into five clades: I, II, III, IV and V. *E. coli* is more closely related to clade I, whereas clade V is reportedly more divergent (52). Both phylogroup and cryptic clade classification is based on alleles of *arpA*, *chuA*, *yjaA* and the DNA fragment TspE4.C2 (55) .

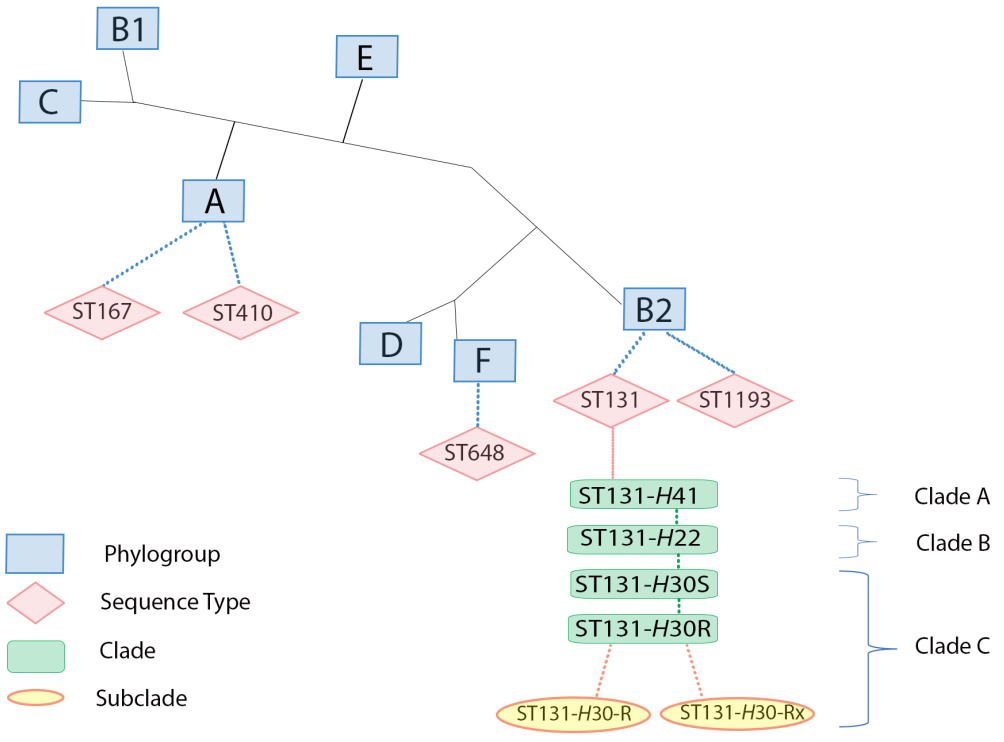


Figure 1.1 *E. coli* Phylogroups, ST, clades and subclades. Example *E. coli* pandemic lineages are displayed in their phylogroups. Petty Classification of ST131 clades described alongside their Fim allele classifications.

Intestinal commensal *E. coli* isolates often fall into phylogroup A and B1 (56) and rarely cause infection, except in some cases where the host is immunocompromised or gastrointestinal barriers are broken. Extraintestinal pathogenic *E. coli* (ExPEC) mainly fall in group B2 and are a major cause of disease in both the community and in hospital and have been linked to increased carriage of antibiotic resistance (57, 58). Uropathogenic *E. coli* (UPEC) and neonatal meningitis associated *E. coli* (NMEC) are important examples of ExPEC *E. coli* (58). Pathogenic *E. coli* clones, like ExPEC, have specific virulence factors which allow them to cause a variety of diseases (56, 59-61). In addition to ExPEC other examples of pathogenic *E. coli* include: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC) (58).

Multiple methods are available to further classify *E. coli* within a phylogroup including classical serotyping, multilocus sequence typing (MLST) and whole genome sequencing (WGS). The Kauffman classification scheme is used for classical serotyping of *E. coli* and is based on somatic lipopolysaccharide (O), capsular (K), and flagellar (H) antigens. Sequencing typing has played a major historical role in tracking outbreaks and transmission patterns, and is still critical in hospital monitoring for hospital infection prevention and control teams. There are several MLST schemes available for typing *E. coli* including Achtman (62), Pasteur (www.pasteur.fr/mlst) and EcMLST (63) schemes. The Achtman scheme (62) is the most widely used and defines *E. coli* lineages by using a set of seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA, recA*). The *E. coli* ST is determined by the alleles found at each housekeeping gene locus

In silico sequence typing using WGS is now more prevalent as WGS has become increasingly accessible and affordable. Advances in sequencing typing in particular over the last few decades have made it possible to identify more specific clones within pathogenic *E. coli* lineages (e.g. *fimH* or *fumC*/*fimH* (CH) typing) and therefore are used to understand how these lineages have and are evolving (64, 65). *fumC* and *fimH* have a higher discriminatory power than MLST (64, 65). *fimH* has been used to determine ST131 clades (66), for example the ST131 clade ST131-*H*30 (Clade C, Petty Classification (67)) is known to be a pandemic multidrug-resistant clone, which has emerged from clade ST131-*H*22 (Clade B) (66, 68).

Multi-drug resistance in *E. coli* is observed within specific clones within lineages, e.g., ST131 Clade C ST131-*H30*R which emerged from a sensitive ST131 Clade C, ST131-*H30*S (Figure 1. 1) (66, 68). Pandemic lineages are strains that have undergone clonal expansion and global dissemination (69). They frequently carry multidrug resistance, including fluroquinolone (FQR) resistance and multidrug resistance plasmids, alongside virulence factors to help them colonise and expand in a niche (66). ST131 is a classic example of a pandemic lineage, but there are other STs that are also pandemic lineages, including ST1193, ST410, ST648, ST167, ST73 and ST69 (57, 66, 69, 70) . All of these STs are common causes of ExPEC infection, e.g., BSIs, UTIs, in the community and in healthcare environments. AMR gene carriage in these pandemic lineages can confer resistance to multiple drug classes and a list of commonly found acquired AMR genes in pandemic *E. coli* lineages can be seen in Table 1.1.

Table 1.1 Drug class resistance driven by antibiotic resistance genes carried in pandemic lineages of *E. coli*

Antibiotic Class	Examples of Resistance Genes which confer resistance to this class	ST Resistance Genes observed in	References*
Aminoglycosides	*aac* genes (e.g. *aac3*)	131, 648, 1193, 410, 167, 69	(71-76)
	ant genes	131, 648, 617, 69	(76, 77)
	aph genes (*e.g. aph(3'')-Ib, aph(6)-Id*)	131, 648, 410, 167, 73, 69	(74, 76-81)
	aad genes	131, 648, 1193, 410, 617, 167, 73, 69	(72, 74-76, 78, 81-84)
	str genes (*e.g. strA*)	131, 648, 1193, 410, 167, 73, 69	(72-74, 76, 78, 82)
Aminoglycosides and quinolones	*aac(6')-Ib-cr*	131, 648, 1193, 410, 617, 167, 69	(72, 74, 78, 79, 85-88)
Chloramphenicol	*cat* genes (e.g. *catA2, catB3)*	131, 648, 1193, 410, 617, 167, 73, 69	(72-74, 76, 78, 79, 83, 84)
	cm1A1	131, 648, 617, 167, 69	(76, 77, 88-90)
	floR	131, 648, 1193, 410, 617, 167, 69	(74, 76, 82, 88, 91-93)
Diaminopyrimidine	*dfrA* genes *(e.g. dfrA14)*	131, 648, 1193, 410, 617, 167, 73, 69	(72-74, 76, 78, 82-84)
Macrolides	*erm* genes	131, 648, 410, 167	(72, 74, 89)
	mph(A)	131, 648, 1193, 410, 617, 167, 69	(72-74, 82, 83, 85, 89)
Monobactam, penicillins and cephalosporins	bla_{TEM}	131, 648, 1193, 410, 617, 167, 73, 69	(72-75, 82, 84, 91)
	bla_{VEB}	648	(77)
Monobactam, penicillin, cephalosporins and carbapenem	bla_{KPC}	131, 648, 410, 617, 167, 69	(74, 77, 78, 80, 81, 91, 94)
	bla_{SHV}	131, 648, 410, 167, 73, 69	(72, 74, 78, 95-98)
	bla_{VIM}	131, 410, 69	(74, 95, 99)
Penicillins, Cephalosporins and Carbapenem	bla_{GES}	131	(100)
	bla_{OXA}	131, 648, 1193, 410, 617, 167, 73, 69	(72, 74, 78, 84, 85, 91, 101, 102
	bla_{NDM}	131, 648, 1193, 410, 617, 167, 69	(74, 78, 91, 101-104)
	bla_{IMP}	131, 648	(105, 106)
Penicillins and cephalosporins	bla_{CTX}	131, 648, 1193, 410, 617, 167, 73, 69	(49, 68, 72, 74, 78, 86, 91, 96)
Penicillins and cephalosporins/cephamycin	bla_{CMY}	131, 648, 1193, 410, 617, 167	(78, 91, 96, 107, 108)
	bla_{DHA}	131, 1193, 410, 167	(78, 91, 101, 107)
Polymyxins	*mcr* genes	131, 648, 1193, 410, 167, 69	(76, 77, 88, 91, 108)
Quinolones	*qnr* genes	131, 648, 410, 617, 167, 69	(72, 74, 81, 87, 96, 109)
	par genes (e.g. *parC, parE*)	131, 648, 1193, 410, 167	(68, 74, 77, 82, 110, 111)
	gyr genes (e.g. *gyrA, gyrB*)	131, 648, 1193, 410, 167	(68, 74, 78, 82, 109-111)
Rifamycin	*arr* genes (e.g. *arr-3*)	648, 167	(77, 89)
Sulphonamides	*sul* genes	131, 648, 1193, 410, 617, 167, 73, 69	(73, 74, 78, 82-85)
Tetracyclines	*tet* genes	131, 648, 1193, 410, 617, 167, 73, 69	(73, 74, 82-85, 88, 112)

*The references that provide examples of these resistance genes carried by the pandemic lineages are references alongside

1.6 Mobile Genetic Elements

Bacteria can acquire resistance (including the genes in Table 1.1) by mutation or by horizontal gene transfer (HGT). This process involves a variety of mobile genetic elements (MGEs). HGT, the movement of genetic information between organisms by transformation, conjugation or transduction, enables AMR spread (113). Transformation is the uptake of naked single stranded DNA where it is taken up into a recipient bacterial chromosome by homologous recombination (114). This transformation is mediated by the recipient bacteria cell. Transduction is the HGT process by which DNA is transferred to a new bacteria recipient by a bacteriophage (114). Conjugation is a horizontal gene transfer mechanism where DNA is transferred from donor cell to recipient cell by direct contact (115) (Figure 1.2). It is a plasmid-mediated process where plasmid encoded pili allow the transfer of a single stranded plasmid to the recipient (115, 116). The single stranded copies of plasmid DNA in both the donor and recipient cells are then replicated so that the donor and recipient cells contain double stranded copies of the plasmid DNA. The conjugation is initiated by binding of plasmid-encoded proteins to the origin of transfer (*oriT*). The ability of a plasmid to undergo conjugation is reliant on the presence of the transfer region (*tra* region). The *tra* region contains transfer genes that encode all the genes required to make the type IV secretion system (T4SS) pilus and the relaxosome which binds to *oriT* to initiate plasmid conjugation. Plasmids can be self-transmissble, where they carry all components required for conjugation or mobilizable(115). Mobilizable plasmids carry only the *oriT* relaxase and proteins needed for nicking. Plasmid retention methods include post-segregational killing where plasmid free cells die. Post

segregation killing can be triggered by suicide mechanisms such as toxin-antitoxin genes(117, 118).

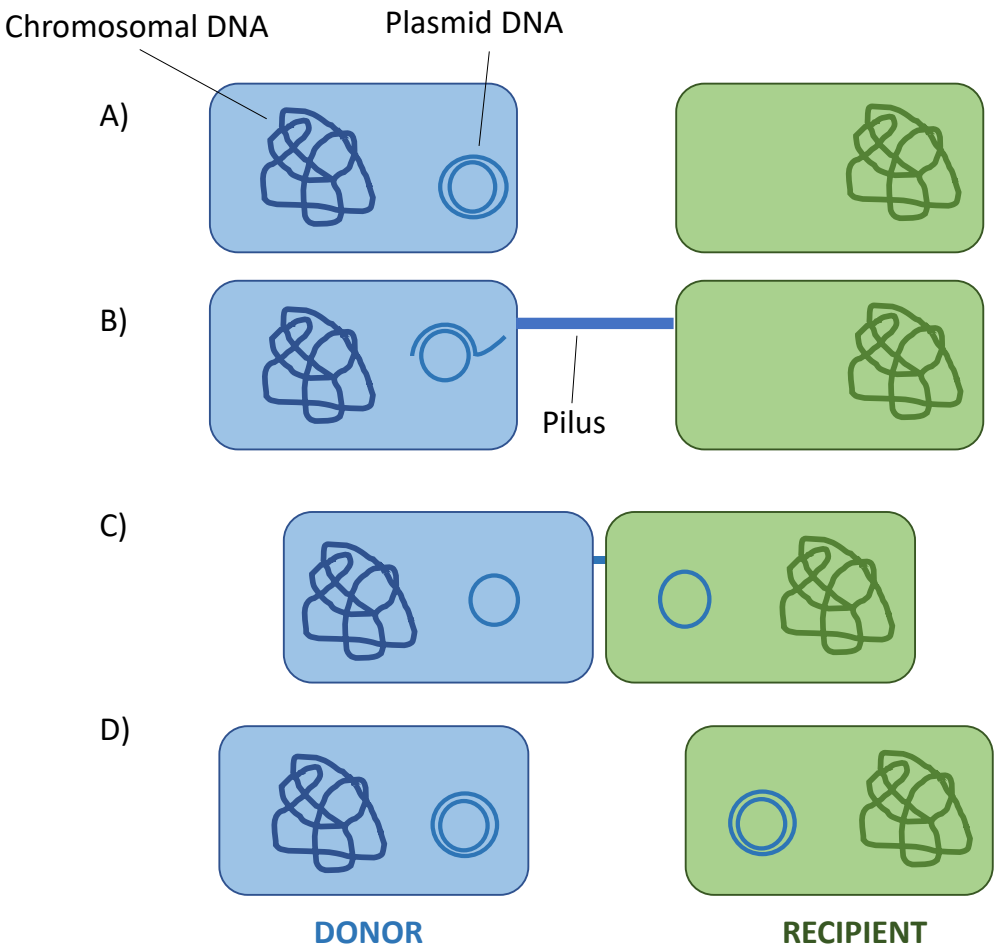


Figure 1.2 Bacterial conjugation. Bacterial conjugation of a plasmid between donor and recipient cells (A), demonstrating the formation of pilus and nicking of DNA (B), ready for transfer of single stranded plasmid DNA to the recipient (C) and duplication to form double stranded plasmids in each cell (D).

Mobile genetic elements (MGEs) contribute significantly to the spread of AMR genes (Table 1.1) by aiding HGT between different bacterial species and also between isolates within these species (44, 45). Examples of MGEs include plasmids, insertion sequence elements (ISs) and miniature inverted repeat transposable elements (MITEs) (119, 120). Plasmids are a key route of transfer for pathogenic and resistance

traits (e.g., AMR genes) between bacteria through HGT, by conjugation (121, 122). Additionally, plasmids can facilitate AMR gene transfer between animal and human reservoirs (123). Plasmids can play an important role in the spread of AMR on a widespread scale, for example, through epidemic plasmid spread, notably in Europe where an L/M type plasmid with *bla*$_{CTX-M-3}$ spread throughout Europe from Poland (124). Plasmid-mediated transmission has been associated with indirect patient contact allowing the spread of AMR in hospital environments, for example a Singapore CPE hospital outbreak (125).

1.6.1 Plasmid Types

Plasmids can be typed by classification of their replicon or mobility genes (126-129). Plasmid types found in *Enterobacteriaceae* include replicon-type F, X, I, H and N plasmids, and these all can potentially carry AMR genes (130). Mobility (MOB) genes can also be used to type plasmids and can include MOB$_F$, MOB$_H$, MOB$_Q$, MOB$_C$, MOB$_P$, and MOB$_V$. These MOB types are categorised by the N terminus of the plasmid relaxase protein (115). Traveller studies have connected resistance genes with specific plasmid types (e.g., *mcr* and IncX4) in colonising *Enterobacteriaceae* (48, 131, 132). F-type plasmids are commonly found to carry resistance genes including ESBLs and carbapenemases (133, 134). They are known to carry multiple replicons (e.g. FII, FIA, FIB) which allows them to be classified into these further subtypes (133). Plasmids of this type are generally 25 – 250 kbp (130), low copy number and often conjugative (133). The globally disseminated ESBL gene, *bla*$_{CTX-M-15}$ is principally carried by F-type plasmids (135), but F-type plasmids frequently carry other *bla*$_{CTX-M}$ genes (e.g. *bla*$_{CTX-M-14}$) (48, 136). Colistin resistance genes have also been found on F-type plasmids (137).

X type plasmids are frequently isolated from *E.coli* sourced from humans and animals (130). They are smaller conjugative plasmids than F-type plasmids, often 30-50 kbp (130). Again, this plasmid type harbours many resistance genes e.g., colistin, carbapenemases, ESBLs, quinolone, tetracycline and trimethoprim (130, 134, 138). Colistin resistance genes, *mcr-1* and *mcr-2,* have been associated with IncX4 (132, 137). *Enterobacteriaceae* also carry other smaller Q-type (5 -15 kbp) and Col plasmids (6-40 kbp) (130), which harbour AMR genes less frequently (119, 130, 139). Q-type plasmids have a broad host range, and can be further typed to IncQ1 and IncQ2 (140). They are frequently mobilized by other self-transmissible plasmids (139, 140). Resistance to multiple antibiotic classes is carried by Q-type plasmids, more commonly sulphonamides, aminoglycosides, cephalosporins, cephamycin and tetracyclines (141-143).

Col plasmids are high copy number plasmids which are non-conjugative and are frequently identified in *Enterobacteriaceae* (144). The bacteriocin, colicin is carried by Col plasmids (145, 146). Bacteriocins are protective to the source bacterial strain as they harm closely related bacterial strains but not the source bacteria (145). Col plasmids, even though small, can carry a variety of AMR genes, although less commonly than larger F-type plasmids (130). One example is ColE which has been reported to carry *bla*$_{\text{CTX-M}}$, *bla*$_{\text{CMY}}$, *sul, tet,* and *mcr* (147-149). Col plasmids are also linked to the spread of quinolone resistance (e.g., *qnrS*, *qnrB19*) (150, 151).

Small plasmids carrying Col replicons, can fall into two categories: those that are theta replicating and those that are rolling circle replicating (e.g., ColBS512). Theta-

replicating plasmids can be protein initiated (θ-Rep) (e.g., Col156) or are θ-RNA initiated (θ-RNA) (e.g., ColRNAI). Co-integration of these small Col plasmid replicons with larger plasmid replicons can be found but little information is available on this co-integration (144). Phages also have the capability to infect Gram-negative bacteria and are known to be found in *E. coli*. Phage plasmids, including p0111 and Y-type plasmids, are known to carry resistance to a broad range of antimicrobials (152, 153).

1.6.2 Other MGEs

Plasmids are not the only mechanism by which AMR can be spread. Movement of transposable elements (e.g., transposons, integrons, insertion sequences) also assists mobility and spread of AMR genes (119, 154-156). Integrons are non-mobile genetic elements which contain gene cassettes. Gene cassettes can be mobilised to other integrons or other regions of the genome. Although integrons are not mobile themselves, they can capture multiple gene cassettes via site specific recombination and contain a promoter to express the genes in the gene cassettes (157) Integrons are known to be associated with antibiotic resistance carried, for example, sulphonamide carrying class I integrons (47, 158). Transposons are DNA segments that can be translocate as a whole from one genomic location (e.g. chromosomal, phage, DNA) to another using a self-encoded transposase enzyme (159).Insertions sequences (IS) are compact transposable elements (< 2500 bp) which only code for transposition activity. Most have terminal inverted-repeat (IR) sequences (10 - 40 bp) (160, 161). IS are categorised by transposase enzyme chemistry (160, 161) and IS elements are frequently associated with AMR genes (161, 162). The transposase mediates IS transposition, where the transposase binds and cleaves at the terminal inverted repeat sequences (IR) (Figure 1.3A). This transposase also then initiates insertion of the IS

element at a new site. Insertion of the IS at a new site frequently creates a target site duplication (TSD) where cleavage of the insertion target site occurs (Figure 1.3B).

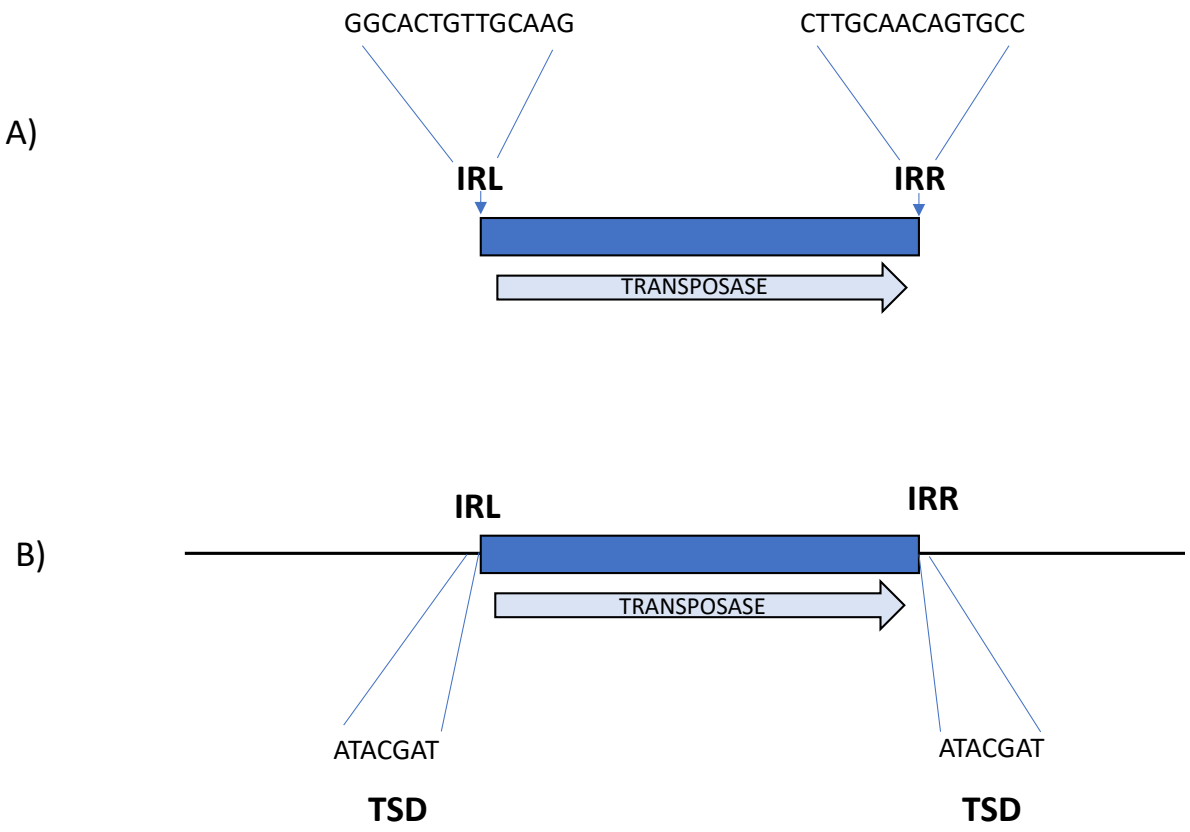


Figure 1.3 Schematic of an IS element, showing A) transposes enzyme with terminal inverted repeats (IRL) and (IRR) and B) target site duplication on insertion of the IS to a new site. The sequences used in this figure are examples only and not linked to any IS elements in particular.

Specific IS elements have been linked to the mobilisation of AMR genes (e.g., *bla*$_{\text{CTX-M}}$ genes). One example is the insertion sequence IS*Ecp1,* which is thought to have contributed to *bla*$_{\text{CTX-M}}$ global spread (163). IS*Ecp1* are frequently found upstream of *bla*$_{\text{CTX-M}}$ (164). IS*26* is also an important insertion sequence associated with a wide variety of AMR genes. Although IS*26* has not been linked to a specific resistance gene it is frequently reported to carry 1-2 resistance genes (161). IS*26* is known to form

arrays of resistance genes as it preferentially inserts adjacent to existing IS*26* sequences (165). This means IS*26* often plays a key role in the accumulation of resistance genes in plasmids and chromosomes. MITEs, miniature inverted repeat transposable elements, play a smaller role in AMR movement, but still provide the ability to transfer genes (120). MITEs are even smaller (256 bp) transposable elements, which can be mobilised by active copies of related transposons (120). Virulence genes and AMR genes can also be disseminated by phage-inducible chromosomal islands (PICIs) which are small highly mobile genetic elements (166).

1.7 Colonising *E. coli* in different environments

1.7.1 Colonisation in travellers

Faecal colonisation with ESBL-producing bacteria has been identified as one route with the potential to be a major worldwide source of the spread of AMR in the environment and between individuals (5). Increased global travel has assisted the spread of antimicrobial resistant organisms including ESBL-producing organisms and CPEs, through increased exposure to these resistances, and transient or persistent colonisation (5, 29). There are several important studies that monitor gut colonisation in travellers using pre and post travel samples at different time points (131).Traveller studies have found that travellers can be colonised with ESBLs, CPEs and colistin resistant organisms (48, 50, 167, 168). Colonisation does not necessarily cause infection and can simply be carriage of MDR organisms in the gut, which is a risk as it facilitates the spread of AMR around the globe. One important large study, The **C**arriage **O**f **M**ultiresistant **B**acteria **A**fter **T**ravel (COMBAT) study, looked at pre and post travel stool samples for individuals (n=2001) and the non-travelling members of their households (n=215) (169). 34% of COMBAT study travellers who were ESBL

negative before travel returned colonised with ESBL *Enterobacteriaceae,* including 9 that had colistin resistance (170). 95% of this colonisation was linked to international travel, with 75% of study participants visiting South Asia. They reported 6.1% of travellers were colonised with ESBL *Enterobacteriaceae* pre-travel. Participant questionnaires suggested that antibiotic use in the three months prior to travel and history of travel outside of Europe in the last year were strong predictors for carriage with ESBL *Enterobacteriaceae* before travel (50).

Another study (132) focussed specifically on the concerning incidence of colistin resistance and compared the whole genome sequences of isolates with colistin resistance using isolates from several different sources including traveller isolates. These sources included some of the traveller isolates from the COMBAT study (six *mcr-1* gene positive *E. coli*) along with 22 human and chicken isolates from Vietnam, and 65 whole genomes sequences from across the globe found in literature reviews. Investigation of this dataset found that *mcr-1* gene was linked to two *E. coli* lineages, with ST10 being the largest lineage, even though the overall *E. coli* population was diverse. This study found 13 plasmid incompatibility types linked to the *mcr-1* gene. IncHI2, IncI2 and IncX4 plasmids were the mobile genetic elements (MGEs) most commonly containing the *mcr-1* gene (132). The study suggested that it was through these plasmids that the *mcr-1* gene was disseminated both locally and globally.

A prospective observational cohort study of ESBL resistance in travellers to South Asia by Bevan et al. (48) investigated the dynamics of acquisition and loss of CTX-M producing and non-producing *E. coli* in travellers. This cohort study investigated changes in the faecal microbiome of 18 participants by analysing their pre- and post-travel isolates. A total of 483 isolates were collected from these 18 individuals. The

study found both extensive colonisation of participants with ESBL-producers during travel and a wide diversity of ESBL *E. coli* within many participants. Results demonstrated 89% of individuals became *bla*$_{CTX-M}$ positive after travel. The two predominant *bla*$_{CTX-M}$ types were *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-15}$. Participants showed polyclonal colonisation with ESBL *E. coli*, with the mean number of STs acquired being 3 STs with *bla*$_{CTX-M}$ (48). Both the COMBAT study and Bevan et al study showed the potential for onward spread of ESBL colonisation after travel, highlighting the concerning issue of MDR colonisation and spread in returning travellers.

1.7.2 Colonisation in the community

AMR colonisation in the community is not well documented in the UK and has been mainly focussed on ESBL-producing organisms. One of the few UK studies was reported in 2014 by McNulty et al and showed that 7.3% healthy individuals surveyed (n=2430) were CTX-M ESBL positive. The highest prevalence for colonisation with an ESBL was linked to the individual being born in South Asia and found in those travelling to certain areas including South Asia (171). A previous smaller scale study in Birmingham carried out by Wickramasinghe et al made similar links to population subsets where individuals from the subset of Middle East/South Asia origin had a significantly higher carriage rate of *bla*$_{CTX-M}$ positive isolates than those from European origin (172). These participants with Middle East/South Asia origin also carried a significantly higher carriage rate of *bla*$_{CTX-M-15}$ specifically. Overall, the prevalence of *bla*$_{CTX-M}$ carriage in the Birmingham sample population was 11.3%. Community colonisation with ESBLs has also been studied in Europe. A 2008 Spanish study highlighted that 16.7% of household contacts of patients with community-acquired

infections caused by ESBLs were also colonised. This indicates a reservoir of ESBL-producing organisms in the community (173).

ESBL-producing organism colonisation in the community can also occur as a result of returning travellers transmitting isolates to members of their household. The COMBAT trial found that 7.7% of travellers returning colonised with ESBL *Enterobacteriaceae* transmitted this to other household members. Travellers were shown to be a potential source of longer-term transmission to other household members, for at least 12 months in some cases (131). These results were supported by the Bevan et al study which showed stable colonisation of faecal samples with ESBL *E. coli* on return from travel, in some cases for periods of up to 7 months. This again highlights the long-term risk and potential for transfer of AMR, including ESBLs, in the community (48). These studies indicate that the community can be a reservoir for ESBL carriage and that although not particularly high overall, global origin can affect likelihood of ESBL carriage. Global travel can be a risk factor for CPE carriage in the community. There is limited data on community carriage of CPE but it is likely that the same is true for CPE as for ESBL and that community can be a reservoir for CPE carriage(174, 175).

1.7.3 Colonisation in hospital settings

AMR is a serious problem amongst travellers and in the community, but the effects of AMR have become an important problem in hospital settings worldwide (1, 5, 176). Colonisation with MDR organisms in the ICU can lead to infection and in turn poor patient outcomes (4, 177). It is well known that patient drug treatment regimes, for example broad spectrum antibiotics likely prescribed in the ICU, disrupt the microbiota in the gut of adults and that interventions, for example feeding tubes, can have the same effect (178). Critically ill patients in ICU are also more likely to be susceptible to

opportunistic pathogens such as *E. coli*, and to require antimicrobial therapy. ICU patients are therefore more likely to be colonised with AMR organisms and to develop drug resistant infections which can lead to poor patient outcomes (179, 180). The infections which occur are increasingly MDR infections. It is therefore important to understand the colonisation dynamics of AMR in order to combat the spread of AMR in the ICU. This information can potentially help to reduce the incidence of AMR especially MDR organisms in critically ill patients.

Infection with drug resistant bacteria is linked to poor patient outcomes and increased treatment costs (4). Studies have shown that infections caused by ESBL-producing isolates are more likely to be linked to treatment failure than those caused by non-ESBL-producing isolates. The consequences of infections caused by ESBL-producing isolates include extended hospital stays, additional longer courses of antimicrobial therapy and specialist treatment for more complex needs, all of which contribute to the increased cost of treating this patient group (181). Our health service in the UK is acknowledged to be overstretched and financially pressured but has a strong ethic of patient centred care so there is a clear motivation to understand more about the impact of colonisation with MDR bacteria to help improve patient outcome and reduce costs.

The Queen Elizabeth Hospital, University Hospitals Birmingham NHS Trust is particularly interested in decreasing the impact and spread of AMR. In a previous study Ravi et al. reported decreased gut diversity in the commensal gut flora over an inpatient stay (182). Monitoring of antimicrobial therapy during the study period in 24 patients showed the differing effects on the gut microbiota after treatment with different broad-spectrum antibiotics. This study confirmed that broad-spectrum antibiotics such as meropenem can alter colonising isolate profile and potentially cause a loss of diversity

in the gut microbiota (182). Studies from hospitals elsewhere in Europe have also demonstrated dysbiosis of the microbiome after patient admission to the ICU and increased likelihood of more severe complications in the critically ill e.g., sepsis and nosocomial infection (182, 183). In one Dutch study colonisation with drug resistant *E. coli* significantly increased with increasing length of stay (184). This study also indicated that ICU patients were at higher risk of colonisation by drug resistant organisms than those on more general wards.

Although colonisation with a drug resistant isolate itself is not directly a problem, it can lead to drug resistant infection. It has been reported that patients who have rectal colonisation with a specific bacterial species (e.g., *E. coli, Enterococcus*) at ICU admission have subsequent infections with the same bacteria (185). This shows the importance of previous hospital stays or community carriage with ESBL, and how this can drive infection and spread of AMR in the ICU. Across the globe recent isolate level ICU colonisation studies have also reported high levels of colonisation with MDR *E. coli*, *Klebsiella* species and *Acinetobacter* species (186-188). This raises cause for concern that UK hospitals may also be experiencing increased levels of drug resistant colonising Gram negative isolates. These studies also indicate the importance of transmission of colonising isolates in causing subsequent infection of multiple ICU patients. The knowledge of transmission can aid the introduction of prevention methods to reduce incidence of infection in this vulnerable patient group.

1.8 Historical infection landscapes

The importance of *E. coli* and other Gram-negative organisms colonising patients in the ICU environment has been established (Section 1.7.3) and helped to provide information about the key STs that are found in circulating in the ICU. These studies

have frequently been short-term studies which could not provide the in-depth knowledge that can be gained from long term studies. Long term historical studies provide a strong resource for tracking critical infection causing organisms over time. There are several major historical datasets that, as well as highlighting critical infection causing STs at different points in time, allow the detection of the emergence and fluctuation of STs in the everchanging AMR landscapes in clinical patient populations.

Multiple studies in European countries have conducted long-term reviews on invasive *E. coli* populations, frequently with a focus on BSIs (85, 189, 190). One significant factor that has emerged from these studies is that these datasets frequently acknowledge the key role of pandemic lineages, especially ST131 in driving bloodstream infection in hospital patients. ST131 is generally recognised as an important pathogenic lineage and in-depth analysis has shown that the clades of ST131 driving infection vary. A Norwegian longitudinal study (190) reported the role of ST131 clade A which contrasted to the ST131 clade C found in an English BSI study (85). This comparison highlights changes in STs of clinical infection *E. coli* isolates across European countries in adult populations. All of these longitudinal studies highlight how prevalent STs vary over decades and the important role pandemic lineages play in invasive disease. They allow the tracking of emerging isolates, and how they displace other STs in the overall ST population. Importantly they can also track the emergence of resistance.

1.9 Measures to combat AMR

The identification of the mechanisms and causes of AMR is an important part of the global effort to control and prevent the spread of AMR. Surveillance, education and

information campaigns and measures to stop misuse and bad practice in antimicrobial treatment are simultaneously being used to help combat the spread and development of AMR. In the short term it is essential to understand the extent of AMR worldwide and to perform surveillance studies that ensure the extent of the problem is known. Surveillance provides information about the current situation and can help predict trends in an environment (9, 191, 192). Current and past AMR threats can be detected by historical surveillance studies (85, 189, 190). These studies can detect emerging lineages and provide knowledge of what may cause a pandemic lineage to be successful (66). The study of geographical areas where acquisition of AMR may be high risk can identify potential AMR threats and detect unknown mechanisms of AMR spread e.g., travel to countries with high incidence of AMR or hospital stays.

In both the short and long-term, antimicrobial stewardship can be used to protect the selection of antimicrobials accessible to combat infection. Antimicrobial stewardship involves healthcare wide processes and systems to promote the appropriate use of antimicrobials in order to ensure they remain effective in the further. These systems and processes also involve the monitoring of antimicrobial usage. It encourages the following of local and national guidelines which are aimed to ensure the prescription of the most appropriate dose, length of course and route of administration where possible using a narrow spectrum antibiotic. In the long-term research and development is an important part of global efforts to control and prevent the spread of AMR. Studies are investigating both the mechanisms of AMR evolution within a species and also the way in which AMR is then spread.

1.10 Study rationale and Aims

There is global concern about the increase in AMR and the ultimate possibility that some infections may no longer be able to be treated by antimicrobials. An understanding of the dynamics of colonisation and infection of pathogens like *E. coli,* which are known to be significant carriers of AMR and assist in the spread of AMR could potentially provide information to help control or prevent further spread of AMR.

My overall aim in this thesis is **to understand the dynamics of colonisation by MDR organisms in differing but relevant settings, and the dynamics of MDR infection in the absence of antibiotic selection**. This thesis provides the opportunity to learn more about colonising and infection causing *E. coli* in different scenarios and to understand the spread of AMR in different environments, age groups and learn more about the mechanisms involved in this spread. To achieve my aim, this thesis examines three datasets of *E. coli* where the participants and patients in the study populations have **not** been selected based on the use of antimicrobials, so reflecting different real-world situations without bias to any particular antimicrobial treatment.

This allows me to address my overall aim asking three distinct questions:

1) How does MDR *E. coli* colonise healthy travellers?

2) How does MDR *E. coli* colonise ICU patients?

3) How does MDR *E. coli* infect neonates who have had minimal previous exposure to antimicrobials?

Neither the ICU patient nor traveller populations studied to examine their colonising *E. coli* were selected on the basis of antibiotic prescription. The cohort of travellers to

Laos were healthy individuals (Chapter 3). The ICU patients (Chapter 4) were selected for based on sudden admission (e.g., trauma, neurology, cardiology) to the ICU with the aim of patients being admitted to the ICU with as close to community gut microbiota as possible. In addition, the final dataset of mainly neonatal patients (Chapter 5) studied to explore their invasive infection causing *E. coli* isolates were not selected on the basis of specific antimicrobial prescription. Due to the age of the patient dataset set there is likely limited opportunity for prolonged antimicrobial prescription prior to the infection isolate. This neonatal dataset was also sourced from the Netherlands, a country with known low AMR levels and good antimicrobial stewardship policies (7, 193). This project allows a unique insight into a neonatal invasive *E. coli* population, a population of ICU *E. coli*, and a detailed strain and plasmid level analysis of *E. coli* colonising travellers during their stay in Laos.

Overall Aim: Understanding the dynamics of colonisation by MDR organisms in differing but relevant settings, and the dynamics of MDR infection in the absence of antibiotic selection.

Chapter Aims:

Chapter 3 – The dynamics of colonising MDR E. coli and the highly diverse plasmid population found in these *E. coli* colonising travellers to Laos and their role in antimicrobial resistance gene carriage

There is minimal knowledge on the gut colonisation of travellers at an isolate level, as most previous reports have been using metagenomic studies. This chapter aims are to:

1) Explore the pattern and dynamics of AMR gut colonisation during a traveller stay in Laos.
2) Explore the role of MGEs within a traveller dataset to understand the role they may play in the spread of AMR in this dataset.

Chapter 4 - Gut colonisation of Intensive Care Unit Patients with Multidrug resistant *E. coli*

Gut colonisation in an ICU settings has mainly been investigated using metagenomic sequencing techniques. This chapter aims to explore UK ICU patient gut colonisation with MDR organisms at an isolate level. This chapter aims are to:

1) Investigate *E. coli* diversity and colonisation dynamics in the ICU environment.
2) Track prevalent *E. coli* STs including transmission between different areas of the ICU.
3) Study AMR genes in ICU patients including effects of antibiotic prescribing on the gut microbiota.

Chapter 5 SENTINEL: SequENcing of hisTorical clinIcal *Escherichia coli* from the NEtherLands

This third chapter focusses on a historical dataset of clinical infection causing *E. coli* in a neonatal patient dataset with low level of antibiotic usage. The previous chapters report the characteristics of colonising *E. coli* isolates in different settings of healthy travellers and ICU patients with datasets collected over short time periods. In contrast this chapter investigates of *E. coli* causing invasive disease in a unique mainly neonatal dataset for over 40 years. This chapter aims to:

1) Study the *E. coli* ST population causing infection in invasive disease patients in a historical dataset from the Netherlands between 1975-2021.
2) Study resistance patterns infection in *E. coli* causing invasive disease in a historical dataset from the Netherlands between 1975-2021.
3) Explore ST567, a key ST causing infection in early part of dataset that is predominant.

2. Methods

2.1 Sampling

This thesis investigates three different collections of *E. coli* isolates from three different clinical studies. Different sampling methods were used to obtain each *E. coli* collection as listed below.

2.1.1 Laos *E. coli* sampling

E. coli were isolated from stool samples from travellers to Vientiane, Laos. Study participants (n=23) and their contacts were attending a medical course that ran from19th September to 9th October 2015. Baseline samples were taken on participant arrival in Laos, daily during the 23-day study and at departure. This study has been published (29) and the study protocol was approved by the Ethics Committee of the Helsinki University Hospital, Helsinki, Finland, and the Ethics Committee of Northwest and Central Switzerland, Basel, Switzerland. Participants gave written informed consent to participate in the study.

Stool samples were screened for extended spectrum beta-lactamase (ESBL) Gram negative bacteria on CHROMagar ESBL agar plates (CHROMagar, Paris, France) at the Microbiology Laboratory of Mahosot Hospital, Vientiane, Laos. Suspected ESBL isolates were sent to the Swiss Tropical and Public Health Institute, Basel, Switzerland, and then to the University of Helsinki, Helsinki, Finland and where they were subcultured on chromogenic media, ChromID ESBL agar (BioMérieux, Marcy-l'Étoile, France). These isolates were then sent from Helsinki to University of Birmingham (UoB) for culture on UTI chromogenic agar and sequencing by MicrobesNG facility.

After Illumina short read sequencing data was received from MicrobesNG the *E. coli* from Kantele et al (n=219) (29) were first sequence typed using mlst (v2.15) (https://github.com/tseemann/mlst). Isolates were then selected for Oxford Nanopore (ONT) MinION sequencing (n=49) by deduplication of longitudinal patient samples, and by using ABRicate (v0.8.10) (https://github.com/tseemann/abricate) to identify unique elements such as plasmid replicons (PlasmidFinder (v0.8.10)(194) and antibiotic resistance genes (ResFinder (v0.8.10) (195) from the short read assemblies generated with SPAdes (v3.13.0) (196). The objective of this method of isolate selection was to capture a diverse set of plasmids carrying multidrug resistance in *E. coli* (197).

2.1.2 IMPACT2 sampling

All bacterial isolates in the IMPACT2 study were obtained from the intensive care unit (ICU) at Queen Elizabeth Hospital Birmingham (QEHB), University Hospitals Birmingham, NHS Foundation Trust (UHB). Isolates were collected from stool samples of 24 patients between 24/05/2021- 01/12/2021. Ethical approval was received for this IMPACT2 study. This project was IRAS approved at main committee 20/YH/0067 and was given IRAS ID 268838. Study inclusion criteria were that patients must be aged over 18 years and admitted to the ICU with the expectation they would remain there for greater than 48 hours. Study exclusion criteria included patients aged under 18 years, patients admitted to the ICU who were not expected to remain there for greater than 48 hours and patients who had opened their bowels whilst on ICU before enrolment to this study. Patients were recruited and consented to the study by research nurses after discussion with an ICU consultant about the likelihood they would remain on the ward for over 48 hours. The first stool sample passed by the patient was stored.

Patients then remained in the study for the duration of their stay and their stool samples were collected until discharge or patient death on the ICU. Metadata was collected including sex, age, admission date to ward, departure date from ward, bed history, microbiology diagnostic test results and antibiotic prescribing history.

Fifty-nine patients were recruited to the IMPACT2 study and 25 patients were withdrawn due to illness or failure to produce a stool sample. Of the remaining 34 patients, 24 patients were included in the study reported here and metadata is shown in Appendix A1. The remaining 10 patient samples were stored but not processed and sequenced due to the time limitations on this project caused by COVID-19 delays.

There was high awareness of the additional risks of carrying out this study at a time in the COVID-19 pandemic and to prevent processing samples from patients who potentially might be asymptomatically shedding *SARS-CoV-2,* stool samples were kept at -80^{o}C for two weeks before processing. Patient metadata was checked for history of infection and illness, including positive *SARS-CoV-2 tests* and gastrointestinal infection before sample transfer to UoB. Stool samples were stored at -80^{o}C on arrival at UoB.

2.1.3 SENTINEL Sampling

All SENTINEL *E. coli* isolates were obtained from Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM), Amsterdam UMC, Netherlands from a historical collection of clinical *E. coli* isolates. These *E. coli* dated from 1975-2021. Regional laboratories in the Netherlands send *E. coli* isolates to the NRLBM reference laboratory on a voluntary basis for all *E. coli* isolated in the CSF, bloodstream and other locations of patients with bacterial meningitis. The NRLBM also recommend *E. coli* isolates are

sent from patients with an invasive infection but not meningitis, and for patients less than 1 year old with invasive infection. These are the current criteria for isolate submission but in earlier years only cerebrospinal fluid (CSF) samples would be submitted to the NRLBM.

The NRLBM provided metadata including source of isolate (e.g., CSF), patient sex, patient age, patient number and, where possible date, of isolation in the local laboratory and date of receipt at the NRLBM. Patient number is a designated number given on sample receipt in the NRBLM to help track samples from the same patient. Any samples collected within 30 days from the same patient are given the same patient number but will have different sample numbers.

All isolates from 1975-2015 were directly sent from NRLBM to MicrobesNG for short read Illumina sequencing and those from 2016-2021 were sequenced in house at the NRLBM. The earliest ST567 isolate (NEL0010_750318_1_F_C_75) was sent to MicrobesNG for their enhanced sequencing service, providing long and short read data.

Separately nine *E. coli* isolates were selected and sent to the UoB for long read sequencing. These nine *E. coli* were selected based on their position in the dated ST567 phylogenetic tree. They were chosen as representatives of isolates with differing core gene profiles based on Panaroo gene presence absence, different plasmid replicons, different AMR genes and differing virulence factor profiles. The isolate selected were: NEL1052, NEL1053, NEL1045, NEL1019, NEL0929, NEL1400, NEL1503, NEL0153 and NEL0156.

2.2 Culture

Culture methods for isolates and stool samples processed varied according to the project.

2.2.1 Laos *E. coli* culture

All *E. coli* isolates sourced from Laos were cultured overnight on UTI Chromogenic agar (Sigma) at 37°C and plates checked for purity before any DNA sequencing was carried out. After purity had been confirmed single colonies were subcultured overnight in LB Broth (Miller) (shaking at 200 rpm, 37°C) in preparation for long read sequencing using Oxford Nanopore MinION technology.

2.2.2 IMPACT2 culture

On receipt from QEHB IMPACT2 stool samples were immediately processed to reduce the number of freeze thaw cycles they experienced. 0.1 g of each stool sample was set aside for use in culture experiments. The remainder of each stool sample was divided, half was frozen neat and half was frozen in 30% glycerol at -80°C, for future reference and use.

In order to isolate *E. coli* colonies of interest the 0.1g of each stool sample previously set aside was incubated in 10ml of LB (Miller) overnight (shaking 200 rpm, 37°C). Once incubated overnight stool broths were diluted in Phosphate Buffered Saline (PBS) to concentrations ranging from 10^{-1} to 10^{-6}. Initially 100 µl from each dilution (from neat to 10^{-6}) was spread plated on both MacConkey agar (Sigma) and ESBL Chromoselect agar (Sigma). This range of dilutions was reduced to neat, 10^{-1}, 10^{-3} and 10^{-6} over the course of the study. Dilution plates of MacConkey and ESBL agar were both incubated overnight at 37°C and representative populations of all bacterial isolates (not just *E.*

coli) were saved in 25% glycerol. All these glycerol stocks were checked for purity on UTI Chromogenic agar (Sigma) with overnight culture at 37°C.

2.2.3 SENTINEL

The nine SENTINEL isolates specifically selected for long read sequencing were cultured overnight at 37°C on UTI Chromogenic agar (Sigma) to check for purity prior to sequencing.

2.3 DNA isolation

DNA was isolated for long read sequencing experiments in both the Laos *E. coli* dataset (Chapter 3) and the SENTINEL dataset (Chapter 5). A variety of methods (Sections 2.3.1-2.3.3) were used to extract DNA from the Laos *E. coli* dataset (Phenol chloroform, phenol chloroform with Cetyltrimethylammonium bromide (CTAB) and Monarch genomics). In the SENTINEL study only Monarch genomic kit was used to extract DNA.

2.3.1 Phenol Chloroform

2 ml overnight culture from Laos *E. coli* was pelleted and the supernatant discarded. The pelleted *E. coli* cells were resuspended in 750 µl Qiagen B1 buffer and 10 µl Proteinase K. These samples were heated at 55°C for 1 hour, with inverting every 10 minutes. Lysed samples were added to 750 µl Phenol:Chloroform:Isoamyl alcohol in a 5PRIME heavy gel phase lock tube (Quanta bio), and mixed using Hulamixer (Invitrogen) for 5 minutes at 80 rpm. After mixing, samples were centrifuged (13,400 rpm for 10 minutes). Supernatant was transferred to 750 µl Phenol:Chloroform:Isoamyl alcohol in a new 5PRIME heavy gel phase lock tube (Quanta bio), then as before mixed (5 minutes at 80 rpm) and centrifuged (13,400 rpm for 10 minutes). The resulting supernatant was added to 100 µl 10 mM ammonium acetate and then 3-5 ml of ice

cold 100% Ethanol added to precipitate out DNA. DNA strands were washed in 80% ethanol. Excess ethanol was left to dry off. DNA was then resuspended in 10 mM Tris buffer and stored at 4°C overnight.

The method described above is an optimised method, initially 500 µl of Qiagen B1 buffer and 500 µl Phenol:Chloroform:Isoamyl alcohol was used. A variety of volumes

of overnight culture were also trialled (1 ml and 3 ml). In addition, a trial Phenol-Chloroform extraction was used to directly compare DNA yields from both a control *E. coli* strain, MG1655, and an isolate from the database known to be difficult to extract, LA133, to verify experimental method and techniques.

2.3.2 Phenol Chloroform and Cetyl trimethylammonium bromide (CTAB) DNA extraction

A high level of cellular debris, assumed to be carbohydrates, was seen in the phase lock tubes described in 2.3.1. This led to the trial of Cetyl trimethylammonium bromide (CTAB) to aid DNA extraction. Method 2.3.1 was repeated but 200 µl 5 M sodium chloride was added to the lysed sample followed by 100µl of 10 % CTAB before adding the lysed samples to Phenyl:Chloroform:Isoamyl. After mixing well and then incubating at 65°C for 10 minutes, Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added and Method 2.3.1 was then followed as above except with the following additional cooling step. Between the two Phenol:Chloroform:Isoamyl alcohol steps the phase lock tube was left on ice for 30 minutes to help separate the cellular debris from DNA.

2.3.3 Monarch Genomic DNA Extraction kit

DNA was extracted from the majority of Laos *E. coli* isolates using the Monarch Genomic DNA Extraction kit (NEB) using an adapted version of the NEB protocol for extracting DNA from Gram negative bacteria (NEB #T3010), but in some instances a

lower quantity and quality yield was obtained. In these instances, atypical precipitates were noticed, and extraction was carried out using the phenol chloroform with CTAB method (Section 2.3.2).

NEB #T3010 Monarch Genomic DNA Extraction kit protocol for extracting Gram negative bacteria genomic DNA binding and elution stages were altered to optimise DNA extraction from Laos *E. coli* isolates. Here one minute spin stages were extended to 90 seconds. An additional spin step was added after the second gDNA wash step, spinning at maximum speed for three minutes to remove all wash liquid from the filter. DNA samples were eluted in 60 µl 10 mM Tris HCl and there was a 5 minute incubation step with this preheated 10 mM Tris HCl before the final spin stages.

In cases where isolates had a low yield of DNA, overnight culture of *E. coli* were subcultured and grown until mid-log optical density to reduce capsular growth and then DNA extraction proceeded as described above.

2.4 DNA quantification

Once isolated DNA concentration was quantified using Qubit ™ Broad Range double stranded DNA kit (Invitrogen™) and Invitrogen™ Qubit 4 fluorometer. The concentrations of any further DNA dilutions required for sequencing were also checked with this method.

2.5 Whole genome sequencing

2.5.1 Illumina

All short read sequencing for Laos *E. coli* isolates (Chapter 3) and IMPACT2 (Chapter 4) was performed by the MicrobesNG facility. Libraries were prepared using the Nextera XT kit (Illumina, San Diego, CA, USA) and sequenced on the Illumina HiSeq

2500 platform over 250 cycles (Illumina). Short read sequencing data for Laos *E. coli* (29) are publicly available under NCBI BioProject accession number PRJNA558187.

In the SENTINEL dataset 1790 isolates dated from 1975-2021. 1388 isolates dated from 1975-2015 sent directly from NRLBM to MicrobesNG for WGS. 402 isolates dated from 2016-2021 were whole genome sequenced in NRLBM and the assembly pipeline is publicly available (https://github.com/NRLBM/assembly). This pipeline included: Trimmomatic to filter and trim fastq files; Shovill to assemble data; Quast for quality control and mlst was used for additional species ID. Once isolates were identified as *E. coli* using this pipeline the untrimmed read files were shared with UoB, and were then processed as described below in section 2.5.3.

2.5.2 Oxford Nanopore Technology MinION long read sequencing

Long read sequencing was used to investigate both the Laos *E. coli* and SENTINEL *E. coli* datasets. Laos *E. coli* DNA was sequenced using MinION (Oxford Nanopore Technology), over four runs using R9.4.1 flow cells (with 12 or 24 isolates per flow cell). Three runs were prepared using Ligation Protocol (LSK-SQK109), and one run with the Rapid Barcoding Sequencing Kit (SQK-RBK004), with both protocols modified to a one-pot implementation. Samples were pooled after barcode ligation, before the first AMPure bead clean up stages. All steps from this point were on the pooled sample. SENTINEL *E. coli* DNA was prepared for MinION sequencing using Ligation Protocol (LSK-SQK109) one-pot implementation protocol and R9.4.1 flow cells. Sequencing data for the Laos study is now publicly available and presented here BioProject PRJNA853172. Complete plasmid sequences have been uploaded to GenBank with accession numbers (OP242215-OP242244, OP156879).

2.5.3 Trimming Read Files

All short read sequencing data provided by MicrobesNG was provided as trimmed read files. MicrobesNG used Trimmomatic (v0.3) with a sliding window quality of Q15, to remove adapters from sequences. Read files provided by the NRLBM directly were trimmed with Trimmomatic (v0.38).

2.5.4 Quality

Quast (v5.0.0) and FastQC (v0.11.7) were used to check the quality of short read sequences of *Laos E. coli* and to spot anomalies in N50 and check that genome size was consistent with *E. coli*. Quality acceptance criteria for the SENTINEL data set was as per NRLBM normal workflow pipeline. This exclusion criteria included Quast (v5.0.2) of N50 greater than 2000, minimum genome size criteria (genome size > 4000000 bp), maximum genome size criteria (genome size < 6000000 bp) and maximum number of contigs greater than or equal to 1000 bp (number of contigs < 500 bp). Nanoplot (v1.28.2) was run to check sequencing quality of each MinION including: mean length and N50 scores (198). Bandage (v0.8.1) view assembly quality and identify plasmid contigs.

2.5.5 Genome Assembly and Annotation

2.5.5.1 Short read assembly and annotation

Genome assemblies for all isolates were created from their trimmed read files using the de Novo assembler SPAdes which is designed for bacterial datasets and can be used with paired-end Illumina reads (Laos *E. coli* v3.12.0 and SENTINEL/IMPACT2 v3.13.0) (196). All assemblies were annotated using Prokka, a tool allowing quick annotation of a draft bacterial genome that is compatible with many downstream programs (Laos *E. coli* v1.13 and SENTINEL/IMPACT2 v1.14.6) (199).

2.5.5.2 Long read and hybrid assembly and annotation

MinION data for both SENTINEL and Laos *E. coli* was basecalled using Guppy, the ONT basecalling software (Laos *E. coli* v0.5.1 and SENTINEL v6.0.1) https://github.com/nanoporetech/pyguppyclient). In read files where coverage was high (coverage > 100x), filtlong (v0.2.0) (https://github.com/rrwick/Filtlong) was used to select the best-read files available and the coverage was limited to 100X. Barcodes were trimmed using qcat (v1.0.1) (https://github.com/nanoporetech/qcat), which at the time was the recommended by ONT for trimming. Hybrid assemblies using the Illumina reads were created with Unicycler (Laos *E. coli* v0.4.7 and SENTINEL v0.4.8) (200) and visualised in Bandage (v0.8.1) (201). Unicycler was unable to fully resolve the assembly (generating only circular sequences) for some Laos *E. coli* genomes. In these cases a long-read first approach was taken, using another de Novo assembler Flye (v2.6) (202) to assemble the long read set into a gfa file that was then provided to the Unicycler pipeline. The nine SENTINEL hybrid assemblies were created using Unicycler bold mode. All hybrid assemblies were annotated using Prokka (Laos *E. coli* v1.13 and SENTINEL/IMPACT2 v1.14.6) (199).

2.5.6 Phylogroup Allocation and MLST Profiling

IMPACT2 and SENTINEL *E. coli* isolates were allocated to a phylogroup using ClermonTyping (53). Sequence data was analysed using mlst (https://github.com/tseemann/mlst) (Laos *E. coli* v2.15 and SENTINEL/IMPACT2 v2.11) to determine the species and sequence types present in the isolate population.

2.5.7 Kleborate

The *Klebsiella* isolates from IMPACT2 dataset were run through Kleborate (v2.2.0) for speciation and clone typing. Kleborate output files were run through the Kleborate-viz web app (https://kleborate.erc.monash.edu/) (203).

2.5.8 Resistance, plasmid replicon and virulence factor identification

In all three datasets all assemblies were analysed using ABRicate (Laos *E. coli* v0.8 and SENTINEL/IMPACT2 v1.0.1) with the ResFinder (195) and PlasmidFinder (194) databases to detect acquired AMR genes and plasmid replicons. SENTINEL analysis was further investigated using AMRFinderPlus (v3.10.20) (204) to detect inherent fluroquinolone resistance genes (*par* and *gyr* genes).

Virulence genes in SENTINEL and IMPACT2 were identified using ABRicate (v0.4.8) and the vfdb virulence factor database (205). EasyFig (v2.2.2) (206) was used for further exploration of the type I fimbriae (Fim) virulence region in SENTINEL isolates that had been long read sequenced.

2.5.9 Capsule investigation

The Illumina assemblies of 16 Laos *E. coli* isolates were serotyped *in silico* using the Center of Genomic Epidemiology SerotypeFinder2.0 (207) as it was particularly difficult to extract their DNA. These isolates were also serotyped using ABRicate (v0.8.10) with the EcOH serotyping database.

In the same way ABRicate (v1.0.1) was used to identify capsule types in Laos *E. coli,* SENTINEL *E. coli* and IMPACT2 *E. coli.* SENTINEL *E. coli* phenotypic capsule typing results were provided by NRLBM and were defined as K1 or non-K1 capsule type. The settings of *kps* genes in SENTINEL isolates that had been long read sequenced were explored using EasyFig (v2.2.2) (206).

2.5.10 Core gene alignment and Core SNP alignments

Core gene alignment used in SENTINEL and IMPACT2 projects were created using Panaroo (v1.2.10).

Snippy was used to call single nucleotide polymorphisms (SNPs) between the reference Prokka annotated GenBank (gbk) file and isolates of the same ST. this method was selected as it is possible to calls for SNPs across STs in the population, producing a high-resolution SNP phylogeny. High-resolution single nucleotide polymorphism analysis used the first isolate of strain as a reference to conduct strain sharing dynamic analysis. Core single nucleotide polymorphism alignments were generated using from Snippy-core (Laos *E. coli* v4.3.6 and SENTINEL/IMPACT2 v4.6.0).

SNP distances for all species were calculated using snp-dists (Laos *E. coli* v0.6 and SENTINEL/IMPACT2 v0.8.2). Within participant or patient comparisons used the earliest isolate as the reference.

2.5.11 Phylogenetic Trees

Phylogenies of the whole Laos *E. coli* dataset were constructed using IQ-TREE (v2.0.3) under the GTR-GAMMA model (v0.6.0), using a core single nucleotide polymorphism alignment from Snippy-core (v4.3.6). IQ-TREE was selected as it is able to produce fast and robust maximum likelihood trees. *E. coli* CFT073 was used as a reference in this tree (NC_004431.1). This phylogeny was midpoint rooted and displayed alongside metadata using Phandango (v1.3.0) (208).

ST131 clade analysis used Mashtree (v0.36.2) to compare all isolates designated as ST131 by mlst to ST131 to a collection of 3186 ST131 assemblies (209)

All further phylogenetic trees for all datasets were constructed using IQTREE (v2.0.3) on either Panaroo core.aln files or Snippy core SNP alignment files as specified in results chapters.

IQ-TREE dated tree flag (--date) was used for ST567 time tree and branch dates. Root-to-tip analysis was performed using TempEst (v1.5.3).

2.6 Plasmid Annotation

The AMR plasmids identified in both the Laos and SENTINEL datasets were annotated using Prokka (Laos *E. coli* v1.13 and SENTINEL v1.14.6) (199). ABRicate (Laos *E. coli* v0.8.10 and SENTINEL v1.0.1) was used to identify plasmid replicons with the PlasmidFinder database and AMR genes were identified with ResFinder (85% coverage and identity cut offs). In situations where PlasmidFinder did not provide a type, plasmid contigs were compared to known plasmid replicons for type assignments using BLAST. Where possible, plasmid replicons were sub-typed using the PubMLST database (https://pubmlst.org/) (133, 210). SnapGene (v5.2.4) was used to visualise Prokka-annotated plasmid contigs. ISFinder (211) was used to identify IS elements. NCBI BLAST (v2.5.0+) was used to compare plasmids to reference plasmids (Table 2.1) and allowed annotation of key genes. The NCBI non-redundant nucleotide database was queried with BLASTn to determine whether plasmids identified here had been seen elsewhere. BLASTn and tBLASTn were used to compare similar plasmid regions and to identify homologs of known plasmid genes.

Table 2.1 Reference Plasmids used in plasmid annotations

Plasmid type	Name	GenBank accession number
FII-2	p39R861-3	MK092064.1
IncX1	pSRC22-2	MN256104
IncQ	RSF1010	M28829
FII-18	pCERC3	KR827684
IncY	P1	AF234172
ColRNAI	pCERC7	KX356458

Additional analysis was carried out on the Laos plasmid dataset (Chapter 3). ISEScan (v1.7.2.3) was used to quantify IS elements in plasmid assemblies at an element-family level (212). The wider Laos *E. coli* Illumina dataset was searched for small plasmids identified in Laos *E. coli* hybrid assemblies. NCBI BLAST (v2.5.0+) was used to search draft Illumina genomes for plasmid-specific 100 bp signature sequences (Table 2.2). Signature sequences, as described previously by Hu Ya et al. (213), are comprised of 50 bp each of a mobile genetic element and its immediately adjacent sequences such that they span the specific junction generated by a given insertion or deletion event. This facilitated the detection of specific plasmid lineages whether they were represented by single or multiple contigs in the Illumina dataset (29).

Table 2.2 DNA sections searched for in the Illumina dataset

Section Number	**Section looked for in Illumina dataset (29)** to look for circulating ColE1-like plasmid (pLAO84) and IncQ1 plasmid (pLAO60)
ColE1-like plasmid (pLAO84) MITESen1 to backbone section 1	TTAAATAATGCGCTTAACGTACAAAAAATTCCGATCTCCA AACTGACCCCTTCTTCGCCTCTTATGTTTCTCGAATGAAT CGATGGGGACAGGAAATGTT
ColE1-like plasmid (pLAO84) MITESen1 to backbone section 2	ATTTATTTTTCATGAAGTTGCGATAAAAATCGCAGCTGCG TTAGGTGTATGGGGTCAGTTTGGATATAGAGAATTATTGT ACGGTAAGCCTGTTTTTTGA
IncQ1 Section from pLAO60	CCGTTAACTGTCACGCCCCCCCGTTAACTGTCACGAACC CCCCGTTAACTGTCACGCCCCCCGTTAACTGTCACGAAC CCCCCGTTAACTGTCACGCCCCC

Additional analysis was performed on SENTINEL study on a ST567 F-type plasmid, FII-13 : FIB-40-like plasmid. NCBI BLAST BLASTn was used to compare a selection of regions in the plasmid to the short read assemblies of all ST567 isolates to confirm likely presence or absence of the plasmid in ST567 short read assemblies. Only isolates that contained sequences 100% coverage and identity to both replicons were classed as same plasmid replicon type. Further comparison of *tra* region, *iro* region and *iuc* regions from FII-13 : FIB-40-like plasmid to the ST567 short read assemblies were used to see if these isolates were likely to contain this exact plasmid.

2.7 Data Visualisation

Box plots to show average number of antimicrobial genes in a genome per year in the SENTINEL dataset were created using ggplot2 R Package (v3.3.5) and Rstudio (v 1.4.1717) Code used is specified in the appendix (Appendix A2).

ST and phylogroup population plots were plotted based on R code previously used by Dr S.J.Dunn to generate a similar figure in previous publication (Figure 2 (214)) which

was adapted for use in this study. These plots used ggplot2, reshape (v0.8.8) and RColorBrewer (v1.1.3) R Packages. Code used is specified in the appendix (Appendix A2).

Prism (v9.2.0) was used to generate SENTINEL bar charts and Laos *E. coli* plasmid ST bar charts and ISEscan heatmaps.

2.8 Yeast Agglutination assays

Isolates representing different parts of the tree were selected to see if there were any difference in phenotypic presentation caused by the genotypic differences to the Fim region. Yeast agglutination experiments were performed as in Roe et. al (215).

3. The dynamics of colonising MDR *E. coli* and the highly diverse plasmid population found in these *E. coli* colonising travellers to Laos and their role in antimicrobial resistance gene carriage

This chapter contains work from two papers.

My first author publication work presented in this chapter is published in Microbial Genomics (216)

'***Snaith AE, Dunn SJ, Moran RA, Newton PN, Dance DAB, Davong V, et al. The highly diverse plasmid population found in Escherichia coli colonizing travellers to Laos and its role in antimicrobial resistance gene carriage. Microb Genom. 2023;9(5).'***

Author contributions: 'AM and JC conceived the idea for this analysis. AK, EK, DABD, PNP, VD collected samples, isolated the strains and provided the isolates and epidemiological data in Laos. AS and SD performed long-read genomic sequencing and sequence processing. AS, SD and RM performed genomic analysis, data interpretation and data visualisation. AS, RM and SD drafted the article. AM discussed results and edited draft. JC, AK, PN and DD critically reviewed the draft. All authors read and approved the final manuscript'.

This chapter also includes my contributions of genomic analysis from another publication (29):

'Kantele A, Kuenzli E, Dunn SJ, Dance DAB, Newton PN, Davong V, et al. Dynamics of intestinal multidrug-resistant bacteria colonisation contracted by visitors to a high-endemic setting: a prospective, daily, real-time sampling study. Lancet Microbe. 2021;2(4):e151-e8.'

Author contributions: 'AK and EK conceived of, designed, and organised the study. AK, EK, DABD, PNP, VD, SM, SHP, AN, and CH collected samples and epidemiological data. SJD, AS, TK, JC, and AM did the genomic analyses. AK, EK, SJD, AS, JC, and AM interpretation the data. SJD and AS visualised the data. AK, EK, SJD, TK, JC, and AM drafted the Article. DABD, PNP, VD, SM, SHP, AN, CH, and AS critically reviewed the draft. All authors read and approved the final manuscript. All authors had full access to all the data and the final responsibility to submit for publication'.

3.1 Introduction

Infections caused by antimicrobial-resistant organisms are harder to treat, lengthen hospital stays, increase mortality rates, and place a significant financial burden on healthcare institutes (217). Colonising MDR organisms have the potential to cause serious consequences for any individual who contracts infection from MDR colonising organisms, as treatment could be limited and patient outcome poorer. Increased intestinal colonisation by antimicrobial resistant organisms and their global spread is closely associated with international travel (5, 48, 50).

AMR is already a global concern and has compromised treatment options for many bacterial pathogens, especially for pathogens carrying ESBL and carbapenem resistance genes (7). It is therefore increasingly important to characterise the mechanisms that allow AMR to spread worldwide. Clinically relevant AMR determinants are commonly found in Gram-negative bacteria colonising travellers returning from regions (frequently low to middle income countries) with high AMR prevalence, including South-East Asia (7, 29, 48, 131). Travellers to S.E Asia are acknowledged to be at particularly high risk of colonisation with MDR Gram negative bacteria, even during a short period of travel (1-5 days), and this colonisation can last for months (218-221). Travellers colonised with MDR Gram negative bacteria are frequently and most often colonised with ESBL *E. coli* (50, 222, 223). Common *E. coli* STs found to be colonising travellers to S.E. Asia include ST131 (48, 224). Bevan at al. in a previous traveller study report that when travellers to SE Asia were colonised with MDR *E. coli* STs, the pre-existing intestinal commensal strains were retained in a minority. On return from travel these pre-existing intestinal commensals were once again detectable as the majority population after the loss of colonising MDR *E. coli* STs (48).

The chapter reports a daily, real-time sampling study which investigated MDR *E. coli* sourced from 23 travellers to Laos who volunteered to provide daily stool samples over a three-week period. The study characterised both the dynamics of acquisition of multi-drug resistant Gram-negative organisms and the role of MGEs, particularly plasmids, in the acquisition of drug-resistance. An important factor in the real time study in this chapter is that it was carried out using stool samples collected daily, during the stay of travellers to Vientiane, Laos. This sampling method revealed the real-time colonisation

dynamics of participants who acquired MDR Gram-negative bacteria during travel so that the drug resistant *E. coli* populations colonising study participants could be monitored and tracked continuously. Continuous monitoring allowed the detection of sequential and transitory colonisation with MDR Gram negative organisms, showing the diversity of the colonising species as well as demonstrating strain sharing between participants. This real-time colonisation study also showed that MDR Gram-negative organisms had a surprisingly high co-prevalence of ESBLs and colistin resistance genes (29). Fine scale genomic analysis of this colonising *E. coli* dataset additionally showed the diversity of STs, circulating strains and dynamics of the colonisation process during a 21-day period in Laos.

The AMR carriage in *E. coli* from this traveller cohort was explored first using short read sequencing and then long-read sequencing was used to understand the contexts of AMR genes and the role of plasmids in the acquisition of drug resistance by colonising *E. coli* in a region of high AMR prevalence. Continuous sampling on a daily basis facilitated the examination of common and circulating plasmids in the *E. coli* population, across a variety of sequence types (STs) and at different study time points. This data shows the diversity and widespread distribution of numerous distinct AMR plasmids acquired by this group of travellers to Vientiane, Laos and the multiple different potential routes of AMR spread by plasmids and highlighted the complex nature of plasmids carrying both ESBL resistance and colistin resistance (*mcr)* genes.

Creation of hybrid assemblies enabled the elucidation of the numerous different types of plasmids carrying AMR genes in colonising *E. coli* isolates found in travellers to Vientiane, Laos. The findings of this plasmid study have been added to known databases of AMR plasmids contributing data on these MDR plasmids found in South

East Asia, an important area of high AMR incidence. Novel AMR plasmids including complex ESBL (*bla*$_{CTX-M}$) and colistin (*mcr*) resistance co-carriage plasmids were characterised, emphasising the potential exposure of travellers to Laos to a wide variety of MGEs that may facilitate global AMR spread. This in-depth study revealed further detail of the numerous factors that may influence AMR transfer, therefore suggesting potential novel routes of AMR spread internationally, and is a step towards finding methods to combat AMR spread.

3.2 Methods

3.2.1 Strain level analysis of the Illumina dataset

E. coli isolated from the 23 participants daily faecal samples were processed as described in Section 2.1.1. *E. coli* were characterised based on ST, AMR genes and plasmid replicons. Isolates from the same ST were compared using Snippy to look for circulating strains within the Illumina dataset. Two participants who produced less than 4 stool samples were excluded from this strain analysis as they would not provide an adequate representation of the strain dynamics over the study period. A total of 219 *E. coli* isolates were identified using mlst, with 48 selected as a representative dataset and long read sequenced (Figure 3.1).

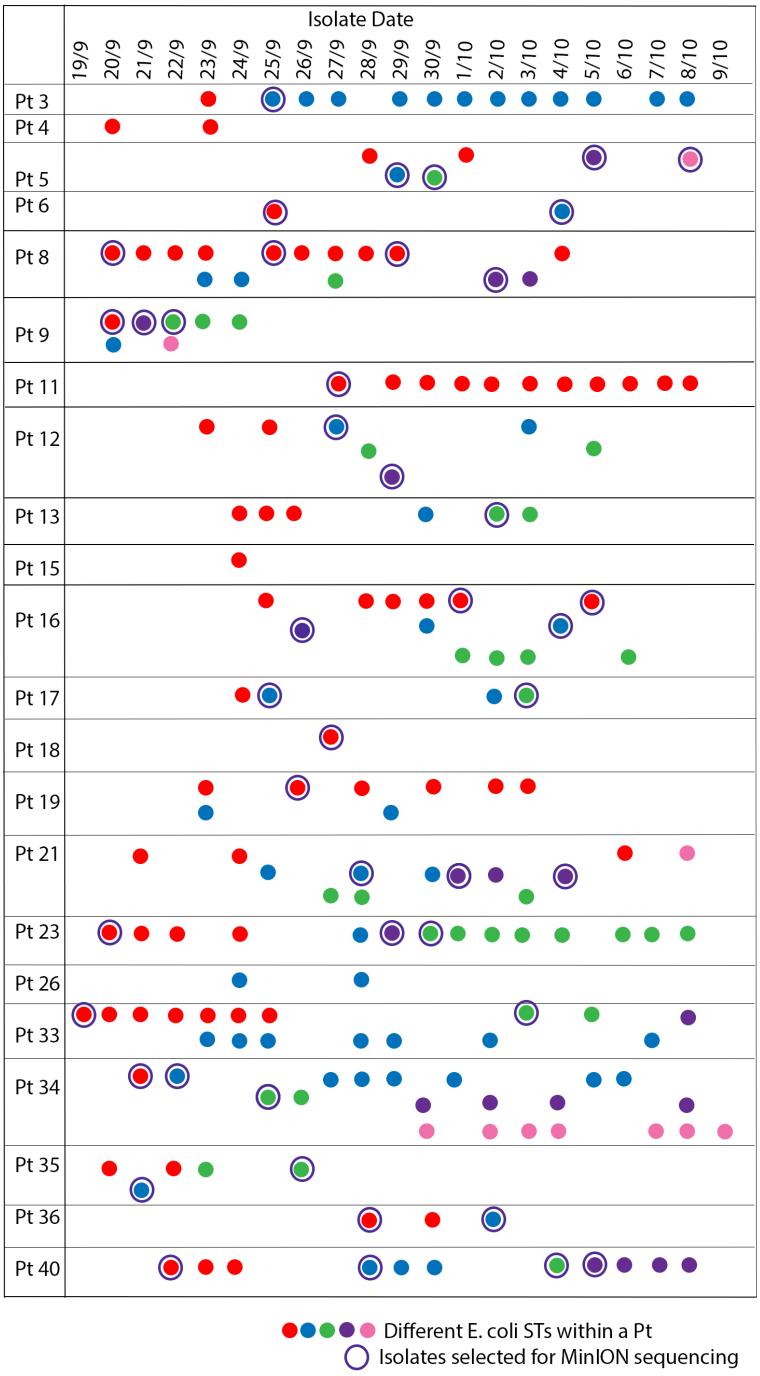


Figure 3.1 Timeline of all E. coli isolates over 21-day collection period by participant (Pt) showing 48 isolates selected for sequencing. Participant isolates within the study period are shown by date per participant where different colours of symbols indicate different strains of *E. coli.* (Isolates described as mixed ST were determined using mSWEEP (v 1.1.0) where mlst could not define a sequence type, if mixed sequence type matched an existing ST they appear as the same colour). Purple circles highlight isolates sequenced using MinION. This plot does not show the 2 isolates MinION sequenced from participant contacts.

3.2.2 DNA Isolation for long read sequencing

DNA extraction of the 48 representative *E. coli* isolates used several methods (Section 2.3) as there was difficulty precipitating DNA using the Phenol-Chloroform method (Section 2.3.1). The first choice of alternative extraction method was the Monarch Genomic DNA extraction kit and where this was unsuccessful Phenol-Chloroform with the addition of CTAB was used to extract DNA. CTAB at 5 M salt concentration allows the separation of polysaccharides and genomic DNA. Genomic DNA is soluble where the polysaccharides are not.

3.2.3 Long read Sequencing and plasmid analysis

Long read sequencing using ONT MinION technology was carried out on the DNA extracted from the selected 48 *E. coli* isolates (Section 2.5.2). These isolates carried a variety of AMR genes and plasmid replicon types as characterised by PlasmidFinder across differing STs in different participants (Section 2.5.5). Illumina genomes and ONT MinION long read genomes were used to create hybrid assemblies. Plasmid contigs were split from these hybrid assemblies, annotated and investigated to understand the plasmid population in these Laos *E. coli* isolates (Section 2.6). This included checking relatedness of AMR and non-AMR plasmids with the same plasmid type using NCBI BLAST.

The summary workflow for plasmid analysis can be seen in Figure 3.2.

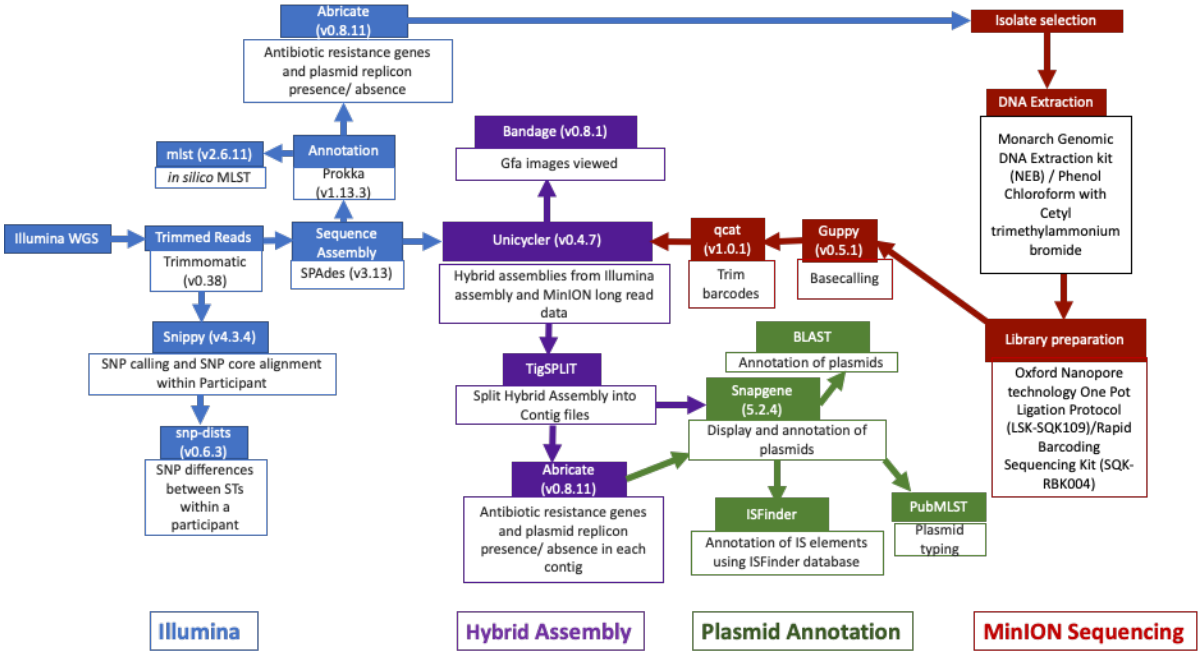


Figure 3.2 Overview of methods used to create hybrid assemblies and identify and annotate plasmids of the Laos dataset

3.3 Results

3.3.1 High prevalence of MDR *E. coli* colonising travellers during their stay in Laos

219 *E. coli* isolates were found in 23 participants recruited to the study and their 22 contacts with all but one having at least one *E. coli* isolated during their stay in Laos. These *E. coli* isolates were from thirty-four different STs, with ST101 and ST195 the most predominant STs (Figure 3.3). Only one ST131 was isolated during this study, this from a contact of a participant (C11B). SNP analysis on isolates from all participants with greater than 4 samples allowed deduplication of strains within a sequence type so only one example of a strain in a patient was counted revealing the incidence of each individual strain of an ST and providing a more accurate account of ST frequency throughout the study period.

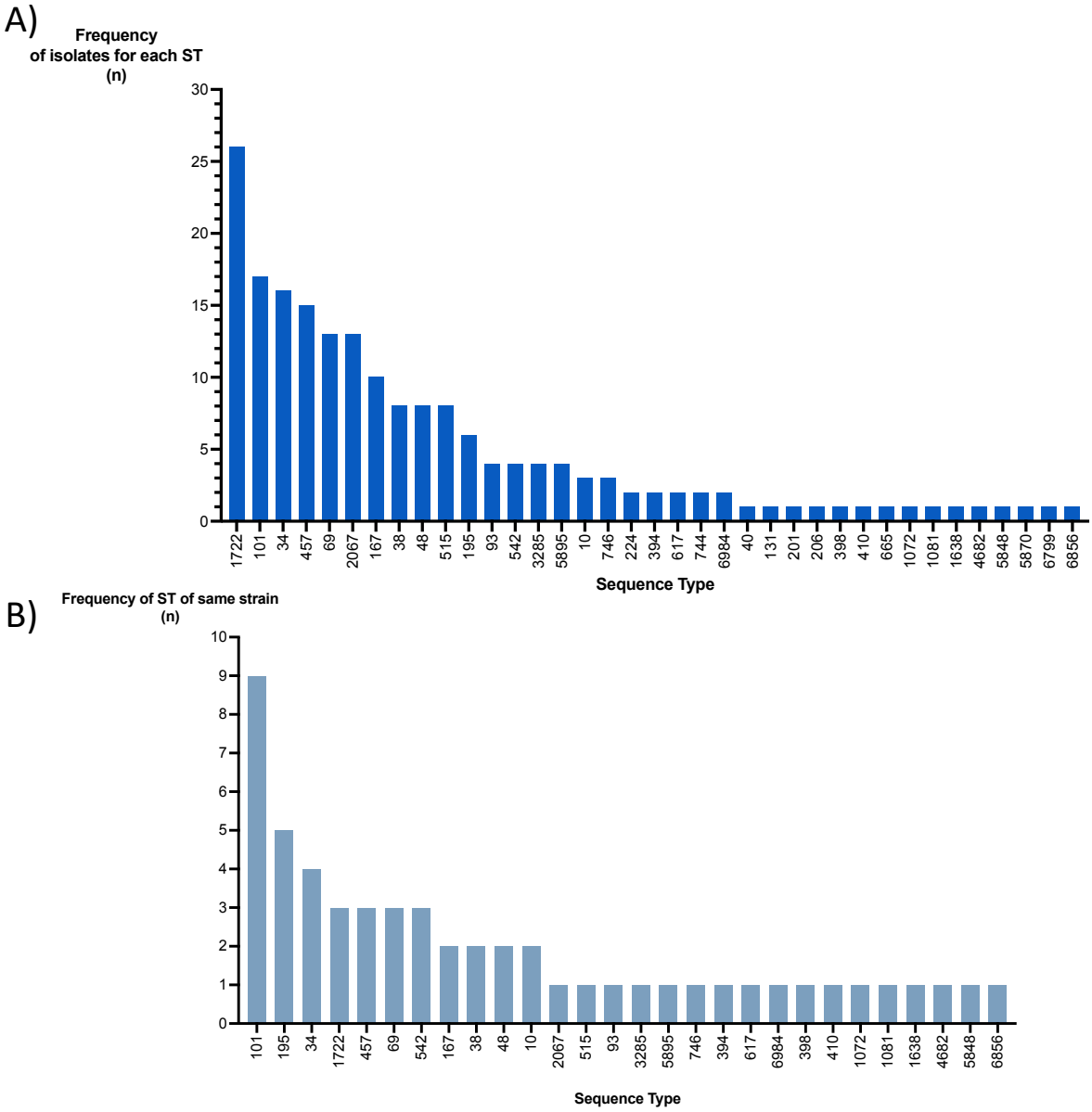


Figure 3.3 Bar chart showing A) frequency of sequence types (STs) for all 219 E. coli isolates in the study and B) frequency of ST deduplicated by specific strain in a ST to remove bias of repeat counting of the same strain from participants with more than 4 samples in the study period. Frequency is displayed as number (n) of isolates.

Participants often had multiple STs over the duration of their stay (Figure 3.4), with participants most likely to be colonised by a single strain which could be transiently interrupted by another strain. This study showed that in many cases a participant was colonised persistently with multiple strains. A good example of this is in participant 21 where four persisting strains were seen from 21/9 - 6/10 followed by a single instance of a separate strain on 8/10 (Figure 3.4).

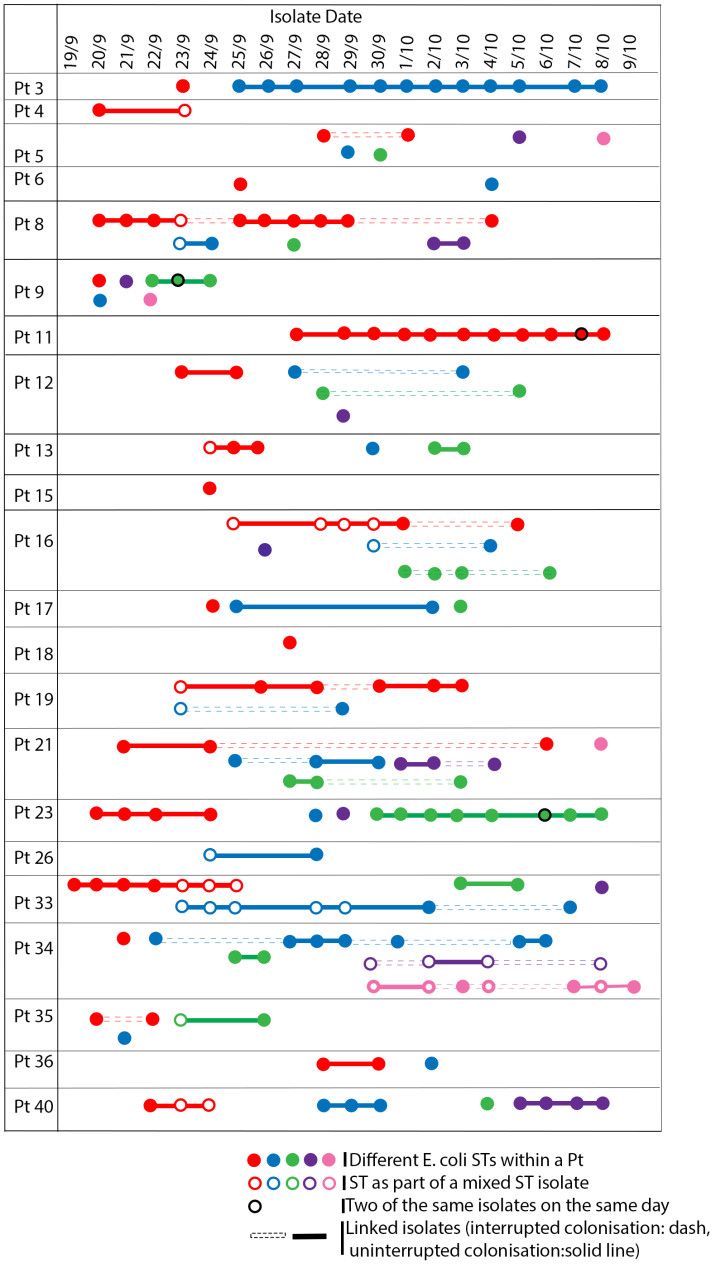


Figure 3.4 Timeline of *E. coli* isolates from participants. This shows participant isolates within the study period per participant. Each participant should be read as a distinct line, colours do not show related STs across participants. Different colours of symbols indicate different strains of the same species within a participant. The same colour may be used for a different ST in different participants for clarity as there are many different STs across the participant population and insufficient distinctive colours for every ST. Dashed lines indicate a single strain over multiple days that has been interrupted by the presence of another strain or instances of no growth between isolation of the same strain. This shows the isolates only from participants, not those from their contacts that are also included in the 219 in this study.

3.3.2 Strain sharing dynamics between participants

Not only were multiple STs and persistent colonising strains found within a participant, in several cases the same ST was found across multiple study participants (Figure 3.5). High-resolution SNP analysis across STs showed little diversity within a strain. There were five sequence types (ST38, ST3285, ST2067, ST515 and ST34) where SNP analysis found strain sharing between study participants and contacts (Figure 3.5). A ST515 strain was found circulating in the greatest number of participants (n=4), followed by a ST38 strain (n=3). In most cases shared strains identified across participants were identical, with 0 SNPs difference within a strain. However, ST38 and ST3285 SNP analysis showed examples of strains with 1-5 SNPs difference between participants. Interestingly ST2067 was found to be persistently colonising Pt11 and this persistent stain was found in the only sample provided by their contact, C11B.

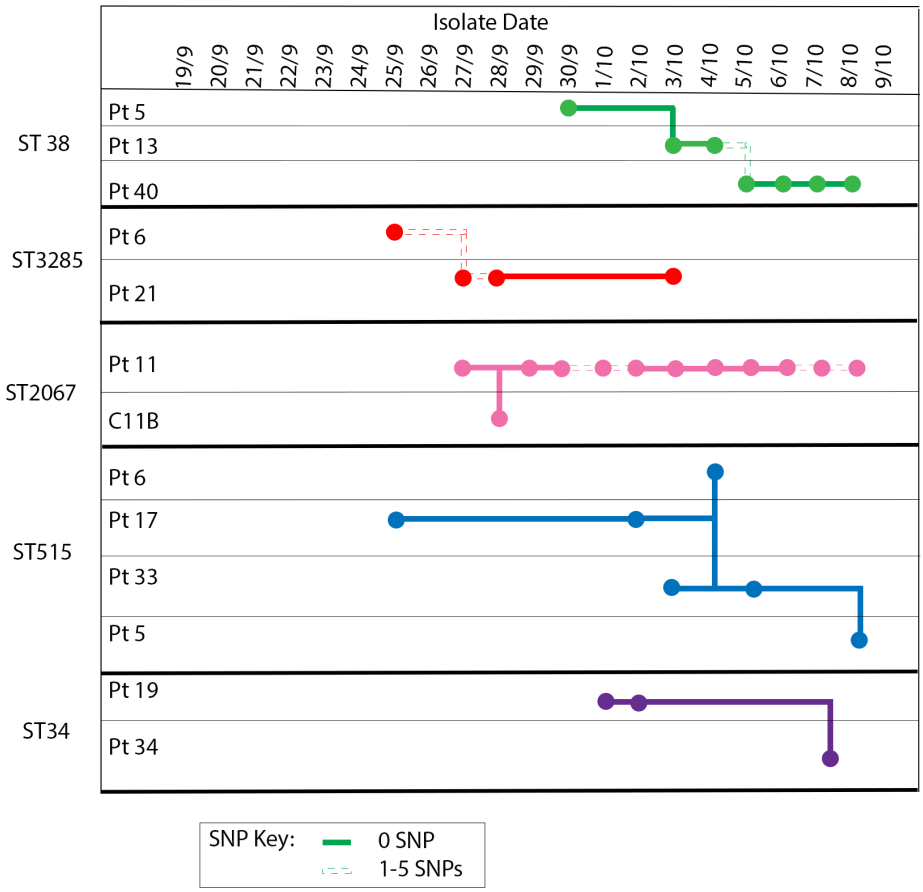


Figure 3.5 Timeline of five circulating STs in the Laos dataset identified using Snippy and snp-dists. The solid lines show 0 SNPs different between strains and dashed lines demonstrated where1-5 SNPs were seen within a ST strain.

3.3.3 Isolates from Laos contain a broad diversity of plasmids and resistance genes

In this wide variety of *E. coli* STs, all isolates carried at least one AMR gene (Figure 3.6). Concerningly the majority of *E. coli* isolates (n=209) carried at least one *bla*$_{\text{CTX-M}}$ gene. The most prevalent *bla*$_{\text{CTX-M}}$ genes in this study were *bla*$_{\text{CTX-M-55}}$ (n=63), *bla*$_{\text{CTX-M-14}}$ (n=58), *bla*$_{\text{CTX-M-159}}$ (n=56), *bla*$_{\text{CTX-M-15}}$ (n=28), and *bla*$_{\text{CTX-M-102}}$ (n=25), as expected in S.E. Asia(48). Isolates were found to carry more than one type of *bla*$_{\text{CTX-M}}$ gene. Even more concerningly many isolates carrying *bla*$_{\text{CTX-M}}$ gene also carried *mcr* with the potential to confer colistin resistance (n=80). Although *bla*$_{\text{CTX-M}}$ genes were the most

prevalent beta-lactamase gene other beta-lactamase genes were identified including: *bla*$_{ACT}$, *bla*$_{ADC}$, *bla*$_{CMY}$ and *bla*$_{VEB}$.

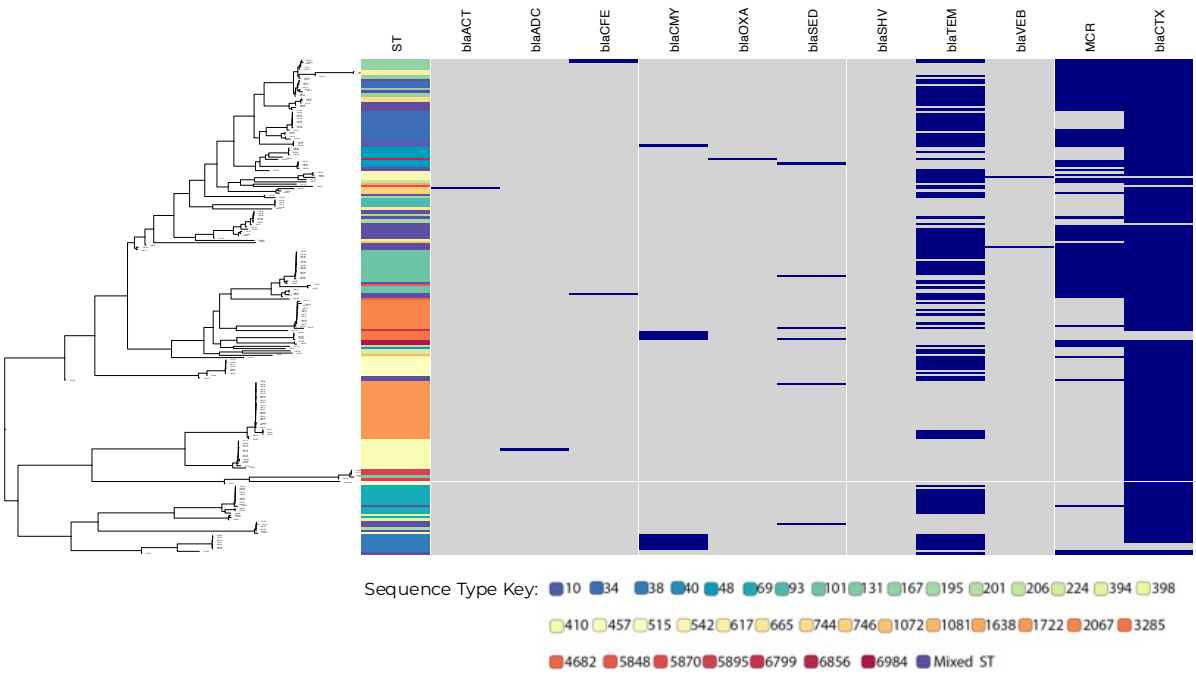


Figure 3.6 Phylogeny of *E. coli i*solates (n=219) shown with presence of observed beta-lactamase genes and colistin resistance Blue = gene present, Pale Grey = gene absent. Phylogeny reconstructed using IQ-TREE, based on a Snippy core SNP alignment, midpoint rooted with increasing nodes and visualised in Phandango. CFT073 *E. coli* was used as a reference and has no metadata attached.

A wide range of plasmid replicons were identified in these *E. coli* (Figure 3.6- 3.7A), suggesting potential for plasmid-based AMR movement. Common plasmid replicons identified in these *E. coli* included theta-replicating plasmids that initiate replication with RNA primers (θ-RNA) or replication proteins (θ-Rep), rolling-circle plasmids as well as F-type and X-type plasmids. They also had a wide range of virulence genes (Appendix A3.1) the most common including csg, *fep, ent, fli* and *fim* genes (Figure 3.7B). All but seven are classified as ExPEC *E. coli* (Figure 3.7B). These seven isolates were ST40 and ST48 and were found to likely be EPEC having virulence genes including *bfp*.

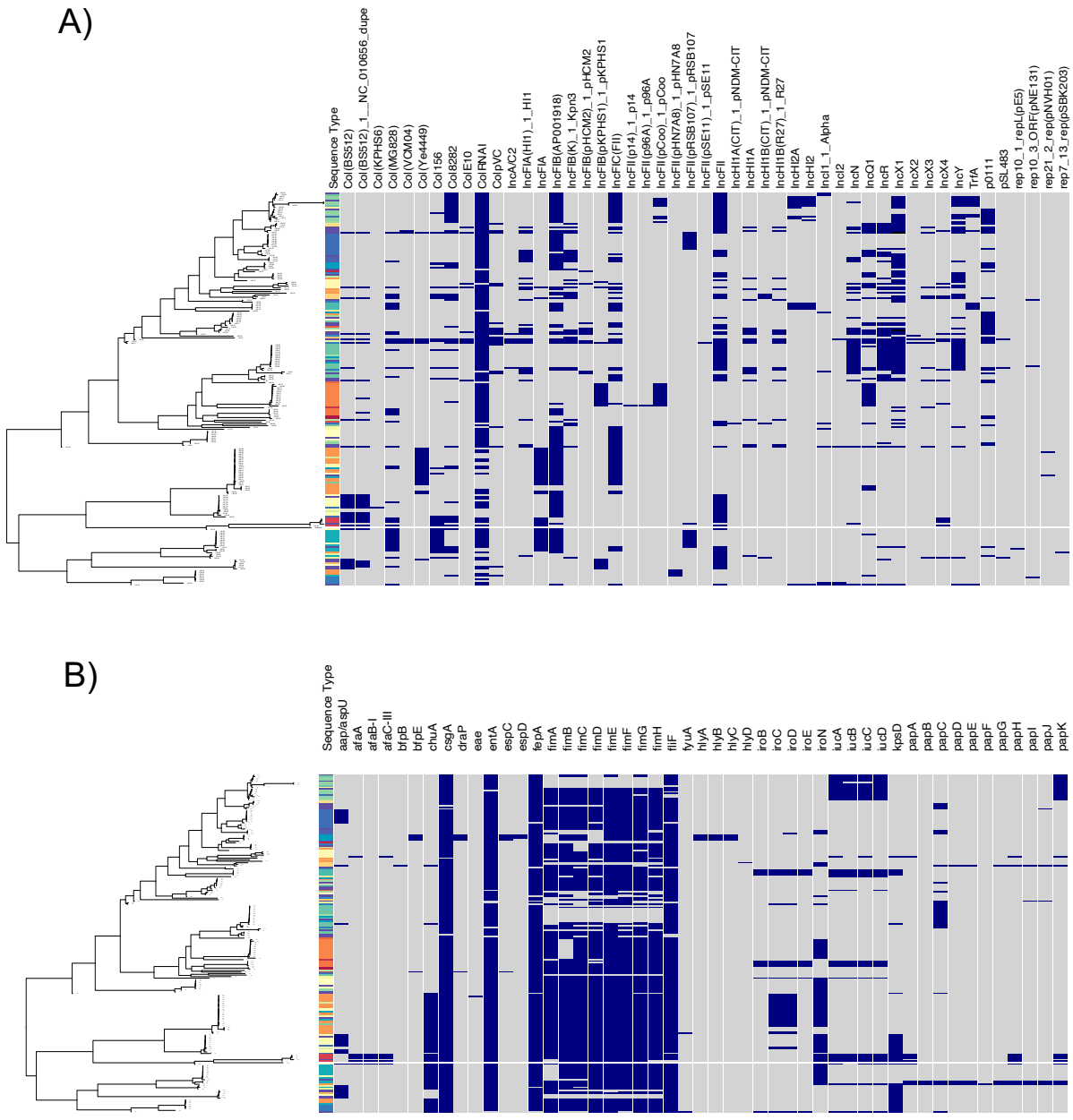


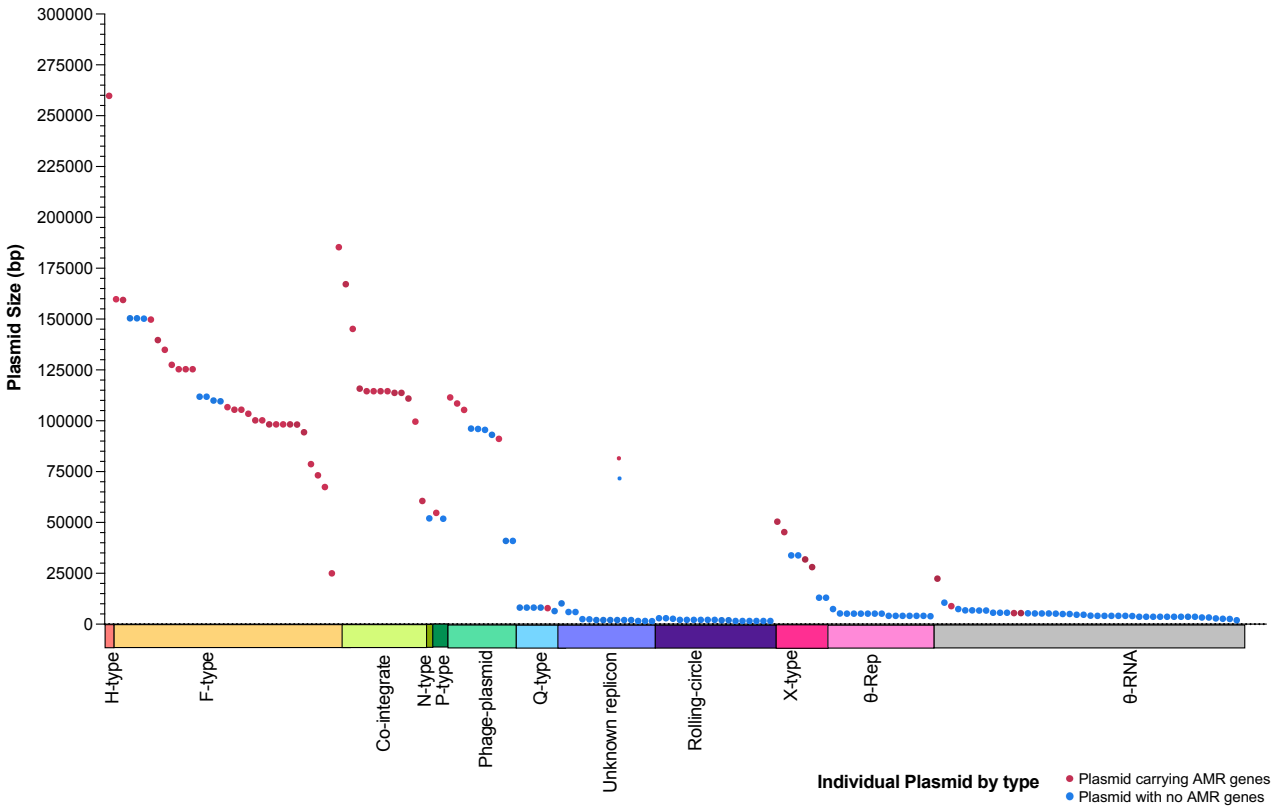
Figure 3.7 Phylogeny of *E. coli* isolates (n=219) shown with presence of A) observed plasmid replicons and B) virulence genes. Blue = gene present, Pale Grey = gene absent. Phylogeny reconstructed using IQ-Tree, based on a Snippy core SNP alignment, midpoint rooted with increasing nodes and visualised in Phandango. CFT073 *E. coli* was used as a reference and has no metadata attached.

3.3.4 The complex and diverse plasmids in *E. coli* from travellers to Laos

Illumina genome analysis showed the co-occurrence of many plasmid replicons and AMR genes suggesting that plasmids may play a role in AMR gene carriage in this set of MDR *E. coli* (Figure 3.7A). Plasmids found in the 48 hybrid assemblies were analysed to investigate carriage of their AMR genes. These isolates were selected to cover the whole study period, across participants, sequence types, resistance genes and plasmid replicons to give the widest possible representation of plasmids and AMR carriage in this study population.

163 complete plasmids were obtained from the hybrid-assembled genomes (Appendix 3). Isolates harboured 1 to 8 plasmids each, except for a single ST1722 isolate that did not contain any plasmids. The 163 plasmids were typed by size, replicon type and AMR gene carriage, and identical or almost-identical plasmids (Appendix A3 and Figure 3.8) were deduplicated, resulting in a total of 105 distinct plasmids. These 105 plasmids, named pLAO1-pLAO105 (Appendix A3.2) ranged in size from 1,531 – 259,739 bp, with around half (53.3%, n=56) smaller than 25 kb. Forty of the 105 plasmids (38.1%) contained one or more AMR genes.

A)



B)

		Sequence Type																												
Plasmid Type	Total Number	10	34	38	40	48	69	101	131	167	195	201	298	410	457	515	542	617	665	1072	1081	1722	2067	3285	4682	5848	5895	6856	6984
θ-RNA	44	3	2	1	1	1	3	7	-	4	1	1	1	-	-	3	6	4	-	-	-	-	1	1	-	-	1	1	2
F-type	32	2	2	4	1	-	4	5	-	-	-	-	-	-	1	4	2	-	1	-	1	1	1	1	-	-	1	1	-
Rolling-circle	17	1	-	-	1	-	4	-	-	-	-	1	-	-	1	-	3	-	-	1	1	-	-	1	-	1	2	-	-
θ-Rep	15	-	1	-	-	-	5	-	1	1	-	-	-	-	1	1	1	1	-	-	-	1	-	-	-	-	2	-	-
Unknown	14	2	1	-	-	2	3	1	-	-	-	-	-	-	-	1	3	1	-	-	-	-	-	-	-	-	-	-	-
Co-integrate	13	2	1	-	-	-	1	4	1	-	-	1	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	1	-
Phage-plasmid	10	2	-	-	-	-	-	4	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	1
X-type	8	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	1	1	-	1	-	-	-	1	-	-
Q-type	6	-	1	-	-	1	-	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
P-type	2	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-type	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H-type	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 3.8 An overview of plasmid diversity showing plasmid size and AMR gene carriage (A) and plasmid type within sequence type (B) for all plasmids identified (n=163).

Thirty-eight different replicon types were identified in the collection of 105 plasmids (Appendix A3.2). These replicon types can be split into groups of both small (<25 kb) and large (>25 kb) plasmids and were given overarching plasmid types (Table 3.1). Small plasmids included theta-replicating plasmids that initiate replication with RNA primers (θ-RNA, n=26) or replication proteins (θ-Rep, n=9), rolling-circle plasmids (n=6), and Q-type plasmids that replicate by strand-displacement (n=3). Amongst large plasmids, F-types (n=23) were dominant, and their replicons were sub-typed using the PubMLST database (Appendix A3.2). Nine large plasmids were co-integrates, which included multiple replicons of disparate types and all contained one or more AMR genes (Figure 3.8, Table 3.1). In total, 76.3% (n=29) of plasmid types contained AMR genes, with the most prevalent being FII-46 : FIB-20 (n=4), X1 (n=3) and θ-RNA (n=3) (Figure 3.8, Table 3.1, Appendix A3.2). AMR plasmids were present across all participants throughout the study (Appendix A3.3), with carriage occurring sporadically and transiently during the study period.

Table 3.1 Overview of frequency of overarching plasmid type of 105 unique plasmids in 48 E. coli hybrid assemblies.

Plasmid Type	Frequency	Percentage
θ-RNA	26	24.8
F-type	23	21.9
Unknown	10	9.5
Phage-plasmid	9	8.6
θ-Rep	9	8.6
Multi-replicon co-integrate	9	8.6
Rolling circle	6	5.7
X-type	6	5.7
Q-type	3	2.9
P-type	2	1.9
N-type	1	1.0
H-type	1	1.0

* Plasmid replicons were categorised into broader categories e.g., F-type. Phage-plasmid category includes both Y and p0111 type plasmids. Unknown category includes plasmids that could not be assigned a replicon type by PlasmidFinder.

Plasmids that did not carry AMR genes were generally smaller (mean size: 23,554 bp), than those that did (mean size: 100,563 bp). The ColE1-like plasmids, pLAO10, pLAO78 and pLAO84 were notable exceptions, carrying 1-5 AMR genes each and ranging in size from just 5,540 bp to 22,368 bp (Appendix A3.2). The most frequently detected plasmid types not carrying AMR genes included: θ-RNA (n=40), rolling circle (n=17) and θ-Rep (n=14) (Figure 3.8) A comparison was made between plasmids from the same replicon types which did and did not carry AMR genes to see if AMR gene

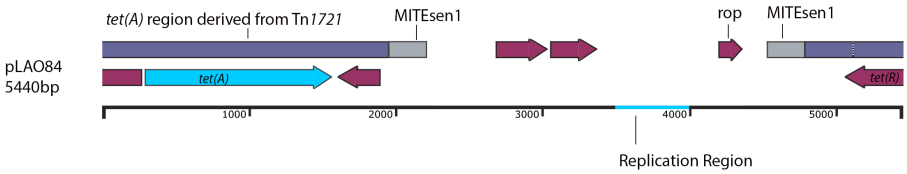
acquisition was captured in this dataset. There was no instance of the same plasmid without AMR genes being found elsewhere carrying AMR genes.

3.3.5 Notable AMR plasmids within the dataset

3.3.5.1 Circulating Plasmids

Evidence was found to suggest the circulation of plasmids (pLAO84, pLAO60) within Lao *E. coli* in the traveller population. The ColE1-like plasmid pLAO84 (GenBank accession: OP242239) carries the tetracycline resistance determinant *tet*(A), and was found two different STs, ST195 and ST34, acquired by two different participants (Pt33 and Pt40) (Figure 3.9A). Mapping contigs assembled from Illumina data against the complete pLAO84 sequence confirmed plasmid circulation. The complete or fragmented pLAO84 sequence was detected in seven additional *E. coli* isolates (Figure 3.9) obtained from six different participants (including Pt33 and Pt40) over a 10-day period. It is not possible to determine where or when pLAO84 transferred between its hosts.

A)



B)

MITSen1 and MITSen2 Blast Contig Hit	Findings
LA239	Original known ColRNAI with tetracycline genes
LA193	Plasmid found complete on one contig
LA191	Plasmid found across two contigs
LA189	Original known ColRNAI with tetracycline genes
LA169	Plasmid found across two contigs
LA166	Plasmid found across three contigs
LA139	Plasmid found across two contigs
LA132	Plasmid found across two contigs
LA092	Plasmid found across three contigs

Figure 3.9 A) Annotated ColE1-like plasmid (pLAO84) harbouring tetracycline resistance with Tn1721 region (purple) and MITESen1(grey)* and B) isolates in Illumina Laos Dataset (29) that contain this same ColE1-like plasmid. *Other Prokka annotated genes are represented with maroon arrows.

The Q1 plasmid pLAO60 (GenBank accession OP242237) carries aminoglycoside and beta-lactam resistance genes (Appendix A3). pLAO60 (Figure 3.10) was present in the complete genome of the ST542 isolate (LA124) and in one genome (LA230) from Pt35 in the wider Illumina dataset. LA230 was allocated a mixed ST by mSWEEP (ST101 + ST34). The Q1 plasmid in isolate LA230 was missing a 101 bp segment that appears to have been lost in a homologous recombination event.

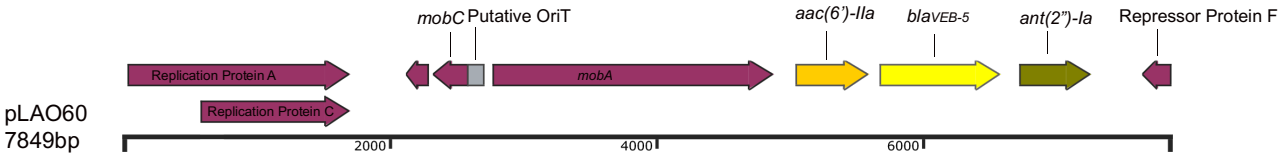


Figure 3.10 Annotated IncQ1 plasmid map from pLAO60 indicating the location of bla_{VEB} Other Prokka annotated genes are represented with maroon arrows. All other brightly coloured genes are antibiotic resistance genes.

3.3.5.2 Phage and Virulence AMR plasmids

Phage and virulence plasmids were other notable AMR plasmids in this collection. A Y-type phage-plasmid, pLAO59, appears to have lost key genes for phage body synthesis removing the potential for this phage-plasmid to become a phage, probably in deletion events mediated by IS*Kpn26* and IS*1294* (Figure 3.11). This detailed comparative analysis to P1 indicated presence of *OriR, repA, parA, parB,* interrupted *mlp* and head gene, 23 in pLAO59. Other key phage gene regions coding for sheath, head structure and processing, tail, packing, baseplate, and tail tube were absent in pLAO59. Instead pLAO59 carried genes that confer resistance to six antibiotic classes (penicillins, tetracyclines, aminoglycosides, lincosamides, quinolones, sulphonamides and macrolides), but no $bla_{CTX\text{-}M}$ and *mcr* genes. In addition to AMR genes this pLAO59 carried metal resistance genes including a mercury resistance region matching the *mer* module from Tn2670 and a completed *cus* operon conferring silver and copper resistance.

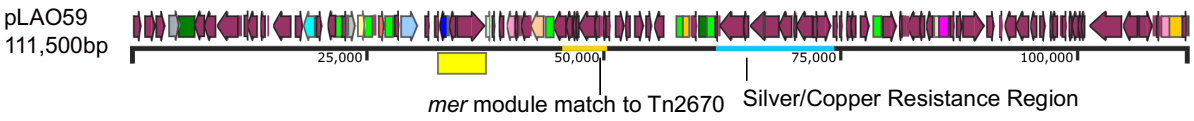


Figure 3.11 Annotated Y-type plasmid map (pLAO59) identifying metal resistance regions and P1-like region. All maroon genes are Prokka-annotated genes. All other brightly coloured genes are antibiotic resistance genes. TEs (e.g., transposons, IS elements) are displayed as brightly coloured boxes. Metal resistance regions marked for copper and silver resistance(blue) and mercury resistance (orange).

A notable virulence-resistance FII-18:FIB-1 plasmid (pLAO32) was identified and contained virulence genes, including those for the aerobactin and Sit siderophore systems, in addition to multiple drug resistance genes (Figure 3.12), but lacked the genes for ColV. This FII-18:FIB-1 pLAO32 is related to a previously reported colicin V (ColV) virulence-resistance plasmid pCERC3 present in *E. coli* isolated from the stool sample of a healthy Australian adult (225).

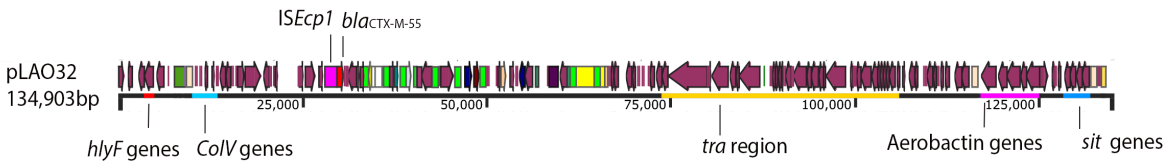


Figure 3.12 Annotated FII-18 plasmid map (pLAO32) showing virulence genes and bla_{CTX-M}. All maroon genes are Prokka-annotated genes. All other brightly coloured genes are antibiotic resistance genes TEs (e.g., transposons, IS elements) are displayed as brightly coloured boxes. Notable IS elements are IS26 (bright green), ISEcp1 (bright pink). *tra* region is highlighted with the yellow outline.

3.3.6 Variation within individual plasmid types, with diverse and complex resistance regions

Long-read sequence data also revealed diversity amongst plasmids of the same type (Appendix A3), in addition to the variety of different plasmid types. AMR plasmids carrying the FII-2 replicon showed considerable genetic diversity including within their resistance regions (Figure 3.13).

FII-2 plasmids pLAO44 and pLAO37 in ST69 and ST101 strains only contained FII-2 replicons, while FII-2 plasmid pLAO82 in an ST34 isolate carried an additional θ -RNA replicon and plasmids pLAO100 (ST40) and pLAO103 (ST457) carried an additional FIB-10 replicon (Figure 3.13 & 3.14). The FII-2 plasmid pLAO37 that carried multiple AMR genes (Appendix A3.2) was present across four different study participants, but always in the same ST101 strain (0 SNPs distance, Figure 3.13B). The FII-46 : FIB-20 AMR plasmid (pLAO69) (Appendix A3.2) was found in the same ST10 strain from one participant at different time points.

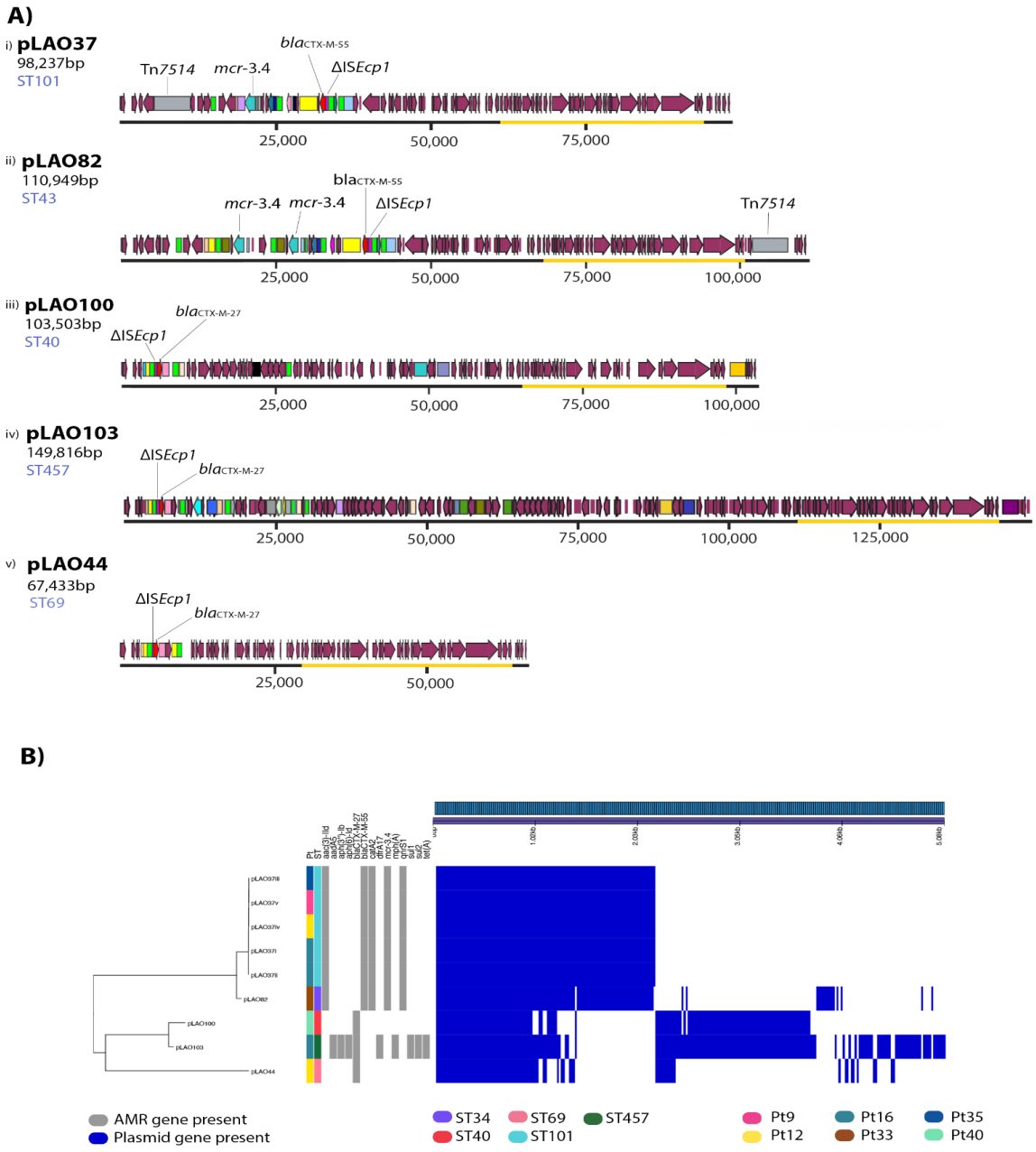


Figure 3.13 Diversity within FII-2 plasmid replicon group showing variable plasmid sizes and resistance genes. A) Annotated FII-2 plasmid group maps indicate the location of *bla*$_{CTX-M}$ and mcr genes. Maroon arrows represent Prokka-annotated genes. All other brightly coloured arrows represent antibiotic resistance genes. Transposable elements are displayed as brightly coloured boxes. Notable elements are IS*26* (bright green), IS*Ecp*1 (bright pink), Tn*1721* (pale blue) and IS*Kpn26* (lavender). The plasmid transfer (*tra*) region is highlighted with an orange outline. B) IQ-tree of FII-2 plasmids core alignment showing AMR genes and gene/presence absence matrix. The participant (Pt) and ST of the isolate from which the plasmid was identified are displayed in different colours. Resistance genes identified using ABRicate (grey = present) are shown alongside the gene presence/absence profile from Panaroo (royal blue = present).

Diversity also occurred in the resistance regions of these plasmids (Figure 3.12- 3.13). FII-2 plasmids carried multiple resistance genes, including various combinations of *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{CTX-M-55}}$ and *mcr-3.4* (Figure 3.13 & 3.14). Plasmid types FII-2 (pLAO37), FII-2:θ-RNA (pLAO82) and FII-46:FIB-like (pLAO69) incorporated resistance regions with an accumulation of multiple resistance genes and co-carriage of *mcr* and *bla*$_{\text{CTX-M}}$ genes (Figure 3.13B). pLAO82 demonstrated more complexity with further accumulation of resistance genes as it carried an additional *mcr-3.4* located between copies of IS*26* and IS*Kpn40* (Figure 3.14). There is widespread global distribution of the resistance regions found in these FII-2 plasmids which have been identified in multiple countries, some of which are not local to Laos (Appendix A3.4). There was no link found between the ST of the isolate and the type of plasmid AMR genes carried by that ST (Figure 3.14A).

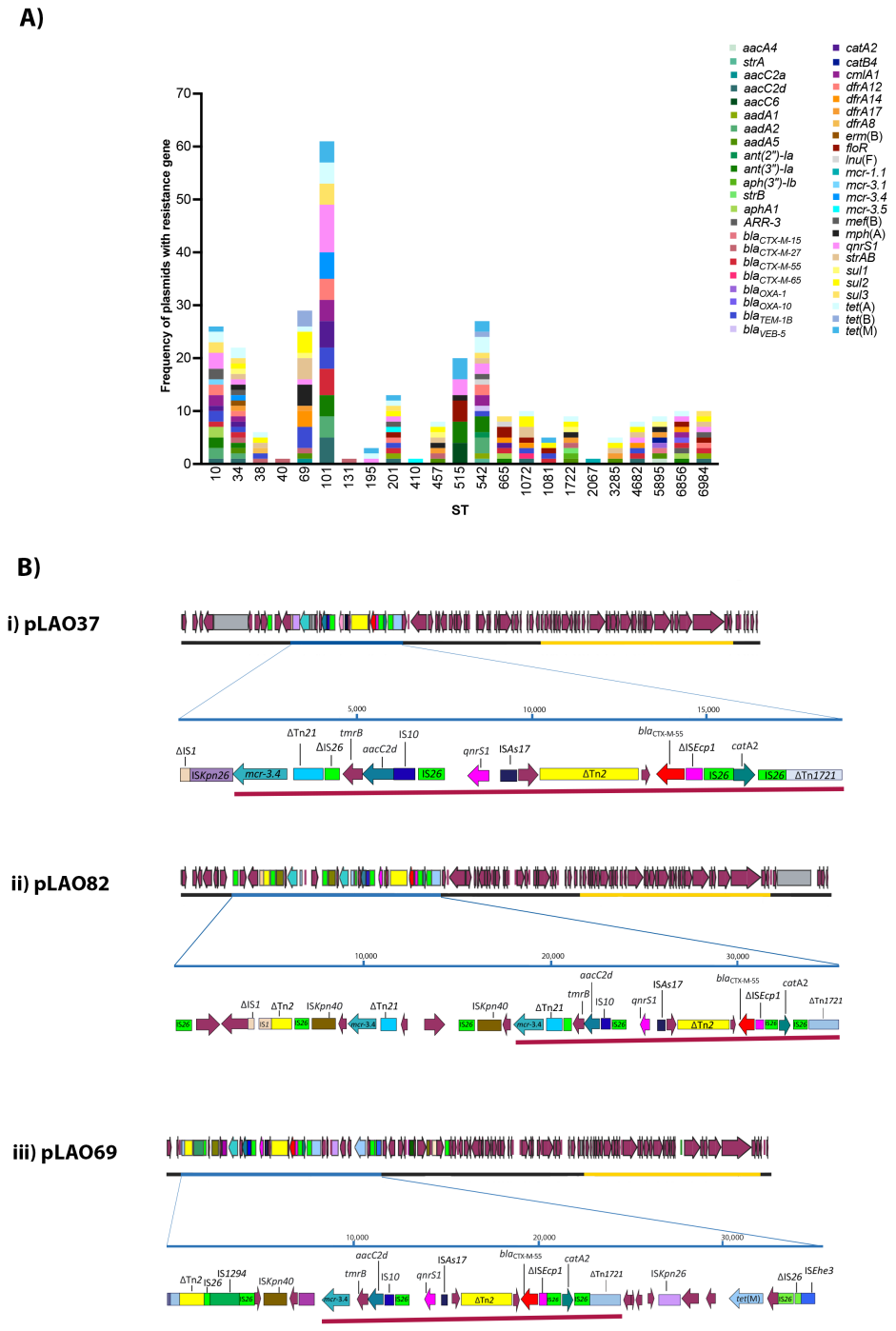


Figure 3.14 Antimicrobial resistance gene carriage diversity by A) ST and B) within *mcr* and *bla*CTX-M co-carriage plasmids. All maroon genes are Prokka-annotated genes. All other brightly coloured genes are AMR genes. Transposable elements (e.g., transposons, ISs) are displayed as brightly coloured boxes. The plasmid *tra* region is highlighted with an orange outline. Red line indicates region of similarity in co-carriage plasmid resistance region.

3.3.7 Co-carriage of *bla*$_{CTX-M}$ and *mcr* genes occurs in multiple resistance region configurations

Three plasmids in this collection carried both *bla*$_{CTX-M}$ and *mcr* genes (Figure 3.14B) and all were F-types, but they differed in size and plasmid replicon type. pLAO37 (98,237 bp) carried only an FII-2 replicon and was found in five ST101 *E. coli* isolates. pLAO82 (110,949 bp) was a multi-replicon co-integrate plasmid carrying both FII-2 and θ-RNA replicons and was found in one ST34 *E. coli* isolate. pLAO69 (105,425 bp) carried FII-46 and FIB-20 replicons and was found in two ST10 *E. coli* isolates. All three plasmids had typical F-type plasmid backbones that included genes for replication, stable maintenance, conjugative transfer and establishment in new hosts (225, 226). Each plasmid contained a complete and uninterrupted transfer region, suggesting all three have the capacity for self-mediated conjugation (227). Comparison of the FII-2 plasmids pLAO37 and pLAO82 showed that their backbones were almost identical apart from a recombination patch (approximately 7 kb) suggesting that they belong to the same lineage that is derived from a recent common ancestor. Variation in their resistance regions account for the differences in sizes of pLAO37 and pLAO82, but there are some similarities. AMR genes in both pLAO37 and pLAO82 are located in a complex region bounded by IS*26* at the left end and Tn*1721* at the right end (Figure 3.14B). These flanking regions differ and are comprised of sequences from multiple mobile genetic elements with distinct origins and include genes that confer resistance to beta-lactams (*bla*$_{CTX-M}$); colistin (*mcr-3.4*); aminoglycosides (*aacC2d*); chloramphenicol (*catA1*) and quinolones (*qnrS1*).

pLAO37 and pLAO82 have the same FII-2 *repA1* gene. An additional θ-RNA replicon in pLAO82 is part of a small plasmid that has been captured and incorporated into the resistance region. pLAO37 and pLAO82 contain an extra partial Tn*21* and partial IS*26* region inserted between *tmrB* and *mcr* gene (Figure 3.14B). The pLAO82 resistance region also includes an additional copy of *mcr-3.4.* The *mcr-3.4* in pLAO37 was truncated by IS*Kpn26*, which removed the terminal 32 bp of the gene. This configuration of IS*Kpn26* and *mcr-3.4* is not present in any other sequence deposited in the GenBank non-redundant nucleotide database. One further distinguishing feature identified by fine-scale comparison was a novel transposon, Tn*7514*, present in both pLAO37 and pLAO82. However, Tn*7514* has inserted in different backbone locations, in both cases flanked by different 5 bp target site duplications (Figure 3.13). Tn*7514* is 5,711 bp and encodes a putative ABC transporter ATP-binding protein in addition to its transposase and resolvase. NCBI database searches identified Tn*7514* in many plasmids including those found in *E. coli* and *Citrobacter* species, from multiple countries including New Zealand, China and Switzerland (e.g., CP032937.1, MZ342958.1, CP063344.1). A search for Tn*7514* in the plasmids identified in this study found it not only in the co-carriage plasmids, but also in a ColRNAI plasmid, pLAO78 which carries *aadA2*, *ant(3'')-Ia*, *cmlA1*, *dfrA12*, *tet(A)*. Partial sections of Tn*7514* were found in seven other plasmids (Table 3.2).

Table 3.2 Novel transposon Tn7514 in other pLAO plasmids that contain complete or partial Tn*7514*

Plasmid	Query Cover (%)	Percentage Identity (%)	Interpretation
pLAO82	100	100	Tn*7514* present
pLAO37	100	100	Tn*7514* present
pLAO78	100	99.98	Tn*7514* present
pLAO86	47	92.97	Related Tn present
pLAO55	47	92.70	Related Tn present
pLAO11	47	92.70	Related Tn present
pLAO71	36	99.95	Partial or recombinant Tn*7514* present
pLAO61	36	99.95	Partial or recombinant Tn*7514* present
pLAO10	36	99.95	Partial or recombinant Tn*7514* present
pLAO93	39	97.24	Related Tn present

*This table shows query cover and percentage identity of Tn*7514* found in these pLAO plasmids. This novel transposon has been registered with the Transposon Registry and was allocated the name Tn*7514*.

Although largely syntenic, the nucleotide identity of the pLAO69 backbone differs significantly from that of pLAO37 and pLAO82, consistent with its distinct replicon type. The FII-46 *repA1* gene of pLAO69 is only 94% identical to FII-2 *repA1* of pLAO37 and pLAO82. The pLAO69 transfer region also differed significantly matching only 89% of pLAO37 and pLAO82 transfer region with an overall identity of 97.2% in a BLASTn comparison. Although pLAO69 carried *mcr-3.1,* differing from the *mcr-3.4* found in pLAO37 and pLAO82, comparison of the resistance region all three plasmids revealed they were largely comprised of the same confluence of mobile elements (Figure 3.14B). In addition to their identities, the configuration of these elements was the same in pLAO69 and pLAO37/pLAO82. An example is that the sizes of regions flanked by IS*26*, and the junctions between elements such as Tn*1721* and IS*26* were identical

across resistance regions of all three plasmids, suggesting that they have been subject to the same molecular events and share an origin.

Overall, the differences between pLAO69 and pLAO37/pLAO82 suggest that pLAO69 is likely to have been an independent F-type plasmids lineage that has acquired the same confluence of mobile elements and AMR genes that was acquired by pLAO37/pLAO82 lineage (Figure 3.13 & 3.14). It appears that this collection of elements found in two distinct plasmid backbones is responsible for the co-occurrence of the AMR genes present in both.

3.3.8 Insertion sequence type, abundance and diversity is associated with AMR gene carriage

The prominence of IS*26,* a member of the IS*6* family,in the *bla*$_{\text{CTX-M}}$/*mcr* co-carriage plasmids prompted a detailed exploration of IS presence in the dataset, specifically to investigate any association with particular IS families and AMR genes.

The vast majority (n=36) of the 40 AMR plasmids contained one or more IS. Three exceptions were plasmids that contained AMR genes found in association with gene cassettes (*bla*$_{\text{VEB}}$ in pLAO60 and *aadA2*, *ant(3'')-Ia*, *cmlA1*, *dfrA12* in pLAO78), or MITEs (*tet*(A) in pLAO84). The remaining plasmid, pLAO41, contained *mcr-1* but no detectable IS elements. AMR plasmids carried almost three times more unique families of IS than their non-AMR counterparts (Figure 3.15A), with an average of 6.75 unique IS families in AMR plasmids (range 1-11), vs 2.4 in non-AMR plasmids (range 1-8). Despite their reduced IS family diversity, non-AMR plasmids were not dominated by a particular type. IS*3* was the most common, occurring in just 35% (n=6), followed jointly by IS*1* and IS*5*, which were present in 29% (n=5). Plasmids without drug-resistance genes had a lower frequency of IS carriage and many did not contain any ISs.

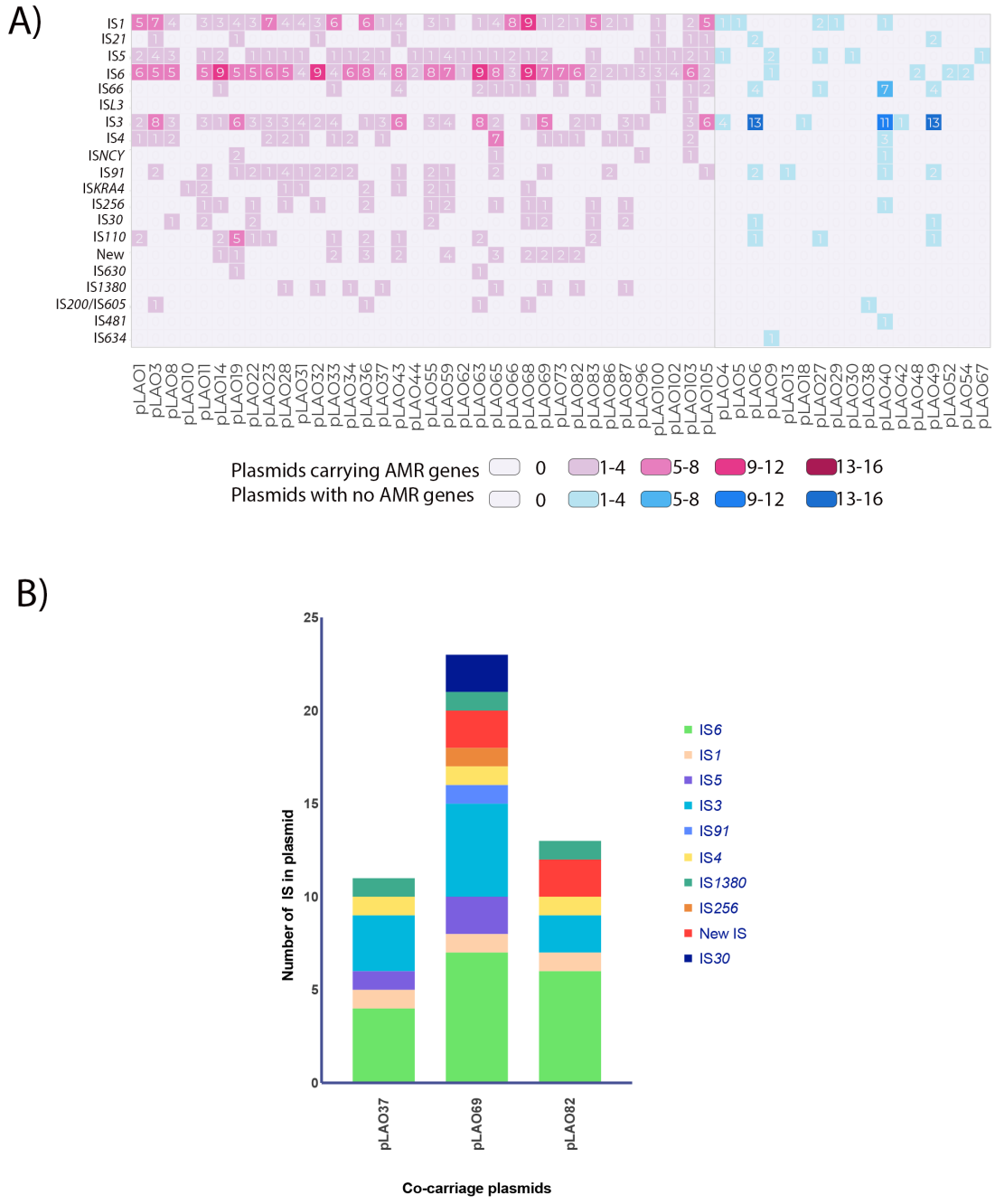


Figure 3.15 IS families present in A) all plasmid types displaying AMR gene presence/absence and IS linked to B) *mcr* and *bla*$_{\text{CTX-M}}$ co-carriage plasmids.

AMR plasmids contained an abundance of IS*6*/26 and IS*1*-family elements. Of the 36 AMR plasmids that did carry IS elements, all but one carried at least one IS*6*-family

element (Figure 3.15A), and IS*1* family was present in 83% (n=30). IS*3* and IS*5* were also common, present in 75% (n=27) and 78% (n=28) of AMR plasmids, respectively.

Differences in IS*6* family element carriage were seen between AMR and non-AMR plasmids, with AMR plasmids containing a mean of five IS*6* family elements per plasmid (range 0-9). In contrast, only 24% (n=4) of non-AMR plasmids carried IS*6* family elements, with only 1-2 IS*6* elements per plasmid (Figure 3.15A). IS6-family elements were present in all plasmids that co-carry *bla*$_{\text{CTX-M}}$ and *mcr* genes (Figure 3.15B), where they were the most prevalent IS family. There was no clear association between IS families and co-carriage of the *mcr* and *bla*$_{\text{CTX-M}}$ genes (Figure 3.15B). In plasmids where there was co-carriage of *mcr* and *bla*$_{\text{CTX-M}}$ genes, the most abundant IS families were IS*6* and IS*3*, with all co-carriage plasmids additionally carrying IS*1380*, IS*1* and IS*4*. In each co-carriage plasmid, more than six different IS families were identified, and of the IS identified between 30%-46% were IS*6* family (Figure 3.15B) with IS*26* the most prominent element.

3.4 Discussion

This chapter reports the first study of the real time dynamics of the acquisition of Gram-negative bacterial colonisation during travel to Laos, specifically focussing on the *E. coli* isolated and the multiple transient colonisation events that occur (29). This study demonstrated that pre- and post-travel sampling methods used in previous studies did not capture all colonisation events during a traveller stay as colonisation is not always persistent but is frequently transient. The daily sampling method used in this study showed all study participants acquired ESBL-producing Gram negative organisms during their stay and was very important as it revealed that a traveller was exposed to a far greater range and number of different MDR organisms than previously suggested

by other traveller studies. This study has improved the understanding and published record of intestinal colonisation experienced by travellers staying in a region which has high prevalence of antimicrobial-resistant organisms and detected a diverse range of plasmids that may contribute to AMR spread.

The very diverse collection of colonising STs identified (Figure 3.3) were previously unreported in Laos (aside from ST38 (228)), and have not commonly been reported to cause infection. These *E. coli* are still a cause of concern as they are MDR (Figure 3.6) and mainly ExPEC (Figure 3.7) so have the potential to be pathogenic and have vectors contributing to the global spread of AMR. Specifically, the predominant STs identified, including ST34 and ST195, have not frequently been reported as infection causing clinical ESBL-producing isolates. The detection of ST38 and ST101 was unsurprising as both are known ESBL-producers which colonise and cause infections and have been isolated from animals and humans, (229-231). ST131 was present but only colonised a single contact of a study participant (C11B). More ST131 isolates had been expected as previous studies have reported the importance of ST131 as a drug resistant *E. coli* in Laos and there is known global dissemination of ST131 MDR clones (e.g., ST131-H30R1 and ST131-H30Rx) (68, 232).

Colonisation, both between species and within a species, was more competitive than previously recognised. It was an extremely dynamic process and constantly changed in the three-week period with various differing combinations of 34 STs found across numerous participants in this relatively short time period (Figure 3.4). This colonisation was complex with multiple STs of MDR *E. coli* found on the same day in the same participant, some colonisation was transient, and some was persistent. Some

participants were also colonised with multiple organisms at one time (29), including MDR *Klebsiella* species and *Citrobacter* species.

There were multiple opportunities in the study period for AMR to spread between colonising isolates, both within a specific *E. coli* ST and across colonising species. The dynamic nature of this colonising population was seen in the strain sharing identified between participants, with some strains colonising up to four participants (0-5 SNPs difference) (Figure 3.5). Strain sharing demonstrated the speed and frequency at which changes in strain colonisation occur, which may be an important factor in rapid MDR spread due to increased opportunities for exposure. Clonality of these shared strains (e.g., ST101) suggests that exposure to a common environmental reservoir was unlikely to have caused the colonisation events as there was not the great strain diversity that would be expected if this was the source (233-235). Consistency of strain clonality (Figure 3.5) suggests that transmission is more likely to have occurred through direct personal contact or common exposure of participants to shared sources of food and drink. This study cannot provide evidence of direct transmission but the clonality has helped narrow down causes of strain sharing.

High levels of $bla_{\text{CTX-M}}$ and *mcr* gene carriage were detected (Figure 3.6), but no carbapenem resistance genes were identified, supporting findings of previous studies in Vientiane, Laos that reported a low carriage rate of carbapenem resistance genes (236). Although there is an increasing presence of carbapenem-resistant *Enterobacteriaceae* (CREs) reported in S. E. Asia this does not appear to be an issue currently in Vientiane, Laos. In contrast to CPEs, the high levels of colistin resistance genes across a range of *E. coli* STs was unexpected and very concerning. Colistin usage levels are not easy to quantify, but the increased level of colisitin resistance

might imply colistin is used more widely in Laos than carbapenems, The detection of so many different STs transiently colonising participants shows the increased risk spread of resistance genes of this last resort antimicrobial therapy. A possible explanation for this increase incidence of *mcr* genes is that *E. coli* strains carrying mobile colistin resistance genes have been spread by global travel to this region in Laos (167).

It should be noted that the sampling method used in this study may have affected the detection of STs and resistance genes in the isolate population colonising study participants. The culture methods and selection of a single isolate of a species to be sequenced may not represent the entire colonising population of a species. This could explain why colonisation appears to be disrupted by an alternative ST (Figure 3.4) and it is probable that the interrupted ST is also in fact still colonising the participant alongside the interrupting ST. Detection of STs is also dependant on the non-invasive sampling method used, a participant passing stool, so it is of course possible that a strain may remain undetected so colonisation may be continuous, this has been taken into account in the study (Figure 3.4). Despite the minor limitations just mentioned, this method still provides an excellent insight into the colonisation dynamics of a traveller during their stay and provides data never reported before. This study provided the best available insight into gut colonisation of travellers to Laos and gave an improved insight when compared to previous pre- and post- traveller colonisation studies.

The ever-changing dynamics of the gut colonisation of travellers to Laos and the wide variety of STs found to be transiently and persistently colonising travellers highlighted some potential routes for AMR spread, but exploration of their plasmids provided additional concerning insights into potential AMR transfer and spread. In-depth

investigation of the role plasmids played in the alarmingly high levels of AMR in *E. coli* colonising the GI tract of travellers to Laos revealed an enormous diversity in the plasmids, particularly in their resistance regions, many of which contained multiple AMR genes (Figure 3.8, Figure 3.14). Plasmids containing AMR genes were numerous, 38.1% (n=40) of the 105 distinct plasmids carried AMR, emphasising their importance as AMR carriers in this *E. coli* population. Concerningly, the majority of the 40 AMR plasmids (n=30, 75%) contained ESBL genes, a colistin resistance gene or both. The accumulation of multidrug resistance to WHO 'watch' and 'reserve' antibiotics (237) on a single plasmid, with functional transfer regions is alarming. The addition of carbapenemase resistance to these plasmids would confer drug resistance making an infection with *E. coli* carrying these plasmids very difficult to treat. Future studies of the movement of the resistance regions of these plasmids on to the chromosome and vice versa, would build on the plasmid analysis work in this study and help to establish if there is conclusive evidence for this type of AMR transfer. This would help to understand potential routes of AMR transfer in this dataset and therefore this risk of the introduction of carbapenemase resistance into these MDR *E. coli*.

MDR plasmids were identified in *E. coli* colonising healthy individuals who were not taking antibiotics (29). Consequently, there was no obvious selection pressure present in the source monitored in the study to drive or explain the accumulation of multiple resistance genes, although environmental sources and pressures must be considered. This concerning finding is contrary to expectations, especially as usage of antimicrobials in Laos is lower than surrounding areas (238). Data on antimicrobial usage in Laos is however limited making the situation difficult to assess (238). The risk of AMR is also higher in Laos due to lack of compliance with international standards

for prescriptions and antimicrobial usage (239). Antimicrobial usage in animals in Laos must be considered, especially use in the veterinary antibiotic supply chain and for growth promotion. There are reported examples of the usage of critically and highly important antibiotics, including colistin, on pig or chicken farms in Laos (240).

The abundance of plasmid types F-type, X-type, Q-type and ColE1-like plasmids revealed their importance as vectors for AMR gene spread in *E. coli* in Laos. F-type plasmids are known to carry AMR genes (including ESBLs, *mcr*) (241) and were major contributors to the high incidence of AMR carriage in this study, accounting for 42.5% of the 40 AMR plasmids identified. Although four plasmid types dominated, the AMR plasmid population in this region of Laos was extremely diverse with 29 different plasmid types, including phage and virulence plasmids, found to carry AMR genes. Long read sequencing revealed the diverse population of apparently previously unidentified AMR carrying plasmids in Laos and these have now been added to the publicly available GenBank database.

A variety of less anticipated vehicles for AMR genes, notably small ColE1-like θ-RNA plasmids, were also identified in these Laos *E. coli* by long read sequencing methods. θ-RNA are common in *E. coli* (130, 242) and were the most prevalent plasmid in the collection (n=26), followed by F-types (n=23) (Figure 3.8). In contrast to F-type plasmids, only a small number of θ-RNA plasmids (n=3) carried AMR genes. Although θ-RNA AMR plasmids are relatively uncommon, this is consistent with previous findings that ColE1-like plasmids occasionally carry resistance genes (144). The importance of small, high-copy number plasmids to AMR should not be underestimated, as these can serve as platforms for the evolution of new resistance phenotypes (144, 243), although at present little evidence is available they carry more

extensive AMR determinants. The *tet*(A)-carrying ColE1-like plasmid pLAO84 demonstrated the capacity these small plasmids have for disseminating resistance genes. pLAO84 was found in multiple E. coli STs, suggesting that it was circulating in the local E. coli population before being acquired by multiple study participants (Figure 3.9). Additionally, the presence of a plasmid almost identical to pLAO84 in GenBank (Genbank accession CP057097.1) indicates this ColE1-like plasmid lineage has already spread internationally, as it was present in *Escherichia fergusonii* isolated from pig faeces in the UK.

A Y-type phage-plasmid (pLAO59, Figure 3.11) highlighted an additional opportunity for AMR spread. pLAO59 not only appeared to have lost key phage genes required for the lytic lifestyle (244), apparently as a result of deletions by insertion sequences, but it carried multiple AMR genes and heavy metal resistance genes, potentially enabling co-selection of this MDR plasmid. Co-resistance and cross-resistance can cause co-selection of bacteria carrying metal resistance and AMR genes, with the metal resistance gene causing maintenance of the AMR gene (245, 246). Silver and copper can co-select for various AMR genes in *E. coli* including tetracycline and sulphonamide resistance genes, which were also found in pLAO59 (247, 248). In Vientiane, Laos environmental samples sourced near municipal solid waste landfill showed heavy contamination with heavy metals including copper at levels higher than WHO permissible standards (249), indicating the possibility that co-selection by metal resistance genes is a real environmental pressure in the Vientiane area from which these isolates were collected.

Multiple combinations of AMR resistance genes were found in multiple genetic contexts associated with various mobile genetic elements. *mcr* genes for example

were found next to multiple TEs (Figure 3.14, Appendix S3) and mainly on F-type plasmids, consistent with literature indicating that *mcr-3.4* is associated with the FII-type (250, 251). IS*Ecp1* is known to contribute to the spread of *bla*$_{CTX-M}$ (163), but interestingly only one complete IS*Ecp1* was identified (pLAO32) as part of a *bla*$_{CTX-M-55}$ containing transposition unit flanked by the 5 bp target site duplication TAACA. Most *bla*$_{CTX-M}$ genes in this collection were associated with complete copies of IS*26* and partial IS*Ecp*1 (Figure 3.11- 3.14, 3.15). IS*26* from the IS*6/26* family is known to play a key role in AMR gene dissemination (165, 252-254). IS*26* carriage predisposes plasmids to insertion of additional IS*26* and any associated AMR genes, which facilitates the accumulation of AMR genes at single sites leading to multi-drug resistance phenotypes (165, 255). This appears to be the case with pLAO37, pLAO82 and pLAO69 featuring IS*26* and based on what is known of IS*26* behaviour (165), IS*26* is likely to have played an important role in the assembly of their complex co-carriage resistance regions (Figure 3.14).

Overall, this study showed the dynamics of the *E. coli* population colonising travellers to Laos. It demonstrated transient colonising nature of the diverse range of ST colonising travellers in a short time and provided evidence for strain sharing amongst participants. This colonising *E. coli* population had a high frequency and diverse range of AMR genes and plasmid replicons. Analysis of plasmid contigs from *E. coli* hybrid assemblies confirmed the vast and diverse genetic context of AMR and highlighted that as well as multiple unique colonising strains, there are multiple distinct plasmids present in this dataset. A concerning convergence of ESBL and colistin resistance determinants was found. Long-read sequencing has been critical in highlighting the key role IS may play in the formation of this complex set of MDR plasmids that risk

spreading AMR. This previously unreported cohort of MDR plasmids offers the alarming prospect that one of these will create a stable configuration for the creation of a successful pandemic MDR plasmid. Even with the great diversity of known plasmid types and large volume of publicly available *E. coli* genomes, ten plasmids with unknown replication mechanisms could not be typed (Figure 3.8). This highlights the importance of sequencing diverse *E. coli* from wide geographical areas and diverse sources to improve publicly available databases that are currently largely biased towards clinical isolates from high-income Western nations.

4. Gut colonisation of Intensive Care Unit Patients with Multidrug resistant *Escherichia coli*

4.1 Introduction

The increasing spread of AMR affects individuals all over the world and has a particularly significant impact in the healthcare setting (4, 256). AMR causes increased morbidity and mortality in both the immunocompetent and immunocompromised (4). Procedures and interventions, including major surgery and organ transplants, depend on antimicrobials to protect high risk patients from infection and help assist their recovery (177). Widespread incidence of AMR could severely impact the success of these lifesaving treatments. Any incidence of antimicrobial resistant infection will mean higher treatment costs, not just for additional drug treatment if first line treatment fails, but because of the increased likelihood of longer hospital stays and recovery time (4, 8).

Patients in an ICU are extremely vulnerable to infections with a wide range of interventions, including urinary catheters, mechanical ventilators, and intravenous lines. They frequently have a weakened immune response, chronic pathologies or trauma and may already suffer from persistent infections. These patients also receive many medications (e.g., painkillers, steroids) and antimicrobials may be more likely to be prescribed in the ICU (179, 180). Increased use of antimicrobials can select for drug resistant isolates present in the hospital environment. Bacteria which are known to colonise patients and have the potential to cause infection in the ICU include: *E. coli,*

Klebsiella pneumoniae, Enterococcus species, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (257, 258). These organisms can cause a wide range of nosocomial infections, including pneumonia, urinary tract infection (UTI) and surgical wound infection. Fungal species such as *Candida albicans* also colonise patients (259). Colonisation of patients frequently occurs in their upper respiratory tract, gut and on their skin (260-262).

Reports have shown that bacterial isolates found colonising ICUs differ between countries and are especially different in both the type and amount of AMR seen on the ICU (18, 177, 186). However, some colonising organisms are commonly seen both in the UK and globally, these include *S. aureus*, *E. coli*, *Klebsiella* species and *Pseudomonas aeruginosa* (18, 257). There are also reports of the persistent presence of carbapenem-resistant *Acinetobacter* species detected in countries outside of the UK, for example in China (186). Although carbapenem-resistant *Acinetobacter* species are a significant concern globally, they are not currently frequently reported by UKHSA voluntary surveillance in UK (186, 263, 264). Outbreaks of infection reported in neonatal ICUs (NICUs) and ICUs have included *E. coli* outbreaks causing meningitis, pneumonia and UTIs (265-267). Globally, outbreaks of infection and transmission of drug resistant organisms are monitored in ICUs by international agencies e.g. ECDC (268). The purpose of this monitoring is to lead to improved infection prevention and control. Effective infection prevention and control is especially important in areas of high staff to patient ratio where many medical interventions are likely to be required by patients. Interventions and frequent contact with multiple hospital staff can increase the chances that a patient could become colonised with an AMR organism.

Long stays in hospital environments, including the ICU, can lead to disturbed microbiomes with overgrowth of species (e.g., *Candida*, *E. coli*) and colonisation with organisms not frequently seen in healthy patients (e.g., *Acinetobacter*) (182, 269). Colonisation does not always cause infection and so may not be a concern in all cases, but can be a risk in vulnerable ICU patients, who may have multiple interventions that may provide routes for infection after colonisation with an antimicrobial resistant organism. ICU patients, due to their vulnerability are therefore more likely to develop secondary or breakthrough infections after colonisation with an opportunistic pathogen (270). If these opportunistic pathogens carry AMR these breakthrough infections would be harder to treat and then might not be treatable with standard antimicrobial therapy.

Gut colonisation is one potential source of infection in the ICU and a potential route of AMR transmission. Previous studies in ICUs have shown dysbiosis of the gut microbiome, which has led to reduced microbiota diversity after hospital stay and increased likelihood of serious nosocomial infections e.g., sepsis (183, 271). There is also evidence that patients in ICU have a significant increase in colonisation by an antimicrobial resistant organism (e.g., ampicillin and/or cephalosporin-resistant *E. coli*) at discharge compared to admission (184). In contrast this same study also showed that patients staying on a general ward did not see a significant increase in colonisation with antimicrobial organisms demonstrating the effect of the difference in environment, severity of illness and extent of treatment experienced by patients in the ICU (184).

The Queen Elizabeth Hospital, University Hospitals Birmingham NHS Trust (QEHB) ICU constantly monitors patient outcomes and aims to ensure no possible opportunity is missed to maintain their high standards and quality of care. The QEHB ICU team is currently interested in understanding more about antimicrobial resistant organisms in

their ICU, including whether they are present, circulating and if gut colonisation plays a role in AMR spread. QEHB want to improve patient outcomes and wish to understand more about the colonisation dynamics of ICU patients to gain insights that could help reduce the incidence of ICU acquired AMR. AMR colonisation awareness can potentially prevent or reduce AMR transmission and would be beneficial to the patient, reducing the chances of infection by antimicrobial resistant organisms. QEHB have previously investigated changes in the patient gut microbiome during the patient ICU stay in a prospective observational shotgun sequencing study on 24 QEHB ICU patients in 2017 - 2018 (182). Study results demonstrated that, as anticipated in an ICU, gut microbiome diversity dropped, and patient commensal gut flora changed during the ICU stay. Patients saw overgrowth of *Candida*, *Enterococcus faecium* and *E. coli* (182). The study also showed the effect of antimicrobial treatment on the gut microbiome. Key findings were that meropenem caused a loss of diversity in gut flora but piperacillin-tazobactam did not have a real effect on the gut flora.

This chapter reports the investigation of *E. coli* and other colonising species isolated from QEHB ICU patient stool samples. This investigation was carried out at isolate level, allowing characterisation of sequence types (STs), AMR genes and plasmid replicons. Isolate level detail allowed the monitoring of species and strain diversity both within patient and between patients during their ICU stays. These details allowed the detection of acquisition and transmission events, and circulating strains, which it was not previously possible to detect from microbiome data. Patient prescribing metadata was also investigated in order to identify any links between prescribing patterns and antimicrobial resistance carriage. This knowledge can be fed back to ward staff and provide information about colonising strains found in the ICU which could help inform

future infection prevention and control practice on the ward to prevent AMR transmission. The IMPACT2 study has contributed understanding of the dynamics of colonisation, including transmission and acquisition, by MDR *E. coli* on a strain level in a UK healthcare setting, where patients have not been selected on the basis of prescription of a specific antibiotic. It has allowed the comparison of colonising *E. coli* in a UK hospital to studies seen abroad where high levels of MDR organism acquisition have been reported in ICUs.

4.2 Methods

Forty-four ICU patients were recruited to the study between May 2021 and December 2021, although 21 were excluded, as they did not produce stool samples during their ICU admission or withdrew from the study. Stool samples were collected from study participants by trained research nurses after patient consent had been received (Section 2.1.1). Overall, 188 stool samples from 23 patients were processed between May 2021 and December 2021 (Figure 4.1).

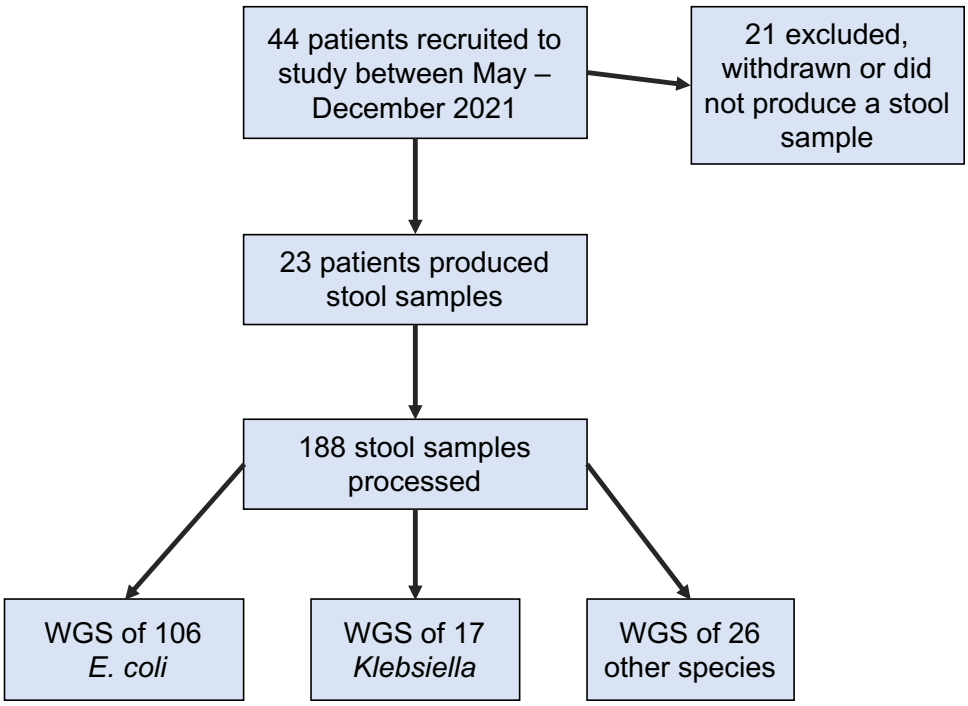


Figure 4.1 Workflow of the study showing the number of patients recruited and the number of isolates whole genome sequenced.

Bacterial isolates, mainly *E. coli*, were selected for WGS using bacterial culture methods (Section 2.2.2). Single isolates from each sample were selected for WGS. Bioinformatic methods (Figure 4.2) were used to characterise isolates and track colonisation patterns across several months in the QEHB ICU. Patient metadata including patient characteristics and antimicrobial prescribing history during the patient ICU stay were collected and analysed.

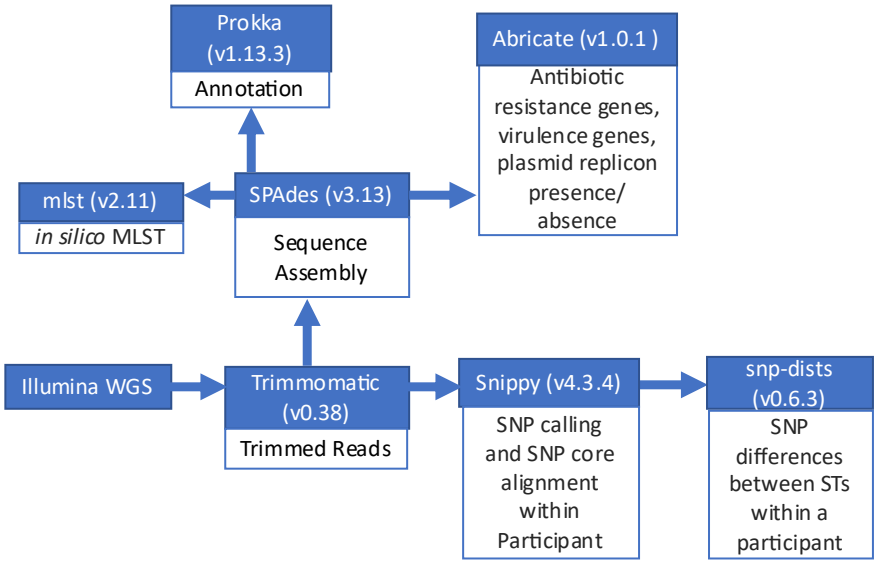


Figure 4.2- Bioinformatic workflow for patient stool sample bacterial isolates selected for WGS

4.3 Results

4.3.1 Patient Overview

Twenty-three QEHB ICU patients recruited between May 2021 and December 2021 produced stool samples and were included in the IMPACT2 study. Inpatient stays ranged from 3 – 55 days. In total 149 colonising isolates were whole genome sequenced. Isolates sent for sequencing focussed on those that identified phenotypically as *E. coli* and *Klebsiella* species (Figure 4.1). Of the 23 patients that produced stool samples, 18 were colonised with *E. coli* at some point during their ICU stay and 6 were colonised with *Klebsiella*. Patients were on the ward for a mean of 7 days (3 – 14 days) before they produced a stool sample, ranged in age between 20 and 80 years old and were admitted to the ICU for a variety of reasons, be it after planned elective surgery or as a result of other trauma or need for critical care (Table 4.1).

Table 4.1 Patient characteristics including sex, age, length of ICU stay and reason for admission*

Patient Characteristic	n (%)
Total Number of Patients	**23**
Male	15 (65.2%)
Female	8 (34.8%)
Age (years)	
Mean	53
Median	54
Range	20 - 80
Length of ICU stay (days)	
Mean	18
Median	14
Range	3 - 55
Reason for admission	
Neurology	5 (21.7%)
Trauma	5 (21.7%)
Cardiovascular	4 (17.4%)
Hepatic	2 (8.7%)
Oncology	2 (8.7%)
Transplant	2 (8.7%)
Burns	1 (4.3%)
Gastroenterology	1 (4.3%)
Elective surgery	1 (4.3%)
Respiratory	1 (4.3%)

*Detailed reason for admission can be seen in Appendix A1.

4.3.2 Overview of colonising species

The focus of this study was to investigate *E. coli* which is a common commensal Gram-negative organism that causes infection and may lead to invasive disease so is of particular concern in hospital settings, especially in the ICU. *Klebsiella* species were also investigated as they are known commensal organisms found in an ICU setting, which can also cause infections (e.g., pneumonia, BSIs) that can be life threatening. It is important to understand the population characteristics and dynamics, including acquisition and transmission, of these species to identify the risks of these Gram-negative organisms in the UK healthcare setting. Stool culture selection for *E. coli* and *Klebsiella* isolates however also revealed other colonising species such as

Cronobacter, *Citrobacter* and *Enterobacter* that are part of the normal gut flora. These species were sequenced in addition to *E. coli* and *Klebsiella* due to their phenotypic similarities to *E. coli* and *Klebsiella* on culture plates. WGS identified 106 *E. coli*, 17 *Klebsiella* species, 8 *Citrobacter,* 8 *Enterobacter* and 10 *Cronobacter* isolates from those phenotypically identified as *E. coli* and *Klebsiella* species. In this chapter colonisation refers to the presence of a specific commensal species, as these organisms are likely selected for in the ICU setting because of antibiotic use, transmission events or the impact of other co-morbidities, making them the predominant species.

The majority of patients were colonised with one or more Gram negative organism identified by WGS (n=20) during their stay. Figure 4.3 demonstrates the incidence of Gram-negative organisms, focusing on *E. coli* and *Klebsiella,* and shows the days on which a patient was colonised during their ICU stay.

18 patients were colonised with *E. coli* during their stay. Six of these patients were also colonised with *Klebsiella* species at some point during their stay. In five patients the colonising *Klebsiella* was identified in the same sample as the *E. coli*. Patients were frequently concurrently colonised with multiple species for several days (e.g., IMP2-20, IMP2-24). Three patients (IMP2-11, IMP2-15, IMP2-28) were not colonised with species sent for WGS. These three patients were colonised with other species given a presumptive ID of *Staphylococcus, Enterococcus* or *Pseudomonas* using the selective media in this study.

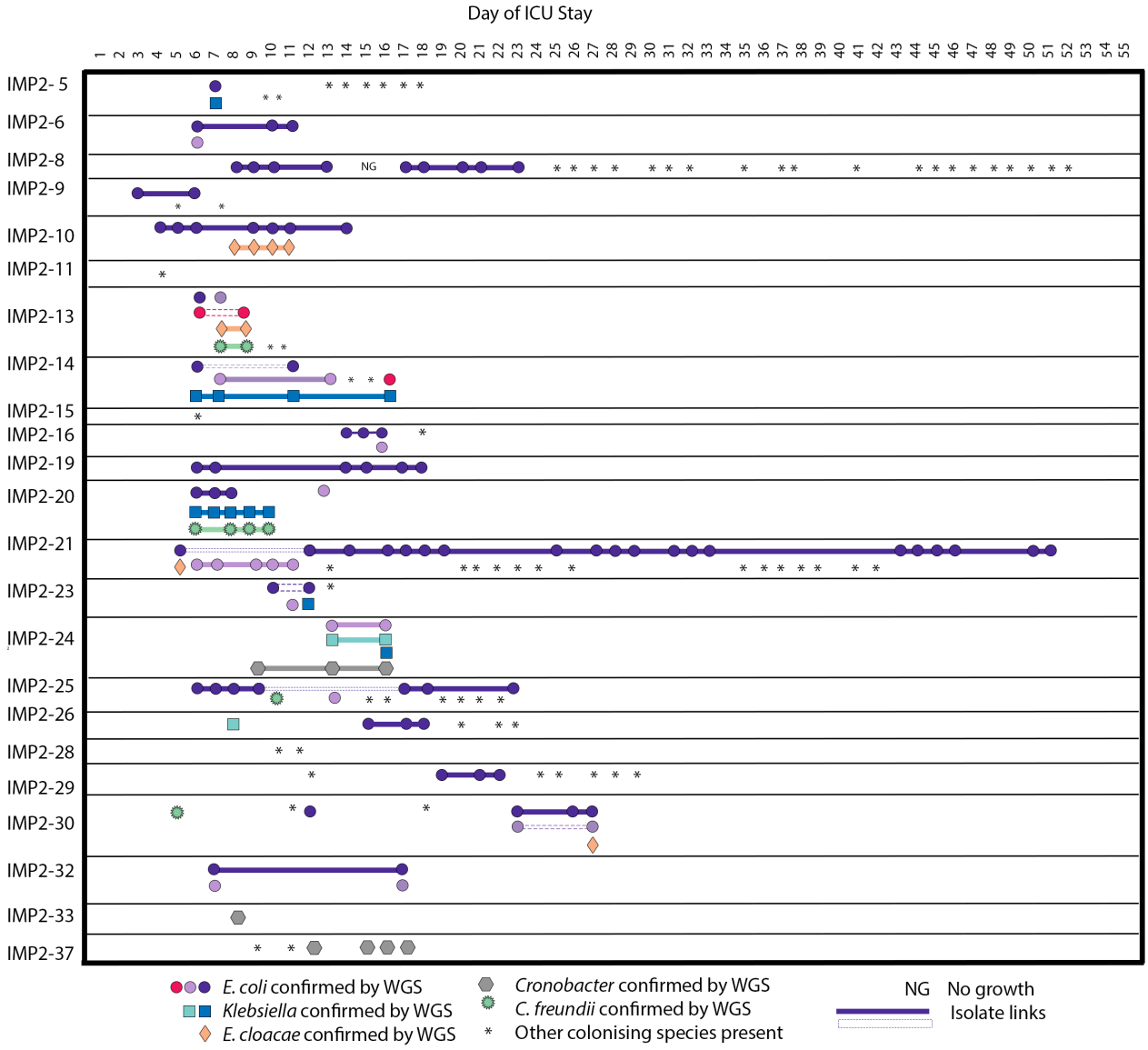


Figure 4.3 Patient timeline showing the differing colonising commensal species during the patient stay. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used. In cases were species aside from *E. coli*, *Klebsiella*, *Enterobacter*, *Cronobacter* and *Citrobacter* were identified an asterisk is used to show these colonising isolates were present.

4.3.3 Colonising *E. coli* population

4.3.3.1 *E. coli* sequence types

Twenty-two different *E. coli* sequence types were identified by WGS (Figure 4.4) for 106 colonising *E. coli* isolates from 18 patients in the QEHB ICU. The two most abundant STs identified were ST69 and ST58. Patients carried a maximum of three different *E. coli* STs during their stay (Figure 4.3). Eleven patients carried more than 1 ST during their stay.

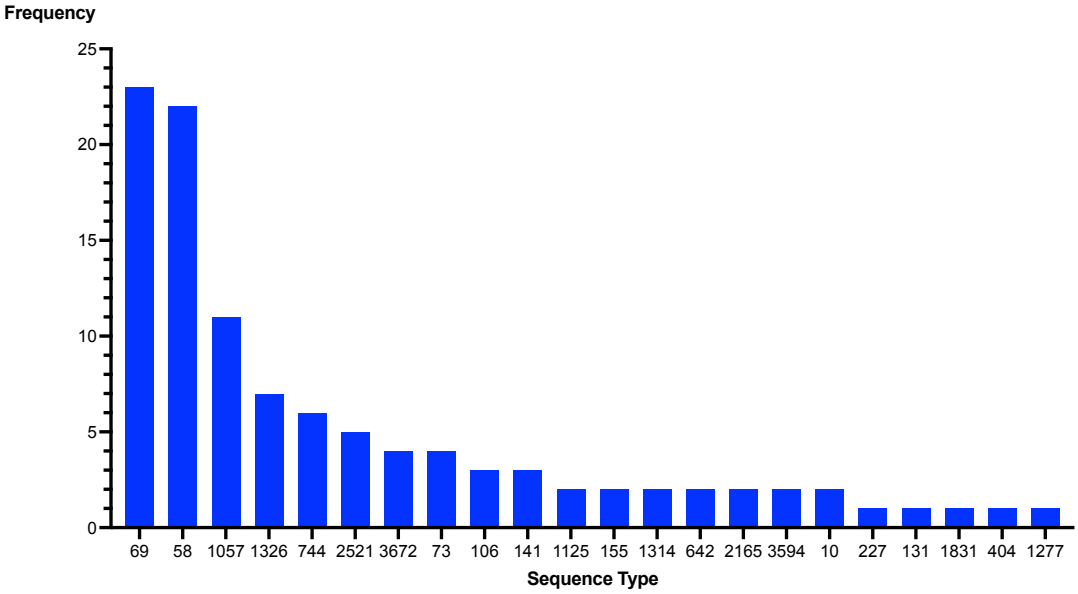


Figure 4.4 Breakdown of all STs for all *E. coli* isolates (n=106) across all patients (n=21).

Comparison of core gene alignment phylogeny of all *E. coli* demonstrated variation between and within sequence types (Figure 4.5). In some cases of high frequency of ST, (e.g., ST58), the high frequency was driven by many isolates of that ST identified from multiple stool samples of one patient.

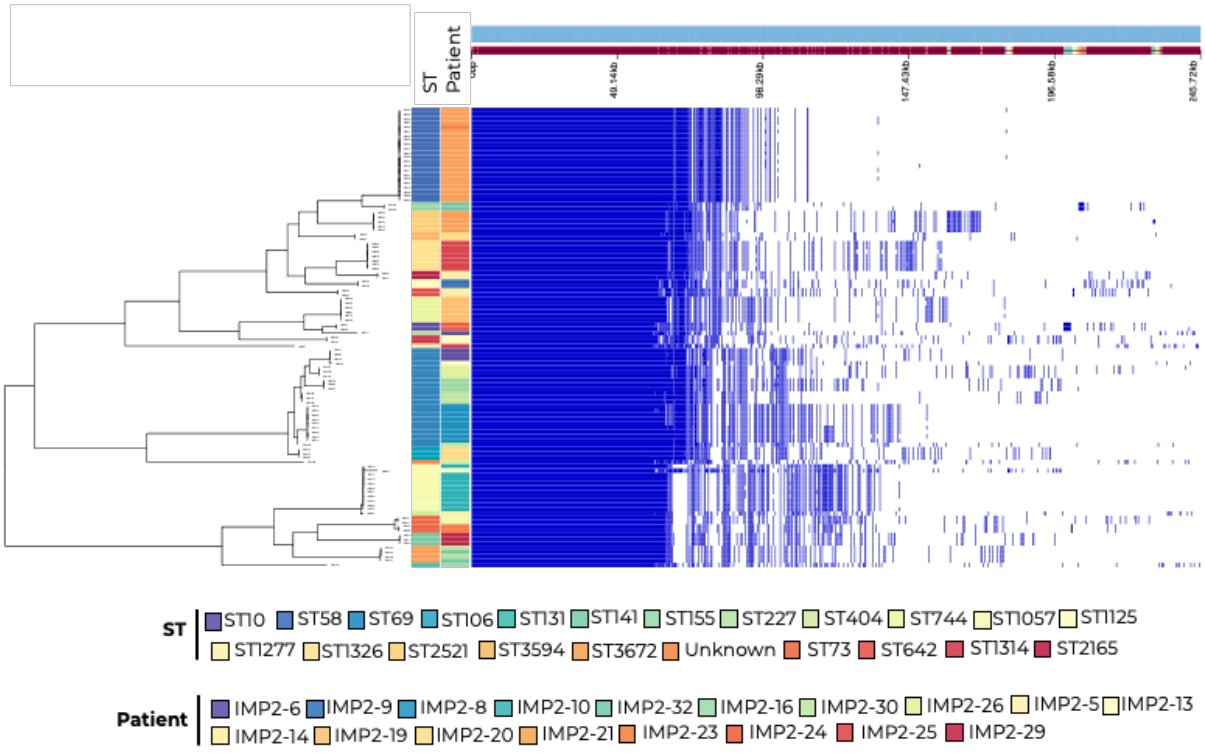


Figure 4.5 *E. coli* core gene alignment phylogeny with Panaroo core gene presence absence profile displayed by patient number and *E. coli* ST . Unknown sequence types are categorised as '-'.

4.3.3.2 *E. coli* resistance, plasmid and virulence factor profiles

Resistance genes were found in the majority of isolates colonising patients but not all colonising *E. coli* isolates carried acquired resistance genes; 34 isolates (32%) did not carry any acquired resistance genes (Figure 4.6). The most common drug resistance genes found in the 106 colonising isolates were those giving resistance to aminoglycosides (*aph(3'')-Ib*/*strA* (n=43, 41%), *aph(6)-Id*/*strB* (n=54, 51%), *aad*A5 (n=23, 22%)) and sulphonamides, *sul1* and *sul2*, (n=87, 82%). Resistance patterns appear similar within a ST as most STs represent a single strain. However, there is variation seen within a few STs where there are multiple strains, for example in ST69 strains. Some, but not all, ST69 strains carry *strAB*, and there is variation between the

types of *sul* and *dfr* genes types carried e.g. *sul1*/*sul2* and *dfr12*/*dfr17*. None of the colonising *E. coli* carried carbapenemase resistance genes or colistin resistance genes. Forty-eight isolates (45%) carried bla_{TEM} resistance genes, and only 10 isolates (9%) carried either $bla_{CTX\text{-}M\text{-}15}$ or $bla_{SHV\text{-}102}$ genes.

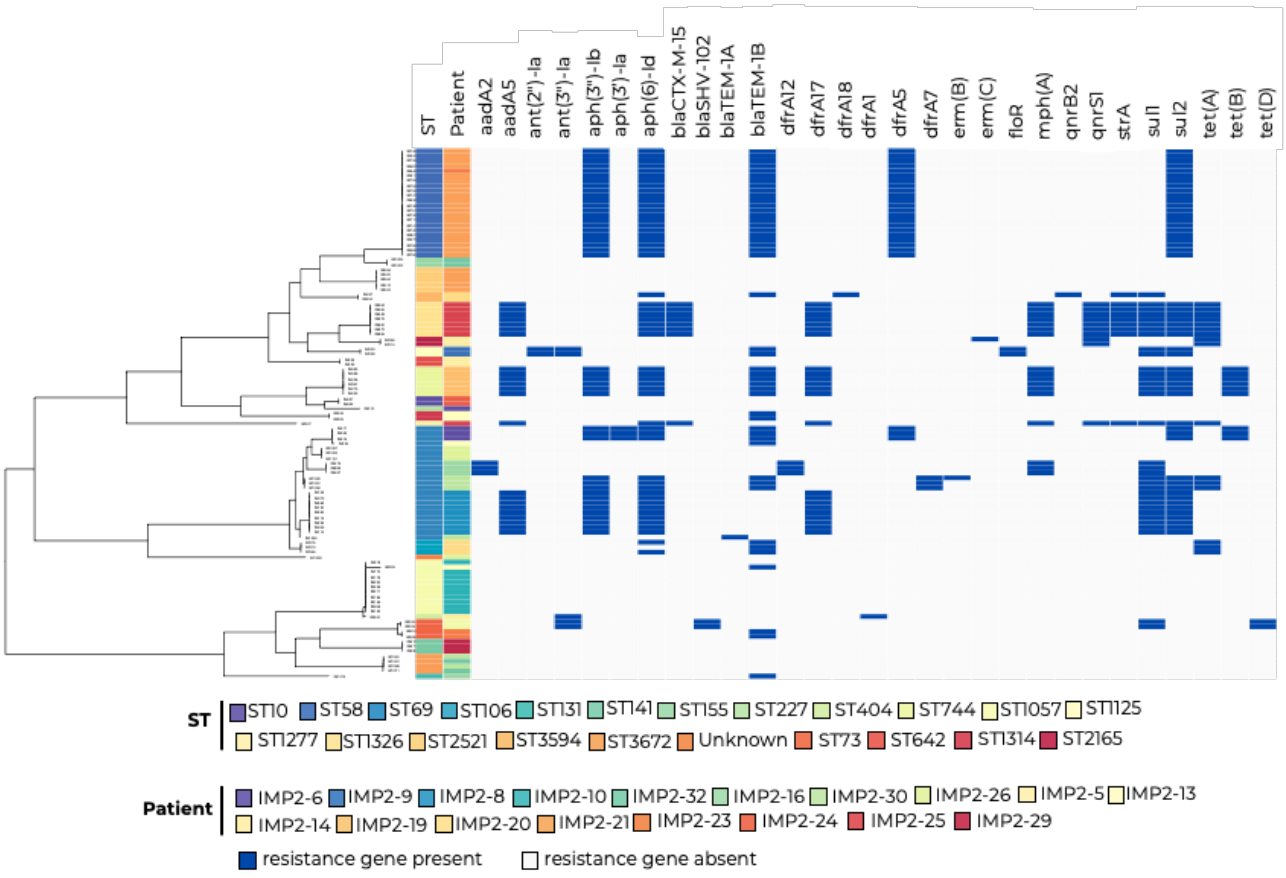


Figure 4.6 Resistance gene profile for all colonising *E. coli* displayed alongside *E. coli* ST and patient number. Navy indicates resistance gene presence and grey resistance gene absence. Unknown sequence types are categorised as '-'.

The resistance gene profiles of the colonising *E. coli* suggest that AMR plasmids may be present as bla_{CTX}, bla_{TEM}, *strA/strB*, *sul* and *tet* resistance genes are frequently found on plasmids (Chapter 3). The plasmid replicons present in the isolates ranged from replicons found on small Col plasmid (e.g. ColRNAI) to larger F-type plasmids

(e.g. IncFII, IncFIB(AP001918)) (Figure 4.7). Replicons from phage-plasmids (e.g., IncY, p0111, pESA2) were also present in eight isolates. An investigation of the carriage of F-type replicon and resistance genes showed that of the 43 isolates that carried *strAB* 37 isolates carried at least one F-type replicon. Similarly, of 59 isolates that carried either or both *sul1* and *sul2* 43 carried at least one F-type replicon, and of 27 isolates carrying *tet*(A), *tet*(B) or *tet*(D) 12 carried at least one F-type replicon. 37 of 48 bla_{TEM} genes were found in isolates which also carried at least one F-type replicon. The strain that carried the only bla_{CTX} gene present in this dataset also carried IncB/O/K/Z and ColRNAI replicons.

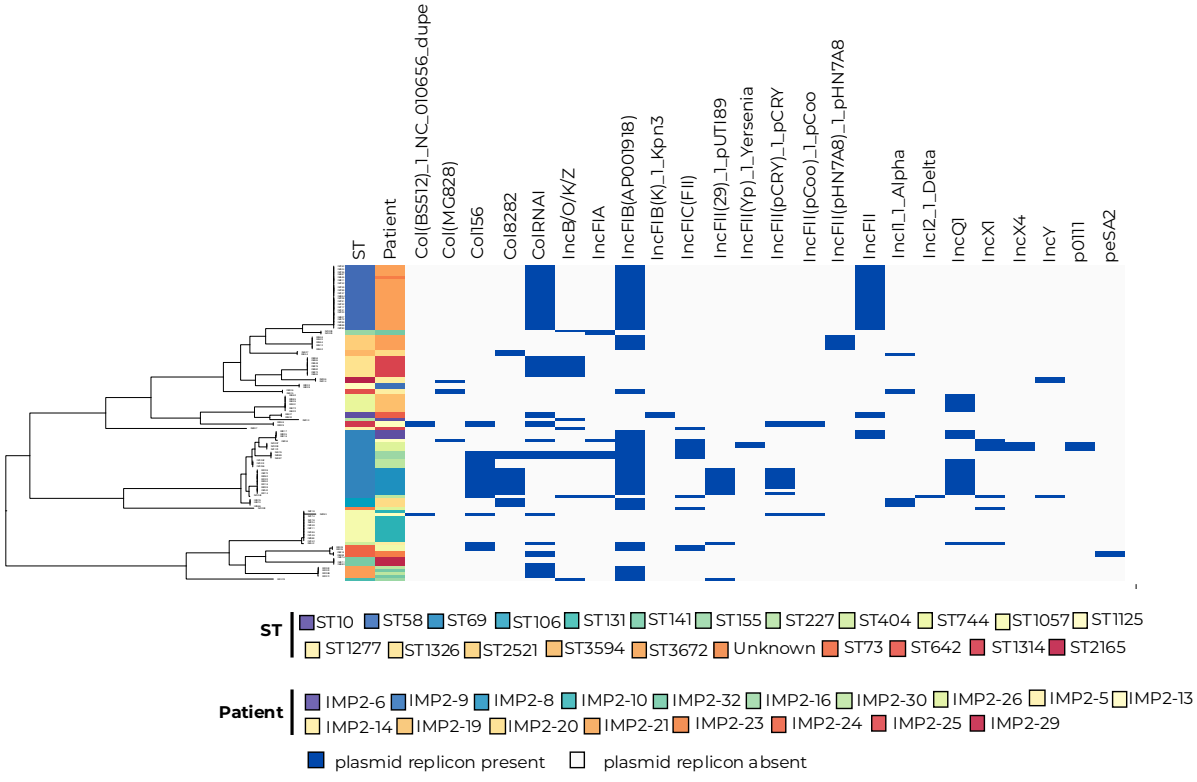


Figure 4.7 Plasmid replicon profile for all colonising *E. coli* displayed alongside ST and patient number. Navy indicates plasmid replicon gene presence and grey plasmid replicon gene absence. Unknown sequence types are categorised as '-'.

Virulence gene analysis highlighted the wide range of virulence associated genes present in the *E. coli* isolates a selection of these are shown in Figure 4.8. *E. coli* carried capsule VF genes, siderophores, haemolysins, P-fimbriae, aerobactin and Type I Fimbriae.

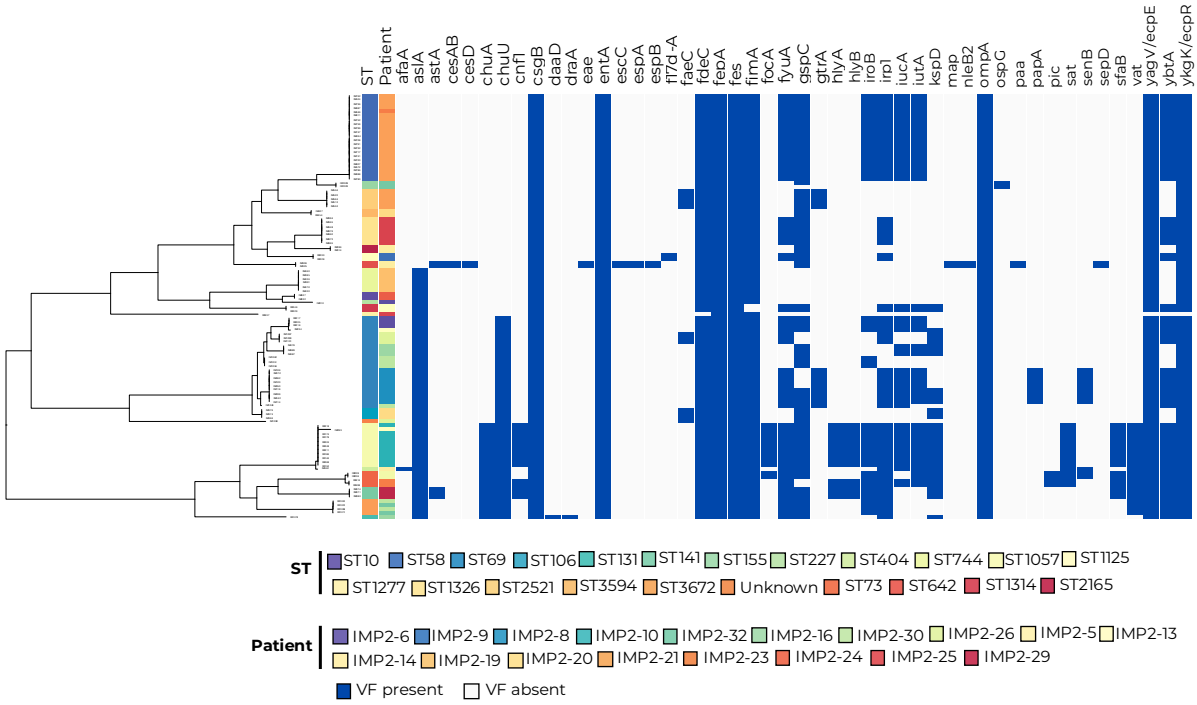


Figure 4.8 Virulence factor profile for all colonising *E. coli* displayed alongside ST and patient number. Navy indicates virulence gene presence and grey virulence gene absence.

4.3.3.3 Within *E. coli* sequence type diversity

The majority of cases where the same ST appears multiple times in the dataset are of persistent colonisation of a single patient (Table 4.2 - 4.3), rather than multiple examples of the same ST in different patients (Figure 4.4). In the most abundant sequence types ST69 and ST58, there are examples of multiple different strains of the same ST, with strains differing between patients rather than a single strain inhabiting

the ICU. This confirms that *E. coli* are most probably resident commensal strains of the patient and are not being acquired during their ICU stay. A focus on the most frequently isolated ST of *E. coli* seen on the ICU between May- December 2021, ST69, demonstrated that 7 distinct strains of ST69 were detected in 6 patients (Table 4.2). All closely related isolates (< 13 SNPs different) were found within the same patient, providing multiple examples of persistent colonisation with a patient-specific single strain in all 6 patients. IMP2-30 was colonised by two distinct strains of ST69 which were over 2000 SNPs different, giving the only example of colonisation with multiple ST69 strains.

Table 4.2 SNP difference matrix of ST69 strains*

Isolate	Patient	Isolation Date	IM005	IM016	IM017	IM039	IM042	IM050	IM072	IM062	IM106	IM114	IM116	IM120	IM1049	IM1051	IM1056	IM1062	IM1101	IM1093	IM1097	IM234	IM476	IM487	IM889
IM005	IMP2-6	27/05/2021	0	3	0	7581	7579	7582	7581	7582	7583	7583	7582	7581	7929	5798	5797	5798	6295	6299	6300	406	7563	7563	7563
IM016	IMP2-6	31/05/2021	3	0	3	7584	7582	7585	7584	7585	7586	7586	7585	7584	7932	5801	5800	5801	6298	6302	6303	409	7566	7566	7566
IM017	IMP2-6	01/06/2021	0	3	0	7581	7579	7582	7581	7582	7583	7583	7582	7581	7929	5798	5797	5798	6295	6299	6300	406	7563	7563	7563
IM039	IMP2-8	29/05/2021	7581	7584	7581	0	4	3	4	3	4	9	3	4	1691	3782	3781	3782	6366	6370	6371	7834	8095	8095	8095
IM042	IMP2-8	30/05/2021	7579	7582	7579	4	0	5	6	5	6	11	5	6	1689	3780	3779	3780	6364	6368	6369	7832	8093	8093	8093
IM050	IMP2-8	31/05/2021	7582	7585	7582	3	5	0	1	0	1	8	4	1	1692	3783	3782	3783	6367	6371	6372	7835	8096	8096	8096
IM072	IMP2-8	03/06/2021	7581	7584	7581	4	6	1	0	1	2	9	5	2	1691	3782	3781	3782	6366	6370	6371	7834	8095	8095	8095
IM062	IMP2-8	07/06/2021	7582	7585	7582	3	5	0	1	0	1	8	4	1	1692	3783	3782	3783	6367	6371	6372	7835	8096	8096	8096
IM106	IMP2-8	08/06/2021	7583	7586	7583	4	6	1	2	1	0	9	5	2	1693	3784	3783	3784	6368	6372	6373	7836	8097	8097	8097
IM114	IMP2-8	10/06/2021	7583	7586	7583	9	11	8	9	8	9	0	10	9	1698	3784	3783	3784	6368	6372	6373	7836	8097	8097	8097
IM116	IMP2-8	11/06/2021	7582	7585	7582	3	5	4	5	4	5	10	0	5	1692	3783	3782	3783	6367	6371	6372	7835	8096	8096	8096
IM120	IMP2-8	13/06/2021	7581	7584	7581	4	6	1	2	1	2	9	5	0	1693	3782	3781	3782	6366	6370	6371	7834	8095	8095	8095
IM1049	IMP2-30	29/10/2021	7929	7932	7929	1691	1689	1692	1691	1692	1693	1698	1692	1693	0	2363	2362	2363	6072	6076	6077	8201	6766	6766	6766
IM1051	IMP2-30	09/11/2021	5798	5801	5798	3782	3780	3783	3782	3783	3784	3784	3783	3782	2363	0	1	0	3953	3957	3958	6044	4701	4701	4701
IM1056	IMP2-30	12/11/2021	5797	5800	5797	3781	3779	3782	3781	3782	3783	3783	3782	3781	2362	1	0	1	3952	3956	3957	6043	4700	4700	4700
IM1062	IMP2-30	13/11/2021	5798	5801	5798	3782	3780	3783	3782	3783	3784	3784	3783	3782	2363	0	1	0	3953	3957	3958	6044	4701	4701	4701
IM1101	IMP2-26	11/10/2021	6295	6298	6295	6366	6364	6367	6366	6367	6368	6368	6367	6366	6072	3953	3952	3953	0	4	9	6562	6045	6045	6045
IM1093	IMP2-26	13/10/2021	6299	6302	6299	6370	6368	6371	6370	6371	6372	6372	6371	6370	6076	3957	3956	3957	4	0	13	6566	6049	6049	6049
IM1097	IMP2-26	14/10/2021	6300	6303	6300	6371	6369	6372	6371	6372	6373	6373	6372	6371	6077	3958	3957	3958	9	13	0	6567	6050	6050	6050
IM234	IMP2-13	13/07/2021	406	409	406	7834	7832	7835	7834	7835	7836	7836	7835	7834	8201	6044	6043	6044	6562	6566	6567	0	7651	7651	7651
IM476	IMP2-16	26/07/2021	7563	7566	7563	8095	8093	8096	8095	8096	8097	8097	8096	8095	6766	4701	4700	4701	6045	6049	6050	7651	0	0	0
IM487	IMP2-16	27/07/2021	7563	7566	7563	8095	8093	8096	8095	8096	8097	8097	8096	8095	6766	4701	4700	4701	6045	6049	6050	7651	0	0	0
IM889	IMP2-16	28/07/2021	7563	7566	7563	8095	8093	8096	8095	8096	8097	8097	8096	8095	6766	4701	4700	4701	6045	6049	6050	7651	0	0	0

*Isolates with less than 13 SNPs different are highlighted in red. Patient number and isolation date is displayed alongside SNP differences. White and grey banding is used to display different patients by rows.

The second most common *E. coli* ST isolated was ST58 and all ST58 isolates were the same strain with ≤ 2 SNPs difference (Table 4.3). This ST58 strain was found colonising two patients IMP2-21 and IMP2-23. IMP2-21 arrived on the ward colonised with ST58, whereas IMP2-23 did not have it in their first sample and acquired the strain during their stay on the ICU. IMP2-23 arrived on the ward after IMP2-21 and was discharged from the ward before IMP2-21. Both were inpatients at the same time on the same ICU ward, WCCB but did not have beds located near each other or ever stay in the same bed location.

Table 4.3 SNP difference matrix of ST58 strains*

Isolate	Patient	Date	IM504	IM572	IM646	IM688	IM694	IM708	IM717	IM732	IM736	IM741	IM747	IM752	IM759	IM762	IM780	IM786	IM791	IM799	IM807	IM811	IM687	IM700
IM504	IMP2-21	24/08/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM572	IMP2-21	31/08/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM708	IMP2-21	02/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM688	IMP2-21	05/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM694	IMP2-21	06/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM717	IMP2-21	13/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM732	IMP2-21	15/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM736	IMP2-21	16/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM741	IMP2-21	17/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM646	IMP2-23	18/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM747	IMP2-21	19/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM752	IMP2-21	20/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM759	IMP2-21	21/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM762	IMP2-21	21/09/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM780	IMP2-21	01/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM786	IMP2-21	02/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM791	IMP2-21	03/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM799	IMP2-21	04/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM807	IMP2-21	08/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM811	IMP2-21	09/10/2021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
IM687	IMP2-21	04/09/2021	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	2
IM700	IMP2-21	07/09/2021	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	0

*Isolates with 0 SNPs different are highlighted in yellow. Patient number and isolation date is displayed alongside SNP differences. White and grey banding is used to display different patients by rows.

There were a few other examples of multiple patient's being colonised with the same ST. Examples of this were seen in ST1057 (Figure 4.9A) and ST3672 (Figure 4.9B). In both cases, the strains were present on different sections of the ward. IMP2-32 had two distinct strains of ST3672, only one of which was closely related to the ST3672 found in IMP2-30.

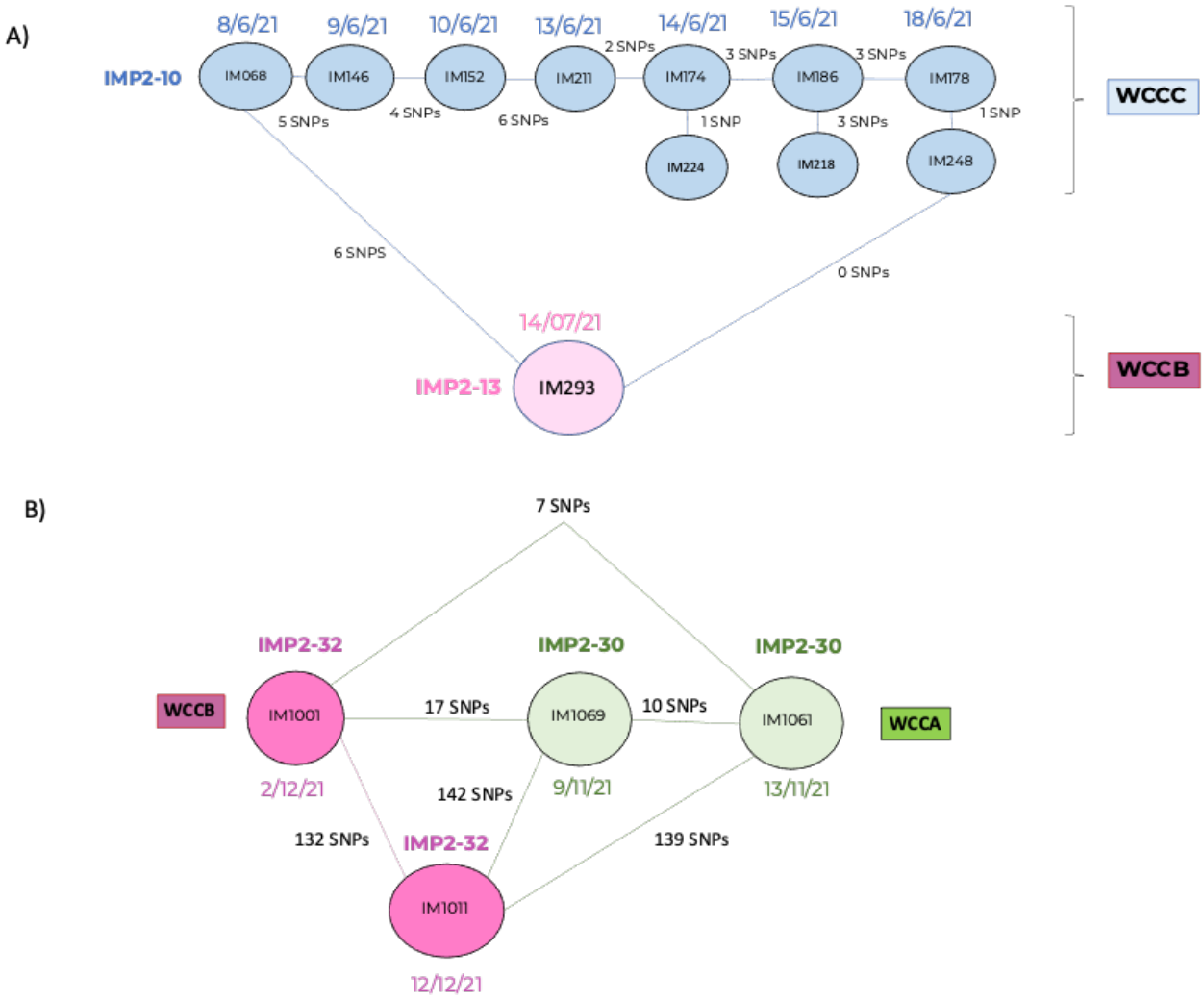


Figure 4.9 - Colonisation of different patients with the same *E. coli* strain of A) ST1057 and B) ST3672. IM number refers to isolate number and IMP2 number is patient number. SNP differences, date of isolation and ward location (WCCA/WCCB/WCCC) are displayed. Ward name where the patient was staying at the time of isolation is linked to the colour of isolate from the ward (WCCA: Green, WCCB: Pink, WCCC: Blue).

An ST1057 strain (11 isolates, 0 - 6 SNPs different) was found colonising both IMP2-10 and IMP2-13 (Figure 4.9A). These patients stayed on the same ward but did not have overlapping stays. There is a possibility both patients acquired this ST1057 *E. coli* whilst in hospital or in the ICU as although they had ST1057 in their first stool samples, both IMP2-10 and IMP2-13 spent over six days on the ICU before their first stool sample, and prior to this ICU stay IMP2-13 spent 13 days on a general ward.

An ST3672 strain was found colonising both IMP2-30 and IMP2-32 on two different wards in the same month. The patient stays of both patients overlapped by 16 days, but they were located on different wards. IMP2-32 had the ST3672 strain in their first stool sample and it was lost, being replaced by another ST3672 strain later in their stay. IMP2-30 acquired this strain 23 days after being on the ICU. Neither patient spent time on a general ward prior to their hospital stay and both were admitted from the community.

4.3.4 Colonising *Klebsiella* isolates

Six different *Klebsiella* strains were found colonising five patients during their stay. *Klebsiella* species were typed using MLST and further analysis using Kleborate allowed sequence typing of isolates that could not be given an ST on MLST, identifying *K. pneumoniae* ST1597-2LV in IMP2-26. Comparison of core gene alignment phylogenies of all *Klebsiella* demonstrated the variation between and within species and sequence types (Figure 4.10). *Klebsiella oxytoca, Klebsiella aerogenes* and *Klebsiella pneumoniae* were all found colonising patients on QEHB ICU. In cases where there were multiple isolates of a single ST, this was always isolated from the same patient. There was also an example of multiple isolates from the same patient on the same day where *Klebsiella* isolates presented phenotypically different on the

culture plate (*K. aerogenes* ST93). Two *K. oxytoca* isolates remained untyped. There were two instances of apparent *Klebsiella* acquisition on the ward (Figure 4.3), this may be due to transmission events or could have been a consequence of the sampling methods used.

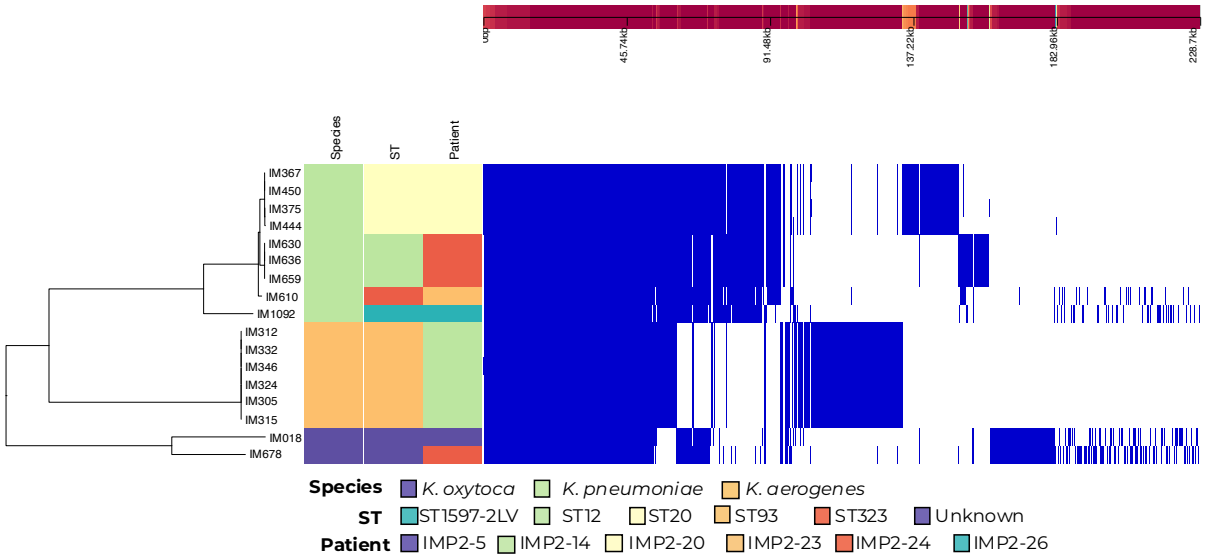


Figure 4.10 *Klebsiella* core gene alignment phylogeny with Panaroo core gene presence absence profile, patient number and *E. coli* ST displayed alongside. Blue is core gene presence and white is core gene absence. Phylogenetic tree is midpoint rooted with increasing nodes.

4.3.4.1 *Klebsiella* resistance, plasmid and virulence factor profiles

Klebsiella species identified in this ICU were not highly drug resistant, with the most prevalent resistance genes being *fosA* and *oqxAB,* which are intrinsic resistance genes in *Klebsiella* (Figure 4.11). Beta-lactamase resistance genes identified were bla_{LEN}, bla_{OXY} and bla_{SHV}. These are the beta-lactamase resistance genes which are found intrinsically in the *Klebsiella* chromosome.

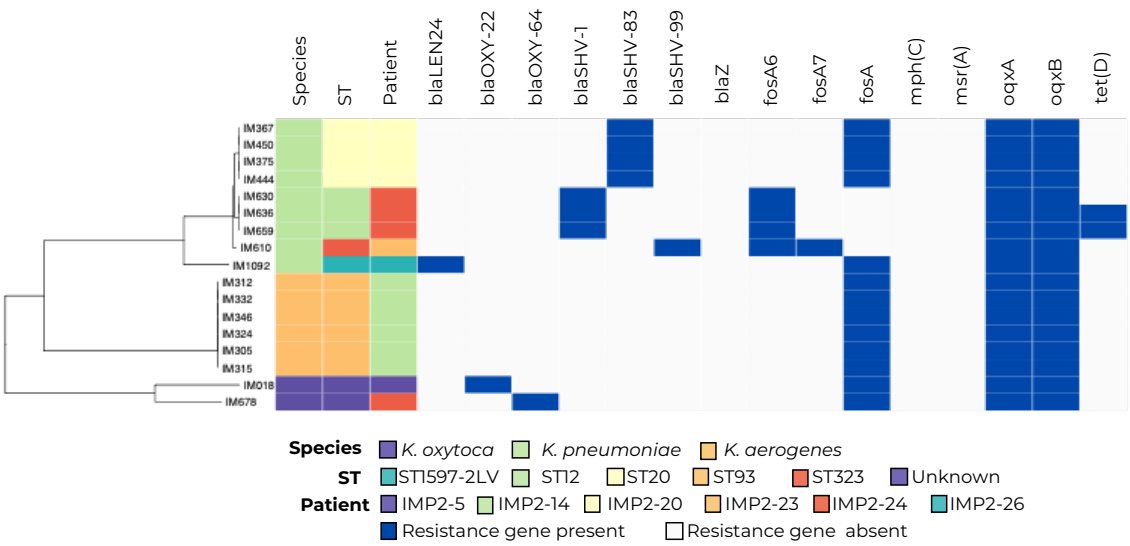


Figure 4.11 Resistance profile for all colonising *Klebsiella* displayed alongside ST and patient number. Navy indicates resistance gene presence and grey resistance gene absence. Phylogeny is midpoint rooted with increasing nodes.

All isolates carried at least one plasmid replicon. *Klebsiella* plasmid replicons, were less diverse than *E. coli* plasmid replicons, but similarly ranged from small Col plasmids to F-type plasmids (Figure 4.12).

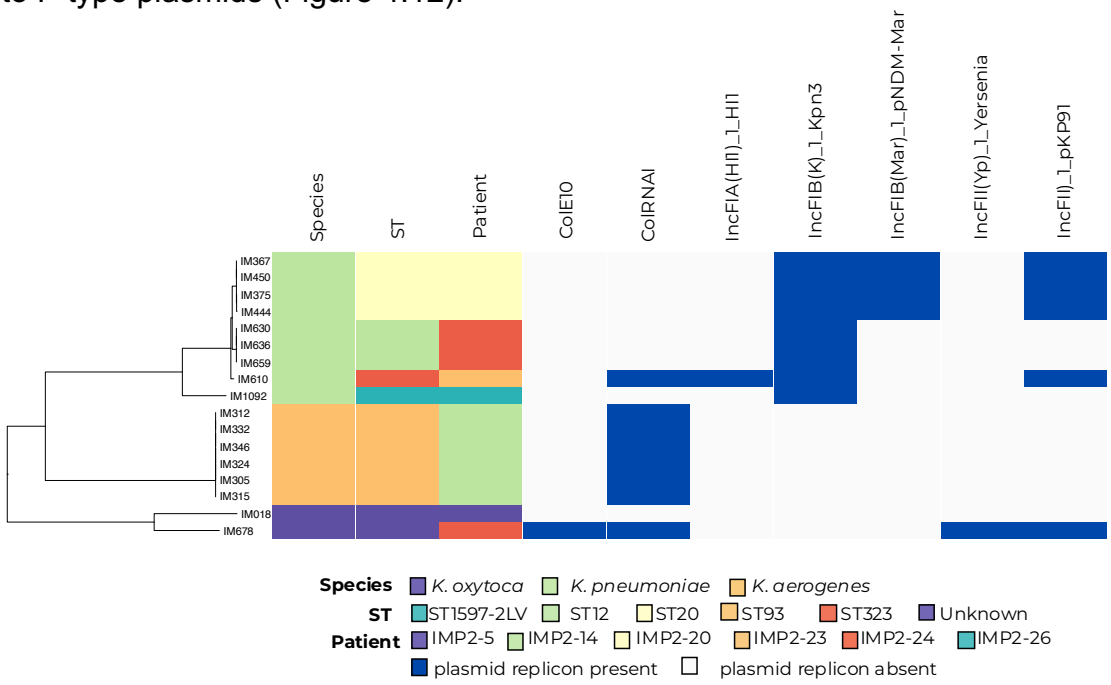


Figure 4.12 Plasmid replicon profile for all colonising *Klebsiella* displayed alongside ST and patient number. Navy indicates plasmid replicon gene presence and grey plasmid replicon gene absence. Phylogeny is midpoint rooted with increasing nodes.

In each *Klebsiella* species virulence factor profiles were similar within ST. All *Klebsiella* have the siderphore *ent*, and other abundant VFs include siderophore *ybt* genes and pilus *yag*/*ect* genes (Figure 4.13). Kleborate results showed that 12 isolates had yersiniabactin, 6 of which also had salmochelin and colibactin genotypes. There was no aerobactin gene presence or high virulent *Klebsiella* isolates identified.

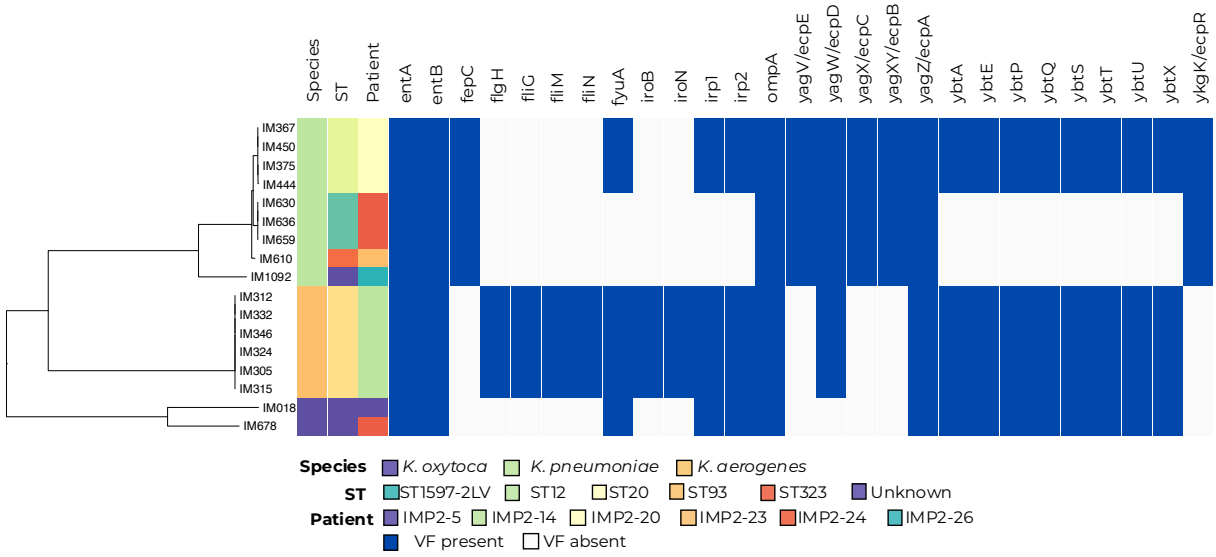


Figure 4.13 Virulence factor profile for all 17 colonising *Klebsiella* displayed alongside ST and patient number. Navy indicates VF gene presence and grey VF gene absence. Phylogeny is midpoint rooted with increasing nodes.

Kleborate allowed serotyping of *Klebsiella species*, which as expected differed between strains (Figure 4.14). All strains had distinct K loci, however *K. pneumoniae* ST20 strain had O1/O2v1 which was the same O loci seen in *K. oxytoca*. This was the only case of a shared loci, and they both had distinct K loci (Figure 4.14).

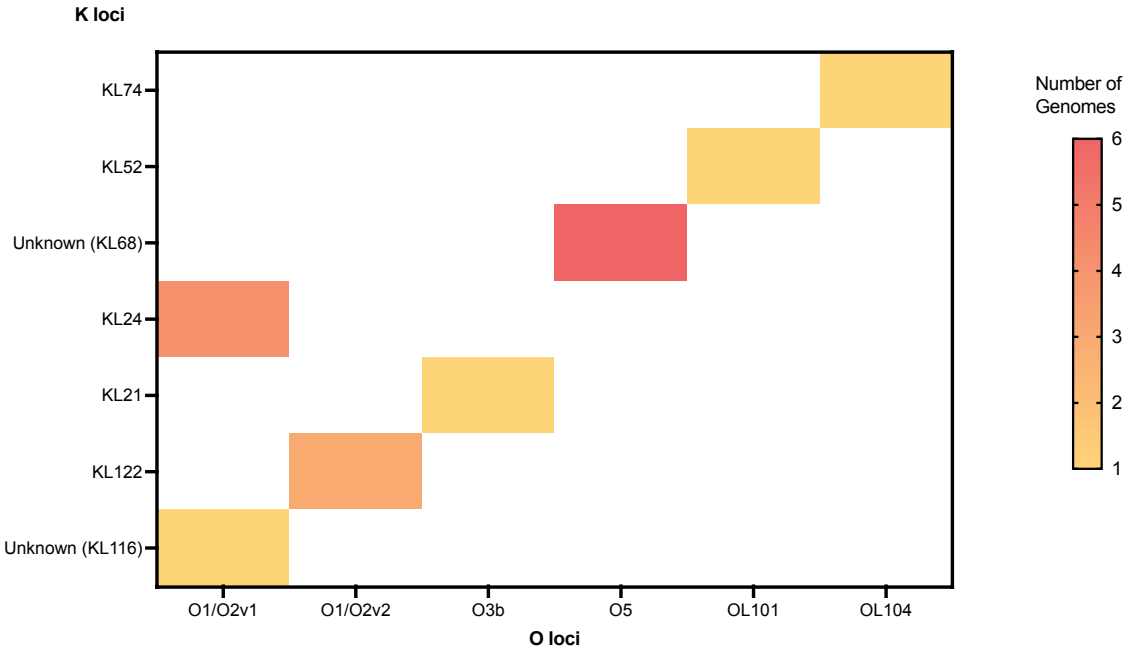


Figure 4.14 K and O loci of all *Klebsiella* isolates as allocated by Kleborate. Number of genomes with the specific K and O loci combination demonstrated by colour gradient.

4.3.4.2 Within *Klebsiella* sequence type diversity

All patients carrying *Klebsiella*, except one (IMP2-24), were colonised with one strain of *Klebsiella*. The only two cross-patient sequence type similarities were the two isolates with a *K. oxytoca* unknown ST found in IMP2-5 and IMP2-24 (Figure 4.10). A SNP difference comparison showed these unknown *Klebsiella* were not related, with > 300,000 SNPs difference. Persistent colonisation with a *K. pneumoniae* ST20 was seen in IMP2-20 for over a month, where isolates were < 2 SNPs different.

4.3.5 Other colonising species

4.3.5.1 Colonising *Citrobacter* species

There were 8 colonising *Citrobacter freundii* identified in this study, six of which could not be assigned a sequence type (Figure 4.15). *C. freundii* was the only colonising species to carry carbapenemase resistance genes. IMP2-20 arrived on the ward colonised with *C. freundii* which carried *bla*$_{KPC-2}$ and remained colonised during their stay. No other patient acquired this strain on their stay in the ward.

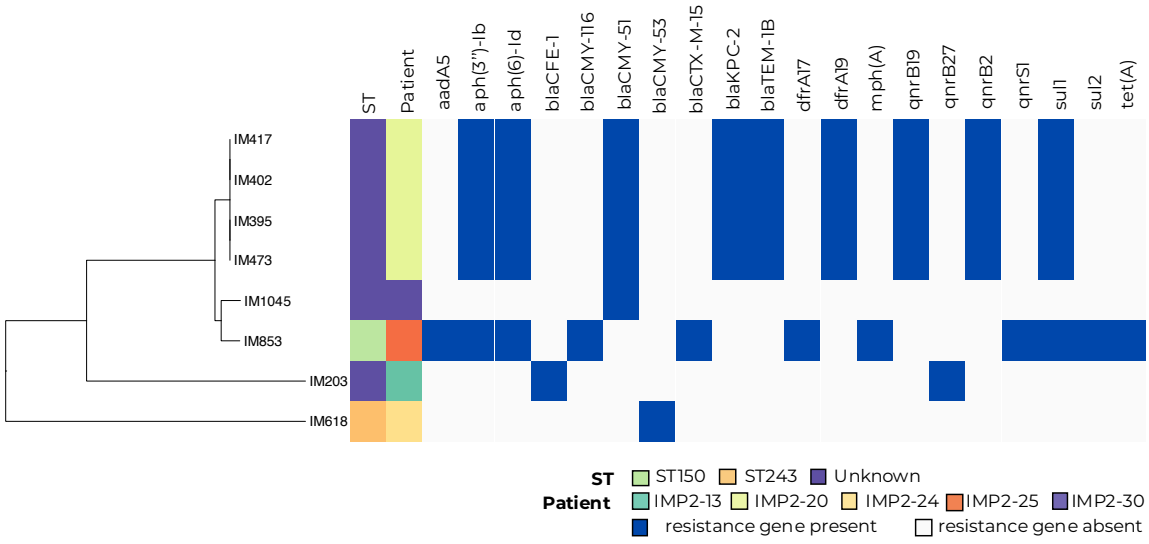


Figure 4.15 Acquired resistance gene profile for all colonising *C. freundii* displayed next to ST and patient number. Navy indicates resistance gene presence and grey resistance gene absence. Phylogeny is midpoint rooted with increasing nodes.

Plasmid replicon analysis indicated the IMP2-20 colonising *C. freundii* strain carried Col, N-type, R-type and H-type plasmid replicons along with their *bla*$_{KPC-2}$ and other drug resistances (Figure 4.16).

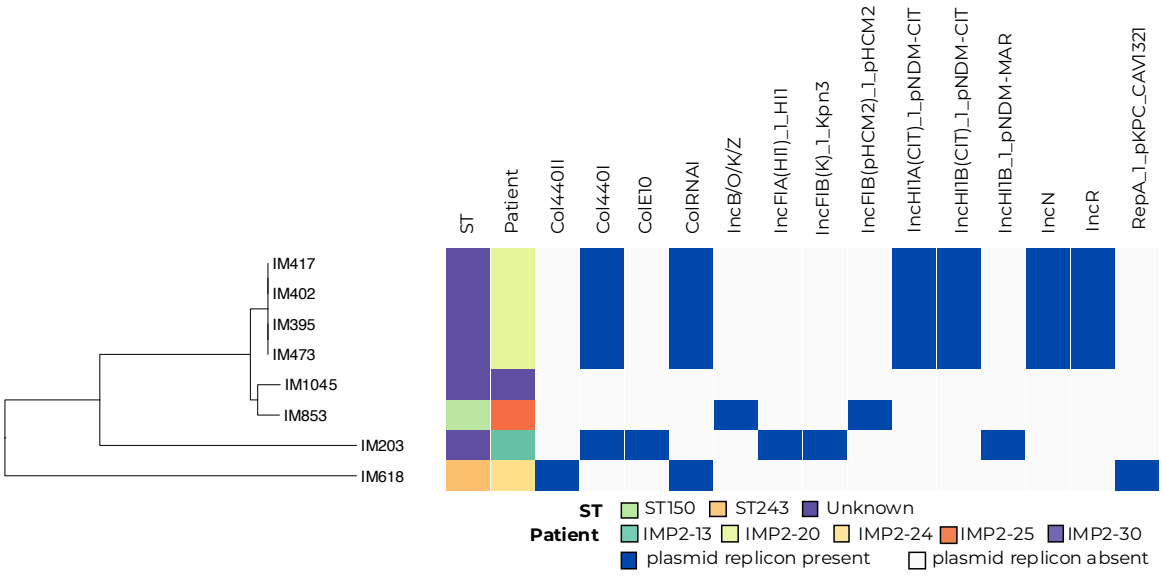


Figure 4.16 Plasmid replicon profile for all colonising *C. freundii* displayed alongside ST and patient number. Navy indicates plasmid replicon gene presence and grey plasmid replicon gene absence. Phylogeny is midpoint rooted with increasing nodes.

4.3.5.2 Colonising *Enterobacter* and *Cronobacter* species

There was little evidence of resistance and plasmid carriage in the small populations of *Enterobacter* and *Cronobacter* species sequenced (Appendix A4). Eight *Enterobacter cloacae* were identified in four patients using WGS. These were ST45, ST555 and two could not be assigned a ST (Appendix A4.1). IMP2-10 was persistently colonised with an ST45 strain with all isolates within 0 - 2 SNPs of each other. *E. cloacae* did not carry many resistance genes, with all carrying the AmpC ß-lactamase *bla*$_{ACT}$ and other resistance genes that are normally found on the chromosome of

Enterobacter. Only three colonising *E. cloacae* carried plasmid replicons, all being F-type (Appendix A4.1). ST45 *E. cloacae* did not carry any replicons.

Sequencing of the 10 *Cronobacter* isolates that were phenotypically similar to *E. coli* showed low levels of resistance gene carriage (Appendix A4.2). These isolates were ST420 and ST421, but although resistance levels were low ST421 carried bla_{ACT-7} and ST420 carried bla_{ACT-15} (Appendix A4.2). bla_{ACT} is normally a chromosomal gene, and plasmid replicon analysis demonstrated ST421 carried no plasmids. ST420 on the other hand carried a small range of Col plasmids and an R-type plasmid (Appendix A4.2).

4.3.6 Patient Colonisation stories

Patients colonised with *E. coli* fell into three categories: acquisition, fluctuation, and loss of STs. Only two patients (IMP2-28 and IMP2-33) who produced multiple stool samples were not colonised with either *E. coli* or *Klebsiella* during their inpatient stay. Three patients (IMP2-11, IMP2-15 and IMP2-33) produced single samples and so did not provide a longitudinal picture of colonisation (Figure 4.3).

4.3.6.1 Acquisition of *E. coli* STs during patient stay

Eight patients appear to have acquired a new *E. coli* ST during their ICU stay. No *E. coli* was detected in the first stool samples of 4 patients and the first stool samples from three patients were colonised with *E. coli* but gained a new ST during their stay which was different to that found colonising their first sample (Figure 4.17). The eighth patient, IMP2-20, was initially colonised with an *E. coli* ST but later in their stay appeared to acquire a different *E. coli* ST which seems to overgrow all other colonising species (Figure 4.19). Factors including time to colonisation, ward location at time of

acquisition, time of ICU admission, antibiotic prescription, and length of stay all varied so there was no obvious linking factor among those patients apparently acquiring *E. coli*. Acquisition of these *E. coli* STs during the patient ICU stay seems the most likely explanation of their occurrence but due to the time limitations on the project, sampling of stools only picked a single colony so it may not have been possible to capture every ST present. Patients who appeared to have acquired *E. coli* STs after their initial stool samples, did not all acquire the same STs. Acquired STs included: ST131, ST2521, ST10, ST1277, ST69, ST141 and ST3672. Two patients, IMP2-26 and IMP2-30 acquired ST69, but these were different strains of ST69. Patients acquired *E. coli* from day 6 – 23 of their stay. The four patients (IMP2-24, IMP2-26, IMP2-29, and IMP2-30) who did not have an *E. coli* in their first stool sample were colonised with an acquired *E. coli* for at least 3 days during their ICU stay (Figure 4.17). Basic comparison of patient timelines drug prescription and subsequent *E. coli* ST acquisition indicated that there was no obvious link between the prescription of an antibiotic and acquisition of *E. coli* (Figures 4.17-4.22).

IMP2-24 (Figure 4.17) acquired *E. coli* ST10, *K. pneumoniae* ST12 and *K. oxytoca*. IMP2-26 showed loss of *K. pneumoniae* followed by transient colonisation with ST69, before an overgrowth with probable *Staphylococcus* species. This patient was transferred from another hospital Trust to the QEHB ICU so could have acquired *K. pneumoniae* in that previous hospital trust environment and not from the QEHB. IMP2-30 acquired two different STs (ST69 and ST3672) during their stay. Four ST69 isolates were found colonising IMP2-30, the day 12 ST69 was a different strain to the three ST69 isolates found after day 23 the three of which were all 0-1 SNPs different from each other.

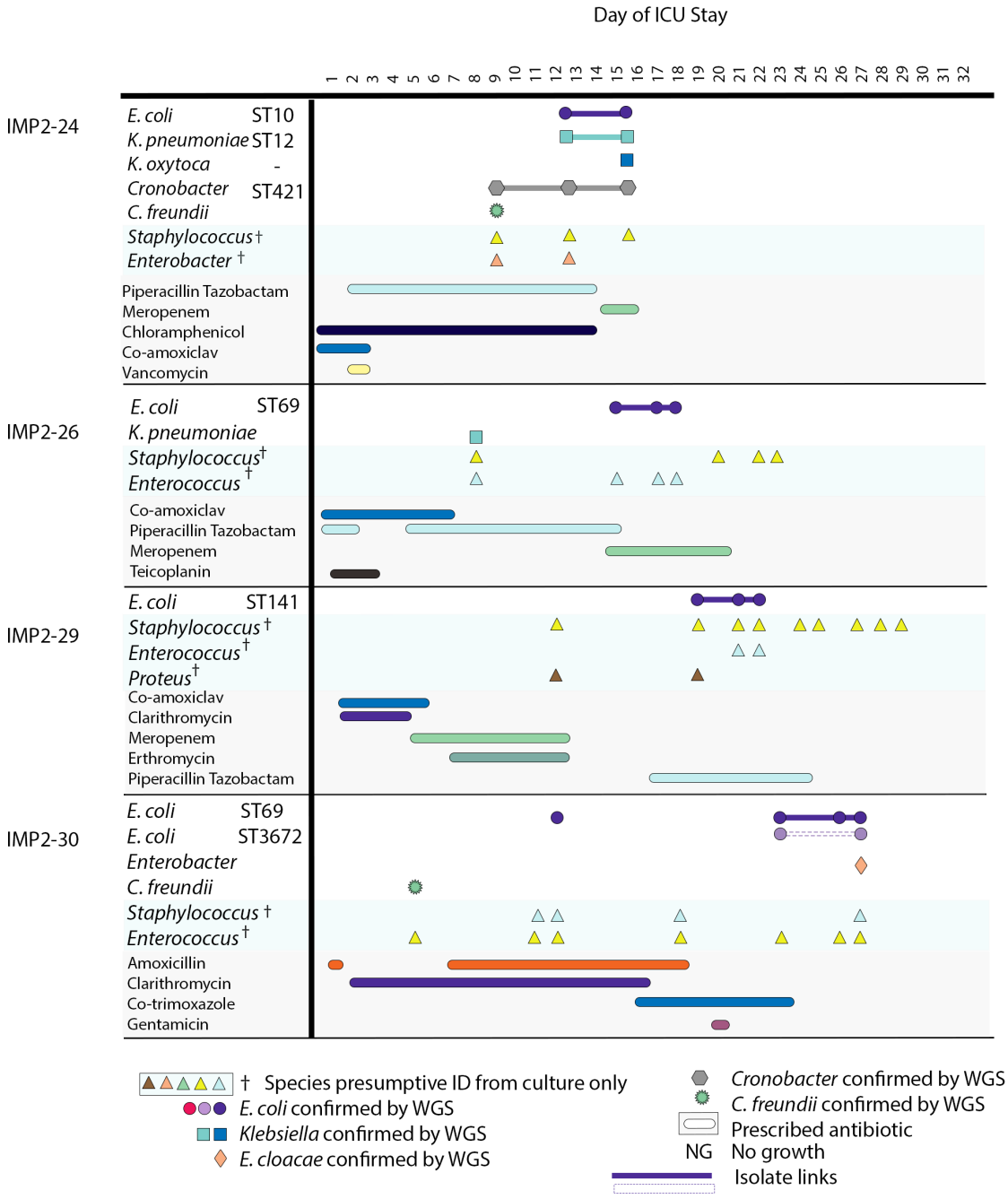


Figure 4.17 Colonising isolate timeline displaying patients who acquired *E. coli* during their stay after having no *E. coli* in their baseline first stool sample. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay are displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used.

Figures 4.17 - 4.18 show patients where multiple colonising species including *Klebsiella, Citrobacter* and *Enterobacter,* were found colonising patients in addition to *E. coli*. Acquisition of *E. coli* did not always mean that *E. coli* was retained, a loss of *E.* coli colonisation did sometimes follow the acquisition of a new *E. coli* strain (e.g., IMP2-16, Figure 4.18).

There were three patients (IMP2-16, IMP2-21, IMP2-25) who acquired a new *E. coli* ST during their stay even though they already had an *E. coli* colonising their first stool sample (Figure 4.18). IMP2-16 showed acquisition of ST131, the only ST131 isolate in the dataset. This ST131 was fim typed as H41, Clade A. This patient had spent time on a ward before admission to the ICU, but was on the ICU for 2 weeks before their first stool sample which was only colonised with ST69. IMP2-25 was initially colonised with ST1326, and acquired ST1277 on day 12. Both the persistently colonising ST1326 and the transient ST1277 carried $bla_{\text{CTX-M-15}}$.

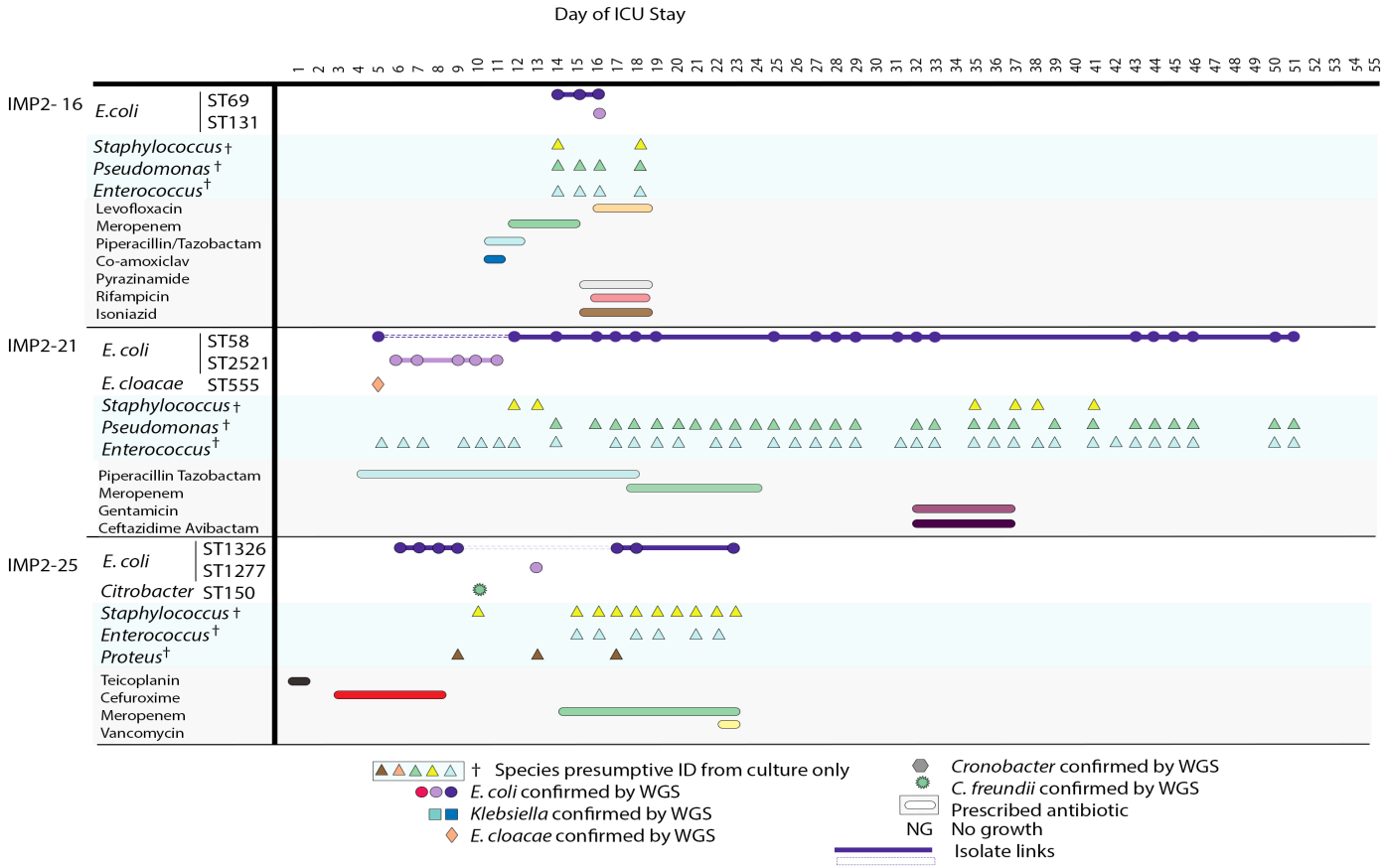


Figure 4.18 Colonising isolate timeline where the patient acquired a new *E. coli* ST during their stay. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay is displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used.

One patient, IMP2-20, had a fluctuating profile of colonisation which resulted in take-over by *E. coli* ST3594 (Figure 4.19). This patient was persistently colonised with an *E. coli*, ST106, a ST20 *Klebsiella* and *Citrobacter* species for several days after which only ST3594 *E. coli* could be isolated. This patient experienced loss of colonising commensal species diversity, resulting in *E. coli* overgrowth. Culture methods alone may not capture the entire bacterial load so it not possible to be certain whether this is total loss of colonising species diversity.

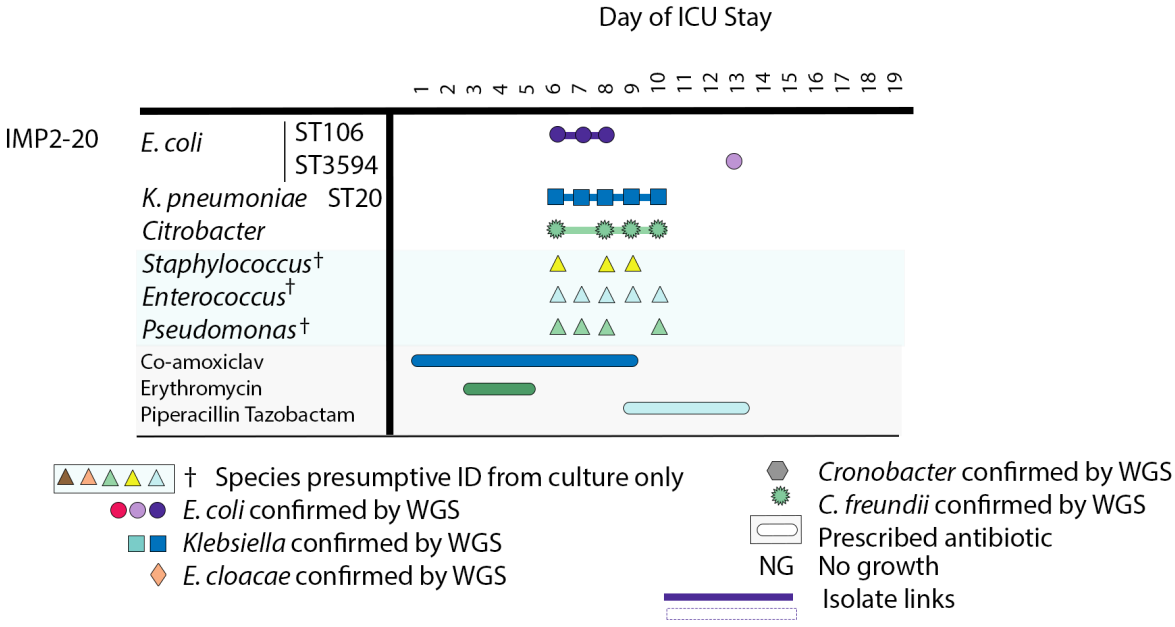


Figure 4.19 IMP2-20 timeline where an acquired *E. coli* appeared to overgrow other initial colonising species. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay is displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used.

4.3.6.2 Fluctuating *E. coli* colonisation profiles

Two patients experienced more complex changes to their *E. coli* profiles during their stay, resulting in acquisition and losses of different STs during their stay. Colonisation with acquired STs was frequently transient, and patients were probably colonised with multiple STs at the same time (Figure 4.20).

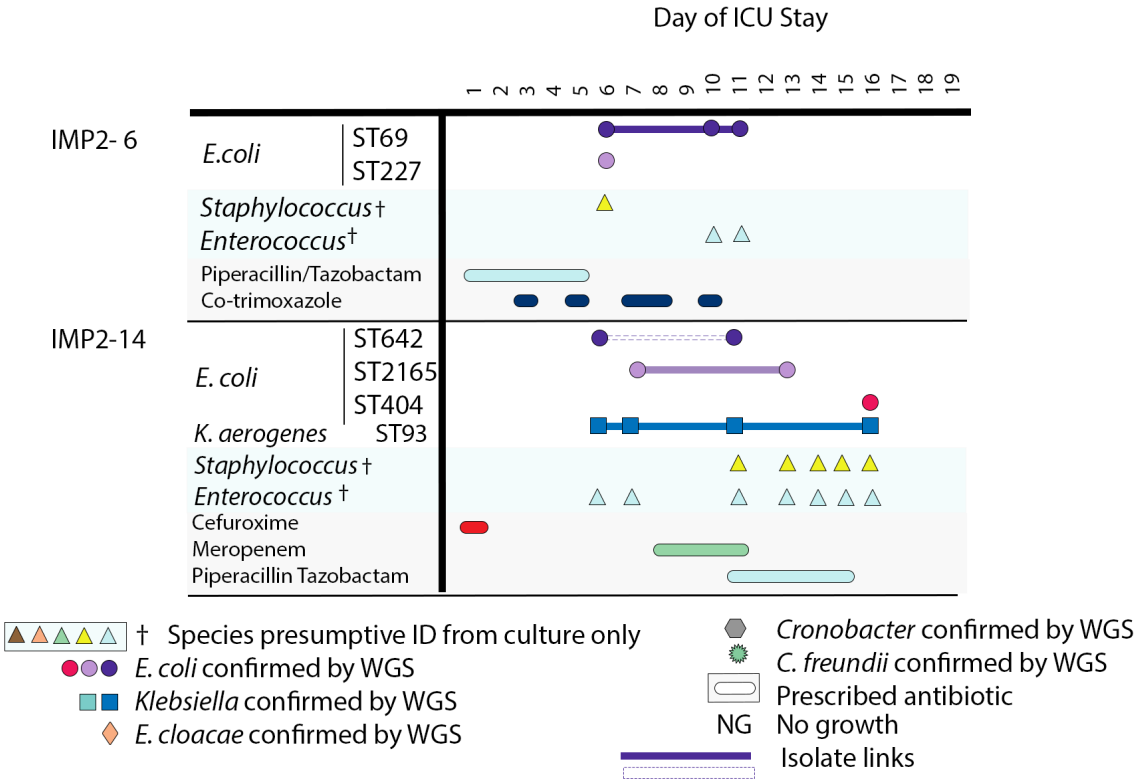


Figure 4.20 Remaining patients with their fluctuating colonisation. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay is displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used.

4.3.6.3 Loss of *E. coli* colonisation during inpatient stay

This dataset revealed four clear examples of loss of *E. coli* colonisation during the inpatient stay of IMP2-5, IMP2-8, IMP2-13 and IMP2-23 (Figure 4.21). IMP2-5 entered the ICU colonised with *E. coli* ST73 in their first stool sample. This disappeared likely due to *Enterococcus* and *Staphylococcus* overgrowth. These patients (Figures 4.17-4.21) demonstrate that colonisation is a fluid process and patients can lose and acquire species and strains during stay (e.g., IMP2-13, IMP2-23).

IMP2-8 had the longest ICU stay of all the patients in the study, with a 55 day stay. Similar to IMP2-5 they entered the ICU colonised with *E. coli*, in this case ST69 and this disappeared on day 25 again likely replaced by *Enterococcus* and *Staphylococcus* overgrowth. The colonisation with ST69 was persistent with ST69 isolates 0-11 SNPs different from each other. IMP2-13 and IMP2-23 demonstrate the loss of multiple STs due to probable *Staphylococcus* overgrowth.

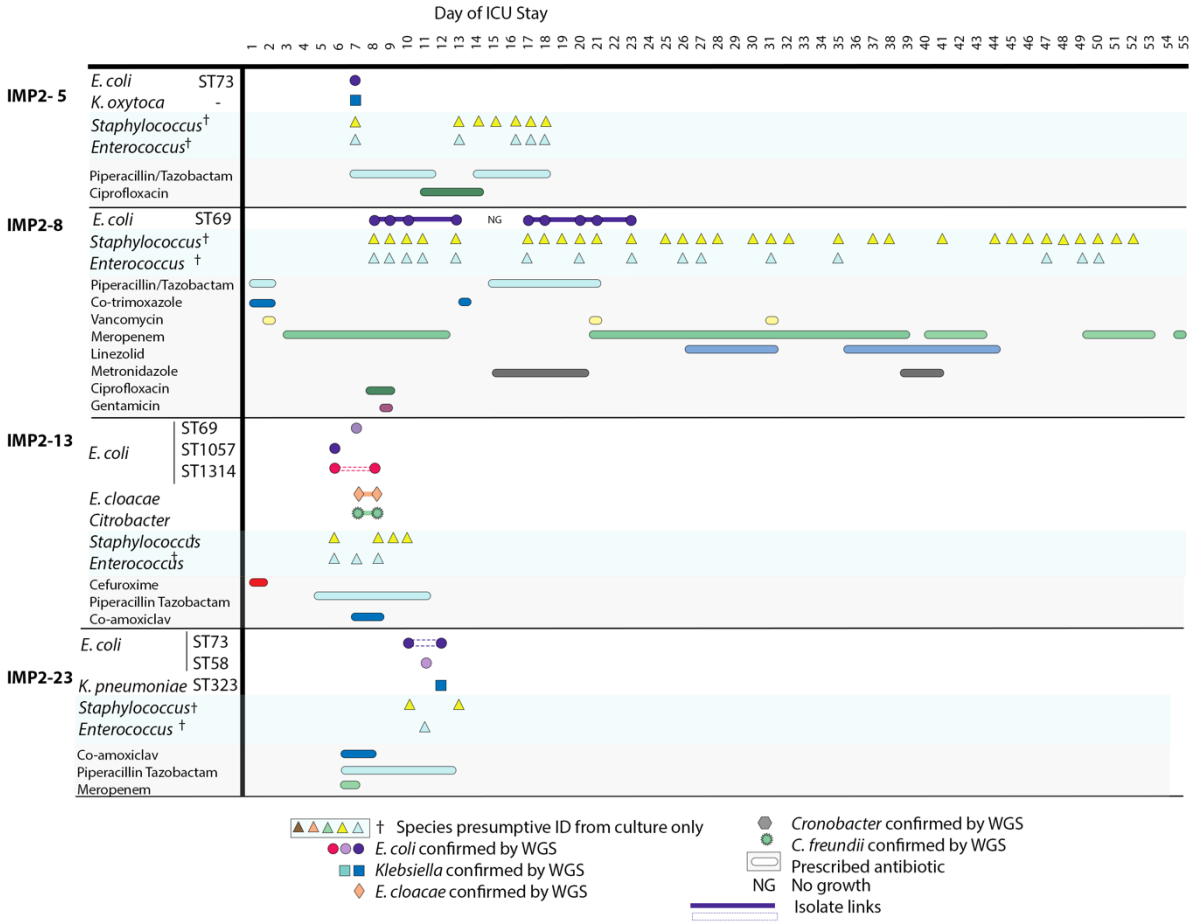


Figure 4.21 Colonising isolate timelines where *E. coli* colonisation was lost during inpatient stay. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay is displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used

4.3.6.4 Stable colonisation with *E. coli* during an ICU stay

Four patients (IMP2-9, IMP2-10, IMP2-19, and IMP2-32) showed stable colonisation with the same *E. coli* strain throughout their ICU stay. Common colonising ICU *E. coli* strains were from ST1057, ST1125, ST774, ST155 and ST3672 (Figure 4.22).

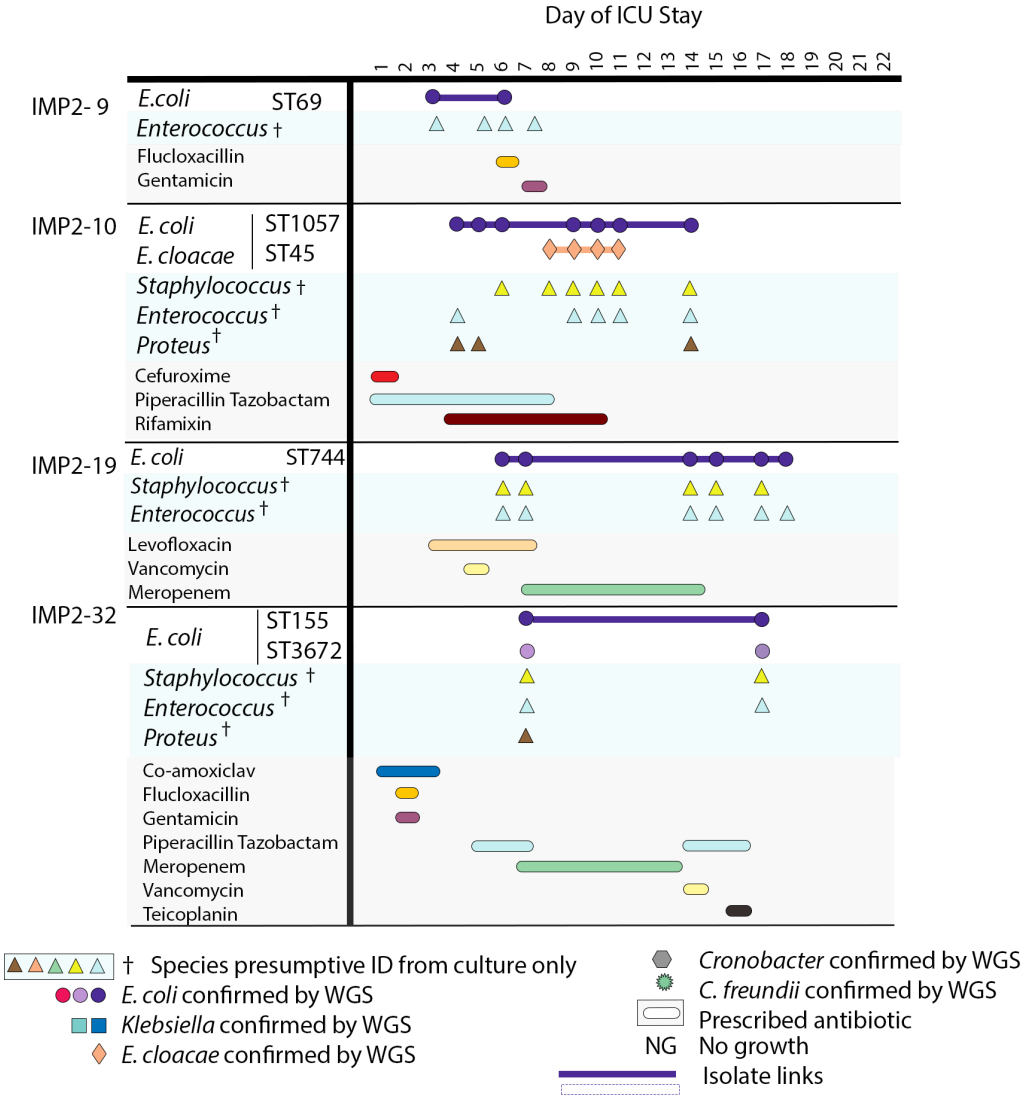


Figure 4.22 Colonising isolates in patients where *E. coli* colonisation was stable during inpatient stay. All species confirmed using WGS are displayed alongside species presumptively identified using culture plates only. Antibiotics prescribed during a patient stay is displayed below. Patients were in the ICU at differing times but for the purpose of this figure the timeline is the inpatient stay day number. In cases where STs are the same they are shown on this figure as the same colour when they occurred in the same patient, but these colours cannot be compared across patients. Isolate links demonstrate with a solid line where colonisation occurs with a solid line and where this ST strain is interrupted by another ST, a dashed line is used.

4.3.7 Colonising *E. coli* isolates and the effect of prescribed antimicrobials

Twenty-two antibiotics were prescribed for the ICU patients in the study in a variety of combinations. Most patients (n=17) in this study were prescribed piperacillin-tazobactam or meropenem or both or at some point during their stay. Therefore, the effect of these two antimicrobials on *E. coli* colonisation was investigated. In some cases, both or one of these antimicrobials appeared to play a role in loss of colonisation with an *E. coli* ST, but there was no clear pattern. Overgrowth of *Staphylococcus or* Enterococcus species frequently replaced the lost *E. coli* ST (e.g., IMP2-8) and on occasion another *E. coli* ST replaced the one that was lost (e.g., IMP2-20) (Figure 4.17-4.22). Some patients were treated with meropenem or piperacillin-tazobactam prior to the detection of the colonising ST, but not at the same time as colonisation (e.g., IMP2-6). The analysis was further complicated because patients were treated with multiple combinations of additional antimicrobials covering different drug classes during their stays (Table 4.4).

Table 4.4 – Colonisation loss of STs that occurred after prescriptions with A) piperacillin-tazobactam and B) meropenem.

A)

Patient	Colonising ST	Colonising ST lost after Piperacillin - Tazobactam prescription
IMP2-8	ST69	X
IMP2-10	ST1057	X
IMP2-13	ST69	✓
	ST1057	✓
	ST1314	✓
IMP2-14	ST642	✓
	ST2165	X
IMP2-20	ST3594	X
IMP2-21	ST58	X
	ST2521	✓
IMP2-23	ST73	✓
	ST58	✓
IMP2-24	ST10	X
IMP2-26	ST69	X
IMP2-29	ST141	✓
IMP2-32	ST155	X
	ST3672*	✓

B)

Patient	Colonising ST	Colonising ST lost after Meropenem prescription
IMP2-5	ST73	✓
IMP2-8	ST69	✓
IMP2-14	ST642	X
	ST2165	✓
IMP2-16	ST69	X
	ST131	X
IMP2-19	ST744	X
IMP2-21	ST58	X
IMP2-24	ST10	X
IMP2-25	ST1326	X
IMP2-26	ST69	✓
IMP2-32	ST155	X
	ST3672*	X

* indicates a single strain of ST3672 was lost after prescription with meropenem.

4.3.8 Colonising isolates and clinical infection isolates

A review of patient medical history identified clinical infection isolates from all microbiology samples and their related phenotypic antibiotic sensitivity profiles. Patient medical history allowed a comparison of species found colonising a patient and any isolates of the same species found causing infection during their ICU stay. This study found cases where the genotypic resistance profiles of the colonising isolate were consistent with the phenotypic sensitivity profile reported in the clinical infection isolate, potentially indicating they could be the same strain (Table 4.5). Unfortunately, due to the impact of COVID-19 access to the clinical isolates was not possible so whole genome sequenced for direct comparison could not be carried out.

The colonising IMP2-24 ST12 *K. oxytoca* was an example of a colonising isolate that could also be causing infection in a patient during their stay. This *K. oxytoca* carried a genotypic resistance profile that could be linked to the patient's clinical sputum isolate present on day 11. IMP2-5 provided another an example as they had a *K. oxytoca* colonising stool on day 7 and a bloodstream isolate on day 8 of the ICU stay which had genotypic and phenotypic resistance profiles that could be linked (Table 4.5).

These examples of potential matches between genotypic resistance profiles of colonising *E. coli* and clinical isolate phenotypic antibiotic sensitivity profiles, which might indicate that the colonising species and infection isolates were the same, require further confirmation by WGS of clinical isolates, using sequence typing and SNP analysis to prove this link. The need for this is demonstrated in IMP2-25 where colonising ST1326 and ST1277 in IMP2-25 have the same resistance profiles and either or both could potentially be linked to the ESBL positive *E. coli* in clinical isolates from urine (day 7) and on a wound swab (day 14) during their inpatient stay.

Table 4.5 Phenotypic profiles of clinical isolates and colonising species and their genotypic sensitivity patterns.

Patient	Clinical Isolate	Clinical Isolate Source	Clinical Isolate Phenotypic Sensitivity Profile and Notes	Potential Colonising Isolate Match	Colonising Isolate Gentypic Sensitivity Profile
IMP2-5	*E. coli*	Urine	Amoxicillin:R, Augmentin:R, Ciprofloxacin: S, Cephalexin: S, Gentamicin: S, Nitrofuratoin:S, Trimethoprim:S	*E. coli* ST73	*tet*(D), *ant(3'')-Ia, sul1,* $bla_{SHV-102}$
IMP2-5	*K. oxytoca*	Bloodstream	Amoxicillin:R, Augmentin: S, Ciprofloxacin: S, Meropenem: S, Tazocin:S	*K. oxytoca*	$bla_{OXY-2-2}$, *oqxA, oqxB, fosA*
IMP2-10	*E.coli*	Sputum	Amoxicillin:S, Augmentin:S, Ciprofloxacin:S , Gentamicin:S, Meropenem:S and Tazocin:S	*E. coli* ST1057	*No acquired resistance genes*
IMP2-23	*K. pneumoniae*	Urine	Amoxicillin:R, Augmentin:S, Ciprofloxacin:S, Cephalexin:S, Gentamicin:S, Trimethoprim:S	*K. pneumoniae* ST323	*oqxA, oqxB, fosA6, fosA7,* bla_{SHV-99}
IMP2-24	*K. oxytoca*	Sputum	Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Meropenem:S, Gentamicin:S. Tazocin: R	*K. oxytoca*	$bla_{OXY-6-4}$, *oqxA, oqxB, fosA*
IMP2-25	*E.coli*	Wound Swab	ESBL Positive. Ciprofloxacin: S, Ertapenem:S, Meropenem:S	*E.coli* ST1362/ST1277	*aadA5, aph(6)-Id,* bla_{CTX-15}, *dfrA17, mph*(A), *qnrS1, strA, sul1, sul2, tet*(A)
IMP2-25	*E.coli*	Urine	ESBL Positive. Amoxicillin:R, Augmentin :S, Ciprofloxacin: S, Cephalexin :R, Ertapenem:S, Fosfomycin:S, Gentamicin:S, Mero:S, Nitrofurantoin:S, Trimethoprim:R	*E.coli* ST1362/ST1277	*aadA5, aph(6)-Id,* bla_{CTX-15}, *dfrA17, mph*(A), *qnrS1, strA, sul1, sul2, tet*(A)
IMP2-26	*K. pneumoniae*	Sputum	Augmentin:S, Ciprofloxacin:S, Meropenem:S, Tazocin:S	*K. pneumoniae* ST1597-2LV	*oqxB, fosA,* bla_{LEN24}

4.4 Discussion

This IMPACT2 study of the patient gut colonisation dynamics during QEHB ICU stay revealed limited ST diversity, acquisition, and transmission of colonising *E. coli*. This study is the first report providing strain level information about the species found colonising QEHB ICU patients including their AMR genes and plasmids replicons. It revealed a diverse set of 22 colonising *E. coli* STs, with 11 patients colonised with more than one ST during their patient stay and their colonisation dynamics. The findings of this study were consistent with metagenomic analysis carried out in a previous study (182). Ravi et al. (182) previously reported 5 of 24 QEHB ICU patients had microbiomes where *E. coli* was a peak pathogen, and similarly *E. coli* was the most predominant pathogen in four of these patients, and it clearly remained an important colonising organism in the ICU during the current study. Although the time limitations caused by COVID-19 pandemic on this latest study meant that microbiome analysis

could not be performed on these samples, in future studies this technique should be used to determine overall *E. coli* abundance in cases where *E. coli* colonisation occurs. In the 2017 metagenomic study other important species identified included *E. cloacae*, *Klebsiella* and *Enterococcus*, where these species were found to be peak pathogens and to increase in relative abundance in long stay patients. Enterococcal blooms were observed during the 2017 ICU patient stay and similarly *Enterococcus* together with *Staphylococcus* appeared to play a role in gut colonisation in the current study where their overgrowth apparently reduced the presence of Gram-negative species (182). Further WGS identification of *Enterococcus* species and microbiome analysis would clarify any effect and confirm the overgrowth indicated by the culture-based techniques in the current study.

Analysis of the strain-level colonisation dynamics of the *E. coli* STs detected in the ICU, that had not been previously possible with metagenomic techniques, revealed that although there were some examples of acquisition (Figures 4.17- 4.18) and loss (Figure 4.21) of *E. coli* strains, there was less dynamic activity than was expected from nosocomial transmission events reported around the globe (187, 272-274) and no identifiable pattern. There was no evidence of any circulating strain and there was very little transmission of *E. coli* strains within or between ICU wards. This might be a consequence of the ICU environment at the time of the study being very shielded due to COVID-19 restrictions, raising a question as to whether acquisition or transmission levels would be higher in different circumstances.

There were 15 events of *E. coli* strain acquisition during the patient stay where previously uncolonized patients acquired *E. coli* or a new strain of *E. coli*. There were two cases of *Klebsiella* acquisition by patients, a *K. oxytoca* strain and a *K.*

pneumoniae ST323. Ideally in future an increased number of isolates per sample should be sequenced to give a broader representation of colonising isolates and used confirm acquisition of strains. This would remove the possibility that the colonising isolate selection process could bias selection towards the most abundant strain colonising a patient, and that a strain classified as an acquisition which was present in an initial sample but at a low level so remained undetected. A previous UK haematology ward transmission study also reported that single colony studies may underrepresent transmission events (272) so increased isolate sampling would confirm the lack of transmission seen on the QEHB ICU.

Pandemic lineages are a significant concern of AMR spread (66, 69) and there was evidence of rare acquisition of pandemic lineages during patient stays on QEHB ICU, which was less than expected in comparison to pandemic lineages reported colonising and infecting patients in other countries (273, 275, 276). Pandemic lineages are known to cause healthcare acquired infections and are found in the hospital environment (69) but in this investigation only a few examples of ST69 (n=7) ST73 (n=2), ST10 (n=1) and ST131 (n=1) pandemic lineages strains were identified. Only ST10, ST69 and ST131 were acquired during patient stay on the ICU (Figures 4.17-4.18). ST69 was found in the greatest number of patients in the study and was also the most abundant ST identified colonising ICU patients, however there was no circulating ST69 strain, the acquisitions were distinct strains. In line with reports in ICUs around the globe (187, 277, 278) where ST69 was detected but transmission was not frequently reported, 3 QEHB patients acquired ST69 on their ICU stay and no ST69 transmission was detected. ST69 is a pandemic lineage known to cause invasive disease including meningitis and bacteraemia (85, 189, 279). The consequences of acquisition of ST69

could therefore be very concerning if the multiple ST69 strains (n=7) detected 6 of 18 QEHB patients went on to cause infection in this vulnerable patient population. ST69 is not highly drug resistant normally (69, 280), however drug resistant strains have been seen in ICU studies in China (187) and Spain (277) which report the presence of ESBL-producing ST69 with *bla*$_{\text{CTX-M-24}}$ and a carbapenemase resistant ST69 carrying *bla*$_{\text{OXA-48}}$ on admission respectively. In QEHB only four strains in three patients carried ESBL genes (*bla*$_{\text{TEM-1A}}$ and *bla*$_{\text{TEM-1B}}$) but colonisation is a potential route for resistance spread in the QEHB ICU as two acquired ST69 strains carried ESBL genes. Colonisation with ESBL-producing isolates may facilitate transfer of these ESBL genes to other STs and species, if the ESBL genes are carried on a plasmid or other MGEs and promote the spread of AMR.

The globally disseminated pandemic lineage *E. coli* ST131 is known to be transmitted in the healthcare environment and to cause outbreaks (68, 85, 189, 190, 276, 281) .Hospital infections and outbreaks (187, 276, 282, 283) , including carbapenemases-producing ST131 in China (281), demonstrate the danger of ST131 outbreaks in a hospital setting. Transmission and acquisition of ST131 *E. coli* in hospital has been clearly demonstrated by a follow up of ESBL *E. coli* infections in a Swiss study, including those caused by ST131, which found a 4.5% transmission rate from an index case of ESBL *E. coli* in hospital to another hospital patient (284). In the UK ST131 has been reported as a major cause of invasive infection (85, 189) (190), which is known to carry ceftriaxone resistance. A recent longitudinal Oxford hospital study for example reported over 52% ceftriaxone resistant ST131 isolates (190). Acquisition of ST131 did occur in on QEHB ICU (Figure 4.18), but only in one QEHB ICU patient (IMP2-16). The single ST131 acquired in this QEHB study was identified as a ST131

Clade A isolate which is known to be largely drug susceptible, lacking *bla*$_{CTX-M}$ and FQR genes. Since ST131 clade A has been a prominent cause of infection in other countries, including Norway (189) any ST131 acquisition in the ICU is still a cause for concern even when it is not a pandemic subclade e.g. ST131-H30R1 and ST131-H30Rx (68, 232, 276) (Section 1.5). In this study there was no evidence of ST131 transfer between patients on the QEHB ICU, just one instance of patient acquisition (IMP2-16, Figure 4.18) but in view of the potential threat this situation needs to be monitored.

The third pandemic lineage detected in this study seems to be an example of colonisation which was acquired in the community and brought into the ICU. Different strains of ST73 were found in the first stool sample of two patients who both arrived in the ICU not having spent time in the QEHB general wards and were the only cases of ST73 in the study. In both cases the ST73 strain was lost during the patient ICU stay and was not detected in other patients so was not circulating on or between ICU wards. ST73, like ST69, is reported to be a mainly susceptible ST (69, 280) and cause invasive disease including bacteraemia and meningitis (85, 189, 279, 285) ST73 is a known major cause of community and hospital UTIs in UK (286), but reports of ST73 acquisition in healthcare settings are minimal, with studies mainly focussing on ST73 clinical infection isolates (84, 272, 273). Although the two ST73 strains identified in QEHB ICU were ESBL carriers (*bla*$_{SHV}$ or *bla*$_{TEM}$) neither strain carried a high level of resistance or *bla*$_{CTX}$ genes. ST73 is a pandemic lineage carrying ESBL genes found colonising ICU patients and like ST69 is something to be monitored going forward, to prevent patient acquisition of colonising ESBL isolates during an ICU stay.

ST58, was an example of transient acquisition, it was found colonising the first stool sample of IMP2-21 and was then transiently acquired by IMP2-23. This transient acquisition of ST58 on the QEHB ICU was interesting because ST58 is an emerging uropathogen in humans across the globe (287, 288) with an increase in ST58 infection reported recently in France (289). Colonisation of healthy individuals with ST58 has also been seen in Netherlands (290) and ST58 is a known cause of ExPEC infections (287). ST58 is also of interest as it is in phylogroup B1 which are normally environmental isolates (291), but ST58 is now identified as an infection concern in humans. The QEHB study did not include environmental samples or samples from healthcare staff, so it is not possible to infer transmission direction or source. IMP2-21 could either have arrived in the ICU colonised with ST58 or acquired ST58 during the 6 days before their first stool sample. IMP2-23 likely acquired this strain from IMP2-21, as IMP2-23 entered and left ward during the lengthy ICU stay of IMP2-21. This acquisition is unlikely to have occurred through direct contact between patients as although both patients were stationed WCCB their bed bays were not close to each other. It is therefore likely that the source of any transfer of this strain would be from the hospital environment or staff.

In addition to the strain sharing of ST58 a second non-pandemic strain example of strain sharing was seen with ST1057. Although ST1057 is not frequently reported as a major cause of infection it has been reported to cause invasive disease. A literature search revealed only one study discussing ST1057, where (292) a single isolate carrying *strAB*, *drf*, *bla*$_{TEM}$ and *mdf(A)* caused bacteraemia. However an inspection of bacteraemia data collected in nationally Norway (n=13) (189) and England (n=1) (85) and in Oxford (n=15) (190) indicates that ST1057 is a minor and low level cause of

invasive disease. Therefore, it is important to consider the potential transfer of this ST in an ICU patient cohort. The origin of ST1057 colonisation, like that of ST58, cannot be confirmed due to the absence of environmental and staff sampling.

Other problematic species found colonising ICUs elsewhere include *Klebsiella* species (293-297) which can carry high levels of resistance, including ESBL plasmids (298). *Klebsiella* species colonised only six patients in this study and in contrast to the colonising *E. coli* population there was no evidence of shared patient strains. The identification of a colonising ST20 *K. pneumoniae* is consistent with previous reports where ST20 *K. pneumoniae* high risk clones caused outbreak on hospital wards around the globe (298, 299). The persistent QEHB ST20 strain was in the first sample of IMP2-20 and was not acquired by any other ICU patients, it also did not carry some of the concerning levels of resistance including bla_{CTX} and bla_{NDM} seen in other ST20 clones causing outbreaks reported across the globe (300). Persistent faecal carriage with ST20 has also been reported for long periods of time in healthy individuals so colonisation in the community might explain why this ST appears in the patient's first stool sample (301).

An important feature of this study was that it not only identified acquisition of strains it also detected levels of resistance in colonising organisms on the ward. Multidrug resistance levels were low in all species identified using WGS and only one CPE strain was discovered colonising patients. Although colistin resistance is found in healthcare settings in other countries (302) there was no evidence of colistin resistance on the ICU, this is consistent with the low reported levels in the UK (182). Examples of ESBL outbreaks in ICUs (including NICUs) leading to higher mortality have been seen across Europe (303). In total only 11 QEHB patients were colonised with ESBL *E. coli* isolates

(e.g., carrying *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$) with the majority of these patients (n=9) carrying only *bla*$_{TEM}$ β-lactamase resistance genes. *bla*$_{TEM}$ produces a narrow-spectrum beta-lactamase which can be inhibited by β-lactamase inhibitors (e.g., clavulanic acid, tazobactam) so is treatable and can be controlled if used in conjunction with good IPC precautions on the ICU.

Our study also highlighted the reassuringly low level of CPE carriage in QEHB ICU patients. Importantly the only CPE isolate in this dataset was the *bla*$_{KPC-2}$ *Citrobacter* isolate in IMP2-20. This CPE isolate was only identified in the patient's first ICU baseline stool sample on day 6 and so not likely acquired in the ICU. This CPE was not identified colonising any other patients in the study so there is no evidence for this CPE being spread or circulating in the ICU. Since IMP2-20 had been admitted from home, not another hospital, the *Citrobacter* colonising IMP2-20 was probably acquired in the community or travel, but they spent 3 days on another general ward before admission to QEHB ICU.

A comparison of antimicrobial prescribing data to the presence of colonising isolates and their resistance carriage did not indicate any consistent link between the use of antibiotics and the loss of a colonising species in a patient, differing from what was reported in a previous study (304) or reveal any noticeable change in resistance profiles of colonising STs.

The effect of COVID-19 precautions during the time of sample collection in this study must be recognised to give context to the study. The COVID-19 pandemic had a big impact on the ICU and wider hospital environment, and this must be taken into account where results differ from other previous colonisation studies in QEHB (182) and

elsewhere in the UK (272). COVID-19 precautions meant reduced the opportunities for contact with community colonising isolates once a patient was on the ICU since at the time visitors had very limited access to the ward. Patients were also much less likely to have travelled globally and therefore have entered the ward colonised with an ESBL or CPE. The colonisation detected in the first baseline stool sample is likely to reflect local community carriage in the UK at time of admission rather than a more national or global picture that may have been seen previously. This study provides a snapshot of colonisation of QEHB ICU in very unusual conditions when samples were collected in a shorter timeframe than originally planned and when ICU patients were potentially shielded from more sources of colonisation would be expected in pre-COVID circumstances.

A limitation in this study was that it was not possible to sample the colonising strains in the ICU environment. Additional sampling of bed bays, healthcare staff and other shared equipment in future studies would help to define whether *E. coli* and *Klebsiella* acquisition occurred during the hospital stay before the first stool sample was passed and to identify potential routes for acquisition. Although the examples of shared strains in this study are of very drug susceptible isolates, these isolates still have the potential to cause infection in these vulnerable patients and their shared occurrence highlights a potential route for transmission of drug resistant isolates between the environment, staff or patient to patient contact.

This overview of specific Gram-negative organisms, including *E. coli* and *Klebsiella,* in this study gave a better understanding of the wider picture of colonisation and colonisation changes on the QEHB ICU ward during a time of COVID-19 restrictions. It helped characterise the AMR carriage found in colonising *E. coli* in a UK ICU and

highlighted the low levels of resistance found compared to other countries around the world. A focus on the colonisation story for each individual patient showed whether these Gram-negative organisms were acquired on the ward and the impact of antimicrobials had on patient colonisation.

5. SENTINEL: **S**equ**EN**cing of his**T**orical clin**I**cal *Escherichia coli* from the **NE**ther**L**ands

5.1 Introduction

E. coli is acknowledged to be an important clinical pathogen globally, and the cause of invasive diseases such as meningitis and bacteraemia. It causes invasive infection in the immunocompetent and immunocompromised, across all age groups both in hospital and community settings. As a consequence in many countries there has historically been significant surveillance of clinical *E. coli* populations at a national level.

Analysis of historical longitudinal datasets of clinical *E. coli* causing invasive disease has been crucial in elucidating the changes to invasive *E. coli* population structures and highlighting the predominant lineages causing infection at specific time points (85, 189, 190). Pandemic lineages and their evolution into more successful pathogens can also be explored using these datasets. In addition, these longitudinal datasets enable study of the emergence and spread of new antimicrobial resistant clones. There have been three major longitudinal studies of invasive *E. coli* national collections in recent years using isolates from England and Norway. A systematic longitudinal survey investigated 1509 *E. coli* isolates collected over 11 years (2001-2012) at a national level by British Society for Antimicrobial Chemotherapy (BSAC) (n=1094) and a local level Cambridge University Hospitals NHS Foundation Trust (CUH) (n=415) (85). A more recent longitudinal study over 10 years (2008-2018) in Oxford investigated 3461 *E. coli* causing bloodstream infections. Another longitudinal study in Norway

investigated 3254 *E. coli* isolates sampled from 22,512 bloodstream infections collected nationally between 2002-2017 (189). Predictably B2 phylogroup caused most of the infection in the English and Norwegian longitudinal collections, with 66-67% of *E. coli* isolates in these three studies belonging to B2. The B2 phylogroup is known to frequently be a source of invasive infection, as it contains extraintestinal pathogenic *E. coli* (ExPEC), including the pandemic lineages ST131 and ST1193 (57, 68, 86).

Across the globe various sequence types have been previously linked to invasive disease (including bacteraemia, meningitis). Since 2000 ST131, ST73, ST95, ST1193 and ST69 (49, 85, 189, 279, 285, 305-307) have been most commonly reported both in short term and longitudinal studies to cause invasive disease. Kallonen et al (85) reported a diverse range of STs causing bacteraemia infection in England with the most frequent STs being ST73 (17%), ST131 (14%) and ST95 (10%). Other key STs in English bacteraemia isolates included ST69 and ST12 (85). This was supported by another English study and a Scottish bacteraemia study where ST131, ST69, ST73 and ST95 were predominant causes of community and healthcare infections (190, 279). In Norway predominant STs causing bacteraemia were similar to those reported in England and included ST73, ST95, ST69, ST131 and ST14 (189). Population analysis in these three longitudinal studies demonstrated a prevalent E. coli ST does not have to be MDR, as susceptible STs remain prevalent and are consistently present in *E. coli* populations over decades (85, 189, 190). Susceptible STs can undergo population expansion at a greater rate than MDR STs, for example, ST73, which is frequently susceptible to antimicrobials, was found to be a predominant cause of infection (85, 189) and so demonstrated that multi-drug resistance was not a critical

factor in the prevalence of a ST in these populations. Norwegian and English studies (85, 189) also reported the importance of the globally disseminated, pandemic lineage ST131 in causing bacteraemia and explored the ST131 population structures over time. Data collected in Norway highlighted the predominance of the susceptible ST131 clade A in the bacteraemia isolate population, this clade was found at much higher levels than in the English ST131 population(189). The BSAC/CUH (85) and NORM (189) datasets additionally demonstrated ST131 population expansion was not driven by the emergence of resistant subclades (e.g., Clade C2).

Several longitudinal European studies (85, 189, 190) only included invasive *E. coli* isolates associated with bacteraemia but not E. coli sourced from other invasive infections, for example meningitis. At present there is limited data from genomic studies that investigate historical *E. coli* populations isolated from CSF causing meningitis. Previous investigations of *E. coli* causing meningitis have been small in size and were not focussed on detailed genomic analysis. These investigations included two French overlapping national studies that have investigated *E. coli* isolates causing meningitis, which reported the key role of the B2 phylogroup and ST95 clones as causes of neonatal meningitis from 1998-2015 (305, 308). Little longitudinal genomic data focussed on invasive *E. coli* isolated from a neonatal population has previously been available. Neither the English nor the Norwegian datasets link study results to the age groups of patients from which the isolates were collected (85, 189, 190).

The SENTINEL study is an investigation of a unique historical collection of clinical *E. coli* isolates causing invasive disease that were collected by the National Reference Laboratory of Meningitis (NRLBM) in the Netherlands over a 47-year period (1975-2021). This is a much longer period of time than investigation in the previous English

(10 (190) and 11 years (85)) and Norway (16 years (189)) genomic studies, providing a much greater opportunity to observe *E. coli* population dynamics. This NRLBM historical collection of 1790 isolates from 1446 patients included CSF isolates (47%) and bloodstream isolates (51%) giving a novel insight into STs important in causing both meningitis and bacteraemia. Another interesting aspect of the SENTINEL dataset is that the majority (69%) of isolates were collected from neonates (0 – 1 month old) allowing for the first time a greater understanding of *E. coli* populations causing invasive disease in this young and vulnerable age group. This SENTINEL dataset explored the fluctuation of *E. coli* ST populations causing invasive disease in the Netherlands over almost half a century and how this related to antimicrobial susceptibility status. It allowed a detailed analysis of the decline and disappearance of an initially predominant lineage, ST567, over the study period. SENTINEL also provided insight into the resistance profile of invasive disease-causing *E. coli* meningitis and bacteraemia in a country with good antimicrobial stewardship and prescribing practices.

5.2 Methods

Short read sequencing and long read sequencing were used to explore this longitudinal *E. coli* collection provided by the NRLBM (Section 2.3.1). The overview of bioinformatic analysis methods used in this study is outlined in Figure 5.1 and full method details are given in Chapter 2.

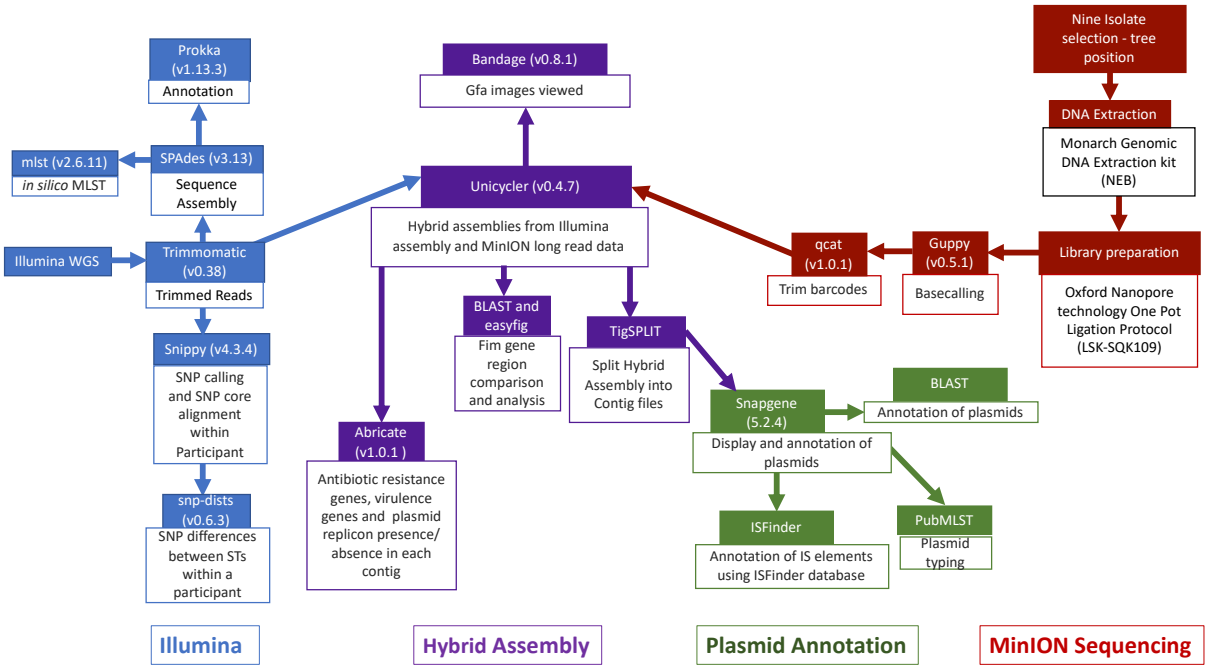


Figure 5.1 *Overview of methods used to create and analyse short read and hybrid assemblies in the SENTINEL dataset.*

Analysis of the ST population over the study period highlighted the decline of ST567. This finding led to further exploration and characterisation of the ST567 dataset (AMR gene carriage, plasmid replicon types and virulence genes), in order to investigate possible reasons for the decline of ST567 population (Method section 2.5). Dated phylogenetic tree analysis was carried out on ST567 (Method section 2.5.11), and the clades of this time tree were explored further by selective long read sequencing (Method section 2.5) and yeast agglutination assays (Method section 2.8).

5.3 Results

5.3.1 Dataset Composition

The study investigated 1790 isolates from 1446 patients. The NRLBM received isolates from an increased number of patients from 2016 onwards (Figure 5.2), reaching a maximum of 93 patients in 2020 compared to 7 patients in 1975.

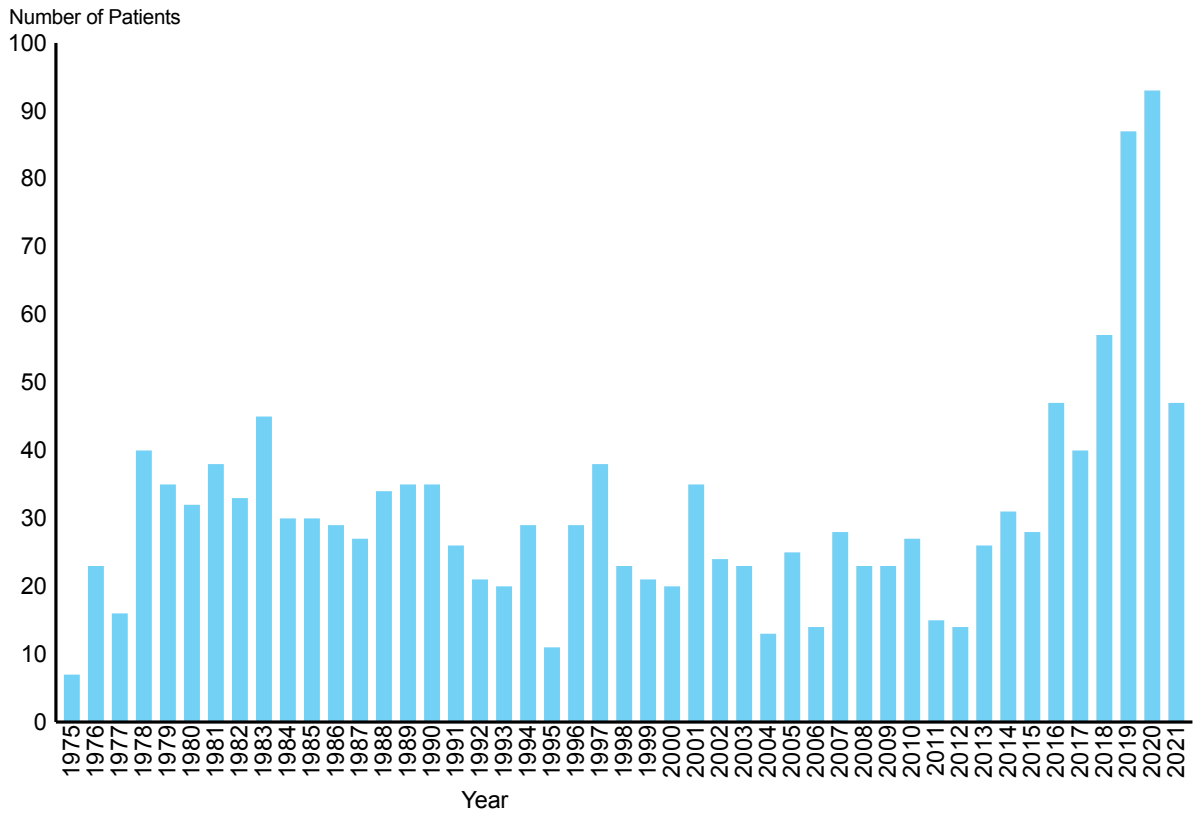


Figure 5.2 Number of different patients submitting one or more isolates to NRLBM per year. Patient is defined by differing NRLBM number (Method section 2.1.3) and in seven cases appear twice where isolates were submitted from the same the patient at different ages.

The isolates that were submitted to NRLBM (n=1790) were collected mainly from neonatal patients that were less than 1 month old (n=1234, 69%). However, the 1446 NRLBM patient ID numbers were linked to patients aged between 0 months and 90 years (median: 0 months). The majority of isolates causing different instances of infection were from patients ranging from 0 - 1 month old (n=1010, 69%) (Figure 5.3). This mainly neonatal dataset also included 18 patients aged 2 - 12 months, 142 were 1 year old and 48 were 2 years old. Only twenty-seven patients were over 18 years old.

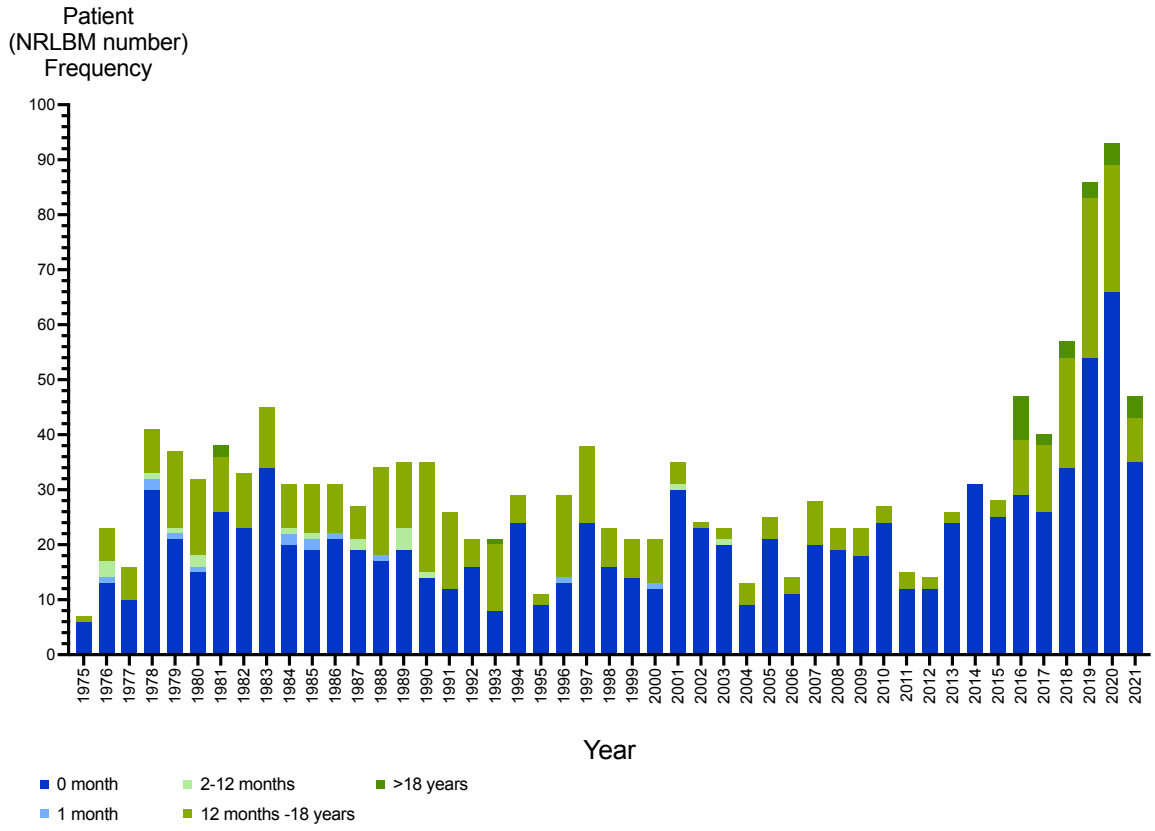


Figure 5.3 Ages of patients submitting one or more isolates to NRLBM per year. Patient is defined by differing NRLBM number. Age categories are split as follows: '0 month' is all patients reported as 0 months, '1 month' is 0 < patient age ≤ 1 months, '2-12 months' is 2 month ≤ patient age < 12 months, '12 months -18 years' is 12 months ≤ patient age < 18 years, and patient age ≥ 18 years is '>18 years'. Seven NRLBM numbers appear twice where isolates were submitted from the same the patient at different ages. One patient here is not included as their age was unknown.

5.3.2 Isolate sources

The largest total number of isolates (n=100) were submitted to NRLBM in 2020 and the lowest number submitted in a year was in 1975 (n=7). The isolate population initially consisted mainly of CSF isolates (Figure 5.4). Over time more bloodstream isolates were submitted to the NRLBM, rising to a maximum of 87% of all isolates collected in 2017 compared to 0% in 1975. From 1998, the majority of isolates collected each year (excluding 2000 and 2004) were sourced from the bloodstream rather than CSF and minority sources. Overall, a total of 916 bloodstream isolates (51%) were included in this study.

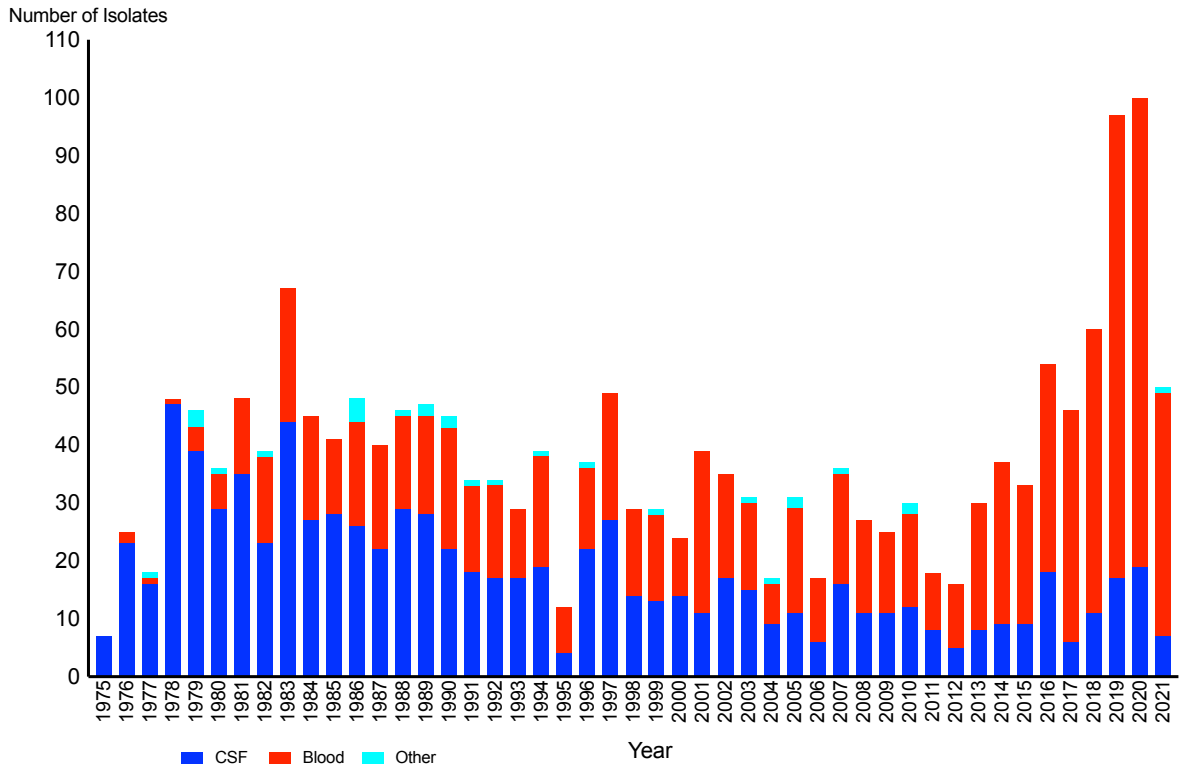


Figure 5.4 Bar chart displaying frequency of isolates per year split into location of isolation. *E. coli* were isolated from CSF isolate (royal blue), bloodstream (red) and other body sites (cyan), including urine/swabs.

5.3.3 A diverse set of STs cause invasive infection

The SENTINEL invasive isolate collection originated from a range of phylogroups and STs (Figure 5.5). Two isolates, NEL0669 and NEL1018, are located on a separate branch from all other isolates, and these identify as cryptic clades III and V respectively. The wide diversity of STs and their phylogroups causing invasive disease can be seen in Figures 5.5 - 5.8.

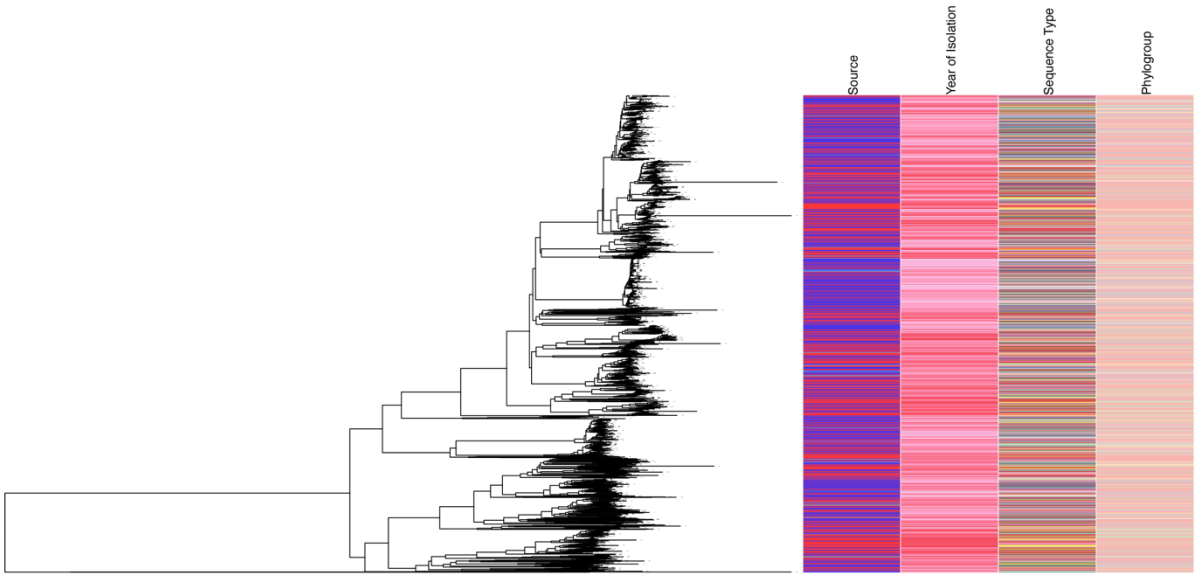


Figure 5.5 Phylogenetic tree created with Mashtree showing all *E. coli* isolates (n=1790) in this dataset. The tree is midpoint rooted and ordered with increasing nodes. Metadata shown alongside includes year of isolation in gradient form (light pink earliest isolates to dark pink most recent isolates) as well as isolate source (CSF-royal blue, blood-red or other-cyan), phylogroup (mixed colours) and ST (mixed colours).

In-depth analysis of STs (Figure 5.6) identified 178 different STs in the 47-years studied. ST95 and ST567 were the most dominant STs over the study period. Pandemic lineages present in this dataset included: ST131, ST1193, ST410 and ST648. Sixty-one isolates could not be sequence typed (Figure 5.6).

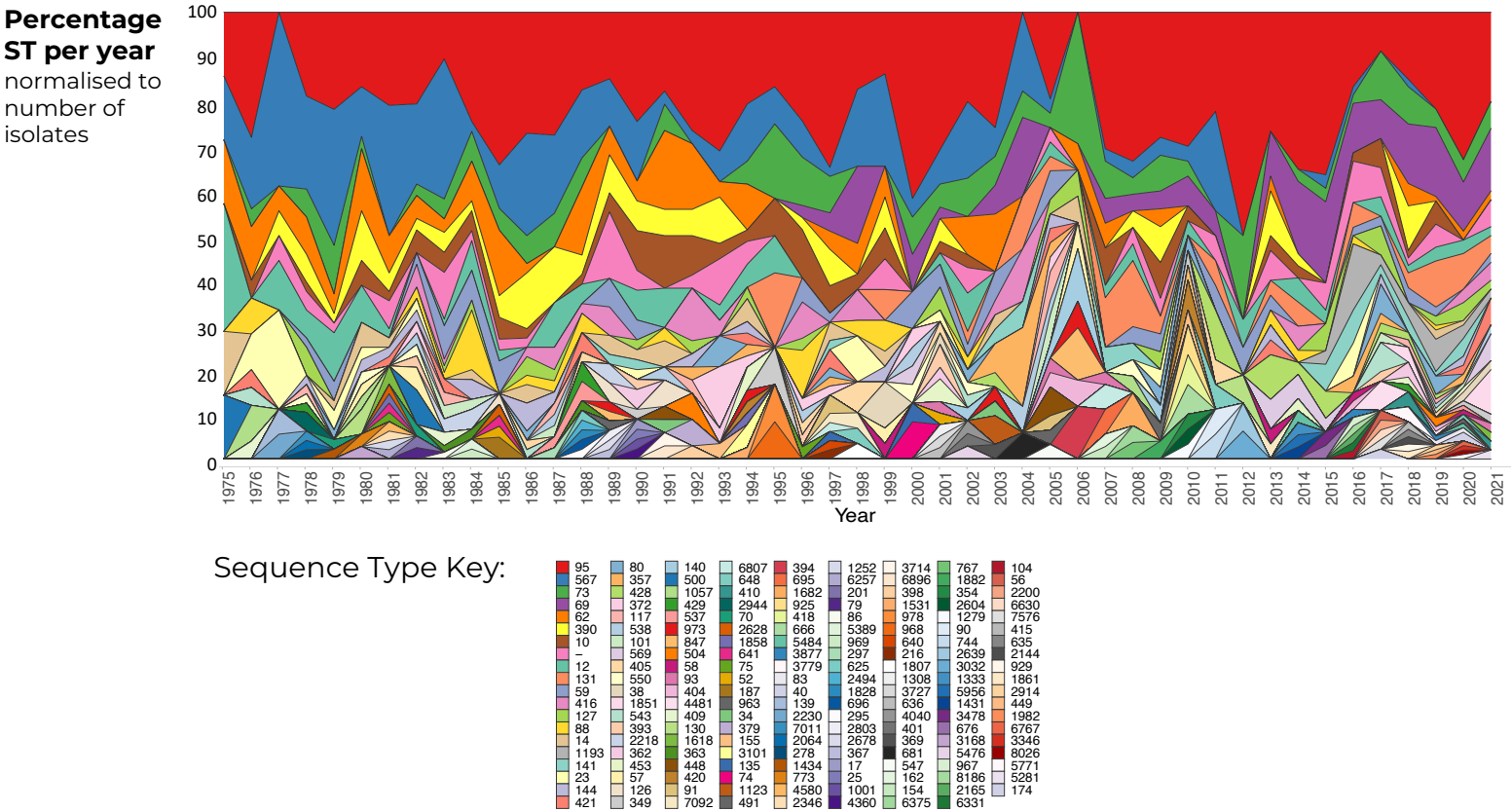


Figure 5.6 Population plot displaying percentage of each sequence type per year normalised to the number of isolates per year. Different sequence types are displayed in different colours. Unknown sequence types are listed under '-' in the key.

The sequence type distribution was further interrogated by focussing on the STs causing 80% of the cases of infection in the SENTINEL dataset. It is now possible to see that as ST567 declines over time and ultimately disappears, ST69 (Figure 5.7, green lineage) and ST73 (Figure 5.7, purple lineage) increase in frequency.

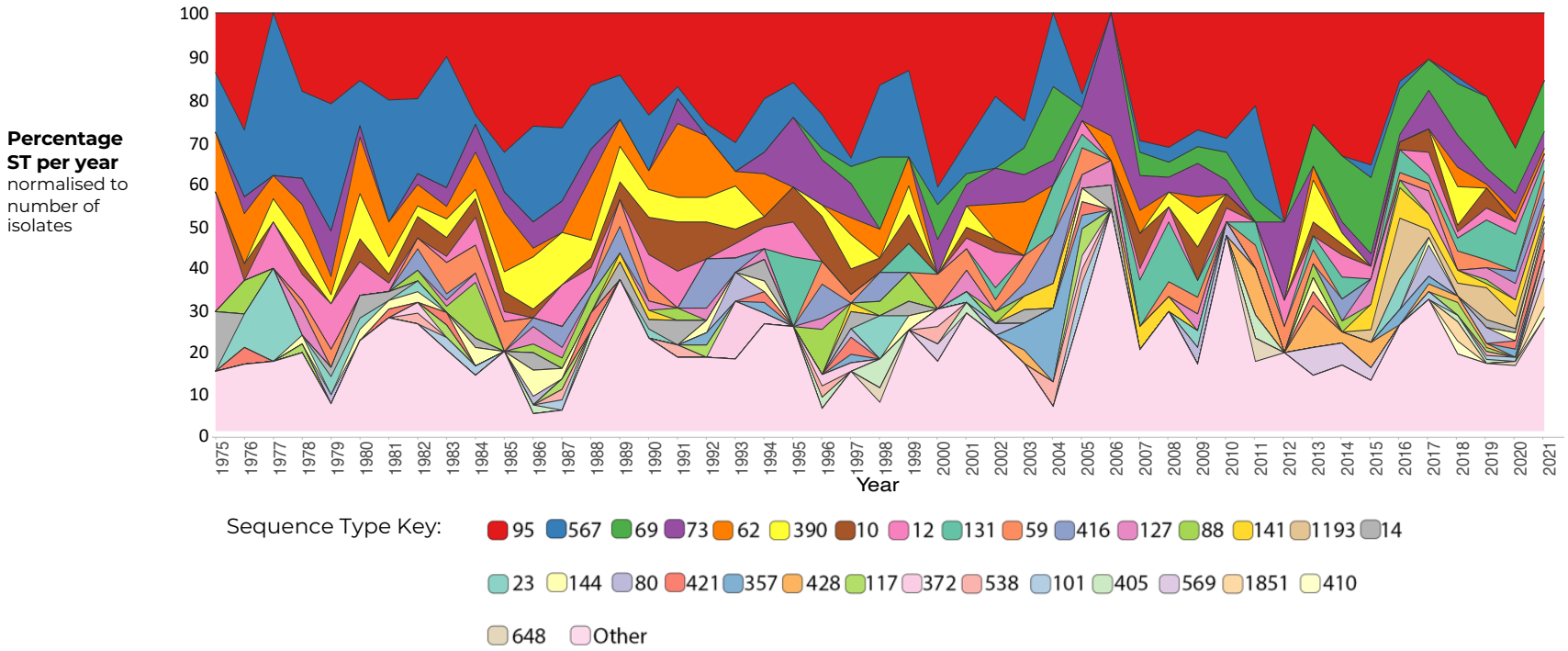


Figure 5.7 Population plot showing sequence type per year normalised to the number of isolates per year shown individually for the 80% most frequent STs in the dataset. Any pandemic lineages not included in the top 80% are also displayed individually (ST410, ST648) and all other STs and unknown STs are described as 'Other'.

Isolates were classified by phylogroup to further characterise the broad range of STs identified in the dataset, especially the uncommon STs. Typing showed that B2 was the most common phylogroup in this collection of invasive *E. coli* isolates (Figure 5.8). In all years B2 accounted for over 50% of all phylogroups identified except 1998 (45%) and 2017 (47%). One isolate could not be allocated a phylogroup.

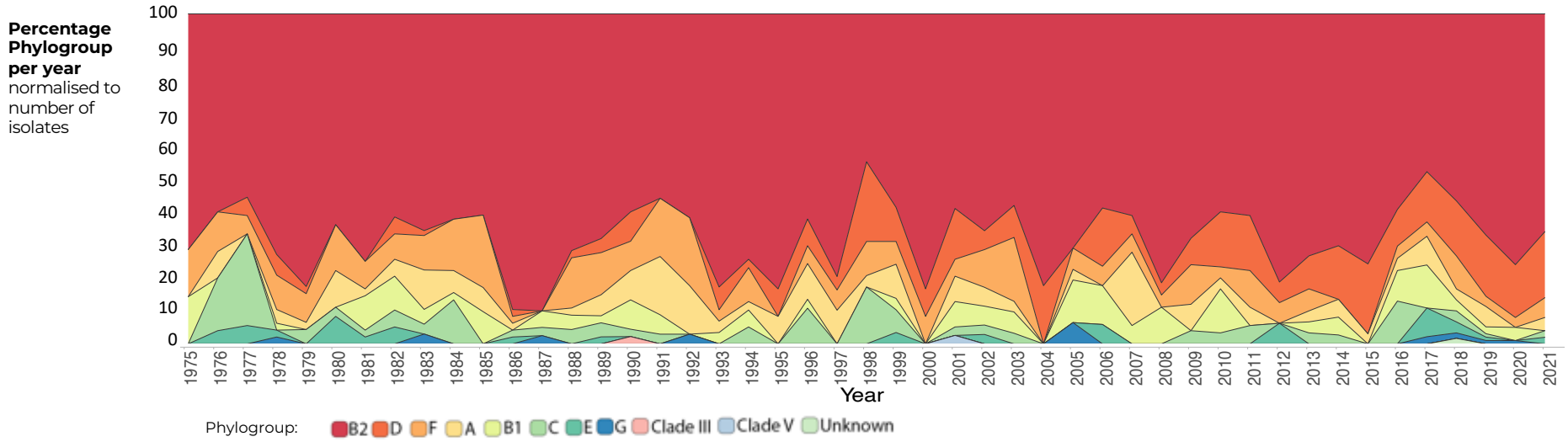


Figure 5.8 Population plot showing phylogroups over the years normalised to the number of isolates per year

One isolate was identified as cryptic clade III (NEL0669) and another as cryptic clade V (NEL1018). Further analysis (Figure 5.9) confirmed the SENTINEL cryptic clade III isolate was located among clade III isolates from environmental and livestock isolates on EnteroBase. The cryptic clade V isolate was located among the clade V isolates from EnteroBase, confirming these cryptic clade allocations for these invasive clinical human isolates are correct.

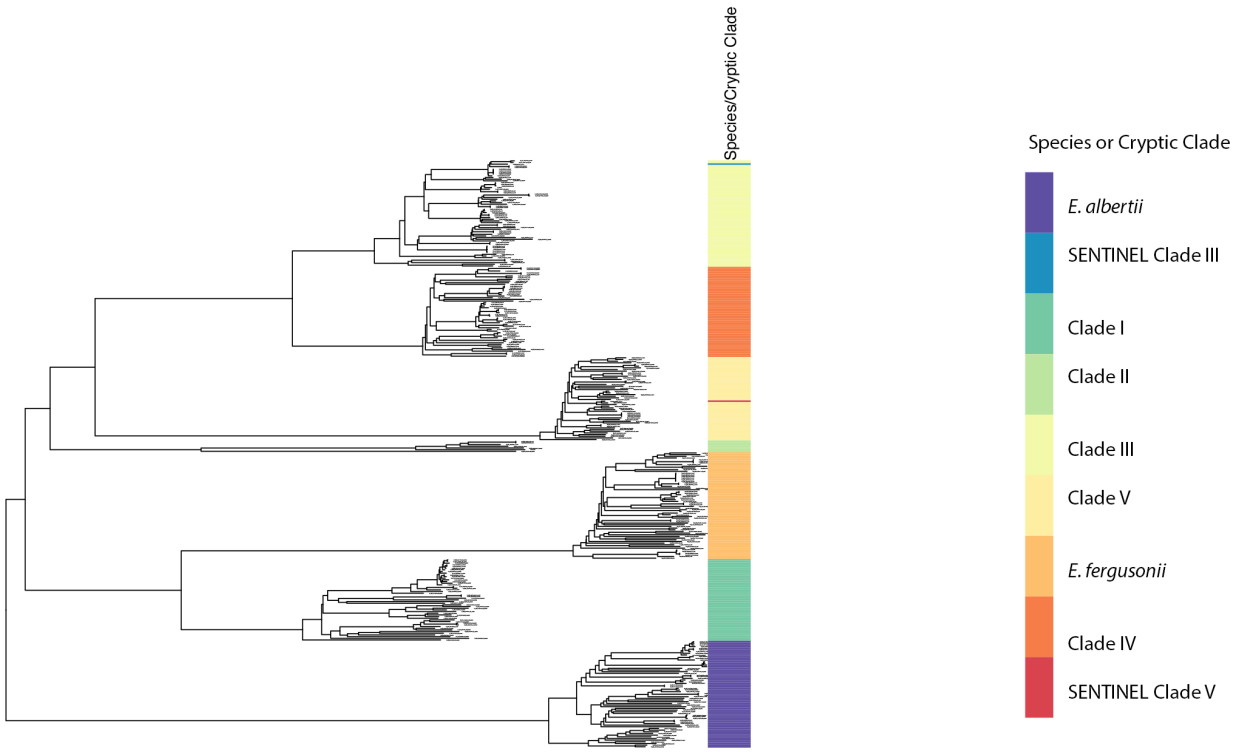


Figure 5.9 Mashtree phylogeny with SENTINEL cryptic clade III and V isolates compared to EnteroBase isolates from Cryptic Clades I-V, *E. albertii and E. fergusonii.*

5.3.4 The search for resistance - SENTINEL invasive *E. coli* resistance gene profiles

5.3.4.1 Antimicrobial resistance carriage was not a driver for the emergence or demise of STs in this invasive *E. coli* population

Fluctuations in ST populations (Figure 5.6), with the emergence of new STs or demise of others, did not affect the amount of overall AMR carriage in these invasive *E. coli*, as there was no trend demonstrating an increase or decrease in acquired resistance genes between 1975-2021 (Figure 5.10). The average number of acquired resistance genes per genome had a median value ranging from 1-5 resistance genes per genome. The highest average number of acquired resistance genes in a genome (n=14) characterised using ABRicate with the Resfinder database, was identified in 2020 and no acquired resistance genes were found in two isolates, one in 1991 and the other 2020. A breakdown of resistance genes from ABRicate (Resfinder) and AMRFinder Plus can be seen in Appendix A5.

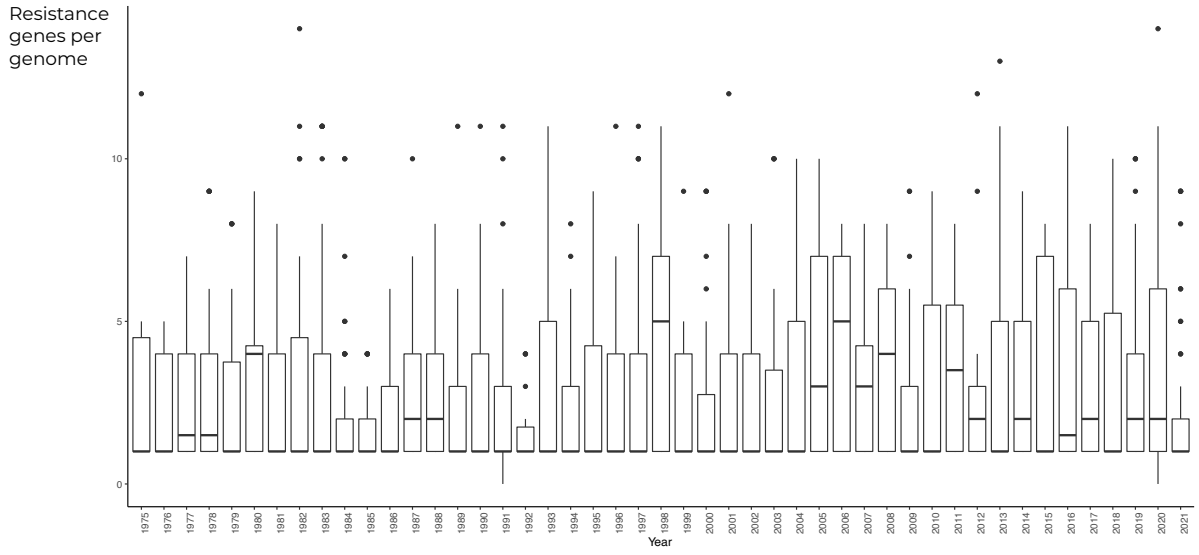


Figure 5.10 Box plot displaying average number of resistance genes per genome per year for all *E. coli* isolates (1975-2021). Bold black line is the median value.

5.3.4.2 Diversity of drug class resistance was not seen to increase in more recent years

The average number of resistance genes per genome did not increase per year, so the type of resistance gene was explored in depth to see if this was impacted by the fluctuations in ST populations. A broad range of resistance genes were identified, and isolates were frequently resistant to multiple drug classes. Similar resistance genes persisted in the population throughout the study period, with no major changes to type of resistance genes observed (Figure 5.11A). If the presence of AMR genes are used as predictors of drug class resistance, in the SENTINEL dataset the most common drug class resistances included: aminoglycoside, tetracycline and sulphonamide (Figure 5.11B). The number of isolates per year does not appear to affect the drug class resistance types observed (Fig 5.11B).

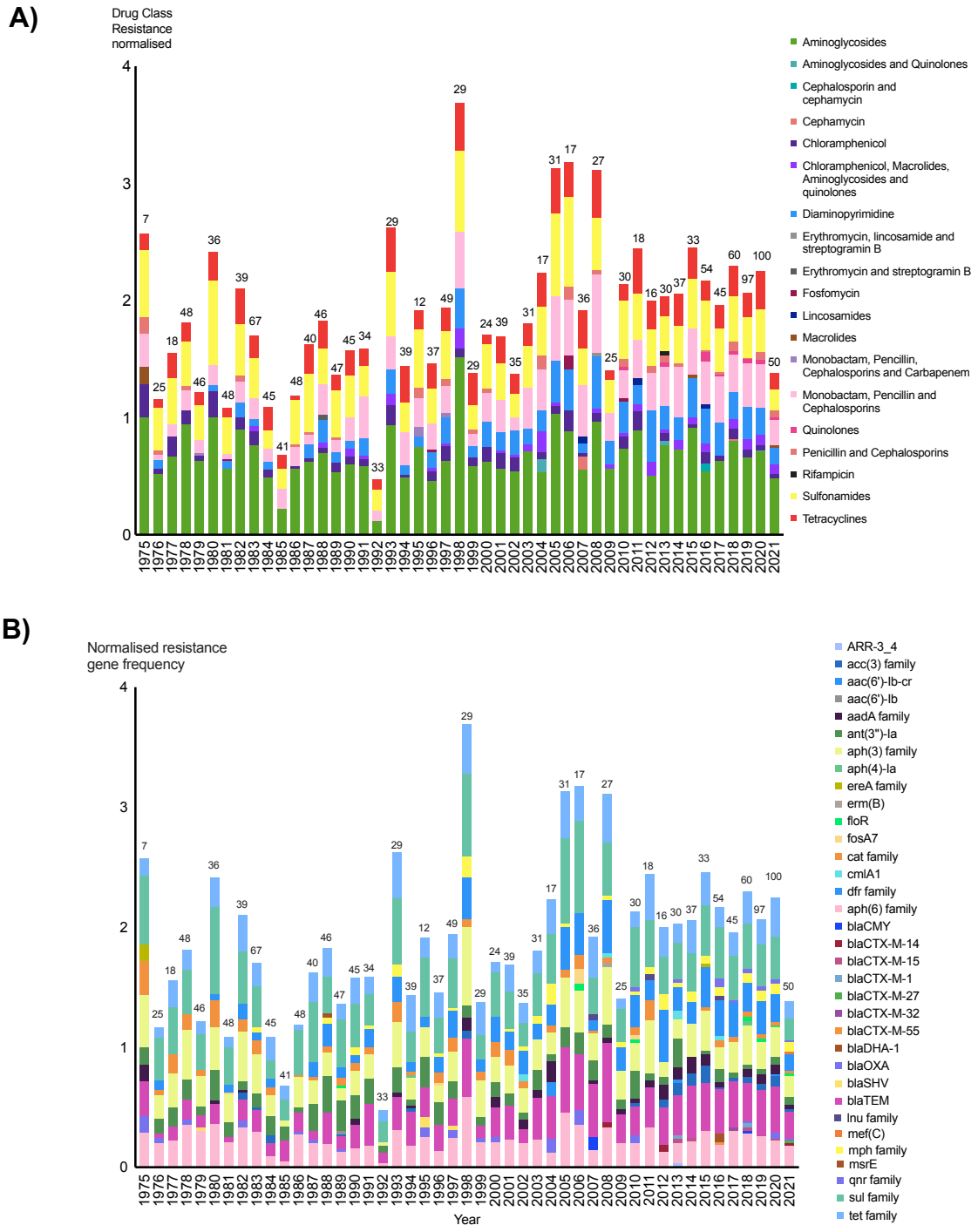


Figure 5.11 Bar charts displaying antimicrobial resistance over the SENTINEL study period showing: A) Frequency of resistance gene normalised to the number of isolates per year. Frequency of isolates in each year can be seen in the black number on top of each bar. Resistance genes are shown in different colours as specified by the key. B) Expected drug class resistance to be conferred by the presence of AMR genes. Frequency of isolates in each year can be seen in the black number on top of each bar. Expected conferred drug class resistance categories are shown in different colours as specified by the key.

5.3.4.3 ESBL-producing *E. coli* were rare

bla$_{TEM}$ resistance genes were predictably common in this dataset (n=469, Figure 5.11B). Surprisingly only a few other beta-lactamase resistance families were identified across the SENTINEL collection (Figure 5.11A, Appendix A5). Between 1970s-2006 only other two types of ESBL genes (*bla*$_{OXA}$ and *bla*$_{SHV}$) were identified in addition to *bla*$_{TEM}$. *bla*$_{OXA}$ genes were the predominant beta-lactamase resistance genes in this period up to 2006, with a maximum of three isolates from two patients in 1998 (Figure 5.12). From 2007 onwards, *bla*$_{OXA}$ genes declined and were replaced by a more diverse selection of beta-lactamase resistance genes, in particular *bla*$_{CTX-M}$ genes. Six types of *bla*$_{CTX-M}$ genes were identified in this dataset from 2008- 2021: *bla*$_{CTX-M-15}$ (n=7), *bla*$_{CTX-M-14}$ (n=3), *bla*$_{CTX-M-1}$ (n=3), *bla*$_{CTX-M-27}$ (n=1), *bla*$_{CTX-M-32}$ (n=1) and *bla*$_{CTX-M-55}$ (n=1). Isolates did not have more than one *bla*$_{CTX-M}$ gene.

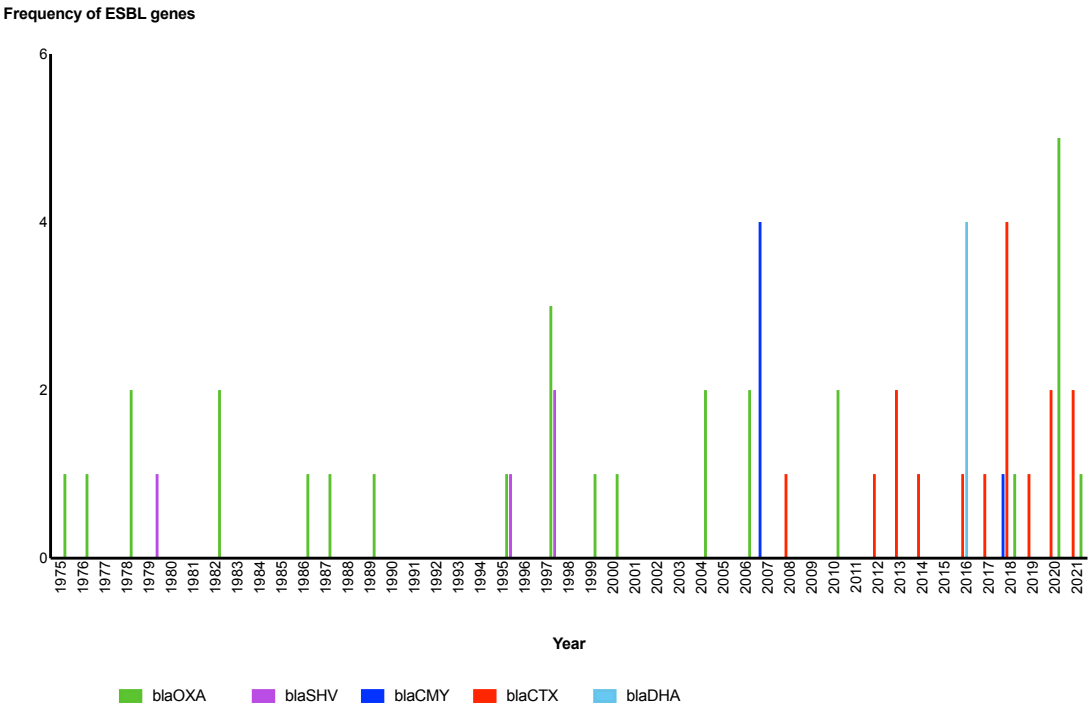


Figure 5.12 Bar chart displaying non-*bla*$_{TEM}$ ESBL genes between 1975-2021. This bar chart shows the different class of beta-lactamase resistance genes per year. Only 57 other *bla* genes were identified alongside the *bla*$_{TEM}$ (n=469) in this dataset.

5.3.4.4 Inherent resistance genes did not drive resistance over the study period

The stability of the acquired AMR gene population over the 47 years led to a search for increases in inherent resistance genes as seen in other longitudinal datasets (e.g., increased fluoroquinolone resistance in Norway (189)). The frequency of mutations in the *par* and *gyr* genes known to confer resistance was explored to determine any potential increases in inherent fluroquinolone resistance between 1975-2021. The prevalence of *par* and *gyr* mutations increased (Figure 5.13A) in parallel with the total frequency of isolates per year (Figure 5.13B), so the increase in resistance genes seen is likely a consequence of this increased isolate frequency.

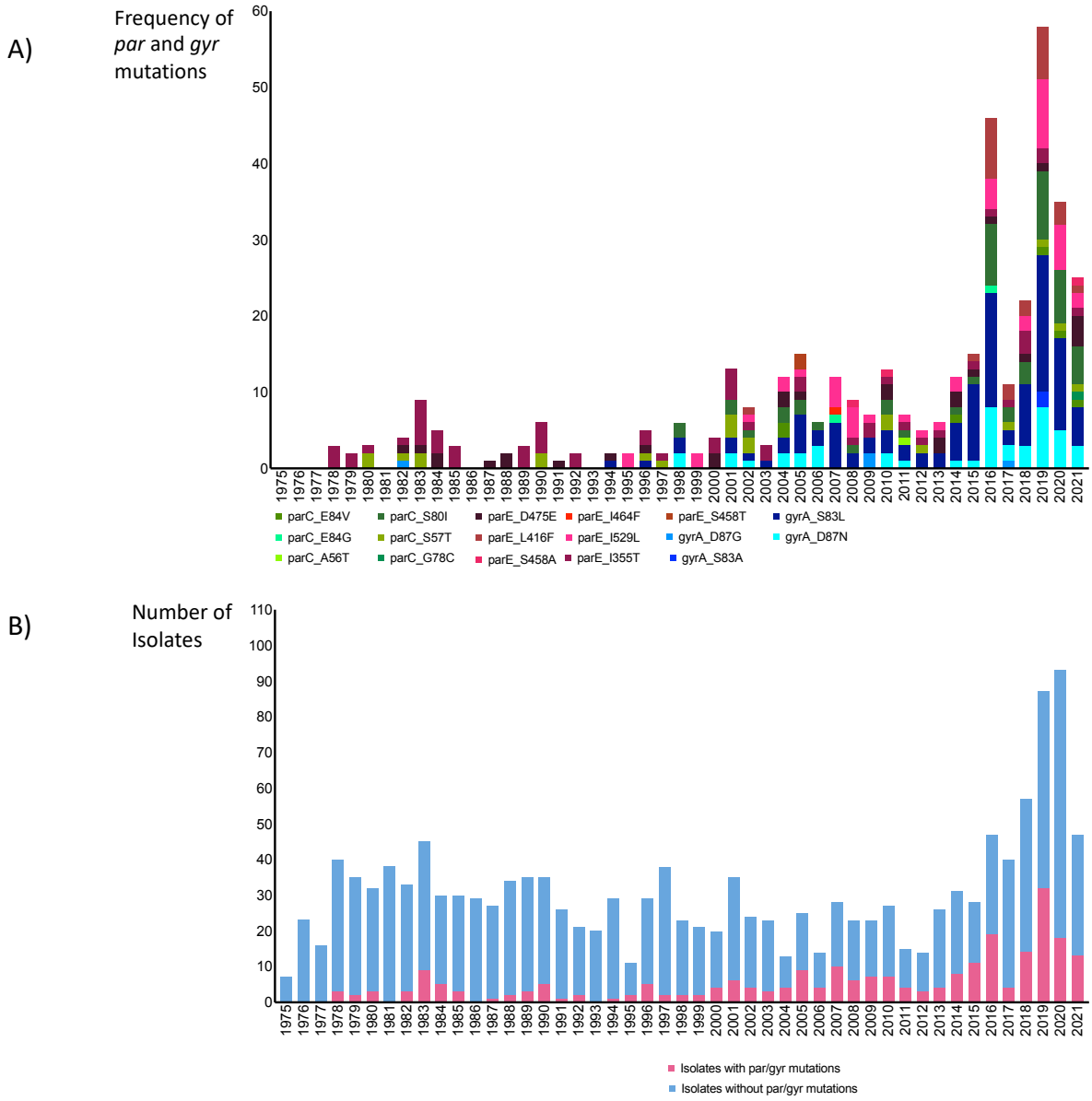


Figure 5.13 Bar charts displaying A) breakdown of type and frequency of *par* and *gyr* mutation genes found per year across all isolates and B) the overview of the total number of isolates with (pink) and without (blue) a *par* or *gyr* mutation per year.

5.3.5 SENTINEL invasive *E. coli* plasmid replicon profiles

This invasive *E. coli* population carried a variety of plasmid replicons (Figure 5.14), including those types known to carry AMR (e.g., F-type), suggesting the potential for AMR gene carriage on differing plasmid replicon types even though resistance gene

frequency was far lower than may have been expected. Plasmid replicon analysis showed that the most common plasmid types were F-type, including IncFIB(AP001918) (n=1346, 75%) and IncFIC(FII) (n=405, 23%), and smaller θ-RNA plasmids, e.g. ColRNAI (n=833, 47%), θ-Rep plasmids, e.g. Col156 (n=614, 34%) and rolling circle plasmid, e.g. Col(MG828) (n=416, 23%).

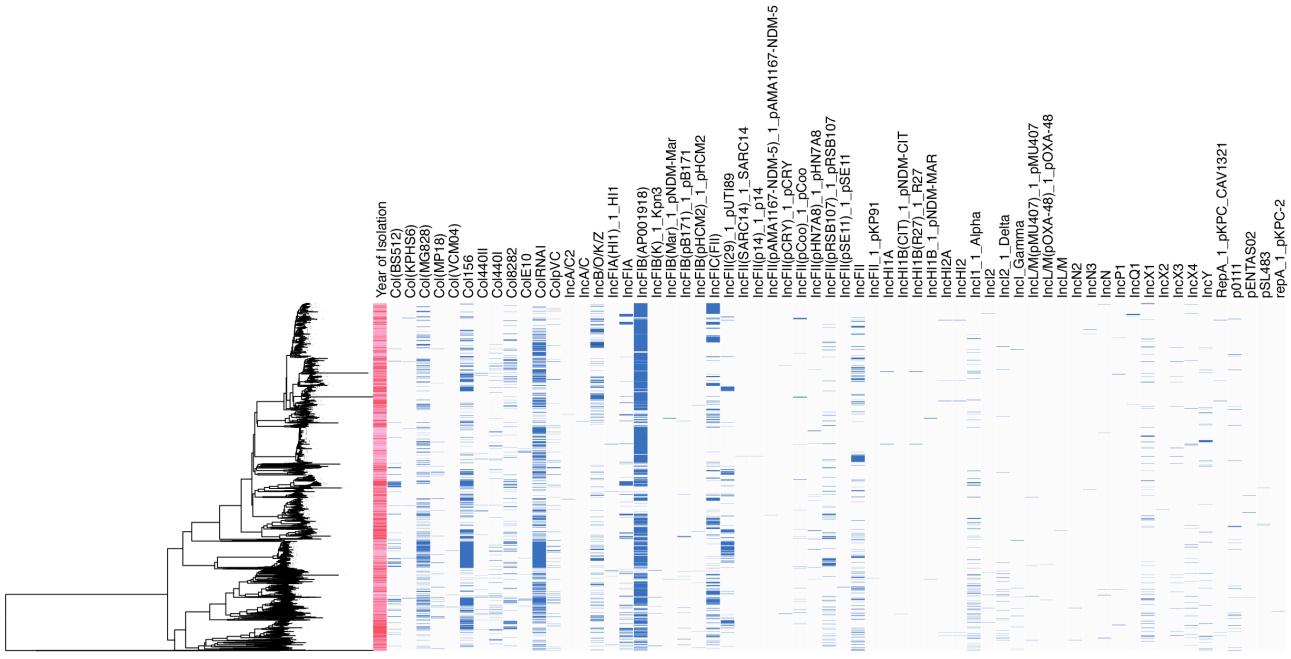


Figure 5.14 Phylogenetic tree created with Mashtree showing all *E. coli* isolates (n=1790) and their plasmid replicons as defined by PlasmidFinder. The tree is midpoint rooted and ordered with increasing nodes. Year of isolation in gradient form is displayed alongside plasmid replicon data. Plasmid replicon presence is displayed by navy and absence by light grey.

5.3.6 ExPEC virulence factors

SENTINEL *E. coli* carried a variety of virulence genes (Figure 5.15, Appendix A5) found in IPEC and ExPEC *E. coli*. The most abundant virulence genes seen were *fim*ABCDE and *fyu*A (Figure 5.15). A focus on ST95, the most abundant ST, showed 412 of the 413 ST95 isolates in this study had capsular polysaccharide virulence genes *kps*D, *kps*M and *kps*T. ST95 had other key virulence genes such as S fimbriae adhesin genes

(*sfa*A) (n=124, 30%), and *ibe*A (n=166, 40%). Interestingly of the 188 ST95 CSF isolates only 66 (16% of all ST95), carried *ibe*A, a VF associated crossing the blood-brain-barrier (BBB). Only 15 of all ST95 isolates were from patients over 1 year old, and this population size was insufficient to make a comparison between the virulence profiles of non-paediatric ST95 isolates found in CSF.

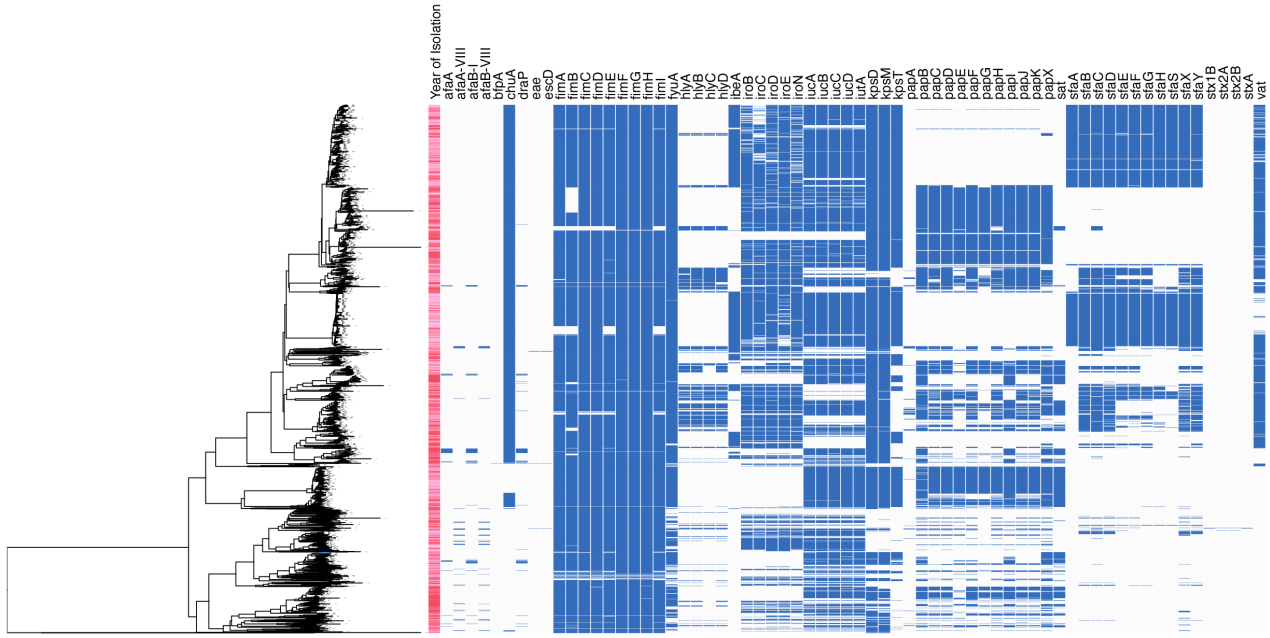


Figure 5.15 Phylogenetic tree created with Mashtree showing all *E. coli* isolates (n=1790) in this dataset with key virulence factors for ExPEC and IPEC displayed. The tree is midpoint rooted and ordered with increasing nodes. Phylogroup and year of isolation in gradient form is displayed alongside virulence gene data. Virulence gene presence is displayed by navy and absence by light grey.

5.3.7 ST131 is not a predominant lineage

Only 46 ST131 isolates were identified in this dataset in 39 patients. It was found that the majority of the SENTINEL ST131 isolates belonged to clade A (n=41) (Figure 5.16) when contextualised in a phylogeny of the ST131 lineage.

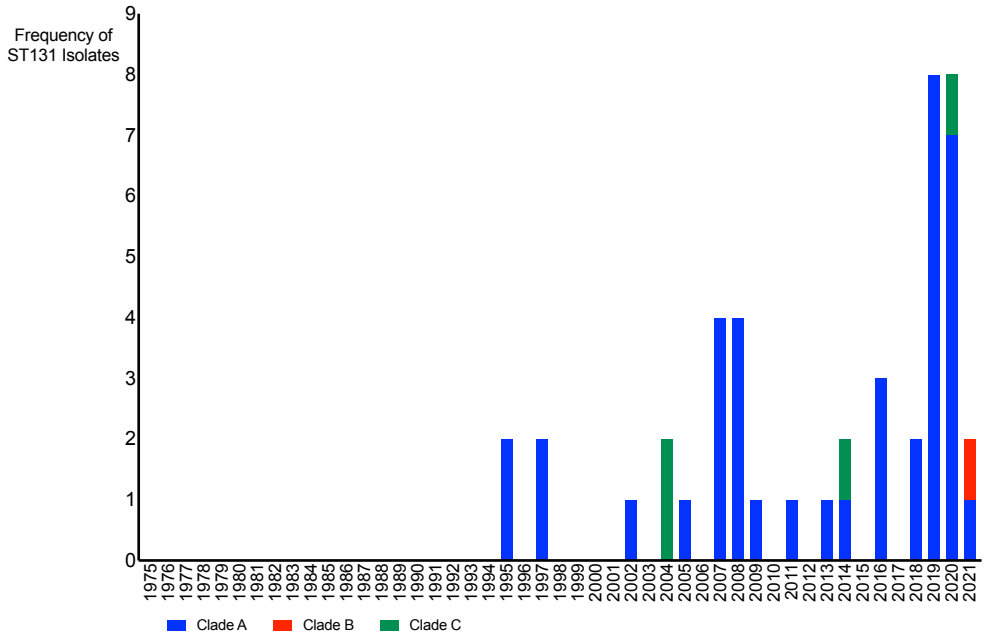


Figure 5.16 Frequency plot showing the breakdown of ST131 clades over the time period.

5.3.8 The disappearance of ST567

The SENTINEL dataset is a primarily neonatal dataset (Figure 5.3) and this is mirrored in the ST567 patient population where 84% of patients (n=108) are infants less than 1 month old (Figure 5.17B). ST567 *E. coli* were found to cause invasive disease in only two patients over 18 years old, but otherwise ST567 was only found in infants who were a maximum of 1 year old. The decline in the isolate frequency of the initially predominant ST567 (Figure 5.17A) and a comparison to patient NRLBM number frequency (Figure 5.17B) demonstrates this decline is not due to patient sample biasing.

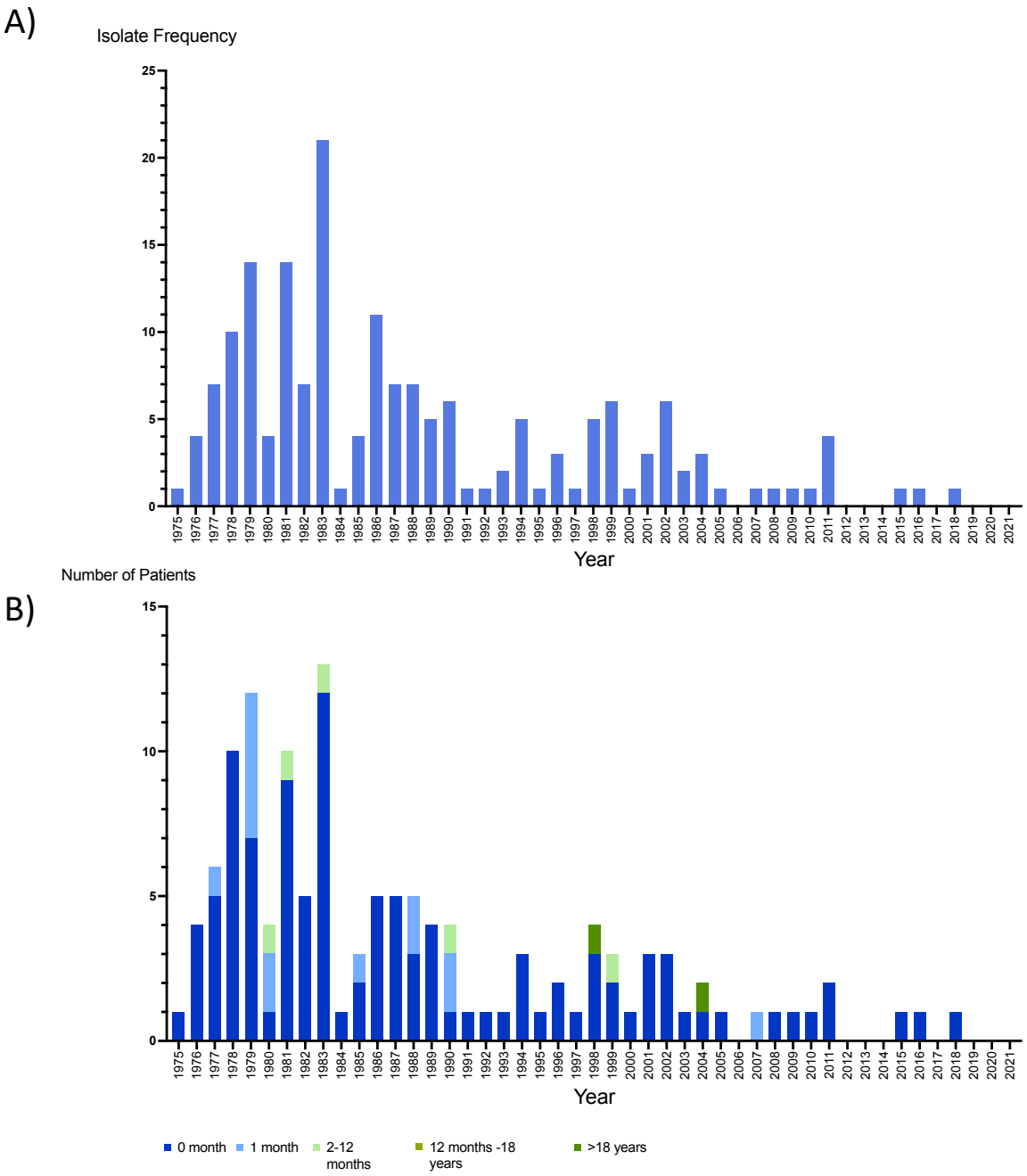


Figure 5.17 Bar charts displaying A) the frequency of ST567 between 1975-2021 and B) the number of patients from which these isolates that caused infection were sourced, colour categorised by patient age. Age categories are split as follows: '0 month' is all patients reported as 0 months, '1 month' is 0 < patient age ≤ 1 months, '2-12 months' is 2 month ≤ patient age < 12 months, '12 months -18 years' is 12 months ≤ patient age < 18 years, and patient age ≥ 18 years is '>18 years'. One patient here could not be included as their age was unknown.

ST567 is not a well-documented ST. ST567 is designated by mlst with the following housekeeping gene alleles: *adk* (13), *fumC* (43), *gyrB* (9), *icd* (37), *mdh* (17), *purA* (37) and *recA* (25). PubMLST was therefore used to investigate if the apparent disappearance of the ST567 lineage could be due to a mutation in a housekeeping gene, which would alter the ST567 allele profile so that it was classified as a different ST. PubMLST searches showed this was not the case and that if 1-2 housekeeping genes were different in ST567 it did not generate STs found in our study (Table 5.1). The closest possibility was a change of 2 housekeeping genes which would transform ST567 to ST126, however only 5 isolates of ST126 were identified in 3 patients (Table 5.1).

Table 5.1 Allelic profiles of STs 1-2 housekeeping genes different from ST567* and their prevalence in SENTINEL dataset

ST	Housekeeping Genes							Number of alleles different to ST567	Total Number Isolates in SENTINEL Dataset
	adk	fumC	gyrB	icd	mdh	purA	recA		
567	**13**	**43**	**9**	**37**	**17**	**37**	**25**	**0**	**175**
126	13	43	**19**	37	17	**25**	25	2	5
430	13	43	9	37	17	**1**	25	1	0
1392	**37**	43	9	37	17	37	25	1	0
2088	13	43	**10**	**20**	17	37	25	2	0
4102	13	43	**10**	**440**	17	37	25	2	0
4581	13	43	**10**	**481**	17	37	25	2	0
4899	**40**	43	9	37	17	37	25	1	0
4900	**40**	43	9	37	**339**	37	25	2	0
6322	13	43	9	37	17	**471**	**2**	2	0
7346	**690**	43	9	37	17	37	25	1	0
8374	13	43	9	37	**715**	37	25	1	0
10275	13	43	9	**1189**	17	**44**	25	2	0
10279	13	**1466**	9	37	**901**	37	25	2	0
12596	13	43	**84**	37	**18**	37	25	2	0

*Bold type in non-ST567 STs shows the difference in housekeeping gene alleles compared to ST567.

5.3.9 Characterisation of ST567 population

The Panaroo gene presence absence profile highlighted the similarity in gene composition of this ST567 population (Figure 5.18). SNP analysis demonstrated that within the core genome isolates were between 0 and 197 core SNPs different.

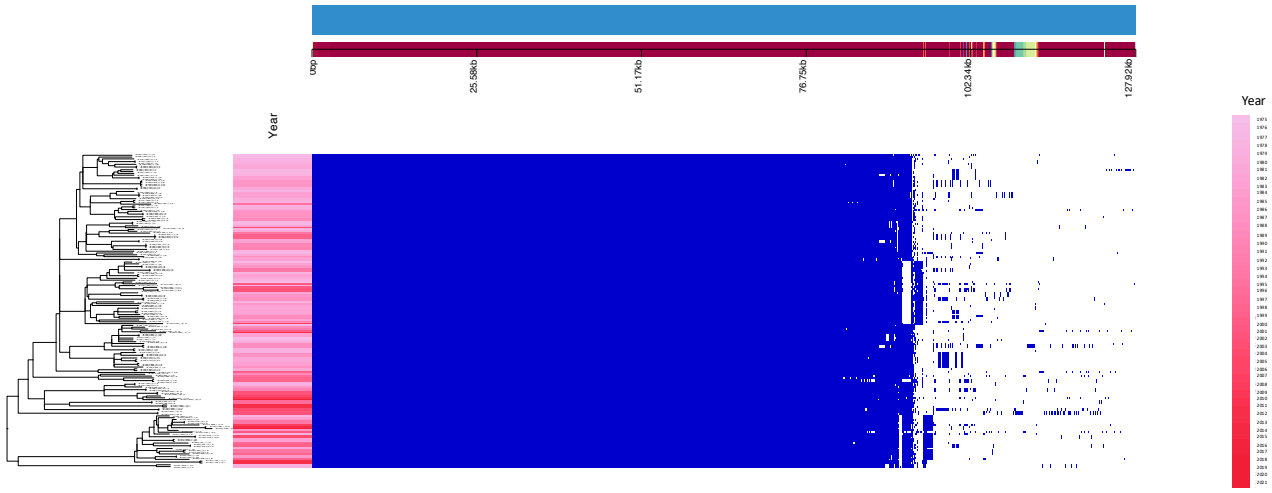


Figure 5.18 Dated Timetree generated with IQ-tree, from core SNP alignment from Snippy, with recombination filtering using Gubbins. Reference isolate was the oldest isolate from 1975, NEL0010.Tree is rooted on 1825 branch and ordered with increasing nodes. Date of isolation is shown in a gradient of pink, earliest (pale pink) to most recent (dark pink). The Panaroo core gene presence output is displayed in royal blue.

Phylogenetic analysis demonstrated that there were two main clades in the SENTINEL ST567 dataset and a dated time tree indicated that these clades split in 1820, with a most recent common ancestor date of 1803 (Figure 5.19).



Figure 5.19 ST567 dated phylogeny generated with IQ-tree, from core SNP alignment from Snippy, with recombination filtering using Gubbins. Blue and red are used to represent the two clades. The isolate name 'Reference' refers to the oldest isolate from 1975, NEL0010.Tree is rooted on 1825 branch and ordered with increasing nodes. IQtree Dating results: rate 0.000209762, tMRCA 1803.45, objective function 1.43975.

Root-to-tip analysis shows the ST567 dataset has a correlation coefficient of 0.77 so positive correlation between root-to-tip divergence and sample date when the mean of the squares of the residuals are minimised in a best fit plot (Figure 5.20). The slope shows an estimate of the rate of evolution in substitutions per site per year, and this is $2.28x10^{-4}$. This method gave a x-intercept, tMRCA, of 1823, similar to that seen in IQtree dating methods (Figure 5.19).

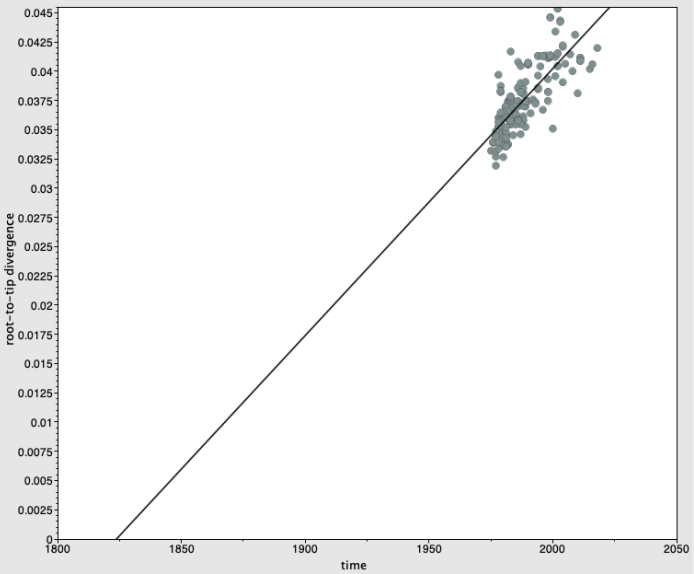


Figure 5.20 TempEst plot displaying divergence of the ST567 population root of tree against time of sampling. Root-to-tip plot function: heuristic residual mean best fit model. Date range: 43 years. Slope (rate) $2.28x10^{-4}$. X-intercept 1823 (tMRCA).

The ST567 population follows trends seen in this dataset with low levels of AMR carriage. Aminoglycoside and sulphonamide resistance were the two most common drug classes resistance observed (Figure 5.21). There appears to be greater diversity of AMR gene types in the smaller of the two ST567 clades (Figure 5.19, blue clade). Although the mean number of resistance gene types is approximately the same in both

clades (larger clade (Figure 5.19, red clade) = 2.7, smaller clade (Figure 5.19, blue clade) = 2.6).

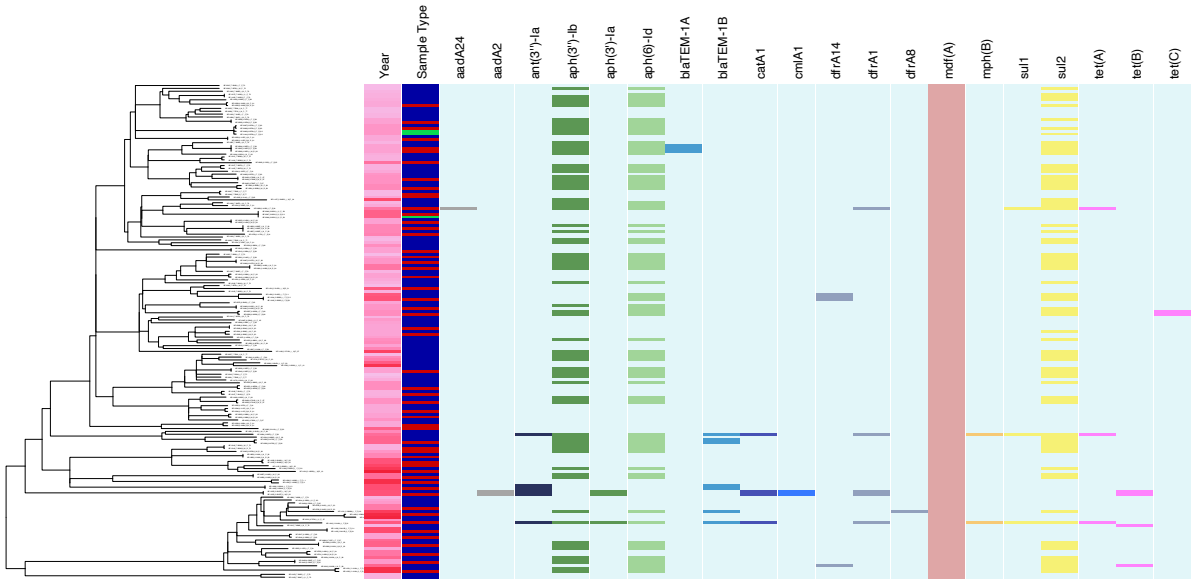


Figure 5.21 ST567 dated phylogeny generated with IQ-tree, from core SNP alignment from Snippy, with recombination filtering using Gubbins showing resistance gene presence in all ST567 isolates. The isolate name 'Reference' refers to the oldest isolate from 1975, NEL0010. Tree is rooted on 1825 branch and ordered with increasing nodes. Metadata displayed shows year of isolation in a gradient of pink, earliest (pale pink) to most recent (dark pink), as in Figure 5.18. Metadata also included: AMR gene presence (differing colours) and absence (pale blue). Sample type is displayed showing CSF isolates (royal blue), bloodstream isolates (red) and other locations of isolation including swabs/urine (green).

5.3.10 ST567 clades have distinct virulence profiles

Although there was no clear differences between resistance gene profiles across the two clades, there was one clear difference observed in VFs. The smaller clade (Figure 5.19 – blue clade) did not have a complete Fim region, as *fimA, fimB, fimE* and *fimI* were missing (Figure 5.22). In contrast the larger clade had a complete Fim region.

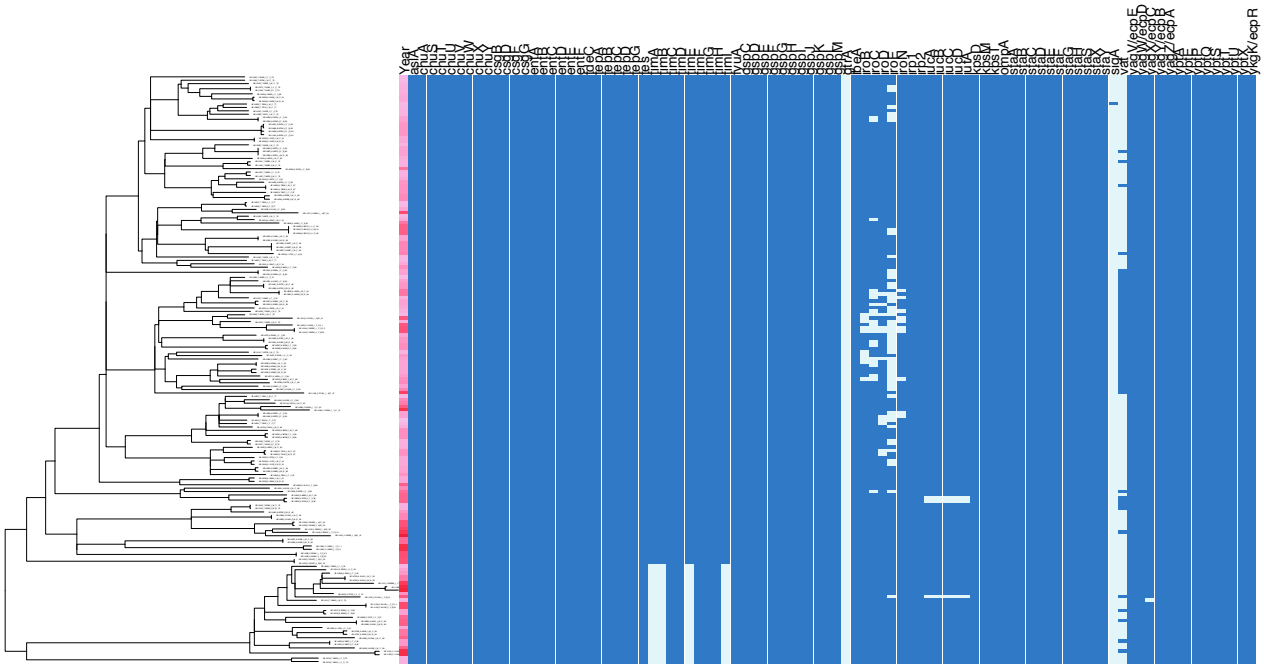


Figure 5.22 ST567 dated tree generated with IQ-tree, from core SNP alignment from Snippy, with recombination filtering using Gubbins showing virulence gene presence in all ST567 isolates. The isolate name 'Reference' refers to the oldest isolate from 1975, NEL0010. Tree is rooted on 1825 branch and ordered with increasing nodes. Metadata displayed shows year of isolation in a gradient of pink, earliest (pale pink) to most recent (dark pink), as in Figure 5.18. Metadata also included: virulence gene presence (differing colours) and absence (pale blue) generated by ABRicate using vfdb database.

5.3.11 ST567 clades have differing *fim* gene regions

The most striking difference between the two clades was seen in the presence and absence of virulence genes. Figure 5.22 shows that the smaller ST567 clade is missing several *fim* genes (*fimA, fimB, fimE* and *fimI)*. Detailed analysis of the Fim region of isolates from different areas of the ST567 tree using BLAST and Easyfig on hybrid assemblies identified a missing region of 891 bp in the smaller clade Fim region (Figure 5.23). This missing region was located between *nanC* and *fimC* in isolates lacking *fimA, fimB, fimE* and *fimI.* ISFinder did not show an insertion of an IS element in this region. Closer inspection of the boundaries of the deleted region showed a 18 bp repeated

region ‘CAGA**C**TGT**G**TATTTCCTG’ (2 SNPs variation in bold). In addition, Easyfig highlighted a region of similarity in *fimE* and *fimB* genes identity (74%) in all isolates with *fimE* and *fimB* genes.

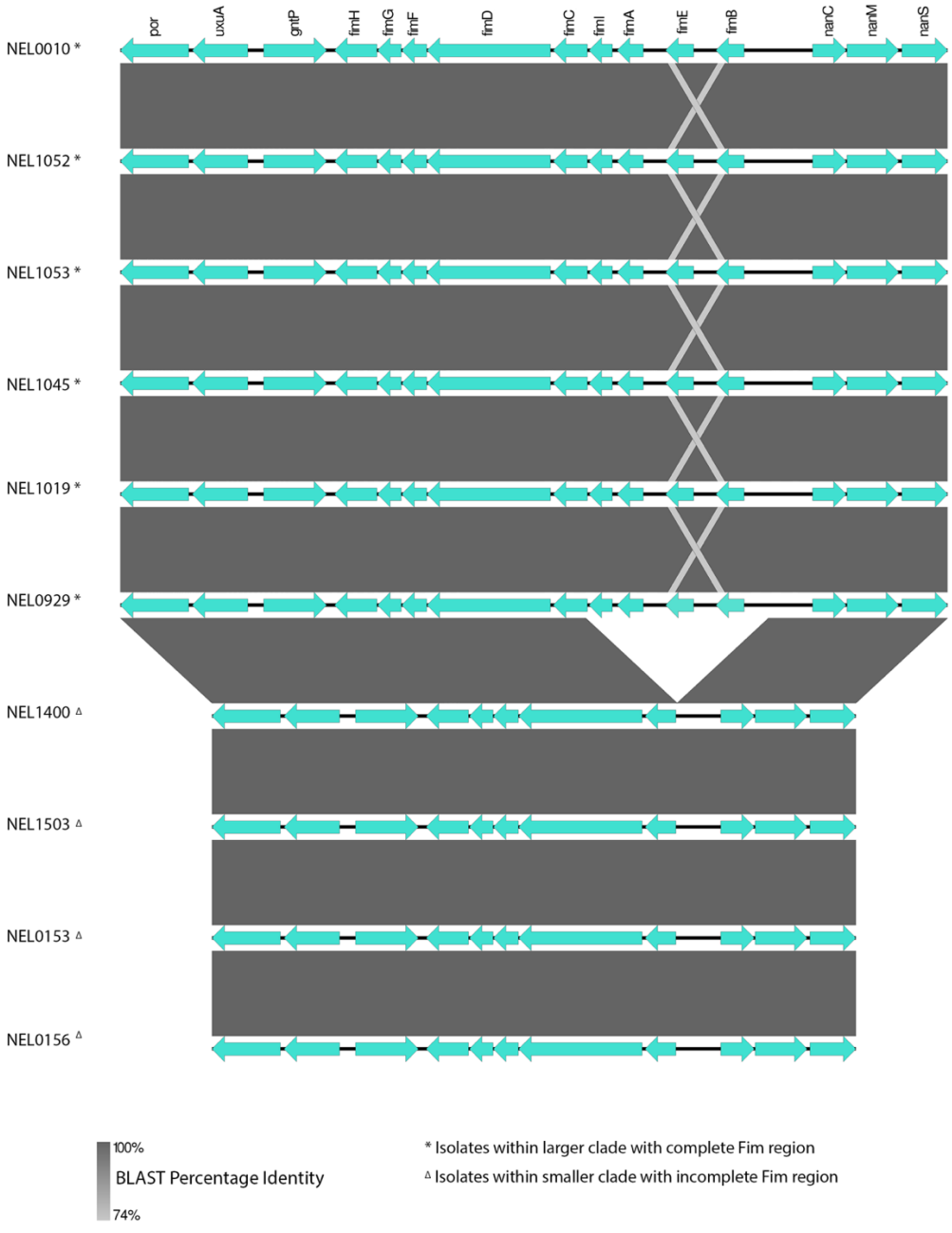


Figure 5.23 Easyfig comparison of hybrid assemblies of the Fim region of ten ST567 isolates from the two distinct ST567 clades, with and without complete Fim region, identified by ABRicate with virulence finder database. Gradient shading shows BLAST identity values provided by Easyfig.

Table 5.2 indicates although the ST567 have very similar core pangenomes there are some areas of difference. Manual checks of Panaroo core gene presence absences profiles (Table 5.2A) and Scoary association traits (Table 5.2B) demonstrated links between the larger ST567 clade to *fimA* and long polar fimbriae (*lpfA*) gene as well as ISs and hypothetical proteins.

Table 5.2 Gene traits associated with ST567 clade containing a complete Fim gene region based on A) Panaroo core gene presence absence profile comparison of clades and B) Scoary trait associate comparison of ST567 clades.

A)

Gene	Non-unique Gene name	Annotation
group_1959	xerD_2;	Tyrosine recombinase XerD;ISKra4 family transposase ISCep1
group_1875	-	hypothetical protein
elmGT_1	elmGT_1	Elloramycin glycosyltransferase ElmGT
group_1832	-	hypothetical protein
pspE	pspE	Thiosulfate sulfurtransferase PspE
lpfA_3	lpfA_3	putative major fimbrial subunit LpfA
fimA	fimA	Type-1 fimbrial protein A chain
group_689	-	ISKra4 family transposase ISCep1

B)

Gene	Non-unique Gene name	Annotation
group_1959	xerD_2;	Tyrosine recombinase XerD;ISKra4 family transposase ISCep1
lpfA_3	lpfA_3	putative major fimbrial subunit LpfA
fimA	fimA	Type-1 fimbrial protein A chain
group_689	-	ISKra4 family transposase ISCep1

**Gene names, non-unique gene names and gene annotations specified by Panaroo. Non-unique gene names are specified where multiple genes in some cases are in multiple groups.*

5.3.12 Phenotypic differences were found in the Type I fimbriae between ST567 clades

The genotypic predictions of which ST567 isolates would have functional type I fimbriae were based on the predicted presence/absence of virulence genes. Genotypic results predicted four of nine the ST567 isolates selected for long read sequencing did not have complete *type I fimbriae* (Fim) gene regions. Yeast agglutination experiments found that there were phenotypic differences of type I fimbriae in these two clades of ST567 isolates which were consistent with genotypic predictions where these genes appear to have been lost from the ancestral lineage. These yeast agglutination experiments suggest that isolates lacking a complete Fim region are unable to agglutinate with yeast as they do not have a functional type I fimbriae (Table 5.3).

Table 5.3 Yeast agglutination assay results for a subset of nine isolates across both ST567 clades.

	Genotypic Result		Phenotypic Result	
Isolate	**Complete Fim region**	**Missing *fimA, fimB, fimE and fimI***	**Agglutinates with 10mg/ml yeast**	**Agglutinates with 10mg/ml yeast and 3% alpha-D-mannose**
NEL0153	x	✓	x	x
NEL1400	x	✓	x	x
NEL1019	✓	x	✓	x
NEL0156	x	✓	x	x
NEL0929	✓	x	✓	x
NEL1053	✓	x	✓	x
NEL1045	✓	x	✓	x
NEL1052	✓	x	✓	x
NEL1503	x	✓	x	x

5.3.13 ST567 isolates carry a variety of plasmids

Investigation into plasmid carriage in the ST567 dataset indicated all ST567 plasmids contained at least one plasmid replicon as defined by ABRicate PlasmidFinder database (Figure 5.24). In the whole ST567 dataset (n=175) the maximum number of plasmid replicons found per isolate was six. Six plasmid replicons were found in two

isolates (NEL1052 and NEL1053) from the same patient (2021557) in their CSF and bloodstream isolates in 2002. Plasmids across both major ST567 clades frequently carried IncFIB(AP001918) (Figure 5.24).

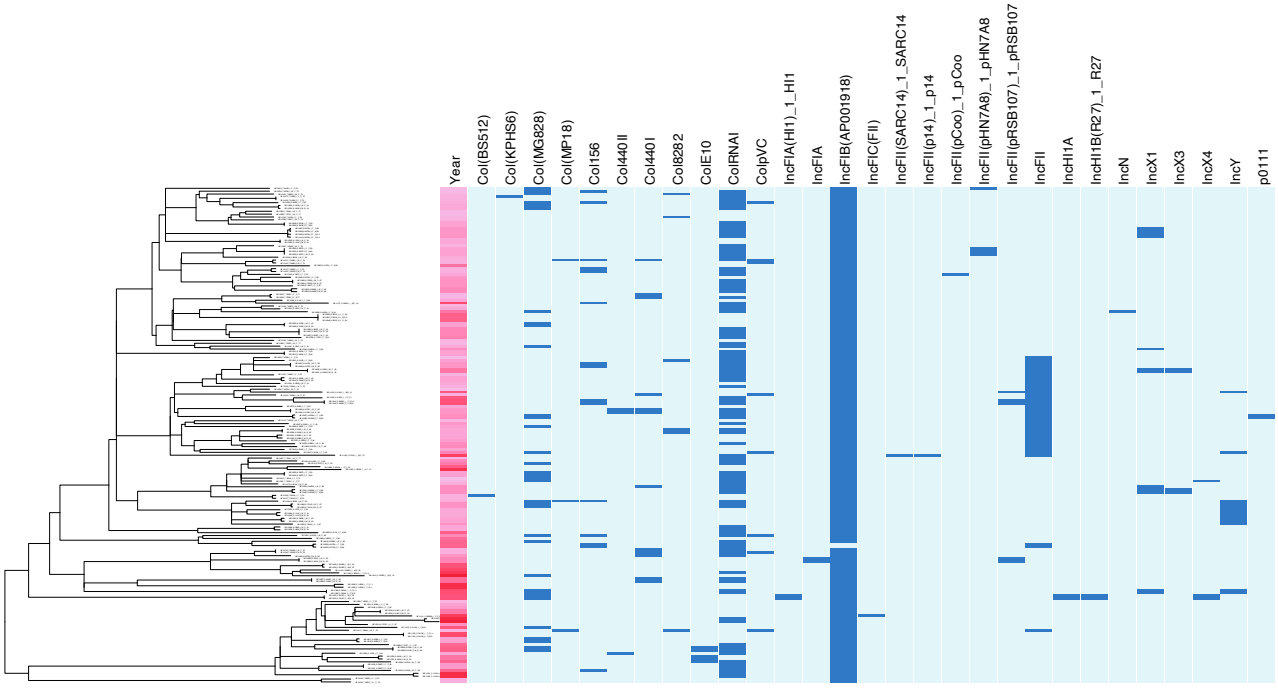
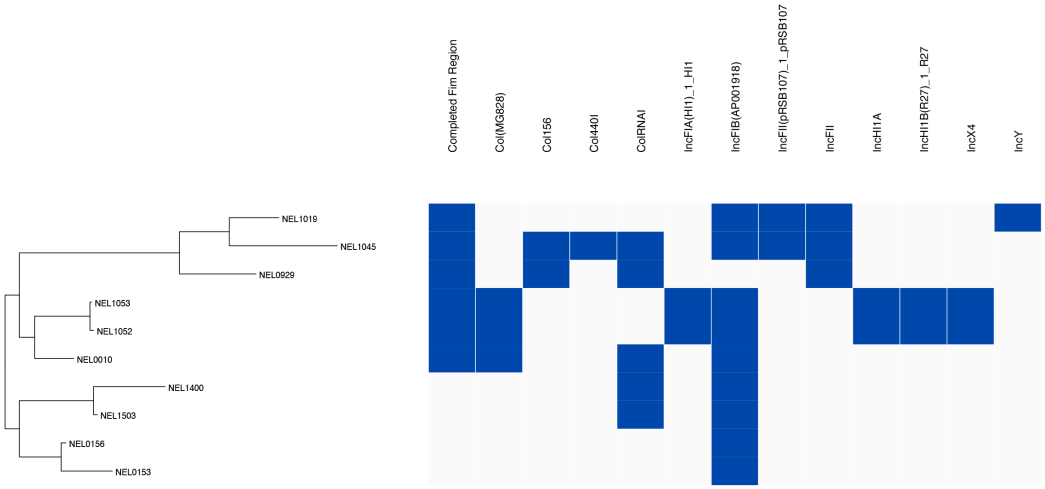


Figure 5.24 ST567 dated tree generated with IQ-tree, from core SNP alignment from Snippy, with recombination filtering using Gubbins showing plasmid replicon presence in all ST567 isolates. The isolate name 'Reference' refers to the oldest isolate from 1975, NEL0010. Tree is rooted on 1825 branch and ordered with increasing nodes. Metadata displayed shows year of isolation in a gradient of pink, earliest (pale pink) to most recent (dark pink), as in Figure 5.18. PlasmidFinder plasmid replicon presence (dark blue) or absence (pale blue) is listed alongside year of isolation.

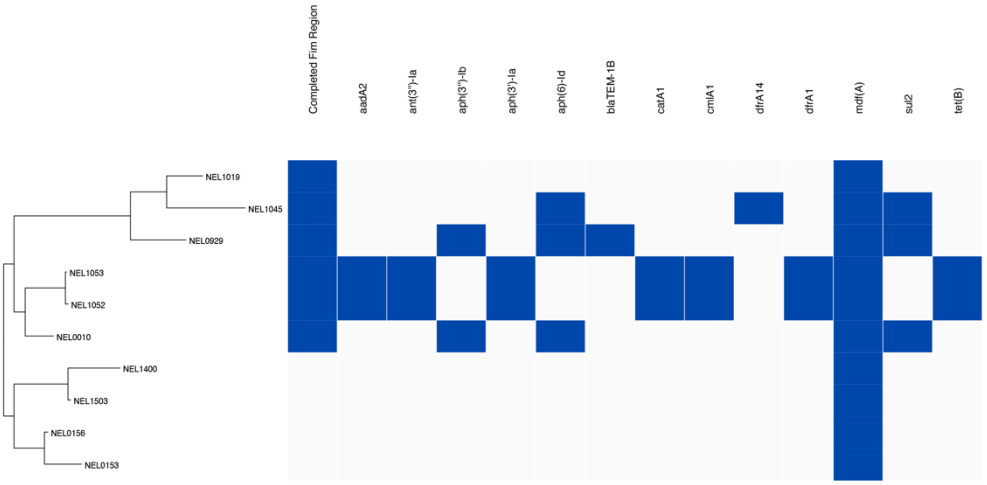
Long read sequencing of a representative subset of isolates in the ST567 dataset (Section 2) confirmed ST567 isolates carried a selection of plasmids, mainly small plasmids that did not contain AMR genes. Plasmid types identified included: F-type, H-type, small ColE1-like and rolling circle plasmids. Complete plasmids ranged in size from 1552 bp - 202,569 bp.

Hybrid assemblies for this small subset showed that in multiple cases where isolates did have a complete Fim region they also carried AMR plasmids (Figure 5.25B) and demonstrated multiple instances of MDR plasmid carriage (Figure 5.25A). These plasmids (Figure 5.25C) commonly carried resistance to aminoglycosides, chloramphenicol, and trimethoprim. None of the small subset of ST567 hybrid assemblies studied that did not have a complete Fim gene region, carried any AMR plasmids (Figure 5.25).

A)



B)



C)

Isolate	Size	PlasmidFinder Replicon	PubMLST Replicon	AMR gene
NEL0010	6,244	ColRNAI	-	*sul2, strA, strB*
NEL0929	76,430	Col440I	-	*sul2, strA, strB, bla$_{TEM-1b}$*
NEL1045	6,790	IncFII and ColRNAI	FII:95-like	*sul2, dfrA14, strB*
NEL1052	202,569	HI1	FIA:8-H1	*tet(B), cmlA1, aadA2, ant(3'')-Ia, dfrA1, aph(3')-Ia, catA1*
NEL1053	202,569	HI1	FIA:8-H1	*tet(B), cmlA1, aadA2, ant(3'')-Ia, dfrA1, aph(3')-Ia, catA1*

Figure 5.25 ST567 hybrid assembly plasmid profiles with a IQtree of Panaroo core gene alignment showing complete Fim gene region presence (navy) and A) PlasmidFinder replicon presence (navy) B) resistance gene presence (navy) and C) table with the breakdown of AMR gene carrying plasmids within the smaller subset of ST567 analysed with long read sequencing.

Long read sequencing of the earliest ST567 isolate in the dataset (NEL0010) showed carriage of only one plasmid that carried AMR genes (Figure 5.26A) and three plasmids that did not carry AMR genes (a rolling circle Col(MG828) plasmid , a F-type FII-13 : FIB-40-like plasmid (Figure 5.26B) and a 4,082bp plasmid which could not be typed by PlasmidFinder).

The 6244bp Col-E1-like AMR plasmid found in NEL0010 (Figure 5.26A) carried a partial region of Tn5393, containing *strAB* aminoglycoside resistance genes, alongside a sulphonamide resistance gene, *sul*2. NCBI Blast searches showed this AMR plasmid had both 100% percentage identity and query coverage matches to pCERC2, a ColE1-like plasmid, found in commensal *E. coli* ST14 strain in faeces from healthy adult, from Australia (NZ_KX291024.1). This ColE1-like plasmid does not contain virulence genes.

In view of the high frequency of IncFIB(AP001918) plasmid replicon found in overall ST567 dataset (Figure 5.24), the 142,433bp F-type plasmid from the earliest isolate was explored further as it was potentially carried throughout the ST567 dataset. It was typed IncFIB(AP001918) by PlasmidFinder and FII-13:FIB-40-like by PubMLST. Only two isolates (NEL0929 and NEL0930) do not carry this IncFIB(AP001918) replicon. These isolates are from the same patient (981709) in 1998 but from different sources, the CSF and bloodstream. This FIB-40 : FII-13 plasmid did not carry AMR genes, however it had ten virulence genes including the aerobactin operon (*iuc*ABCD, *iut*A) and the catecholate sidephore *iro*BCDEN. Comparative analysis using NCBI Blast showed 136 ST567 isolates had 100% matches for the FII-13 replicon and FIB-40-like replicon.

A)



B)

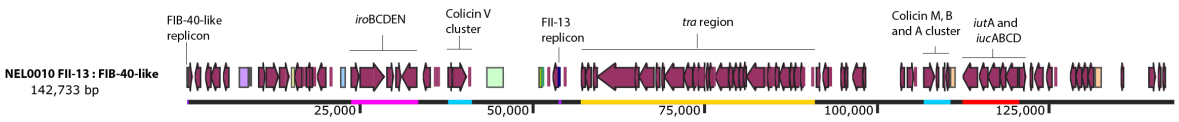


Figure 5.26 Annotated plasmid map of A) ColE1-like ColRNAI plasmid and B) F-type: FII-13 : FIB-40-like plasmid from NEL0010 ST567 isolate. Plasmids drawn using SnapGene annotated with Prokka genes, plasmid replicons and ABRicate vfdb results.

5.3.14 ST567 Capsule region studies

5.3.14.1 ST567 population is predominantly K1

152 of 175 ST567 isolates were given a K capsule serotype by NRLBM and 147 of those given a K capsule serotype were K1 (Figure 5.27). Four isolates (NEL0010, NEL0047, NEL0048 and NEL0834) were typed as K-. One isolate NEL0015 was classified as K2.

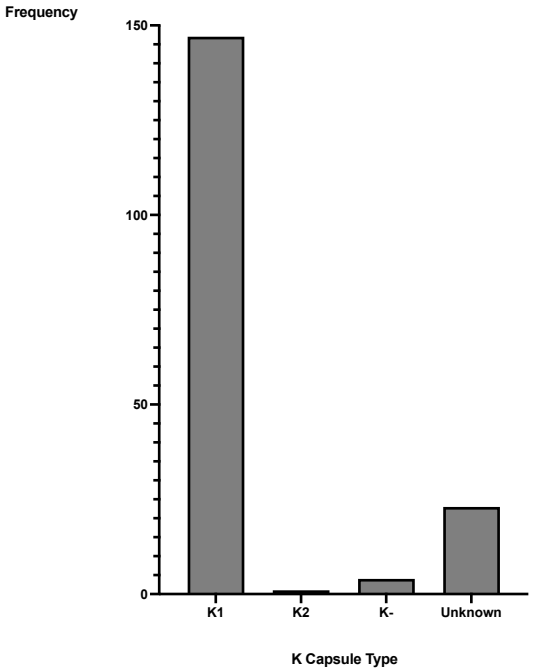


Figure 5.27 Bar chart showing frequency of the different K capsule types present in the ST567 population.

5.3.14.2 *kps* gene regions were identical across ST567 clades

The capsule region of these isolates was explored using long read sequencing to see if there were any changes in the capsular *kps* region between the two ST567 clades. The virulence profile of ST567 (Figure 5.22) indicated all isolates had *kps*DMT and examination of long read sequences demonstrated that there was no difference between the *kps* gene setting on the chromosome in either ST567 clade (Figure 5.28).

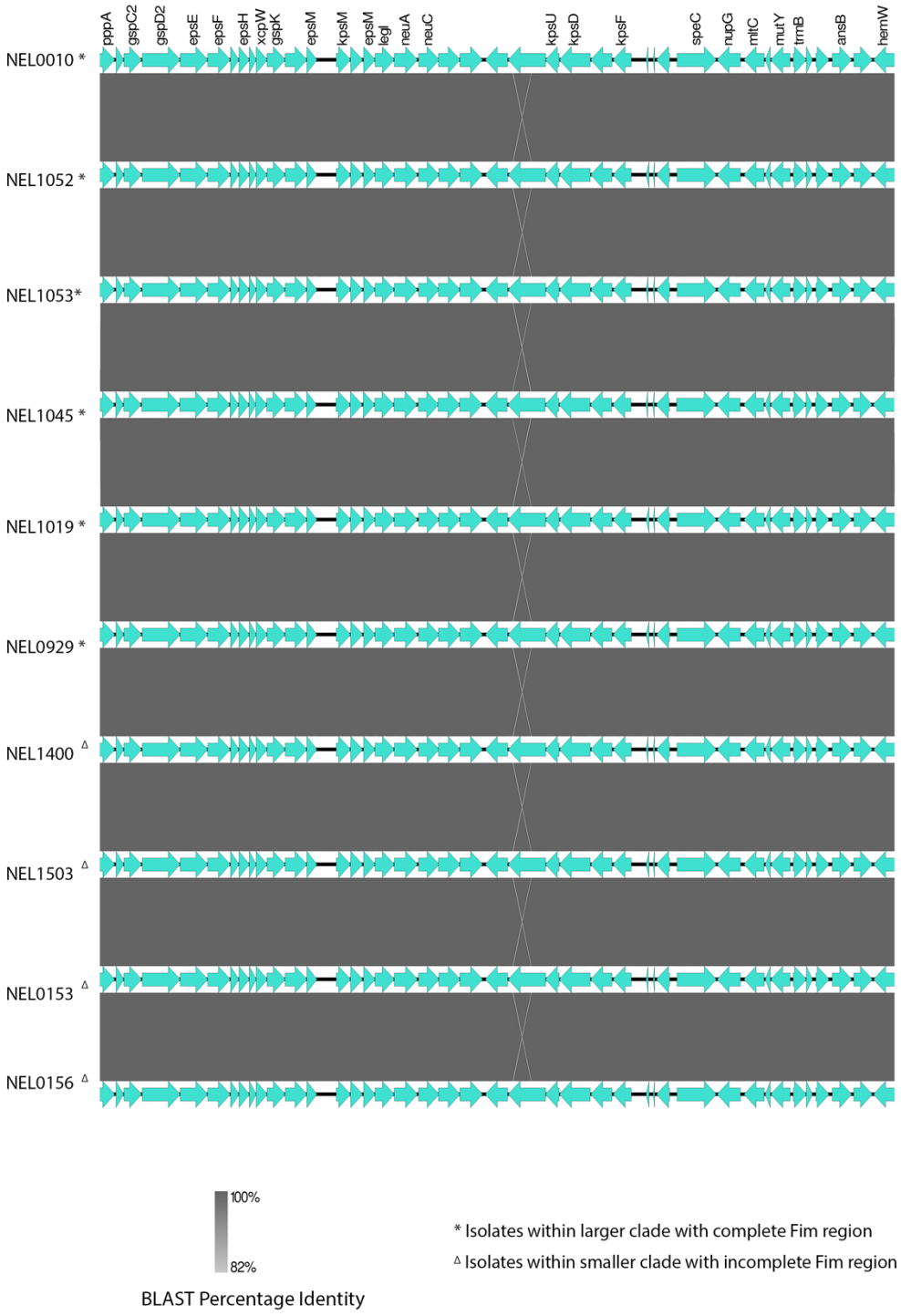


Figure 5.28 Easyfig comparison of hybrid assemblies of the kps gene region of ten ST567 isolates from the two distinct ST567 clades, with and without complete Fim region, identified by ABRicate with VFDB. Gradient shading shows BLAST identity values provided by Easyfig. Gene arrows are characterised by Prokka and those arrows without labels are hypothetical proteins assigned by Prokka.

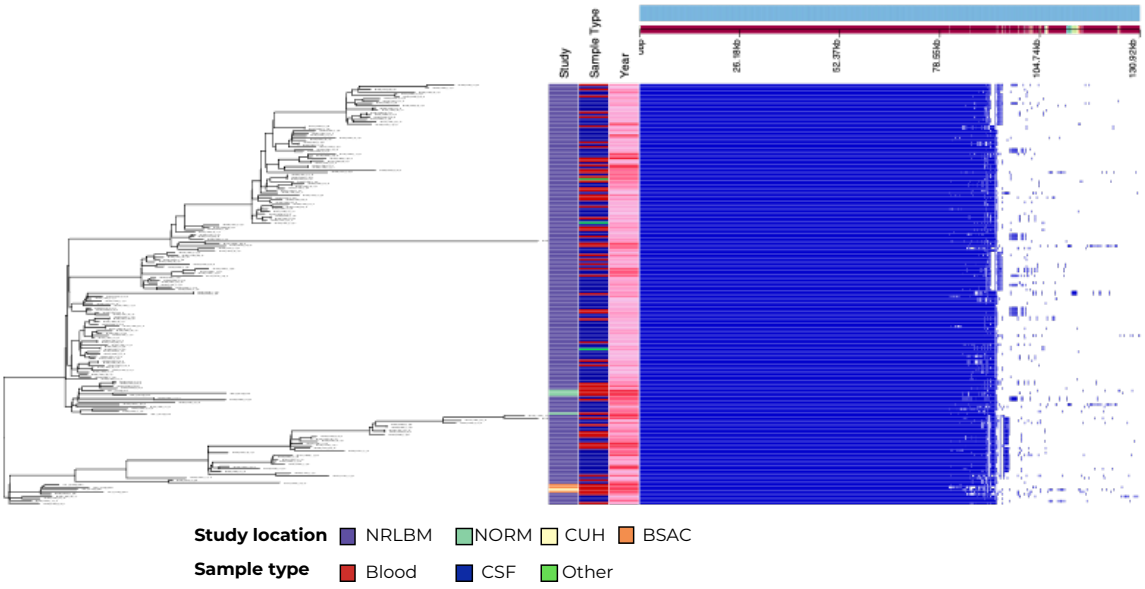
5.3.15 SENTINEL ST567 isolates do not differ drastically from other publicly available ST567 genomes

Other clinical longitudinal datasets were explored to look for the prevalence and frequency of ST567. In the NORM and BSAC/CUH datasets (85, 189) only 8 ST567 isolates were identified. These ST567 isolates from the NORM and BSAC datasets are located within the SENTINEL ST567 major clades (Figure 5.29A). In all cases these NORM and BSAC clinical isolates were collected from the bloodstream between 2004 - 2016 and can be seen in both clades of the ST567 tree (Figure 5.29A).

Additional examples of ST567 isolates (n=56) from both livestock and humans across multiple regions of the globe were identified in EnteroBase and these were also found throughout the SENTINEL ST567 phylogenetic tree (Figure 5.29B). Not all isolates from EnteroBase had known isolation dates, but in the cases where the isolation date was known, isolation dates ranged from 1980-2021. One large cluster of EnteroBase isolates clustered in the SENTINEL ST567 phylogeny on a separate small clade (Figure 5.29B). However other more recent EnteroBase isolates were found throughout the SENTINEL ST567 phylogeny located near SENTINEL ST567 isolates from a similar year of isolation.

ST567 isolates of livestock origin found in EnteroBase clustered amongst the SENTINEL study isolates, and other publicly available isolates collected from humans in other countries in Europe including Netherlands, USA and Australia. The ST567 isolates from livestock collected from EnteroBase were also distributed throughout the phylogeny near the SENTINEL ST567 isolates from a similar year isolation and other isolates from EnteroBase (Figure 5.29).

A)



B)

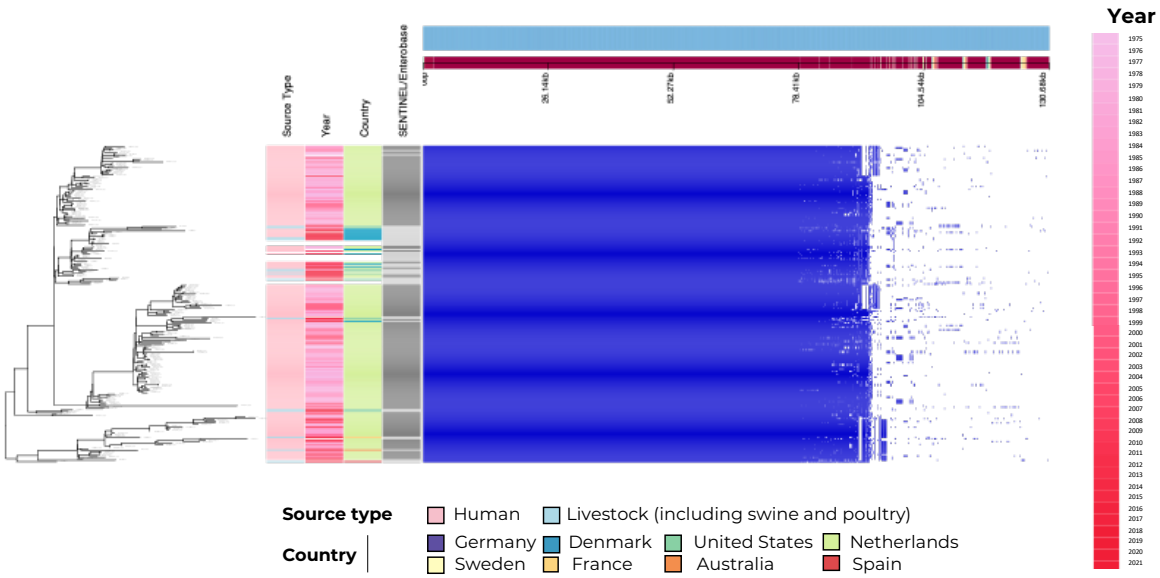


Figure 5.29 IQ-tree generated using Panaroo core gene alignment of SENTINEL ST567 dataset with A) ST567 isolates from BSAC, CUH and NORM longitudinal dataset isolates and B) all ST567 isolates with publicly available assemblies on EnteroBase. For both graphs metadata is displayed next to the tree. The year of isolation uses the gradient key next to (Figure 5.29B). Panaroo gene presence/absence output is displayed in royal blue. On Figure 5.29A the source of the clinical isolate is listed, CSF, bloodstream or other sources. On Figure 5.29 B dark grey indicated the isolate was from the SENTINEL collection and light grey indicates the isolate assembly was downloaded from EnteroBase. Country of origin is displayed alongside whether the EnteroBase isolates were collected from livestock samples or humans. White metadata cells indicates where this information is not available from EnteroBase.

The lack of a complete Fim region seen in the SENTINEL dataset, is also mirrored in the EnteroBase isolates (Figure 5.30) with one exception. This exception, EnteroBase isolate (ESC_WB7828AA_AS) differs from the SENTINEL ST567 isolates it is located closest to as it has a complete Fim gene region. ESC_WB7828AA_AS is actually located on a separate branch from this small SENTINEL clade, dividing it from all SENTINEL ST567 isolates that are missing a Fim region. ESC_WB7828AA_AS was isolated in Spain in 2015 from swine intestinal segments faecal content.

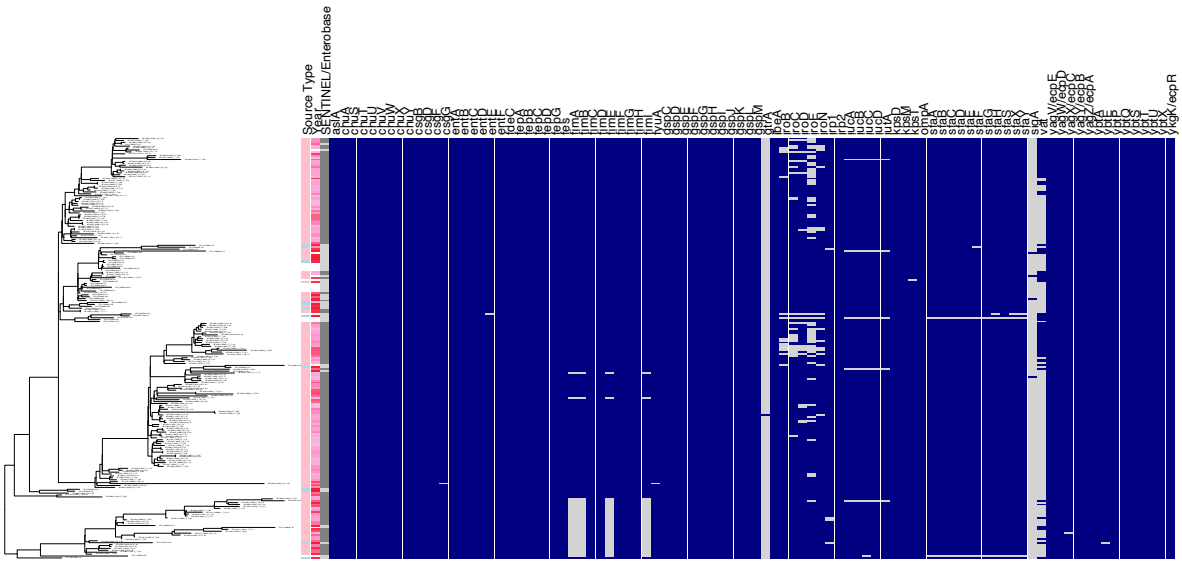


Figure 5.30 Phylogenetic tree of ST567 generated with IQ-tree using panaroo core gene alignment. Metadata displayed shows source type (pink: human, purple: livestock) and whether the isolates were from the Sentinel dataset (dark grey) or EnteroBase (light grey). Year of isolation is displayed as a colour gradient as in Figure 5.18 where darker pink is more recent isolates and paler pink are older isolates. Virulence gene as defined by vfdb database using ABRicate is shown as present (navy) and absent (pale grey).

5.4 Discussion

SENTINEL is a unique longitudinal study as it investigated Dutch clinical isolates from a mainly neonatal population who have had little opportunity for exposure to pathogens and antimicrobials in their environment. This 47-year (1975-2021) unique historical *E. coli* strain collection (n=1790) differed from recently published longitudinal studies in other countries as *E. coli* was isolated from much younger patients and from CSF as well as the bloodstream (85, 189, 190).

Characterisation of phylogroup and ST distribution in the SENTINEL dataset, initially demonstrated the diversity of STs from different phylogroups that cause invasive infection in neonates, supporting findings of previous recent longitudinal studies (85, 189, 190) but also discovering important differences. The high levels of the B2 phylogroup found in other recent longitudinal studies of *E. coli* isolated from older patient populations (85, 189, 190) were mirrored in the SENTINEL dataset. The B2 phylogroup is a known cause of invasive infection and unsurprisingly over the 47-years in SENTINEL over 50% of isolates per annum were classified as B2, in all but two years (1998 - 46.4%, 2017 - 47.8%) (Figure 5.8) confirming that B2 phylogroup is a major cause of invasive infection in all age groups including neonates.

Phylogroup analysis also revealed that two cryptic clade isolates (clade III and V) caused meningitis in infants (6 year-old and 2 month-old respectively) in addition to the range of phylogroups (e.g. B2, D) normally expected to cause infection. This is a rare finding as cryptic clades are usually found in animals or the environment (52, 190), however it is not unique, as for example, Lipworth et al. found at least 1 cryptic clade isolate causing bloodstream infection in English adults (190). SENTINEL shows that although cryptic clade isolates do not usually cause infection, they are still a risk to be

considered if a pregnant individual is colonised with them or a neonate is exposed to them.

The investigation of SENTINEL isolates revealed a difference in ST diversity compared to that reported in older populations (85, 189, 190). SENTINEL isolates were very diverse, considering that they were from a neonatal population (178 STs in 1790 isolates) but were less diverse, with fewer different types of ST, than those found in adult populations over shorter study periods (85, 189, 190). One probable explanation for this difference in ST diversity is that a neonatal population is likely to have less exposure to different STs, since neonates would have exposure limited to colonising isolates from the mother and their surroundings (including hospital settings). Neonates are unlikely to have had exposures that an adult might have through travel and varied food sources.

Another noticeable difference between the STs of Dutch isolates causing infection and STs in Norwegian, Scottish, English studies (85, 189, 190, 279) was that different STs predominated. ST95 and ST567 were the most predominant SENTINEL isolates over the whole study period (Figure 5.6-5.7). Although the most dominant STs identified in other longitudinal studies (ST131, ST73, ST69) (85, 189, 190) were present in SENTINEL they were not such key players in driving infection. Significantly ST131 which had been previously linked to the carriage and expansion of AMR in adult populations only accounted for 2.6% of SENTINEL study isolates. ST131 and other STs known to carry resistance are less likely to be present in this neonatal dataset as a consequence of low-level antimicrobial usage in this patient population. This would cause a lower level of selection for antimicrobial resistant isolates, exposing neonates to a different range of STs. It would be interesting to explore a large historical adult

dataset from the Netherlands to understand if this low level of ST131 and other resistant isolates is seen in the adult population of the Netherlands or if it is just a consequence of the young age of the patients in this study. The young population of patients included in SENTINEL would also be more vulnerable to invasive infection due to their age (309) so more likely to experience invasive disease from STs that would not be so prevalent in causing disease in an adult population (e.g. non-pandemic STs). The change in source of invasive isolates to include CSF in SENTINEL is likely an additional factor which contributes to the variation in ST population profile when comparing SENTINEL to bacteraemia datasets.

Levels of AMR in the Netherlands are low (310) and the mainly neonatal patient source of SENTINEL isolates would have had little exposure to high levels of antibiotic use or contact with the environment. Important consequences of this low level of antibiotic exposure in the neonatal *E. coli* population appear to be that SENTINEL, unlike other longitudinal studies, did not find a heavily multidrug resistant isolate population or observe an expansion of drug resistant isolates present, such as ST131, or other pandemic lineages including ST648, ST410, ST1193. In the absence of antibiotics, susceptible isolates are more likely to survive and be detected and established in the neonatal population. The low level of pandemic lineages is likely to explain why the increase in AMR carriage which would be expected from previous studies did not occur.

SENTINEL did not show an increase in AMR with median carriage of 1-5 types of AMR genes per genome per year in the SENTINEL isolate population throughout the period 1975-2021. In contrast to findings in isolates from an adult Norwegian population there was no real increase in ESBL gene carriage (Figure 5.12) (189). ECDC reports from

2000 to 2021 indicate that the Netherlands is a country of low levels of third generation cephalosporin, CPEs and colistin resistance (310). The SENTINEL isolates submitted on a voluntary basis to NRLBM, agree with those that required mandatory reporting to the ECDC and as expected, no carbapenemase-producing *E. coli* or colistin resistance genes were detected in this neonatal dataset as exposure to *E. coli* from areas of high CPE or colistin resistance (e.g., by travel) was unlikely. The variation from STs seen in other studies was also likely factor in the stability of SENTINEL isolate AMR population size and provided greater opportunities for expansion of more susceptible isolates.

In the absence of a rapid increase in frequency and diversity of acquired AMR genes in SENTINEL (Figure 5.11) inherent resistance genes were explored and this indicated an apparent increase in frequency of inherent resistance genes in more recent years (Figure 5.13). This might be attributable to the increased in number of isolates received by NRLBM per year (Figure 5.13B) or to the increased frequency of ST131 (Figure 5.7) and other pandemic lineages (e.g. ST1193) which carry inherent fluoroquinolone resistance genes (86). There is no evidence for an expansion of inherent resistance gene carriage and there does not appear to be an increase in AMR associated with inherent resistance genes.

Pandemic lineages, including ST131, ST73, ST95 all caused infection in the neonatal population. ST131 was a ST of particular interest as its pandemic clones are known to have played a role in the spread of AMR globally through their carriage of virulence factors, fluoroquinolone resistance and *bla*$_{\text{CTX-M}}$ genes (66, 68, 224). ST131 was detected at a low level (n=48) (Figure 5.6-5.7) but did not drive an increase in AMR carriage in the SENTINEL dataset and was not a major contributor to ceftriaxone

resistance as seen in Norway or England (85, 189). In contrast to SENTINEL (Figure 5.6-5.7), previous longitudinal studies have found rapid ST131 population emergence and expansion, especially in Clade A in Norway (189), and these were accompanied by an increase in the AMR carriage observed. ST131 appeared earlier in the Dutch isolate population (1995) than in the English and Norwegian studies (early 2000s), but did not expand at the same rate (85, 189, 190), did not cause an increase in AMR prevalence or play such a large role in causing disease in a neonatal population as seen in adult populations. The SENTINEL study confirms the importance of ST131 as a carrier of AMR as it demonstrates that, in the absence of an increasing ST131 population, AMR carriage does not increase, particularly *bla*$_{\text{CTX-M}}$ carriage. This supports findings in other longitudinal studies, where an increase in ST131 sees an increase in *bla*$_{\text{CTX-M}}$ carriage (85, 189). The appearance of ceftriaxone resistance in the last few years of SENTINEL was driven by the presence of ST131, ST69 and ST1193 among other STs, not found in early study years and was not due to just an increase in a specific ST131 clone.

In the SENTINEL isolate population, the most conspicuous expansion of STs seen over the 47-year period was in ST73 and ST69. Expansion of the mainly susceptible ST73 (Figure 5.7) likely contributes to the lack of increase in AMR in the SENTINEL dataset and it has previously been reported that multidrug resistance is not a factor required for expansion of the mainly susceptible ST73 population (85). ST73 in this dataset carried low levels of acquired resistance genes, all carried *mdf*(A), with 60% only carrying *mdf*(A) and no other resistance genes. The remaining MDR ST73 isolates carried additional genes to *mdf*(A), with low level of carriage of *bla*$_{\text{OXA}}$ (n=6, 6%) and *bla*$_{\text{TEM}}$ (n=23, 24%). The expansion of the susceptible ST73 population may be

assisted by the low levels of potential competing pandemic MDR lineages e.g., ST131, which is known to expand rapidly, and the decline observed in other STs, notably ST567.

Maternal colonisation with community-acquired and UTI-causing ST69 isolates appears be linked to the high incidence of ST69 in the neonatal SENTINEL patient population affected by bacteraemia and meningitis. ST69 has been a notable pandemic ST in clinical longitudinal datasets across England, Scotland and Norway and has been linked to some community-associated bloodstream infections (279). ST69 is not normally ESBL or *bla*$_{\text{CTX-M}}$ carrying (69), and the emergence and expansion of this ST69 population in the SENTINEL study is consistent with lack of AMR carriage and mirrors ST69 expansion seen in English and Scottish studies in 2000s (85, 190, 279). In the SENTINEL study ST69 also seems to be gradually replacing the decreasing ST567 population (85, 279) alongside ST73. ST69 may benefit from the low levels of competitive and potentially rapidly expanding pandemic lineages e.g., ST131. Both ST69 and ST73 appear to be emerging without association to antimicrobial resistance.

Despite the prevalence of ST95 it proved to be an exception to the link between pandemic lineages and AMR expansion and did not prove a driving force in increasing AMR carriage in the study as the population remained stable with no associated increase in AMR prevalence. ST95 frequently causes meningitis in neonates and was consistently a predominant cause of meningitis (n=66) and bacteraemia (n=99) in the SENTINEL patient population, agreeing with other reported European bloodstream infection studies, where it is a common cause of bacteraemia (85, 189, 190). *E. coli* causing neonatal meningitis require the ability to traverse the blood-brain-barrier (BBB)

and our ST95 isolates have VFs associated with this phenotype, including K1 capsule and *ibeA*, at high levels in this dataset (Figure 5.15). *ibeA* is a VF associated with the invasion of brain microvascular endothelial cells (69) but in this ST95 dataset only 16% of ST95 CSF isolates carried *ibeA*.

ST567 was initially a predominant ST in this invasive *E. coli* population, but surprisingly it declined and almost disappeared from this dataset. ST567 is clearly clinically important historically in causing meningitis and bacteraemia in neonates in the Netherlands and should be recognised as having been a major cause of invasive infection in neonates. Despite this ST567 has not previously been well documented, with only a handful of colonising faecal and UTI *E. coli* isolates being reported (311, 312).

ST567 was found at a very low frequency in *E. coli* populations causing bloodstream infection in English and Norwegian studies (85, 189, 190). Our SENTINEL finding adds to the current scientific knowledge available on ST567 and shows it has been and can be a significant pathogen, especially in a neonatal population. Further exploration of ST567 gave more insight into factors potentially causing meningitis and bacteraemia in neonates.

The striking disappearance of the historically important ST567 from this invasive *E. coli* dataset raised questions as to why such a predominant dataset disappeared. There is no evidence to suggest a ST567 mutation caused the development of a new clade during the study period and caused the ST to be less successful at maternal colonisation, vertical transmission and causing neonatal invasive disease. Time dated phylogenetic analysis showed that although the ST567 population falls into two clades

with highly similar core genomes and different virulence profiles (Figure 5.22), there is no evidence that the clade not containing a complete Fim region (Figure 5.19 - blue clade) emerged from the other more virulent clade (Figure 5.19 - red clade) in the study timeframe and became dominant. In a hypothetical situation of a weaker clade dominating in more recent years, there would have subsequently been a weaker ST567 population, which would likely be less successful and so possibly cause the sudden decline observed in ST567. There were two distinct clades, with both containing isolates from recent study years and so co-existing at the same point in time, and having evolutionary split in the 1800s, so this cannot explain the decline in ST567.

ST567 isolates causing meningitis were found in both clades and phylogenetically dispersed among bloodstream infection isolates. There was no obvious difference between the ST567 isolates that crossed the BBB compared to those that caused bacteraemia. Clearly neither ST567 clade had evolved to be more successful at crossing BBB during the study period, consequently the lower proportion of CSF isolates received by NRLBM in the later study years did not have an impact on or explain the observed disappearance of ST567.

Investigation of several other factors that might cause the decline in ST567 also gave no clear explanation for the decline in ST567. Plasmid replicons and AMR carriage were similar in both ST567 clades and showed no clear trends accounting for the decline of ST567 in this neonatal population. Isolates in both clades carried a 142,433 bp virulence plasmid, and varying AMR plasmids, with no clear difference in AMR plasmid carriage to have impact on either clade.

Resistance gene carriage was at a low-level across the whole SENTINEL *E. coli* dataset, however focussing on the ST567 dataset (Figure 5.21) there does appear to be an increased frequency of type of resistance genes per genome in the more recent ST567 isolates, but not linked to a specific clade There is not a drastic increase that caused a sudden decline in population. Further study of a larger set of long read sequenced ST567 isolates could establish whether uptake of AMR plasmids or AMR genes, plays a role in the disappearance of this ST.

A change that may have affected the colonising success of ST567 *E. coli* was found in the virulence factor profiles. One key difference in virulence potential between the two ST567 clades was the absence of a section of the Fim region (*fim*ABEI) across the smaller clade (Figure 5.19 - blue clade) especially in more recent isolates. Fim genes are involved in adhesion (313). In depth gene association studies (Table 5.2) confirmed this clade difference and highlighted a link between genes for fimbriae structures and the larger clade (Figure 5.19 – red clade).

Long read sequencing demonstrated the missing section of the chromosomal Fim region included *fim*S, the phase switching region as well as *fim*ABEI (Figure 5.23). The missing region was comprised of recombinases that mediate the inversion of the *fim* phase switch (FimB and FimE), the major subunit forming the type 1 *fimbriae (*FimA) and another gene known to be required for fimbriae production (FimI) (314). *E. coli* missing all these genes (*fim*ABEI) would be predicted to have non-functional fimbriae and yeast agglutination assays confirmed this phenotypically, suggesting the *E. coli* in this smaller clade (Figure 5.19 - blue clade) would be less successful at adherence

(315) and therefore less effective at the colonisation of a niche and expansion, compared to the larger (Figure 5.19 - red clade) that had a completed Fim gene region.

The cause of this *fim*ABEI deletion is likely ancestral and due to homologous recombination of the 891 bp region of similarity at two points in the chromosome (Section 5.3.11). Long read sequencing also showed that the subsets of isolates from the two clades had different Fim regions but within each clade the Fim regions were the same (Figure 5.23). All isolates with a non-functional Fimbriae had a deletion in the Fim region which occurred in the same location on the chromosome and was likely responsible for their non-functionality.

Type I fimbriae help *E. coli* adhere to mucosal surfaces and enhance intestinal colonisation (313, 315). Therefore it is likely that non-functional or missing fimbriae would make it harder for *E. coli* adhere, colonise and expand in a niche, but in this case are unlikely to be responsible for the disappearance for ST567 since the loss of Fim genes from ST567 clades occurred in 1800s (Figure 5.19) and is not a recent loss during the study period so may play a factor in the disappearance but cannot explain the loss of both clades. This means both clades are well established, even with the lack of these key virulence gene, so is therefore unlikely that this factor is the main reason for the more recent disappearance of the ST567 in this invasive disease dataset.

Another factor that could have caused the decline in ST567, would be a change to the capsular gene region making ST567 less pathogenic. There was no major difference in capsule typing across clades, as the majority of isolates were typed K1. In depth capsule region studies showed that ST567 had *kps*D, *kps*M and *kps*T in identical

settings in both clades with no disruption to the capsule gene cluster that would affect capsule production and so ST567 decrease virulence and contribute to the decline in ST567 population (Figure 5.29).

The cause of the decline in ST567 in this neonatal population remains puzzling. Although prevalent in the Netherlands for almost 50 years, publicly available datasets show ST567 has only been reported elsewhere over the globe in the past few decades (Figure 5.29B). The earliest reported isolate which had a known isolation date was collected from livestock in 1980. Publicly available datasets also included ST567 isolates obtained from humans since 2000s. A few of these isolates were obtained from the human bloodstream, supporting Norwegian and English findings (85, 189, 190) that ST567 had caused invasive disease in other countries. Despite being present in other countries ST567 only appeared at a much lower frequency than in SENTINEL, with only 56 isolates reported on EnteroBase and so it is not possible to determine if the ST567 decline observed in SENTINEL occurred more widely. Unfortunately, there was no available information on ST567 isolated from CSF, so a comparison with SENTINEL ST567 CSF isolates is not possible either.

The Dutch population of ST567 was similar to other European isolates (Figure 5.29) (85, 189) raising question as to why the ST567 was apparently a more successful invasive ST in the SENTINEL population based on frequency of infection. It seems likely that ST567 is more successful in this neonatal population, than in adult populations, because of the vulnerability of a neonate since there are no significance differences in the ST567 characterised (Figure 5.17). Another factor to consider is that the SENTINEL dataset had an absence of most competing pandemic lineages (e.g., ST131), which may have allowed ST567 more opportunity to cause infection in a

neonatal population. Ideally it would be useful to compare SENTINEL findings with similar large historical collections of isolates from the commensal and environmental population of Netherlands *E. coli* but unfortunately these are not available. One important consideration is the SENTINEL study includes isolates from 1975, much earlier than the other European studies, it is possible that the lower level of ST567 in these other studies is because the decline seen in the SENTINEL population has not occurred solely in the Netherlands and has happened on a wider geographic scale, explaining why the more recent studies have a lower frequency of ST567 isolates.

Analysis of the small number of publicly available ST567 livestock isolates showed these were closely related to human isolates since they fell within the SENTINEL dataset clades so could be a persistent source of ST567 population posing a risk of human colonisation and infection. Publicly available livestock ST567 isolate data suggests that recent ST567 isolates when compared to SENTINEL data have not adapted to be more successful in colonising or infecting livestock than humans causing a shift of ST567 away from humans to livestock. There was no consistent trend in the emergence or distribution of livestock isolates which could account for the decline in ST567 seen in SENTINEL. Livestock isolates were found throughout the ST567 tree, not in a particular clade and where dates were known, isolated from 1980-2021. This therefore ruled out the possibility that ST567 could have adapted to find livestock a preferential host and so this is not a reason for the decline in ST567 seen in SENTINEL.

The reason for the decline of the ST567 population in SENTINEL remains to be determined. Currently the only potential explanation maybe linked to a change in virulence factors and adhesion capability of ST567, preventing the colonisation and

likely expansion in the human host. This however cannot be the only explanation as it cannot account for the decrease in ST567 isolates which do have a complete Fim region.

SENTINEL highlights the low level of AMR gene carriage seen in this *E. coli* population throughout the study period which demonstrates that the prescribing practices and antimicrobial stewardship used at a national level in the Netherlands has been successful in controlling the spread of AMR in this patient population over this 47-year period. The antimicrobial stewardship and surveillance in the Netherlands appear to be preventing the selection and spread of resistant isolates in this neonatal population and therefore probably in pregnant individuals. The older population captured in this study also do not show real differences in resistance profiles, between age groups, showing good stewardship and surveillance in these populations too.

Overall, this study revealed the trends in the presence and diversity of AMR genes and the STs discovered in this majority neonatal population, as well as the diversity of Dutch pathogenic *E. coli* isolates over several decades. It showed the emergence and demise of dominant lineages and found no links between either and antimicrobial susceptibility. Surprisingly ST131 did not play a major role in causing infection in the study population. SENTINEL identified ST567 as a major contributor to invasive neonatal disease and thorough investigation has helped to contribute new knowledge to an area where there was previously little information available, although questions still remain as to why ST567 disappeared in this neonatal dataset in recent years. It is hoped that in the future this dataset will give further insight into how these invasive *E. coli* are spreading in this young, likely nosocomial, population and enable this to be better controlled.

6. Discussion and Future Work

E. coli is known to cause disease in both the immunocompetent and immunocompromised and play an important role in the global spread of AMR. This thesis has provided an insight into the presence of *E. coli* in three settings, chosen to represent where the presence of AMR can have significant and possible life-threatening impacts. It reports investigations of the dynamics of colonisation and infection in the absence of antibiotic selection and the levels of AMR in different environments and age groups as well as the mechanisms involved in the spread of AMR. An important factor in this thesis was to obtain and characterise strain-level detail of *E. coli* dynamics in multiple settings using WGS. This in combination with daily sampling was a powerful tool which for the first time allowed dynamics of colonising isolates to be studied during a traveller stay and during QEHB ICU inpatient stay. It also allowed detection of the presence and absence of circulating strains. In a healthcare environment it is critical to detect and understand the presence of circulating strains as it indicates the quality of IPC precautions in place. The strain level analysis demonstrated the concerning amount of AMR present in colonising *E. coli* in travellers to Laos and gave an insight into those STs present on an ICU ward in the UK. It highlighted the real differences in ST diversity and AMR carriage of colonising STs between healthy travellers to Laos and those found colonising patients in a Birmingham ICU ward during the COVID-19 pandemic.

Colonisation in healthy travellers and in ICU inpatients both demonstrated that colonisation can be persistent, but equally an individual can be colonised with an ST

for as little as a day. This revealed the multiple opportunities for AMR spread. These strain level studies may have sampling limitations, perhaps only isolating and capturing the predominant *E. coli* ST. However, even with this limitation this method captures the variation in colonising *E. coli* STs and so is still an excellent method in providing an insight into previously unseen strain dynamics. Ideally if there was more time and access to sufficient volume of stool samples from both colonisation studies, multiple colonies from same stool sample should be whole genome sequenced. This expansion of isolates sequenced would enable the detection of even more diversity in colonising *E. coli* isolates from travellers to Laos and would potentially identify any smaller ST population, which may have been overlooked with the current sampling method. Expanded sequencing in combination with metagenomic sequencing of samples would detect both the persistent and transient ST at the same time removing selection bias and clarify whether as expected in the Laos plasmid study persistent strains have been interrupted by transient STs. In addition expanded sequencing of the QEHB ICU patient stool samples would help confirm if a patient is colonised by a single ST only, as might be expected from *E. coli* overgrowth seen in a previous QEHB ICU patients (182) and would allow detection of the impact of these changes in colonisation on the whole microbiome. If there are more STs present in the same QEHB stool sample this method would capture less abundant STs undetected by the current sampling method. In both the ICU and in traveller populations further sampling from the stool samples would confirm if there were even more STs present, providing even more opportunities for AMR spread. It would also detect any more AMR carriage already present in any additional less prevalent ST populations.

Strain sharing was demonstrated in both the traveller population and on the ICU, in both cases the speed and frequency of strain changes was identified. This helped to identify potential routes for AMR spread either by the circulation of resistant *E. coli* between patients or with the increased opportunities for exposure to AMR by mixing of *E. coli* populations carrying plasmids that have the potential to spread resistance. In both colonisation studies the importance of the environmental reservoir of AMR in strain sharing was identified and this highlighted the real importance of knowing the colonising populations present in the surroundings of the patient or traveller cohort. In other publications (186, 187, 316, 317) knowledge of the local environmental reservoir of *E. coli* has been linked to routes of strain spread and so potential routes of AMR spread. This environmental information would have been invaluable in the settings in this thesis, especially in a hospital, to monitor and prevent outbreaks of AMR organisms.

A comparison of the *E. coli* STs found in both colonising and infection isolate populations in the absence of selection based on any specific antimicrobial therapy in the three different settings found great ST diversity across settings. The differences in ST found in the differing settings can be explained by the different ages, locations and vulnerability of the study participants in each setting. The healthy travellers in Laos had visited multiple places in Vientiane, Laos, a setting with high increasing levels of AMR reported in the environment (29, 228, 238, 318). This was a far less controlled environment than that of the QEHB ICU patients who had additional shielding due to COVID-19 restrictions in the UK community limited travel and interactions before their admission to ICU and then the COVID-19 precautions in place in the ICU. The combination of COVID-19 precautions and restricted patient mobility due to illness

limited patient exposure to their direct environment on the ward or areas of testing in the hospital (e.g., scanners) and hospital staff, in complete contrast to the freedom of movement seen in the Laos traveller population. It should be noted that the COVID-19 precautions in 2020-2021 may have limited the exposure of neonates and their mothers and therefore affected the STs isolated in SENTINEL study. The SENTINEL patient population, like the ICU patients, again had less direct exposure to highly resistant *E. coli,* as levels of AMR in the Netherlands is known to be low (7, 193), the neonates in this study were probably only exposed to the hospital environment and any colonising *E. coli* from their mother.

Although there was great diversity of ST populations between the three study settings, pandemic lineages were present in each setting. Pandemic lineages played a role in infecting the majority neonatal population in the Netherlands. Interestingly in multiple settings the pandemic lineage ST131 was not as high as anticipated from previous reports (85, 189), perhaps this is because ST131 is more commonly linked to infection rather than colonisation (276). All datasets also highlighted the importance of non-pandemic lineages in the carriage of a variety of resistance genes and plasmids. This thesis demonstrated the significant role of non-pandemic lineages in neonatal infection. One important example was ST567, the disappearing lineage in SENTINEL project, which demonstrated clearly that major causes of disease in vulnerable populations are not always pandemic lineages. Non-pandemic lineages carried concerning levels of MDR in Laos, and the invasive *E. coli* longitudinal dataset provided further reason to acknowledge the role of non-pandemic lineages in causing disease at a national level.

Low or no CPEs were detected in all settings. This was contrary to what would be expected since CPEs were previously reported in an ICU in Vientiane, Laos (319), and in other UK hospitals (39, 189, 190). The prescription of antimicrobials that have the potential to select for a carbapenemases resistant population of *E. coli* are also frequently prescribed in hospital settings and could be a driving force for carbapenemase resistance.

The known increase in AMR levels over the globe reported in recent years and seen in other longitudinal datasets (85) led to the expectation that ESBL *E. coli* levels would be high in the ICU dataset and in the more recent years of the SENTINEL longitudinal study, but this was not the case. In both these patient datasets there was little ESBL carriage aside from bla_{TEM}, and the traveller dataset confirmed the important role of plasmids in carriage of bla_{TEM} in *E. coli*. TEM beta-lactamases are widespread and widely occurrent in colonising *E. coli* (320). bla_{TEM} ESBL infections can be treated with an antimicrobial plus a beta-lactamase inhibitor, so although still AMR, they are less of a concern than CPE and colistin resistance.

SENTINEL was a powerful historical study providing insight into a neonatal population for 40 years, which showed long term infection trends with widely fluctuating ST populations. It was a longitudinal study about a previously understudied cohort, which also provided insight into historical CSF and BSI isolates, in contrast to previous longitudinal studies which are mainly focussed on BSIs (85, 189, 190). Although the important ST, ST567 was well characterised in this study, the cause for the disappearance of ST567 has not yet be identified. Further research into the patient population could help to establish whether there were patterns in the patients infected with this ST (e.g., medical history, prescribed antimicrobial therapy) to identify potential

causes of selection for the ST. Access to the geographical location of the patients could show whether ST567 was localised to any particular region of the Netherlands and whether anything that changed locally could have triggered the decline in ST567 submitted to the NRLBM.

In future long read sequencing could be utilised to further analyse *E. coli* populations from all three settings discussed in this thesis. The characterisation of the Laos plasmid population showed the limitations of current plasmid databases and the diversity of plasmids in this population. Long read sequencing techniques identified a new transposon and allowed detailed plasmid typing not possible using short read sequencing. This technique also helped to compare the differences in the Fim gene region in ST567 clades and give a focussed view of the capsule gene settings in these clades. In the future the remaining colonising isolates from travellers to Laos not yet sequenced with long read sequencing could be explored and the technique also could be used to see the collection of plasmids present on the ICU ward. The ICU study demonstrated the lack of strain movement on the ICU ward and long read sequencing could be used to confirm the strain sharing and learn more about these isolates. In addition it would also be interesting to long read sequence a wider set of isolates from the SENTINEL ST567 dataset to further explore the potential historical carried plasmid and to characterise other historically important sequenced types driving neonatal invasive infection.

Future metagenomic studies could be used to explore the microbiome of patients in the ICU and confirm the actual impact of antimicrobial prescribing during a patients stay. It would also confirm the suspected overgrowth with species such as *Enterococcus* and *Staphylococcus* suggested by culture plating in this thesis.

It would be interesting to repeat sampling on the ICU now community COVID-19 restrictions have been lifted to see if this affected the colonising ST population now that patients are likely to have been travelling more locally and internationally prior to their ICU admission. It would be interesting to contrast transmission levels of colonising *E. coli* now that IPC has changed in the hospital environment and would help to understand if COVID-19 precautions on the ward had reduced transmission of colonising isolates.

This thesis has demonstrated the differences between MDR colonising *E. coli* in travellers and ICU patients, and *E. coli* that cause infection in a neonatal population all in the absence of antibiotic selection. It demonstrated the diversity of multidrug resistance settings in the Laos *E. coli* population and highlighted the lack of MDR in both colonising *E. coli* in ICU patients and *E. coli* infecting the neonatal population in the Netherlands. The strain level sampling method provided a useful tool in identifying circulating strains and dynamics of colonising populations in the ICU patient and traveller populations. It also allowed the monitoring of long-term ST and AMR trends in invasive *E. coli* in the Netherlands. The use of long read sequencing in this thesis allowed a focus on MGEs and their role in spread of AMR in travellers to Laos. In the future additional long read sequencing, metagenomic sequencing and environmental sampling in these three settings could answer remaining questions and potentially further add to existing knowledge the role of *E. coli* in the spread of AMR.

7. Appendices

Appendix A1

Table A1.1 IMPACT2 Patient information

Sample Number	Patient Number	Recruited to IMPACT2	First Stool sample received	Last Date of Stool sample	Gender	Age	Date of ICU Admission	Date of Transferred off ICU	Length of ICU Stay (days)	Admission Reason	Overarching admission category	Admission from
QEHB05	IMP2-05	19/05/2021	24/05/2021	04/06/2021	F	45	18/05/2021	06/06/2021	19	Subarachnoid haemorrhage (SAH)	Neurology	Other hospital
QEHB06	IMP2-06	26/05/2021	27/05/2021	02/06/2021	F	29	22/05/2021	02/06/2021	11	Liver failure	Hepatic	Other hospital
QEHB08	IMP2-08	28/05/2021	29/05/2021	12/07/2021	F	47	22/05/2021	15/07/2021	55	Liver transplant	Transplant	Community
QEHB09	IMP2-09	30/05/2021	31/05/2021	04/06/2021	M	23	29/05/2021	04/06/2021	5	Burns	Burns	Community
QEHB10	IMP2-10	05/06/2021	08/06/2021	18/06/2021	M	41	05/06/2021	18/06/2021	13	Cardiac arrest	Cardiovascular	Community
QEHB11	IMP2-11	22/06/2021	24/06/2021	24/06/2021	M	77	21/06/2021	24/06/2021	3	Severe aortic stenosis	Cardiovascular	4 days on QEHB general ward
QEHB13	IMP2-13	12/07/2021	13/07/2021	17/07/2021	M	50	08/07/2021	18/07/2021	10	Meningioma	Neurology	10 days on QEHB general ward
QEHB14	IMP2-14	13/07/2021	17/07/2021	26/07/2021	F	57	12/07/2021	26/07/2021	14	Craniotomy	Neurology	Other hospital
QEHB15	IMP2-15	25/07/2021	29/07/2021	29/07/2021	M	79	24/07/2021	Died	5	Cardiac arrest	Cardiovascular	Community
QEHB16	IMP2-16	25/07/2021	26/07/2021	30/07/2021	M	80	23/07/2021	30/07/2021	7	Low GCS on ward stay	Neurology	10 days on QEHB general ward
QEHB19	IMP2-19	18/08/2021	18/08/2021	30/08/2021	M	68	13/08/2021	02/09/2021	20	Fall from height	Trauma	Community
QEHB20	IMP2-20	19/08/2021	20/08/2021	27/08/2021	M	73	15/08/2021	28/08/2021	14	Oncology admission due to facial swelling	Oncology	Community
QEHB21	IMP2-21	21/08/2021	24/08/2021	09/10/2021	F	74	20/08/2021	10/10/2021	51	Road traffic collision (RTC) - Polytrauma	Trauma	Community
QEHB23	IMP2-23	10/09/2021	10/09/2021	20/09/2021	F	53	08/09/2021	22/09/2021	14	Cardiac arrest	Cardiovascular	Community
QEHB24	IMP2-24	10/09/2021	17/09/2021	24/09/2021	M	20	09/09/2021	24/09/2021	16	RTC-Polytrauma	Trauma	Community
QEHB25	IMP2-25	27/09/2021	28/09/2021	16/10/2021	F	61	23/09/2021	16/10/2021	24	Malignant peripheral nerve sheath tumour	Oncology	1 day on QEHB general ward
QEHB26	IMP2-26	30/09/2021	04/10/2021	19/10/2021	M	51	27/09/2021	19/10/2021	22	Boerhaave's perforation	Gastroenterology	Other hospital
QEHB28	IMP2-28	16/10/2021	20/10/2021	20/10/2021	F	54	12/10/2021	21/10/2021	9	Elective admission - hepatectomy	Elective surgery	Community
QEHB29	IMP2-29	20/10/2021	23/10/2021	11/11/2021	M	51	15/10/2021	14/11/2021	30	SAH	Neurology	Community
QEHB30	IMP2-30	21/10/2021	22/10/2021	13/11/2021	M	55	18/10/2021	14/11/2021	27	Community acquired pneumonia (CAP)	Respiratory	Community
QEHB32	IMP2-32	29/10/2021	02/11/2021	13/11/2021	M	56	27/10/2021	13/11/2021	17	RTC-Polytrauma	Trauma	Community
QEHB33	IMP2-33	15/11/2021	20/11/2021	20/11/2021	M	25	13/11/2021	21/11/2021	8	RTC -Polytrauma	Trauma	Community
QEHB37	IMP2-37	20/11/2021	20/11/2021	01/12/2021	M	44	16/11/2021	02/12/2021	16	Lung transplant	Transplant	Community

Table A1.2 Positive microbiology tests for ICU patients during their stay.

Patient Number	Date	Sample	Results
IMP2-05	20/05/2021	Urine (Midstream Urine (MSU))	*E.coli* isolated (Amoxicillin:R, Augmentin:R, Ciprofloxacin: S, Cepahlexin: S, Gentamicin: S, Nitrofurantoin:S, Trimethoprim:S)
IMP2-05	22/05/2021	Urine (Catheter specimen of Urine (CSU))	*E.coli* isolated (Amoxicillin:R, Augmentin:S, Ciprofloxacin: S, Cepahlexin: S, Gentamicin: S, Nitrofurantoin:S, Trimethoprim:S)
IMP2-05	25/05/2021	Blood culture (BC)	*K. oxytoca* (Amoxicillin:R, Augmentin: S, Ciprofloxacin: S, Meropenem: S, Tazocin:S)
IMP2-05	30/05/2021	BC	*S.hominis* (Clindamycin: S, Flucloxacin: R, Fusidic Acid: R, Gentamicin:S, Vancomycin:S)
IMP2-05	04/06/2021	BC	*E. faecium* (Amoxicillin: R. Vancomycin:S)
IMP2-05	06/06/2021	Cerebrospinal fluid (CSF)	*S. haemolyticus* (Flucloxacillin:R, Gentamicin:R , Clindamicin:R, Linezolid:S, Vancomycin: S, Rifampicin:R)
IMP2-06	22/05/2021	Blood serum	EBV DNA Quantification <150 IU/mL
IMP2-06	22/05/2021	Blood	CMV PCR < 200IU/mL
IMP2-06	29/05/2021	Blood serum	Serological evidence of past EBV infection, recent EBV unlikely.
IMP2-06	29/05/2021	Blood serum	EBV DNA Quantification <150 IU/mL
IMP2-06	29/05/2021	Blood	CMV PCR < 200IU/mL
IMP2-06	02/06/2021	CSF Culture	Clear colourless, Culture: *S. epidermidis* , *S. capitis* .
IMP2-08	02/06/2021	Sputum	Blood stained, *Candida albicans* (+)
IMP2-08	11/06/221	Tip of cannula (central line)	*Staphylococcus. hominis* (++) (Clindamycin S; Fluclox R; Fusidic Acid R; Gentamicin R; Vancomycin S)
IMP2-08	13/06/2021	Intrauterine contraceptive device	*Candida albicans* (++)
IMP2-08	13/06/2021	High vaginal swab	*Candida albicans* (++)
IMP2-08	18/06/2021	Drain	*Enterococcus faecium* (+++, Amox:R, Vanc:S), S. haemolyticus (+)
IMP2-08	22/06/2021	Blood	CMV DNA 712 cp/ml
IMP2-08	27/06/2021	Blood serum	CMV IgG Pos
IMP2-08	27/06/2021	Blood serum	CMV DNA 1558cp/ml
IMP2-08	04/07/2021	Blood serum	CMV IgG Pos
IMP2-08	14/07/2021	Blood	CMV DNA 392 cp/ml
IMP2-09	29/05/2021	Swab	*Bacillus cereus* (+++) and normal skin flora isolated
IMP2-09	29/05/2021	Swab	*B. cereus* (+++) and normal skin flora isolated
IMP2-09	29/05/2021	Swab	*B. cereus* (+++) and normal skin flora isolated
IMP2-10	07/06/2021	Sputum	Purulent. *E. coli* ++
IMP2-10	08/06/2021	Sputum	*E . coli* (Amoxicillin:S, Augmentin: S, Ciprofloxacin: S, Co-trimoxazole:S, Gentamicin: S, Meropenem:S, Tazocin:S)
IMP2-10	10/06/2021	BC	*S. epidermidis* . (Clindamycin:R, Flucloxacillin:R, Fusidic Acid:R, Gentamicin:R, Rifamipicin:R, Vancomycin:S)
IMP2-10	14/06/2021	Sputum	Mucoid sputum. Purulent. *E. coli* +++ (Amoxicillin: S, Augmentin: S, Ciprofloxacin: S, Gentamicin:S , Meropenem:S)
IMP2-10	16/06/2021	Sputum	Mucopurulent sputum. Purulent. *E. coli* +++, (Amoxicillin: S, Augmentin: S, Ciprofloxacin: S, Gentamicin:S , Meropenem:S) No other *Candida* or fungi isolated. Other respiratory tract flora also isolated at 48 hours.
IMP2-13	13/07/2021	Sputum	Mucopurlent sputum , *Citrobactrer koseri* (Ciprofloxacin:S, Gentamicin: S, Meropenem: S, Augmentin: S), *Acinetobacter ursingii* (Ciprofloxacin:S, Gentamicin: S, Meropenem: S), culture also yielded upper respiratory tract flora at 48hrs.
IMP2-14	17/07/2021	Sputum	Purulent. *Klebsiella aerogenes* (+++). (Augmentin:R; Ciprofloxacin:S, Co-trimoxazole:S, Ertapenem:S, Gentamicin:S, Meropenem:S, Tazocin:S) No *Candida* or fungi isolated
IMP2-14	17/07/2021	Urine (Unspecified site)	*Enterococcus Faecalis* (Amoxicillin:S, Nitrofurantoin:S, Vancomycin:S)
IMP2-15	26/07/2021	Sputum	Mucopurlent. *Pseudomonas aeruginosa* +++ (Ceftazidime:S, Ciprofloxacin:S, Gentamicin:S, Meropenem:S, Tazocin:S, Tobramycin:S) No *Candida* or fungi isolated
IMP2-19	15/08/2021	Sputum	Purulent. *Haemophilus influenzae* +++ (Tetracycline:S, Amoxicillin :S, Augmentin:S), S. aureus ++ (Clindamycin:S, Flucloxacillin:S, Gentamicin:S, Vancomycin:S, Tetracycline:S) *Candida albicans* + Also upper respiratory tract flora at 48hrs.
IMP2-19	21/08/2021	Sputum	Mucoid bloodstained *Candida albicans* +
IMP2-19	22/08/2021	BC	*S. epidermidis* (Clindamycin:R, Flucloxacillin:R, Fusidic acid:R, Gentamicin:S, Vancomycin:S)
IMP2-20	14/08/2021	Pus swab (chin)	Mixed growth including *Pseudomonas* . No anaerobes
IMP2-20	15/08/2021	Pus sample (neck)	Numerous Pus cells. Gram positive cocci seen. *S. epidermidis* + and *Streptococcus intermedius* +++ (Penicillin:S, Amoxicillin :S, Vancomycin :S, Clindamycin:S)
IMP2-20	22/08/2021	Sputum	Purulent. *Pseudomonas +++*
IMP2-20	26/08/2021	Pus swab	*C. albicans* (+++) No anaerobes
IMP2-21	23/08/2021	Bronchoalveolar lavage (BAL)	Numerous Pus cells seen. GPC seen. *S. aureus* +++ (Clindamycin:S; Flucloxaciilin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S)
IMP2-21	23/08/2021	Sputum	Purulent. GPC seen. *S. aureu* s +++ (Clindamycin:S; Flucloxaciilin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S) No fungi or *Candida* isolated.
IMP2-21	24/08/2021	Sputum	Bloodstained GPC seen. *S. aureus +++* (Clindamycin:S; Flucloxaciilin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S) No fungi/*Candida* isolated
IMP2-21	24/08/2021	Sputum	Purulent. GPC seen. *S. aureus +++* (Clindamycin:S; Flucloxaciilin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S) No fungi/*Candida* isolated
IMP2-21	27/08/2021	Sputum	Mucopurulent. +++ *S. aureus* (Clindamycin:S; Flucloxaciilin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S) *Hafnia alvei* ++ (Ciprofloxacin:S, Gentamicin:S, Augmentin:R, Tazocin:S) No *Candida* or other fungi isolated.

Patient Number	Date	Sample	Results
IMP2-21	29/08/2021	Tip of cannula	*C. albicans* +++ (Amphotericin:S, Caspofungin:S, Fluconazole:S, Voriconazole:S) and Mixed organisms including Coliforms
IMP2-21	29/08/2021	Sputum	*Serratia marcescens* + (Augmentin:R, Ciprofloxacin:S, Gentamicin:S, Meropenem:S) *Candida albicans* +
IMP2-21	29/08/2021	Urine	*Candida albicans* >10^5 orgs/ml
IMP2-21	03/09/2021	Sputum	Blood stained. *Serratia marcescens* +++ (Augmentin:R, Ciprofloxacin:S, Gentamicin:R, Meropenem:S) *Pseudomonas* +++ (Ciprofloxacin:S, Gentamicin:S, Meropenem:S, Tobramycin:S)No Candida or fungi isolated.
IMP2-21	05/09/2021	Urine	*C. albicans* >10^5 orgs/ml
IMP2-21	05/09/2021	Urine	Heavy mixed growth
IMP2-21	07/09/2021	Sputum	*E. coli* (Ciprofloxacin:S, Gentamicin:S, Meropenem:S, Tazocin:R, Amoxicillin:R, Augmentin:R), *Pseudomonas aeruginosa* + (Ciprofloxacin:S, Gentamicin:S, Meropenem:S, Tazocin:S) and Candida albicans ++
IMP2-21	12/09/2021	Wound swab (fistula)	*Candida albicans* +
IMP2-21	12/09/2021	Sputum	Mucoid *Pseudomonas aeruginosa* +++ (Ceftazidine R, Ciprofloxacin:I, Gentamicin:s, Meropenem :R, tobramycin:s, Tazocin R) , *Candida albicans* +
IMP2-21	14/09/2021	Sputum	Purulent. *P. aeruginosa* +++ and *Candida species* +
IMP2-21	20/09/2021	Sputum	Mucopurulent Bloodstained. *Pseudomonas aeruginosa* +++, *E.coli* +, No *Candida* or fungi.
IMP2-21	20/09/2021	Sputum	*Pseudomonas* +++
IMP2-21	23/09/2021	Sputum	Purulent. *Pseudomonas aeruginosa* +++ (Ceftazidine:S, Ciprofloxacin:R, Gentamicin:S, Meropenem:R, tobramycin:S, Tazocin:R), *Candida* species +
IMP2-21	28/09/2021	Sputum	Mucopurulent. *Pseudomonas aeruginosa* +++ (Ceftazidine:R, Ciprofloxacin:R, Gentamicin:S, Meropenem :R, tobramycin:S, Tazocin R) *Candida* species + Culture also yielded: Upper respiratory tract flora at 48 hours
IMP2-23	13/09/2021	Sputum	Mucosalivary. *S. aureus* (Clindamycin:R, Flucloxacillin:S, Gentamicin:S, Tetracycline:S and Vancomycin:S) *Candida dubliniens* +
IMP2-23	14/09/2021	Sputum	*Rauotella. ornitholytica* + (Amoxicillin:R, Augmentin:S, Ciprofloxacin:S, Meropenem:S, Gentamicin:S), *S. aureus* (Gentamicin:S, Clindamycin:R, Flucloxacillin :S, Tetracycline:S, Vancomycin:S)
IMP2-23	18/09/2021	Sputum	Purulent. *R. ornitholytica* + (Amoxicillin:R, Augmentin:S, Ciprofloxacin:S, Meropenem:S, Gentamicin:S) No *Candida* or other fungi isolated
IMP2-23	21/09/2021	Urine (CSU)	*E. coli* >10^5 orgs/ml (Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Cephalexin:S, Gentamicin:S, Trimethoprim:S, Tazocin:R, Nitrofurantoin:S)
IMP2-24	10/09/2021	Sputum	Purulent. *Candida albicans* + Culture also yielded: Upper respiratory tract flora at 48 hrs.
IMP2-24	13/09/2021	Sputum	Purulent. *Candida albicans* + Upper respiratory tract flora at 48 hrs
IMP2-24	19/09/2021	Sputum	*Klebsiella oxytoca* + (Amoxicillin:R, Augmentin:R, Ciprofloxacin: s, Meropenem: s, Gentamicin:S. Tazocin: R) *Candida metapsilosis* +
IMP2-25	29/09/2021	Wound swab (lumber spine)	*E. coli* + (Ciprofloxacin: S, Ertapenem:S, Meropenem:S) ESBL positive.
IMP2-25	06/10/2021	Tip of cannula	*S. epidermidis* +++ (Flucloxacillin:R, Gentamicin:R, Vancomycin:S) , *S. haemolyticus* +++ (Flucloxacillin:R, Gentamicin:R, Vancomycin:S)
IMP2-25	06/10/2021	BC	*S. epidermidis* (Clindamycin:R,Flucloxacillin:R, Gentamicin:R, Vancomycin:S)
IMP2-25	06/10/2021	Urine	*E. coli* + (Amoxicillin:R, Augmentin :S, Ciprofloxacin: S, Cephalexin :R, Ertapenem:S, Fosfomycin:S, Gentamicin:S, Meropenem:S, Nitrofurantoin:S, Trimethoprim:R) ESBL positive.
IMP2-25	15/10/2021	Urine (Unspecified)	*C. albicans* >10^5 orgs/ml
IMP2-25	29/09/2021	Drain	*Candida glabrata* + (Amphotericin:S, Caspofungin:S, Voriconazole:S). No anaerobes isolated after 5 days incubation
IMP2-25	07/10/2021	BAL	Herpes simplex type 1 DNA DETECTED by PCR
IMP2-25	07/10/2021	Swab (Chest drain site)	*Klebsiella pneumoniae* +++
IMP2-25	07/10/2021	Swab (unspecified site)	*K. pneumoniae* +++ (Augmentin:S, Ciprofloxacin:S, Meropenem:S, Tazocin:S)
IMP2-25	07/10/2021	Swab (unspecified site)	*K. pneumoniae* ++
IMP2-25	10/10/2021	Sputum	Mucopurulent sputum. *Klebsiella* species (Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Gentamicin:S, Meropenem:S, Tazocin:R)
IMP2-25	16/10/2021	Pus - Oesophageal	Numerous Pus cells, No organisms seen. Culture: *Kocuria varians* +++, *S. haemolyticus* +++, *Streptococcus* species+
IMP2-28	15/10/2021	Tissue	Gram Stain: No Pus cells seen, No organisms seen. *Cutibacterium acnes* ++ No anaerobes isolated after 5 days incubation
IMP2-29	15/10/2021	Sputum	Purulent *Serratia marcescens* +++ (Ciprofloxacin: S, Co-trimoxazole: S, Gentamicin: S, Meropenem :S)
IMP2-29	17/10/2021	Sputum	Purulent, *S. marcescens* ++ (Ciprofloxacin: S, Co-trimoxazole: S, Gentamicin: R, Meropenem :S, Tobramycin:R)
IMP2-29	19/10/2021	Sputum	Mucopurulent bloodstained *S. marcescens* ++ (Ciprofloxacin: S, Co-trimoxazole: S, Gentamicin: S, Meropenem :S, Tobramycin:R)
IMP2-29	30/10/2021	Sputum	Purulent bloodstained. *S. aureus* +++(Clindamycin: S; Flucloxacillin:S; Gentamicin:S; Tetracycline :S ; Vancomycin:S) and *S. marcescens* + (Ciprofloxacin:S , Gentamicin:S; Augmentin: R, Meropenem:S)
IMP2-29	31/10/2021	Sputum	Purulent *S. aureus* +++ (Clindamycin: s; Flucloxacillin:s; Tetracycline :s ; Vancomycin:s) No *Candida* or other fungi isolated
IMP2-29	31/10/2021	BC	*S. epidermidis* (Clindamycin: R; Flucloxacillin:R; Gentamicin:R; Vancomycin:S)

Patient Number	Date	Sample	Results
IMP2-30	30/10/2021	Sputum	Mucoid. *S. maltophilia* - Co-trimoxazole:I
IMP2-30	31/10/2021	Tip of cannula (CVC tip)	*S. epidermidis* +++ (Clindamycin: R, Flucloxacillin:R, Fusidic acid:R, Gentamicin:S, Vancomycin:S) Also normal skin flora
IMP2-30	03/11/2021	Sputum	Mucoid. *S. maltophilia* ++ No *Candida* or other fungi isolated. Culture also yielded: Upper respiratory tract flora at 48 hours
IMP2-30	05/11/2021	Sputum	Mucoid Bloodstained *Enterobacter cancerogenus* ++ and ++ *E. cloacae* complex (Augmentin:R, Ciprofloxacin:S , Gentamicin:S, Meropenem:S) Culture also yielded: Upper respiratory tract flora at 48 hour
IMP2-30	08/11/2021	Sputum	Purulent. *Candida* species
IMP2-32	30/10/2021	Sputum	Purulent. *Morganella* morganii +++(Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Gentamicin :S, Meropenem :S) and *C. albicans* ++ Culture also yielded: Upper respiratory tract flora at 48 hours
IMP2-32	04/11/2021	Tip of cannula (CVC tip)	*S. haemolyticus* ++ (Clindamycin: R; Flucloxacillin: R, Gentamicin:R, Rifampicin:R, Vancomycin: S)
IMP2-32	09/11/2021	Sputum	Purulent. *Morganella morganii* + (Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Gentamicin:S, Meropenem:S) *C. albicans* ++
IMP2-32	11/11/2021	Sputum	Mucoid with purulent flecks. *M. morganii* + (Amoxicillin:R, Augmentin:R, Ciprofloxacin:S, Gentamicin: S, Meropenem :S) *C. albicans* ++
IMP2-32	11/11/2021	Tip of cannula (CVC tip)	*S. haemolyticus* ++ (Clindamycin: R; Flucloxacillin: R, Gentamicin:R, Vancomycin: S.) *Staphylococcus capitis* + (Clindamycin: R; Flucloxacillin: R, Gentamicin:S Vancomycin: S)
IMP2-33	17/11/2021	Sputum	Bloodstained. *C. albicans* ++
IMP2-37	15/11/2021	BAL	Few pus cells. Gram positive cocci seen. Gram positive bacilli seen. *S. aureus* +++ (Clindamycin:S, Flucloxacillin:S; Gentamicin:S;Tetracycline:S;Vancomycin:S) No *Candida* or other fungi isolated Culture also yielded: Upper respiratory tract flora at 48 hours
IMP2-37	16/11/2021	BAL	Few pus cells. Gram positive cocci seen. Gram positive bacilli seen. *S. aureus* +++ (Clindamycin:S, Flucloxacillin:S; Gentamicin:S;Tetracycline:S;Vancomycin:S) No *Candida* or other fungi isolated Culture also yielded: Upper respiratory tract flora at 48 hours
IMP2-37	19/11/2021	Tissue lung	*C. albicans* + (Caspofungin: S, Fluconazole :S, Voriconazole:S), *S. aureus* + (Flucloxacillin:S, Gentamicin:S; Tetracycline:S and Vancomycin:S)

Table A1.3 Bed space movement for IMPACT2 ICU patients

Patient Number	Date Admitted to ICU	Ward admitted to	Bed	Move bed/Leave ward/Died	ICU Ward	Bed	Move bed/leave ward	Ward Move to	Bed	Date Moved	Ward Move to	Bed	Move bed/leave ward	Ward Move to	Bed	Date Moved
IMP2-06	22/05/2021	WCCA	1	28/05/2021	WCCA	7	29/05/2021	WCCA	12	02/06/2021	Left ICU					
IMP2-38	20/11/2021	WCCA	3	27/11/2021	WCCA	7	30/11/2021	Left ICU								
IMP2-39	29/11/2021	WCCA	5	05/12/2021	Left ICU											
IMP2-30	18/10/2021	WCCA	6	14/11/2021	WCCA	29		Left ICU								
IMP2-28	12/10/2021	WCCA	7	21/10/2021	Left ICU											
IMP2-24	10/09/2021	WCCA	9	20/09/2021	WCCA	7	24/09/2021	Left ICU								
IMP2-19	13/08/2021	WCCA	12	23/08/2021	WCCB	19	28/08/2021	WCCB	15	29/08/2021	WCCB	9	02/09/2021	Left ICU		
IMP2-26	27/09/2021	WCCA	12	17/10/2021	WCCA	10	18/10/2021	WCCA	15	19/10/2021						
IMP2-16	23/07/2021	WCCA	20	24/07/2021	WCCA	7	30/07/2021	Left ICU								
IMP2-08	22/05/2021	WCCA	21	14/06/2021	WCCA	10	09/07/2021	WCCA	5	10/07/2021	WCCA	7	14/07/2021	WCCA	13	15/07/2021
IMP2-15	24/07/2021	WCCA	23	29/07/2021	Died											
IMP2-13	08/07/2021	WCCB	1	09/07/2021	WCCB	1	18/07/2021	Left ICU								
IMP2-09	29/05/2021	WCCB	3	31/05/2021	WCCB	3B/3	04/06/2021	Left ICU								
IMP2-21	20/08/2021	WCCB	8	21/08/2021	WCCB	7	20/09/2021	WCCB	1	10/10/2021	Left ICU					
IMP2-32	27/10/2021	WCCB	8	13/11/2021	Left ICU											
IMP2-33	13/11/2021	WCCB	8	17/11/2021	WCCB	23	18/11/2021	WCCB	8	21/11/2021	WCCB	19	21/11/2021	Left ICU		
IMP2-25	23/09/2021	WCCB	13	02/10/2021	WCCB	10	16/10/2021	Left ICU								
IMP2-20	15/08/2021	WCCB	15	28/08/2021	Left ICU											
IMP2-14	12/07/2021	WCCB	20	16/07/2021	WCCB	14	26/07/2021	Left ICU								
IMP2-23	08/09/2021	WCCB	23	16/09/2021	WCCA	27	18/09/2021	WCCA	26	20/09/2021	Left ICU					
IMP2-29	14/10/2021	WCCB	23	15/10/2021	WCCB	19	14/11/2021	Left ICU								
IMP2-05	18/05/2021	WCCB	5	02/06/2021	WCCB	9	06/06/2021	Left ICU								
IMP2-10	05/06/2021	WCCC	13	06/06/2021	WCCB	12	18/06/2021	Left ICU								
IMP2-37	16/11/2021	WCCC	17	28/11/2021	WCCC	10	29/11/2021	WCCC	8	02/12/2021	Left ICU					
IMP2-11	21/06/2021	WCCC	20	24/06/2021	Left ICU											

Appendix A2

A2.1 Boxplot R code

```
# load package

library(ggplot2)

# load list of number of resistances genes per genome and year

averagegeneboxplot <- read.csv("Sentinel_res_genes.csv")

# boxplot creation

averagegeneboxplot$Year<-as.factor(averagegeneboxplot$Year)

boxplot <- ggplot(averagegeneboxplot, aes(x = Year, y = Resistance.Genes.per.Genome)) + geom_boxplot() + theme_classic() +theme(axis.text.x = element_text(angle = 90, vjust = 1, hjust = 1)) #+

  #geom_point()

# boxplot check

print(boxplot)

# boxplot save

ggsave(boxplot, file="sentinel_boxplot.eps", width=14, height=7, device="eps")
```

A2.2 ST Population plot R code

```
# Set up environment

library(ggplot2)

library(reshape)

library(RColorBrewer)


# Get data

# Load data as columns of Year, ST, Frequency isolates with that ST in the year and percentage of that ST in the total no STs per year

data.f <- read.csv("full_data.csv")
```

```
# Load premelt - list of Year and ST for all isolates
individually

premelt <- read.csv("MELT_data.csv")

# Load data grid - Table showing total numbers of each ST per
Year and ST

data <- read.csv("data_all_grid.csv")


# Melt data - organises data in long frame format

melted <-melt (premelt,id=c("Year"))


# Plot data

# Plot based on ggplots geom_area syntax

p <- ggplot(data, aes(x="YEAR", y="PERC", fill="CLASS")) +
geom_area(position="fill", alpha=1) + theme(legend.position =
"none")

print(p)


# Coloursets to be used in plot

colour_set                                                    <-
unlist(lapply(c("Set2","Accent","Paired","Spectral","Pastel1",
"Pastel2","Paired","PiYG", "BrBG", "Dark2","PuBu", "PuOr",
"GnBu", "Purples", "Oranges", "Greys", "Greens", "Blues"),
function(x) brewer.pal(8, x)))


# Plot drawn with manual colour scale

m <- ggplot(melted, aes(x=Year, y=value, fill=variable)) +

  geom_area(position="fill", size=.01, colour="#363636") +

  scale_fill_manual(values=colour_set) +

  scale_x_continuous("YEAR", breaks=seq(1975,2021,by=1)) +

  scale_y_continuous("PERC", breaks=seq(0, 1, by=0.1),
labels=seq(0,100, by=10)) +

  theme_light() +

  theme(panel.grid.major = element_blank(),
```

```
        panel.grid.minor  =  element_blank(),plot.background  =
element_blank()) +

  theme(legend.key.size = unit(1, 'cm')) + #added legend size
comment

  theme(legend.text = element_text(size=30)) +

  theme(legend.title = element_text(size=30)) +

  theme(axis.text.x = element_text(size=20, angle = 90)) +

  theme(axis.text.y = element_text(size=25)) +

  theme(axis.title=element_text(size=25))


#Graph check

print(m)


#Save graph

ggsave(m,  file="sentinel_ST_population_graph.eps",  width=40,
height=10, device="eps")
```

Appendix A3

A3.1 Laos isolate virulence genes

Virulence genes identified in the 219 *E. coli* are listed below and those whose frequency are displayed in Figure 3.7B are listed in **bold**:

aap/aspU, ***afaA***, ***afaB-I***, ***afaC-III***, *agg3A, agg3B, agg3C, algW, aslA,* ***bfpB***, ***bfpE***, *cesAB, cesD, cesD2, cesF, cesL, cesT, cheA, cheR, cheW, cheZ,* ***chuA***, *chuS, chuT, chuU, chuV, chuW, chuX, chuY, clfA, cpsB,* ***csgA***, *csgB, csgC, csgD, csgE, csgF, csgG, daaF, draE2,* ***draP***, ***eae***, *east1, efa1, eltA, eltB,* ***entA***, *entB, entC, entD, entE, entF, entS, escC, escD, escE, escF, escG, escI, escJ, escL, escN, escO, escP, escR, escS, escT, escU, escV,* ***espC***, ***espD***, *espFu/tccP, espG, espH, espJ, espK, espL1, espL2, espL4, espP, espR3, espR4, espT, espW, espX1, espX2, espX4, espX5, espX6, espX7/nleL, espY1, espY2, espY3, espY4, etgA, fabZ, faeC, faeD, faeE, faeF, faeH, faeI, faeJ, fdeC,* ***fepA***, *fepB, fepC, fepD, fepG, fes,* ***fimA***, ***fimB***, ***fimC***, ***fimD***, ***fimE***, ***fimF***, ***fimG***, ***fimH***, *flgB, flgF, flgG, flgM, flhB, flhC,* ***fliF***, *fliH, fliI, fliN, fliS,* ***fyuA***, *gmhA/lpcA, gspC, gspD, gspE, gspF, gspG, gspH, gspI, gspJ, gspK, gspL, gspM, gtrA, hddA,* ***hlyA***, ***hlyB***, ***hlyC***, ***hlyD***, *hsiC1/vipB, invF,* ***iroB***, ***iroC***, ***iroD***, ***iroE***, ***iroN***, *irp1, irp2,* ***iucA***, ***iucB***, ***iucC***, ***iucD***, *kdsA,* ***kpsD***, *manB, map, mgtC, nagK, nleA, nleA/espI, nleB1, nleB2, nleC, nleD, nleE, nleF, nleG7', nleH1, nleH2, p0111_1,* ***papA***, ***papB***, ***papC***, ***papD***, ***papE***, ***papF***, ***papG***, ***papH***, ***papI***, ***papJ***, ***papK***, *pet, rfaE, sdrE, senB, sepD, sepL, sepQ/escQ, sepZ/espZ, shuA, shuS, shuU, shuY, xcpR, yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU and ybtX*

Table A3.2 Overview of Laos Plasmids(197)

#	pLAO number	Isolate Name	Carries AMR or No AMR genes	Sequence Type	Participant	Date Isolated	Plasmid Size (bp)	Plasmidfinder Results	PubMLST Classification	Plasmid Type (Detailed)	Plasmid Type (Overarching group)	Antibiotic resistance genes
1	pLAO1	LA002	AMR	1722	3	25/09	159743	IncFIA, IncFIB(AP001918), IncFIC(FII)	FIA-17 (11SNPs diff):FIB:20	FIA-17-like:FIB-20	F-type	dfrA17, aadA5, sul1, mph(A), sul2, strAB, tet(A), blaCTX-M-27
2	pLAO2	LA002	NO AMR	1722	3	25/09	5284	Col(MGD2)_1, Col(Ye4449)_1	-	θ-Rep (covers both rep types)	θ-Rep	-
3	pLAO3	LA022	AMR	6856	5	29/09	185337	IncX1, p0111	-	X1:p0111	cointegrate (X1:phage-plasmid)	blaCTX-M-55, blaOXA-10, aphA1, ARR-3, tet(A), dfrA14, cmlA1, ant(3")-Ia, qnrS1, floR
4	pLAO4	LA022	NO AMR	6856	5	29/09	111836	IncFIB(pHCM2__1_pHCM2)	-	FIB-like	F-type	-
5	pLAO5	LA022	NO AMR	6856	5	29/09	5231	ColRNAI_1	-	θ-RNA	θ-RNA	-
6	pLAO6	LA023	NO AMR	38	5	30/09	150419	not typed by PlasmidFinder	FII-30	FII-30	F-type	-
7	pLAO7	LA026	NO AMR	542	5	05/10	109626	IncFIB(pKPHS1)_1_pKPHS1	FIB-like	FIB-like	F-type	-
8	pLAO8	LA026	AMR	542	5	05/10	105347	IncY_1	-	Y	phage-plasmid	tet(B), strAB
9	pLAO9	LA026	NO AMR	542	5	05/10	13026	IncX1_1	-	X1	X-type	-
10	pLAO10	LA026	AMR	542	5	05/10	8880	ColRNAI_1	-	θ-RNA	θ-RNA	qnrS1
11	pLAO11	LA028	AMR	515	5	08/10	100198	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC6, ant(3")-Ia, floR, qnrS1, tet(M)
12	pLAO12	LA028	NO AMR	515	5	08/10	4076	ColRNAI_1	-	θ-RNA	θ-RNA	-
13	pLAO13	LA030	NO AMR	542	5B	26/09	8153	IncQ1_1	-	Q1	Q-type	-
14	pLAO14	LA030	AMR	542	5B	26/09	145171	IncFIC(FII)_1, IncR_1	FII-46:R	FII-46:R	cointegrate (FII:46:R)	aadA2, ant(3")-Ia, cmlA1, dfrA12, tet(A), tet(M)
15	pLAO15	LA030	NO AMR	542	5B	26/09	2138	ColpVC_1	-	rolling-circle	rolling-circle	-
16	pLAO16	LA030	NO AMR	542	5B	26/09	1552	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
17	pLAO17	LA030	NO AMR	542	5B	26/09	1531	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
18	pLAO18	LA030	NO AMR	542	5B	26/09	111874	IncFIB(pHCM2__1_pHCM2)	FIB-like	FIB-like	F-type	-
19	pLAO9	LA030	NO AMR	542	5B	26/09	13026	IncX1_1	-	X1	X-type	-
20	pLAO19	LA033	AMR	3285	6	25/09	159431	IncFIB(AP001918)_1	FII-24	FII-24	F-type	aadA5, strAB, dfrA17, sul2, tet(A)
21	pLAO20	LA033	NO AMR	3285	6	25/09	5153	ColRNAI_1	-	θ-RNA	θ-RNA	-
22	pLAO21	LA033	NO AMR	3285	6	25/09	2920	Col(MG828)_1	-	rolling-circle	rolling-circle	-
23	pLAO22	LA035	AMR	515	6	04/10	94395	IncFIB(AP001918)_1 , IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC6, ant(3")-Ia, floR, tet(M)
24	pLAO23	LA041	AMR	69	8	20/09	125325	IncFIA_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	FIA-1:FII-1:FIB-66	FIA-1:FII-1:FIB-66	F-type	blaTEM-1B, strAB, sul2, mph(A), dfrA14, tet(B)
25	pLAO24	LA041	NO AMR	69	8	20/09	5631	ColRNAI_1	-	θ-RNA	θ-RNA	-
26	pLAO25	LA041	NO AMR	69	8	20/09	5167	Col156_1	-	θ-Rep	θ-Rep	-
27	pLAO26	LA041	NO AMR	69	8	20/09	2042	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
28	pLAO47	LA041	NO AMR	69	8	20/09	1549	Col(MG828)_1	-	rolling-circle	rolling-circle	-
29	pLAO23	LA047	AMR	69	8	25/09	125325	IncFIA_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	FIA-1:FII-1:FIB-66	FIA-1:FII-1:FIB-66	F-type	strAB, blaTEM-1B, dfrA14, mph(A), sul2, tet(B)
30	pLAO24	LA047	NO AMR	69	8	25/09	5631	ColRNAI_1	-	θ-RNA	θ-RNA	-
31	pLAO25	LA047	NO AMR	69	8	25/09	5167	Col156_1	-	θ-Rep	θ-Rep	-
32	pLAO26	LA047	NO AMR	69	8	25/09	2042	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
33	pLAO47	LA047	NO AMR	69	8	25/09	1549	Col(MG828)_1	-	rolling-circle	rolling-circle	-
34	pLAO28	LA053	AMR	6984	8	02/10	108526	p0111	-	p0111	phage-plasmid	blaCTX-M-55, sul2, strAB, qnrS1, aacC2d, mef(B), sul3, aadA1, dfrA12, floR
35	pLAO29	LA053	NO AMR	6984	8	02/10	7470	ColRNAI_1	-	θ-RNA	θ-RNA	-
36	pLAO30	LA053	NO AMR	6984	8	02/10	4663	ColRNAI_1	-	θ-RNA	θ-RNA	-
37	pLAO31	LA058	AMR	4682	9	20/09	91136	p0111	-	p0111	phage-plasmid	blaCTX-M-55, blaTEM-1B, sul2, drfA14, aacC2d, strAB, tet(A), qnrS1
38	pLAO32	LA059	AMR	665	9	20/09	134903	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-18:FIB-1	FII-18:FIB-1	F-type	ant(3")-Ia, aphA1, blaCTX-M-55, catA2, dfrA14, floR, lnu(F), floR, sul3
39	pLAO33	LA061	AMR	1072	9	21/09	99625	IncFIB(K)_1_Kpn3, p0111_1	-	FIB(K):p0111	cointegrate (F-type:phage-plasmid)	strAB, blaTEM-1B, dfrA14, sul2
40	pLAO34	LA061	AMR	1072	9	21/09	50395	IncX1_1	-	X1	X-type	aacC2d, strAB, blaCTX-M-65, floR, sul2, tet(A)
41	pLAO35	LA061	NO AMR	1072	9	21/09	1934	ColpVC_1	-	rolling-circle	rolling-circle	-
42	pLAO36	LA063	AMR	101	9	22/09	114563	IncN_1, IncR_1, IncX1_1	-	R:X1:N1	cointegrate (R:X1:N1)	aadA2, ant(3")-Ia, blaTEM-1B, cmlA1, dfrA12, qnrS1, sul3, tet(A), tet(M)
43	pLAO37	LA063	AMR	101	9	22/09	98177	IncFII	FII-2	FII-2	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.4, qnrS1
44	pLAO38	LA063	NO AMR	101	9	22/09	96159	IncY_1	-	Y	phage-plasmid	-
45	pLAO39	LA063	NO AMR	101	9	22/09	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
46	pLAO40	LA069	NO AMR	2067	11	27/09	109992	IncFII(pCOO)_1_pCOO	FII-16	FII-16	F-type	-
47	pLAO41	LA069	AMR	2067	11	27/09	31882	IncX4_1	-	X4	X-type	mcr1.1
48	pLAO42	LA069	NO AMR	2067	11	27/09	10560	ColRNAI_1	-	θ-RNA	θ-RNA	-
49	pLAO43	LA084	AMR	69	12	27/09	167161	Col156, IncFIA , IncFIB(AP001918), IncFIC(FII)	FIA-4:FII-31:FII-36:FIB-10	FIA-4:FII-31:FII-36:FIB-10:θ-Rep	cointegrate(F-type:θ-Rep)	blaTEM-1B, sul1, aadA5, mph(A), sul2, strAB, tet(A), qnrS1, aacC2a, dfrA17
50	pLAO44	LA084	AMR	69	12	27/09	67433	IncFIC(FII)	FII-2	FII-2	F-type	blaCTX-M-27
51	pLAO45	LA084	NO AMR	69	12	27/09	7463	Col156_1	-	θ-Rep	θ-Rep	-
52	pLAO46	LA084	NO AMR	69	12	27/09	4072	Col8282_1	-	θ-Rep	θ-Rep	-
53	pLAO47	LA084	NO AMR	69	12	27/09	1552	Col(MG828)_1	-	rolling-circle	rolling-circle	-
54	pLAO39	LA086	NO AMR	101	12	29/09	3659	not typed by plasmidfinder	-	θ-RNA	θ-RNA	-
55	pLAO36	LA086	AMR	101	12	29/09	114564	IncN_1, IncR_1, IncX1_1	-	R:X1:N1	cointegrate (R:X1:N1)	aadA2, ant(3")-Ia, blaTEM-1B, cmlA1, dfrA12, qnrS1, sul3, tet(A), tet(M)

#	pLAO number	Isolate Name	Carries AMR or No AMR genes	Sequence Type	Participant	Date Isolated	Plasmid Size (bp)	Plasmidfinder Results	PubMLST Classification	Plasmid Type (Detailed)	Plasmid Type (Overarching group)	Antibiotic resistance genes
56	pLAO37	LA086	AMR	101	12	29/09	98233	IncFII_1	FII-2	FII-2	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.4, qnrS1
57	pLAO48	LA086	NO AMR	101	12	29/09	95536	IncY_1	-	Y	phage-plasmid	-
58	pLAO49	LA094	NO AMR	38	13	02/10	150223	not typed by plasmidfinder	FII-30	FII-30	F-type	-
59	pLAO13	LA104	NO AMR	101	16	01/10	8153	IncQ1_1	-	Q1	Q-type	-
60	pLAO39	LA104	NO AMR	101	16	01/10	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
61	pLAO50	LA104	NO AMR	101	16	01/10	2621	ColRNAI_1	-	θ-RNA	θ-RNA	-
62	pLAO51	LA104	NO AMR	101	16	01/10	2046	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
63	pLAO36	LA104	AMR	101	16	01/10	114554	IncN_1, IncR_1, IncX1_1	-	R:X1:N1	cointegrate (R:X1:N1)	aadA2, ant(3")-Ia, blaTEM-1B, cmlA1, dfrA12, qnrS1, sul3, tet(A), tet(M)
64	pLAO37	LA104	AMR	101	16	01/10	98237	IncFII_1	FII-2	FII-2	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.4, qnrS1
65	pLAO52	LA104	NO AMR	101	16	01/10	96015	IncY_1	-	Y	phage-plasmid	-
66	pLAO53	LA108	NO AMR	38	16	04/10	3965	ColRNAI_1	-	θ-RNA	θ-RNA	-
67	pLAO36	LA110	AMR	101	16	05/10	114564	IncN_1, IncR_1, IncX1_1	-	R:X1:N1	cointegrate (R:X1:N1)	aadA2, ant(3")-Ia, blaTEM-1B, cmlA1, dfrA12, qnrS1, sul3, tet(A), tet(M)
68	pLAO37	LA110	AMR	101	16	05/10	98237	IncFII_1	FII-2	FII-2	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.4, qnrS1
69	pLAO54	LA110	NO AMR	101	17	05/10	93150	IncY_1	-	Y	phage-plasmid	-
70	pLAO39	LA110	NO AMR	101	16	05/10	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
71	pLAO55	LA118	AMR	515	17	25/09	106711	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC6, ant(3")-Ia, floR, mph(A), qnrS1, tet(M)
72	pLAO56	LA118	NO AMR	515	17	25/09	3877	not typed by PlasmidFinder	-	-	θ-Rep	-
73	pLAO57	LA118	NO AMR	515	17	25/09	3285	ColRNAI_1	-	θ-RNA	θ-RNA	-
74	pLAO58	LA118	NO AMR	515	17	25/09	2460	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
75	pLAO59	LA124	AMR	542	17	03/10	111500	IncY_1	-	Y	phage-plasmid	ant(3")-Ia, aadA1, aadA2, blaTEM-1B, lnu(F), mef(B), qnrS1, sul3, tet(A), tet(M)
76	pLAO60	LA124	AMR	542	17	03/10	7849	IncQ1_1	-	Q1	Q-type	aac(6')-Ila, ant(2")-Ia, blaVEB-5
77	pLAO61	LA124	NO AMR	542	17	03/10	5269	ColRNAI_1	-	θ-RNA	θ-RNA	-
78	pLAO30	LA124	NO AMR	542	17	03/10	4664	ColRNAI_1	-	θ-RNA	θ-RNA	-
79	pLAO15	LA124	NO AMR	542	17	03/10	2137	ColpVC_1	-	rolling-circle	rolling-circle	-
80	pLAO62	LA130	AMR	410	18	27/09	51823	not typed by PlasmidFinder	-	P-type	P-type	mcr-3.5
81	pLAO63	LA133	AMR	34	19	26/09	139721	IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	FII-1:FIB-33	FII-1:FIB-33	F-type	aadA5, strAB, blaCTX-M-27, dfrA17, erm(B), mph(A), sul1, sul2, tet(A)
82	pLAO64	LA133	NO AMR	34	19	26/09	4073	not typed by PlasmidFinder	-	-	θ-Rep	-
83	pLAO65	LA153	AMR	201	21	28/09	259739	IncFIA(HI1)_1_HI1, IncHI1A_1, IncHI1B(R27)_1_R27	FIA:8	FIA:8	H-type	aacC2d, aadA1, blaCTX-M-55, blaTEM-1B, dfrA12, floR, mef(B), qnrS1, sul2, sul3, tet(A), tet(M)
84	pLAO66	LA153	AMR	201	21	28/09	60565	IncFIB(K)_1_Kpn3, IncX1_1	-	X1:FIB(K)	cointegrate (X1:FIB(K))	mcr-3.5
85	pLAO67	LA153	NO AMR	201	21	28/09	4145	ColRNAI_1	-	θ-RNA	θ-RNA	-
86	pLAO77	LA153	NO AMR	201	21	28/09	2089	Col(BS512)_1, Col(BS512)_1__NC_010656_dupe	-	rolling-circle	rolling-circle	-
87	pLAO68	LA156	AMR	10	21	01/10	113769	IncFIA(HI1)_1_HI1, IncFIB(K)_1_Kpn3, IncN_1, IncX1_1	FIA-18:repN-1:FIB(K):IncX1	FIA-18:N1:FIB(K):X1	cointegrate (FIA-18:N1:FIB(K):X1)	aadA2, ant(3")-Ia, aphA1, blaTEM-1B, cmlA1, dfrA12, mef(B), qnrS1, sul3, tet(A)
88	pLAO69	LA156	AMR	10	21	01/10	105425	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.1, qnrS1, tet(M)
89	pLAO70	LA156	NO AMR	10	21	01/10	40901	not typed by PlasmidFinder	-	-	phage-plasmid	-
90	pLAO71	LA156	NO AMR	10	21	01/10	6024	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
91	pLAO72	LA156	NO AMR	10	21	01/10	4067	ColRNAI_1	-	θ-RNA	θ-RNA	-
92	pLAO68	LA160	AMR	10	21	04/10	113769	IncFIA(HI1)_1_HI1, IncFIB(K)_1_Kpn3, IncN_1, IncX1_1	FIA-18:repN-1:FIB(K):IncX1	FIA-18:N1:FIB(K):X1	cointegrate (FIA-18:N1:FIB(K):X1)	aadA2, ant(3")-Ia, aphA1, blaTEM-1B, cmlA1, dfrA12, mef(B), qnrS1, sul3, tet(A)
93	pLAO69	LA160	AMR	10	21	04/10	105425	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.1, qnrS1, tet(M)
94	pLAO72	LA160	NO AMR	10	21	04/10	4067	not typed by plasmidfinder	-	-	θ-RNA	-
95	pLAO39	LA160	NO AMR	10	21	04/10	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
96	pLAO21	LA160	NO AMR	10	21	04/10	2920	Col(MG828)_1	-	rolling-circle	rolling-circle	-
97	pLAO70	LA160	NO AMR	10	21	04/10	40901	not typed by PlasmidFinder	-	-	phage-plasmid	-
98	pLAO71	LA160	NO AMR	10	21	04/10	6024	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
99	pLAO73	LA165	AMR	5895	23	20/09	127536	IncFIA_1, IncFII_1	FIA-1:FII-2	FIA-1:FII-2	F-type	aacA4, aadA5, blaCTX-M-15, blaOXA-1, catB4, dfrA17, mph(A), sul1, tet(A)
100	pLAO74	LA165	NO AMR	5895	23	20/09	33826	IncX4_2	-	X4	X-type	-
101	pLAO25	LA165	NO AMR	5895	23	20/09	5164	Col156_1	-	θ-Rep	θ-Rep	-
102	pLAO75	LA165	NO AMR	5895	23	20/09	4082	Col8282_1	-	θ-Rep	θ-Rep	-
103	pLAO76	LA165	NO AMR	5895	23	20/09	3649	ColRNAI_1	-	θ-RNA	θ-RNA	-
104	pLAO77	LA165	NO AMR	5895	23	20/09	2102	Col(BS512)_1, Col(BS512)_1__NC_010656_dupe	-	rolling-circle	rolling-circle	-
105	pLAO47	LA165	NO AMR	5895	23	20/09	1546	Col(MG828)_1	-	rolling-circle	rolling-circle	-
106	pLAO13	LA170	NO AMR	542	23	29/09	8153	IncQ1_1	-	Q1	Q-type	-
107	pLAO78	LA170	AMR	542	23	29/09	22368	ColRNAI_1	-	θ-RNA	θ-RNA	aadA2, ant(3")-Ia, cmlA1, dfrA12, tet(A)
108	pLAO25	LA170	NO AMR	542	23	29/09	5164	Col156_1	-	θ-Rep	θ-Rep	-
109	pLAO12	LA170	NO AMR	542	23	29/09	4076	ColRNAI_1	-	θ-RNA	θ-RNA	-
110	pLAO76	LA170	NO AMR	542	23	29/09	3649	ColRNAI_1	-	θ-RNA	θ-RNA	-

#	pLAO number	Isolate Name	Carries AMR or No AMR genes	Sequence Type	Participant	Date Isolated	Plasmid Size (bp)	Plasmidfinder Results	PubMLST Classification	Plasmid Type (Detailed)	Plasmid Type (Overarching group)	Antibiotic resistance genes
111	pLAO77	LA170	NO AMR	542	23	29/09	2101	Col(BS512)_1, Col(BS512)_1__NC_010656_dupe	-	rolling-circle	rolling-circle	-
112	pLAO79	LA170	NO AMR	542	23	29/09	1541	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
113	pLAO74	LA170	NO AMR	542	23	29/09	33826	IncX4_2	-	X4	X-type	-
114	pLAO80	LA172	NO AMR	457	23	30/09	54724	not typed by PlasmidFinder	-	-	P-type	-
115	pLAO81	LA172	NO AMR	457	23	30/09	2651	Col(BS512)_1	-	rolling-circle	rolling-circle	-
116	pLAO82	LA189	AMR	34	33	19/09	110949	Col440I_1, IncFII_1	FII-2:Col440I	FII2:θ-RNA	cointegrate (FII:θ-RNA)	aacC2d, qnrS1, blaCTX-M-55, catA2, mcr-3.4
117	pLAO83	LA189	AMR	34	33	19/09	73226	IncFIB(K)_1_Kpn3, IncFIA(HI1)_1_HI1	FIA-18:FIB(K)	FIA-18:FIB(K)	F-type	mef(B), sul3, ant(3")-Ia, cmlA1, aadA2, dfrA12, blaTEM-1B
118	pLAO13	LA189	NO AMR	34	33	19/09	8153	IncQ1_1	-	Q1	Q-type	-
119	pLAO84	LA189	AMR	34	33	19/09	5440	ColRNAI_1	-	θ-RNA	θ-RNA	tet(A)
120	pLAO57	LA189	NO AMR	34	33	19/09	3285	ColRNAI_1	-	θ-RNA	θ-RNA	-
121	pLAO85	LA189	NO AMR	34	33	19/09	2029	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
122	pLAO11	LA200	AMR	515	33	03/10	100198	IncFIB(AP001918)_1, IncFIC(FII)_1	FII-46:FIB-20	FII-46:FIB-20	F-type	aacC6, ant(3")-Ia, floR, qnrS1, tet(M)
123	pLAO12	LA200	NO AMR	515	33	03/10	4076	ColRNAI_1	-	θ-RNA	θ-RNA	-
124	pLAO86	LA205	AMR	1081	34	21/09	28024	IncX1_1	-	X1	X-type	floR, sul2
125	pLAO87	LA205	AMR	1081	34	21/09	24981	IncFIB(AP001918)_1	FIB:20	FIB:20	F-type	blaCTX-M-55, blaTEM-1B, tet(M)
126	pLAO35	LA205	NO AMR	1081	34	21/09	1934	ColpVC_1	-	rolling-circle	rolling-circle	-
127	pLAO88	LA206	NO AMR	167	34	22/09	6761	ColRNAI_1	-	θ-RNA	θ-RNA	-
128	pLAO89	LA206	NO AMR	167	34	22/09	6675	ColRNAI_1	-	θ-RNA	θ-RNA	-
129	pLAO90	LA206	NO AMR	167	34	22/09	4993	ColRNAI_1	-	θ-RNA	θ-RNA	-
130	pLAO91	LA206	NO AMR	167	34	22/09	4072	Col8282_1	-	θ-Rep	θ-Rep	-
131	pLAO92	LA206	NO AMR	167	34	22/09	2525	ColRNAI_1	-	θ-RNA	θ-RNA	-
132	pLAO88	LA207	NO AMR	617	34	25/09	6761	ColRNAI_1	-	θ-RNA	θ-RNA	-
133	pLAO89	LA207	NO AMR	617	34	25/09	6675	ColRNAI_1	-	θ-RNA	θ-RNA	-
134	pLAO93	LA207	NO AMR	617	34	25/09	5279	ColRNAI_1	-	θ-RNA	θ-RNA	-
135	pLAO90	LA207	NO AMR	617	34	25/09	4993	ColRNAI_1	-	θ-RNA	θ-RNA	-
136	pLAO91	LA207	NO AMR	617	34	25/09	4072	Col8282_1	-	θ-Rep	θ-Rep	-
137	pLAO94	LA207	NO AMR	617	34	25/09	2345	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
138	pLAO15	LA227	NO AMR	5848	35	21/09	2138	ColpVC_1	-	rolling-circle	rolling-circle	-
139	pLAO39	LA232	NO AMR	101	35	26/09	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
140	pLAO37	LA232	AMR	101	35	26/09	98237	IncFII_1	FII-2	FII-2	F-type	aacC2d, blaCTX-M-55, catA2, mcr-3.4, qnrS1
141	pLAO39	LA236	NO AMR	101	36	28/09	3659	ColRNAI_1	-	θ-RNA	θ-RNA	-
142	pLAO95	LA238	NO AMR	298	36	02/10	2857	ColRNAI_1	-	θ-RNA	θ-RNA	-
143	pLAO96	LA239	AMR	195	40	22/09	45300	IncX1_1	-	X1	X-type	qnrS1, tet(M)
144	pLAO84	LA239	AMR	195	40	22/09	5440	ColRNAI_1	-	θ-RNA	θ-RNA	tet(A)
145	pLAO97	LA243	NO AMR	48	40	28/09	10248	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
146	pLAO98	LA243	NO AMR	48	40	28/09	6477	IncQ1_1	-	Q1	Q-type	-
147	pLAO51	LA243	NO AMR	48	40	28/09	2043	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
148	pLAO99	LA243	NO AMR	48	40	28/09	1917	ColRNAI_1	-	θ-RNA	θ-RNA	-
149	pLAO100	LA249	AMR	40	40	04/10	103503	IncFIB(AP001918)_1, IncFII_1	FII-2:FIB-10	FII-2:FIB-10	F-type	blaCTX-M-27
150	pLAO101	LA249	NO AMR	40	40	04/10	5330	ColRNAI_1	-	θ-RNA	θ-RNA	-
151	pLAO47	LA249	NO AMR	40	40	04/10	1552	Col(MG828)_1	-	rolling-circle	rolling-circle	-
152	pLAO6	LA251	NO AMR	38	40	05/10	150416	not typed by PlasmidFinder	FII-30	FII-30	F-type	-
153	pLAO102	LA251	AMR	38	40	05/10	78662	IncFII(pHN7A8)_1_pHN7A8	FII-novel (closest match FII-103)	FII-novel (closest match FII-103)	F-type	strAB, blaCTX-M-27, blaTEM-1B, dfrA8, sul2, tet(A)
154	pLAO23	LA275	AMR	69	8	29/09	125325	IncFIA_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	FIA-1:FII-1:FIB-66	FIA-1:FII-1:FIB-66	F-type	strAB, blaTEM-1B, dfrA14, mph(A), sul2, tet(B)
155	pLAO24	LA275	NO AMR	69	8	29/09	5631	ColRNAI_1	-	θ-RNA	θ-RNA	-
156	pLAO25	LA275	NO AMR	69	8	29/09	5167	Col156_1	-	θ-Rep	θ-Rep	-
157	pLAO26	LA275	NO AMR	69	8	29/09	2042	not typed by PlasmidFinder	-	-	replication mechanism unknown	-
158	pLAO47	LA275	NO AMR	69	8	29/09	1549	Col(MG828)_1	-	rolling-circle	rolling-circle	-
159	pLAO103	LA294	AMR	457	16	26/10	149816	IncFIB(AP001918)_1, IncFII_1	FII-2:FIB-10	FII-2:FIB-10	F-type	aadA5, strAB, blaCTX-M-27, dfrA17, mph(A), sul1, sul2, tet(A)
160	pLAO104	LA294	NO AMR	457	16	26/10	4062	not typed by PlasmidFinder	-	-	θ-Rep	-
161	pLAO105	LA300	AMR	131	19B	21/11	115804	Col156_1, IncFIA_1, IncFIB(AP001918)_1, IncFII(pRSB107)_1_pRSB107	FIA-2:FII-1:FIB-20	FIA-2:FII-1:FIB-20:θ-Rep	cointegrate(F-type:θ-Rep)	blaCTX-M-27
162	pLAO27	LA300	NO AMR	131	19B	21/11	52036	IncN_1	-	N	N-type	-
163	pLAO25	LA300	NO AMR	131	19B	21/11	5167	Col156_1	-	θ-Rep	θ-Rep	-

Table A3.3 – The spread of AMR and non-AMR plasmid replicon types by participant over the study period for 48 *E. coli* isolates (197)

Participant	19/09	20/09	21/09	22/09	23/09	24/09	25/09	26/09	27/09	28/09	29/09	30/09	01/10	02/10	03/10	04/10	05/10	06/10	07/10	08/10	26/10	Post Study 21/11
3							FIA-17-like: FIB-20, θ-Rep															
5											X1:p0111, FIB-like, θ-RNA	FII-30				Y, θ-RNA, FIB-like, X1			FII-46:FIB-20, θ-RNA		
5B								FII-46:R, Q1, rolling-circle, Unknown, FIB-like, X1														
6							FII-24, θ-RNA, rolling-circle									FII-46:FIB-20						
8		FIA-1:FII-1: FIB-66, θ-RNA, θ-Rep, Unknown, rolling-circle					FIA-1:FII-1:FIB-66, θ-RNA, θ-Rep, Unknown, rolling-circle				FIA-1:FII-1:FIB-66, θ-RNA, θ-Rep, Unknown, rolling-circle			p0111 θ-RNA							
9		p0111, FII-18:FIB-1	FIB(K):p0111, X1, rolling-circle	R:X1:N, FII-2, Y, θ-RNA																		
11									X4, FII-16, θ-RNA													
12									FIA-4:FII-31:FII-36:FIB-10: θ-Rep, FII-2, θ-Rep, rolling-circle		R:X1:N1, FII-2, θ-RNA, Y										
13														FII-30								
16													R:X1:N1, FII-2, Q1, θ-RNA, Unknown, Y			θ-RNA	R:X1:N1, FII-2, θ-RNA				FII-2:FIB-10, Unknown	
17							FII-46:FIB-20, Unknown, θ-RNA								Y, Q1, θ-RNA, rolling-circle		Y					
18									P-type													
19								FII-1:FIB-33, Unknown														
19B																						FIA-2:FII-1: FIB-20:θ-Rep, N, θ-Rep
21										FIA:8, X1:FIB(K), θ-RNA, rolling-circle			FIA-18:N1: FIB(K):X1, FII-46:FIB-20,Unknown, θ-RNA			FIA-18:N1:FIB(K):X1, FII-46:FIB-20, θ-RNA, rolling-circle, Unknown						
23		FIA-1:FII-2, X4, θ-Rep, θ-RNA, rolling-circle									θ-RNA, Q1, θ-Rep, θ-RNA, rolling-circle, Unknown, X4	Unknown, rolling-circle										
33	FII2:θ-RNA, FIA-18:FIB(K), θ-RNA, Q1, θ-RNA, Unknown														FII-46:FIB-20, θ-RNA							
34			X1, FIB:20, rolling-circle	θ-RNA, θ-Rep			θ-RNA, θ-Rep, Unknown															
35			rolling-circle					FII-2, θ-RNA														
36										θ-RNA				θ-RNA								
40				X1, θ-RNA						Q1, Unknown, θ-RNA						FII-2:FIB-10, θ-RNA, rolling-circle	FII-novel (closest match FII-103) , FII-30					

Red - Plasmid replicon carrying AMR genes Blue -Plasmid replicon type not carrying AMR genes * Duplicates have been omitted.

Table A3.4 Plasmids available in the Genbank database* that match backbone, resistance regions or whole plasmids in a selection of plasmid replicon types in the pLAO database.

Plasmid Type	Backbone or Resistance Region Match	Country	Source of Plasmid	Host	Genbank Accession	pLAO Name
FII-2	Partial Backbone Region	India	human urine	*E. coli*	CP047407.1	pLAO37
		South Korea	human blood	*E. coli*	CP088774.1	pLAO100, pLAO103
		USA	human	*Klebsiella pneumoniae*	CP069948.1	pLAO44
	Partial Resistance Region	New Zealand	human urine	*E. coli*	CP032937.1	pLAO37
		USA	human rectal swab	*E. coli*	CP054458.1	pLAO100
		UK	human	*Shigella sonnei*	KX008967.1	pLAO44
FII-46:FIB-20	Partial Backbone Region	Hong Kong	pig faeces	*E. coli*	MK134376.1	pLAO69
		China	chicken faeces	*E. coli*	CP019261.1	
		USA	veal	*E. coli*	CP063717.1	
	Partial Resistance Region	Thailand	duck faeces	*E. coli*	MT449722.1	
		New Zealand	human urine	*E. coli*	CP032937.1	
FII-18:FIB-1	Partial Backbone Region	USA	human	*E. coli*	CP069572.1	pLAO32
		Canada	turkey	*E. coli*	CP059989.1	
		-	wetland sediment	*E. coli*	MN158991.1	
	Partial Resistance Region	Taiwan	human urine	*E. coli*	CP021847.1	
Y-type	Partial Backbone Region	China	inhalable particles	*Enterobacter*	CP063951.1	pLAO59
	Partial Resistance Region	Spain	bird	*E. coli*	MT859326.1	
		China	pig	*E. coli*	CP041631.1	
Q-Type	Whole plasmid match	Hong Kong	shrimp	*Vibrio alginolyticus*	KX578338.1	pLAO60
		Hong Kong	shrimp	*Vibrio parahaemolyticus*	KX539265.1	
ColE1-like	Whole Plasmid match	UK	pig faeces	*E. fergusonii*	CP057097.1	pLAO84

* The country, source and host of the GenBank Plasmid Match is listed.

Appendix A4

A4.1Colonising Enterobacter species

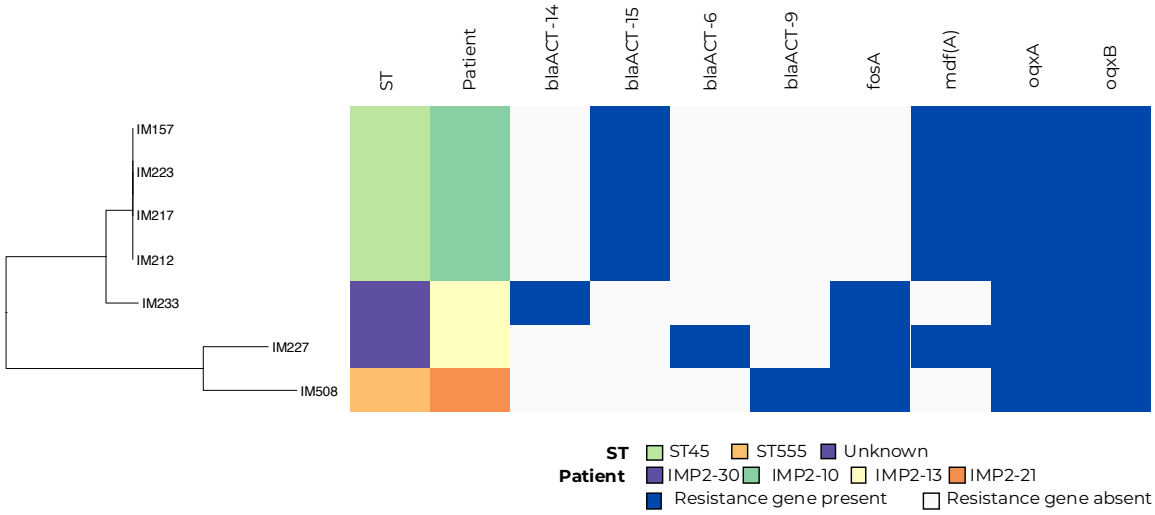


Figure A4.1.1 Acquired resistance gene profile for all colonising *E. cloacae* displayed alongside ST and patient number. Navy - resistance gene presence and grey - gene absence. Phylogeny is midpoint rooted with increasing nodes.

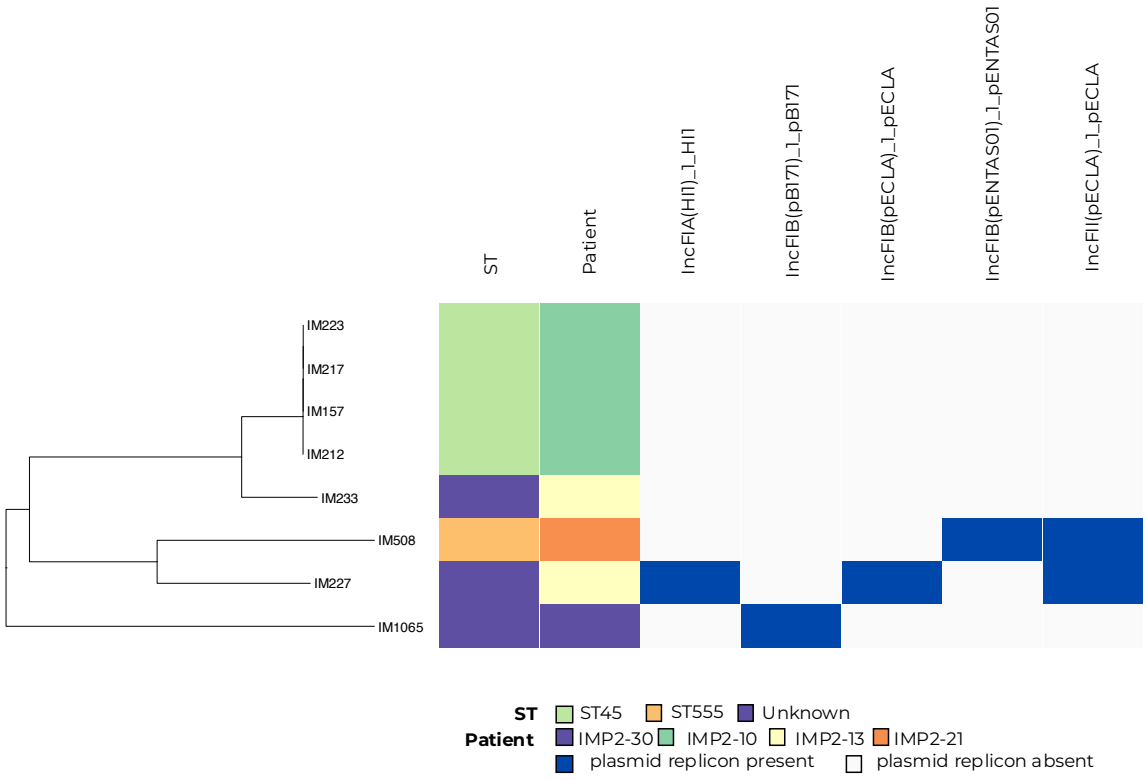


Figure A4.1.2 Acquired plasmid replicon profile for all colonising *E. cloacae* displayed alongside ST and patient number. Navy - plasmid replicon presence. Grey - plasmid replicon absence. Phylogeny is midpoint rooted with increasing nodes.

A4.2 Colonising *Cronobacter* species

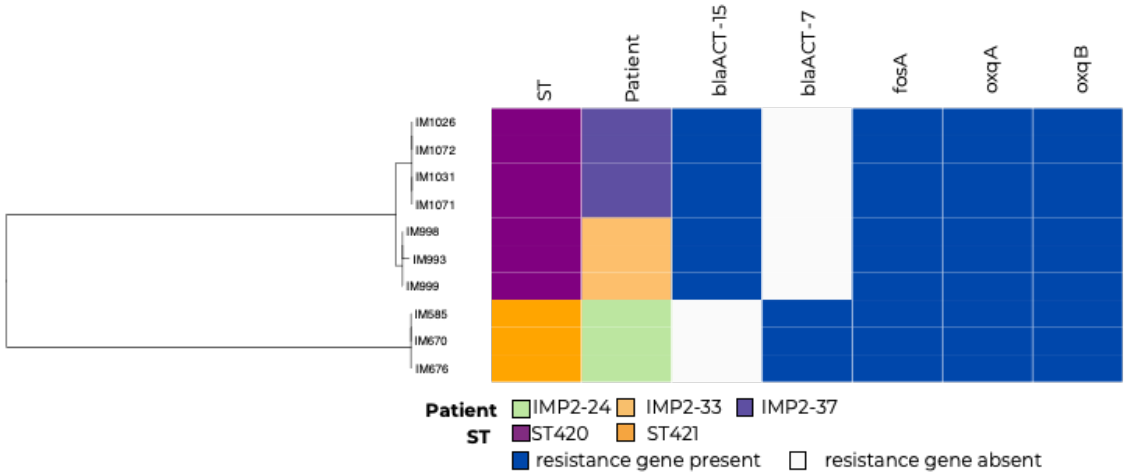


Figure A4.2.1 Acquired resistance gene profile for all colonising *Cronobacter* displayed alongside ST and patient number. Navy indicates resistance gene presence and grey resistance gene absence. Phylogeny is midpoint rooted with increasing nodes.

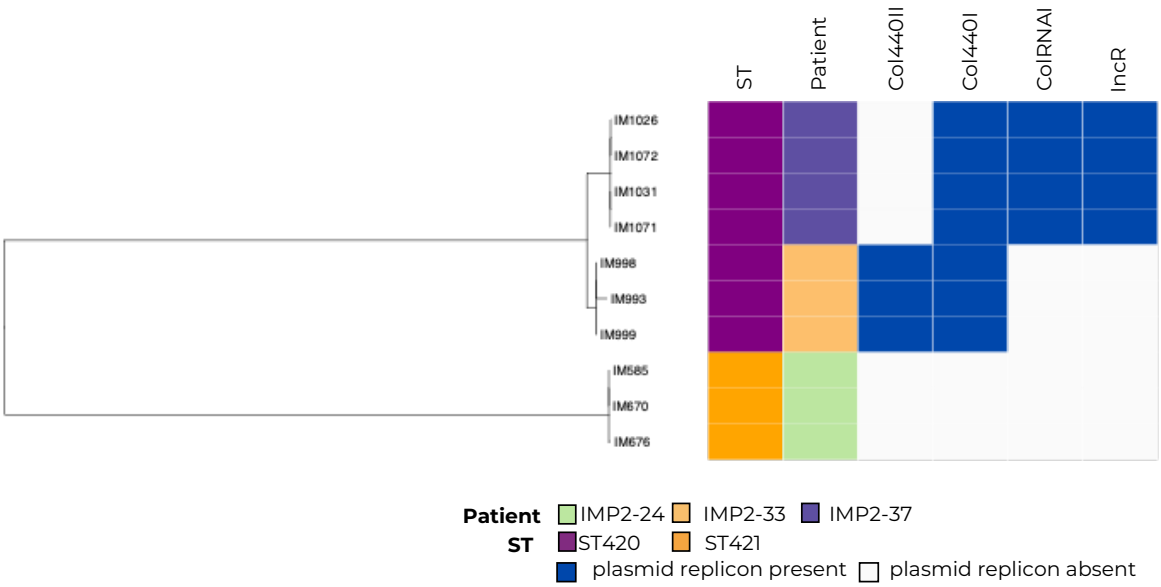


Figure A4.2.2 Plasmid replicon profile for all colonising *Cronobacter* displayed alongside ST and patient number. Navy indicates plasmid replicon gene presence and grey plasmid replicon gene absence. Phylogeny is midpoint rooted with increasing nodes.

Appendix A5 SENTINEL Dataset Isolate Information

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0001_750045_1_F_C_75	750045		0	female	CSF	1975	14	B2		mdf(A)_1
NEL0002_750127_1_F_C_75	750127		2	female	CSF	1975	500	B1		aadA1_4, aph(3'')-Ib_5, aph(3')-Ia_7, aph(6)-Id_1, blaOXA-1_1, blaTEM-1B_1, catA1_1, ere(A)_1, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL0004_750128_1_M_C_75	750128		0	male	CSF	1975	12	B2		mdf(A)_1
NEL0006_750167_1_M_C_75	750167		0	male	CSF	1975	62	F		mdf(A)_1
NEL0007_750245_1_M_C_75	750245		0	male	CSF	1975	12	B2		ant(3'')-Ia_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5
NEL0008_750246_1_M_C_75	750246		0	male	CSF	1975	95	B2		mdf(A)_1
NEL0010_750318_1_F_C_75	750318		0	female	CSF	1975	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0012_760034_1_M_C_76	760034		0	male	CSF	1976	88	C		mdf(A)_1
NEL0014_760090_1_M_C_76	760090	62		male	CSF	1976	95	B2		mdf(A)_1
NEL0015_760180_1_F_C_76	760180		0	female	CSF	1976	567	B2		mdf(A)_1
NEL0016_760187_1_F_C_76	760187	50		female	CSF	1976	1057	B2		mdf(A)_1
NEL0017_760190_1_F_C_76	760190	2		female	CSF	1976	73	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5
NEL0018_760206_1_U_C_76	760206		0	unknown	CSF	1976	62	F		mdf(A)_1
NEL0023_760229_1_M_C_76	760229	2		male	CSF	1976	543	E		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0024_760268_1_M_C_76	760268	1		male	CSF	1976	10	A		mdf(A)_1
NEL0025_760283_1_F_C_76	760283		0	female	CSF	1976	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0027_760288_1_F_C_76	760288	78		female	CSF	1976	88	C		aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1
NEL0028_760378_1_F_C_76	760378		0	female	CSF	1976	95	B2		mdf(A)_1
NEL0029_760391_1_M_C_76	760391		1	male	CSF	1976	95	B2		mdf(A)_1, tet(A)_6
NEL0031_760392_1_M_C_76	760392	9		male	CSF	1976	95	B2		mdf(A)_1
NEL0033_760467_1_U_C_76	760467		0	unknown	CSF	1976	95	B2		mdf(A)_1
NEL0034_760476_1_M_C_76	760476		0	male	CSF	1976	62	F		mdf(A)_1
NEL0036_760536_1_F_C_76	760536	69		female	CSF	1976	1057	B2		mdf(A)_1
NEL0037_760567_1_M_C_76	760567		2	male	CSF	1976	62	F		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0038_760674_1_M_C_76	760674		0	male	CSF	1976	95	B2		mdf(A)_1
NEL0039_760674_2_M_B_76	760674		0	male	blood	1976	95	B2		mdf(A)_1
NEL0040_760718_1_M_C_76	760718		0	male	CSF	1976	23	C		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0041_760718_2_M_B_76	760718		0	male	blood	1976	23	C		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0042_760734_1_M_C_76	760734		0	male	CSF	1976	567	B2		mdf(A)_1
NEL0043_760736_1_M_C_76	760736	0	0	male	CSF	1976	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0044_760737_1_F_C_76	760737		0	female	CSF	1976	409	A		mdf(A)_1
NEL0046_760768_1_M_C_76	760768		0	male	CSF	1976	421	B2		mdf(A)_1
NEL0047_770002_1_F_C_77	770002		0	female	CSF	1977	567	B2		mdf(A)_1
NEL0048_770002_2_F_B_77	770002		0	female	blood	1977	567	B2		mdf(A)_1
NEL0049_770037_1_U_C_77	770037		1	unknown	CSF	1977	12	B2		mdf(A)_1
NEL0051_770044_1_M_C_77	770044	77		male	CSF	1977	23	C		mdf(A)_1
NEL0052_770098_1_F_C_77	770098		7	female	CSF	1977	12	B2		blaTEM-1A_1, mdf(A)_1
NEL0053_770109_1_M_C_77	770109		0	male	CSF	1977	62	F		mdf(A)_1
NEL0055_770294_1_M_C_77	770294		0	male	CSF	1977	567	B2		mdf(A)_1
NEL0057_770301_1_M_C_77	770301		0	male	CSF	1977	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0060_770322_1_M_C_77	770322		1	male	CSF	1977	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0061_770332_1_F_C_77	770332		0	female	CSF	1977	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0062_770416_1_F_C_77	770416		0	female	CSF	1977	-	E		mdf(A)_1, sul2_2, tet(A)_6
NEL0063_770417_1_F_C_77	770417		0	female	CSF	1977	390	B2		mdf(A)_1
NEL0064_770439_1_M_C_77	770439	17		male	CSF	1977	23	C		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL0066_770706_1_M_C_77	770706		0	male	CSF	1977	139	D		mdf(A)_1
NEL0068_770710_1_M_C_77	770710		0	male	CSF	1977	567	B2		mdf(A)_1
NEL0070_770719_1_M_C_77	770719	39		male	CSF	1977	2230	C		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0071_770737_1_M_C_77	770737		0	male	CSF	1977	23	C		ant(3'')-Ia_1, aph(3')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0072_770737_2_M_O_77	770737		0	male	other	1977	23	C		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0073_780011_1_M_C_78	780011		0	male	CSF	1978	7011	A		blaTEM-1A_1, mdf(A)_1
NEL0075_780061_1_F_C_78	780061	65		female	CSF	1978	127	B2		ant(3'')-Ia_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5
NEL0076_780067_1_F_C_78	780067		0	female	CSF	1978	12	B2		mdf(A)_1
NEL0078_780101_1_M_C_78	780101		1	male	CSF	1978	62	F		mdf(A)_1
NEL0081_780155_2_F_B_78	780155		3	female	blood	1978	62	F		mdf(A)_1
NEL0082_780171_1_M_C_78	780171		0	male	CSF	1978	12	B2		aph(3'')-Ib_2, aph(3')-IIa_2, aph(6)-Ic_1, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1
NEL0084_780194_1_M_C_78	780194		0	male	CSF	1978	95	B2		mdf(A)_1, sul2_2
NEL0085_780201_1_M_C_78	780201		0	male	CSF	1978	-	D		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL0086_780237_1_F_C_78	780237		0	female	CSF	1978	429	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0087_780268_1_F_C_78	780268		0	female	CSF	1978	144	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0088_780283_1_F_C_78	780283		0	female	CSF	1978	95	B2		mdf(A)_1
NEL0089_780312_1_M_C_78	780312		0	male	CSF	1978	73	B2		mdf(A)_1
NEL0090_780330_1_F_C_78	780330		0	female	CSF	1978	567	B2		mdf(A)_1
NEL0092_780337_1_M_C_78	780337		0	male	CSF	1978	95	B2		mdf(A)_1
NEL0094_780339_1_F_B_79	780339		0	female	blood	1979	59	F	parE_I355T	mdf(A)_1, sul2_14
NEL0095_780355_1_F_C_78	780355		0	female	CSF	1978	95	B2		mdf(A)_1
NEL0096_780401_1_M_C_78	780401		0	male	CSF	1978	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0097_780429_1_M_C_78	780429		0	male	CSF	1978	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0099_780471_1_M_C_78	780471		0	male	CSF	1978	567	B2		mdf(A)_1
NEL0100_780502_1_M_C_78	780502		0	male	CSF	1978	567	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0101_780584_1_F_C_78	780584		0	female	CSF	1978	59	F	parE_I355T	mdf(A)_1
NEL0102_780655_1_M_C_78	780655		0	male	CSF	1978	567	B2		mdf(A)_1
NEL0104_780665_1_M_C_78	780665	1		male	CSF	1978	2944	D	parE_I355T	ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_7, aph(6)-Id_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0105_780665_2_M_C_78	780665	1		male	CSF	1978	2064	E		blaTEM-1A_1, mdf(A)_1
NEL0106_780673_1_M_C_78	780673		0	male	CSF	1978	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0107_780697_1_F_C_78	780697		0	female	CSF	1978	567	B2		mdf(A)_1
NEL0108_780699_1_M_C_78	780699		2	male	CSF	1978	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0109_780701_1_M_C_78	780701		0	male	CSF	1978	117	G		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0110_780727_1_M_C_78	780727	1		male	CSF	1978	127	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(C)_3
NEL0111_780755_1_M_C_78	780755		0	male	CSF	1978	62	F		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0112_780756_1_M_C_78	780756		0	male	CSF	1978	567	B2		mdf(A)_1
NEL0113_780817_1_M_C_78	780817		1	male	CSF	1978	390	B2		mdf(A)_1
NEL0114_780839_1_F_C_78	780839		1	female	CSF	1978	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0115_780845_1_M_C_78	780845		0	male	CSF	1978	567	B2		mdf(A)_1, tet(B)_2
NEL0116_780934_1_M_C_78	780934	2		male	CSF	1978	73	B2		mdf(A)_1
NEL0117_780934_2_M_C_78	780934	2		male	CSF	1978	550	B2		mdf(A)_1
NEL0118_780971_1_F_C_78	780971	13		female	CSF	1978	95	B2		mdf(A)_1
NEL0119_780979_1_F_C_78	780979	85		female	CSF	1978	73	B2		mdf(A)_1
NEL0120_781025_1_F_C_78	781025		0	female	CSF	1978	567	B2		mdf(A)_1
NEL0122_781047_1_M_C_78	781047		0	male	CSF	1978	-	B2		mdf(A)_1
NEL0124_781073_1_M_C_78	781073		0	male	CSF	1978	62	F		mdf(A)_1
NEL0126_781097_1_F_C_78	781097		0	female	CSF	1978	278	B1		ant(3'')-Ia_1, mdf(A)_1
NEL0127_781123_1_M_C_78	781123		0	male	CSF	1978	10	A		ant(3'')-Ia_1, aph(3'')-Ia_1, aph(6)-Id_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL0128_790016_1_M_C_79	790016		0	male	CSF	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0129_790071_1_F_C_79	790071		0	female	CSF	1979	-	B2		mdf(A)_1
NEL0131_790083_1_F_C_79	790083		0	female	CSF	1979	95	B2		mdf(A)_1
NEL0132_790086_1_F_C_79	790086	78		female	CSF	1979	73	B2		mdf(A)_1
NEL0133_790122_1_M_C_79	790122		1	male	CSF	1979	14	B2		mdf(A)_1
NEL0135_790139_1_F_C_79	790139	1		female	CSF	1979	12	B2		ant(3'')-Ia_1, blaSHV-1_1, mdf(A)_1, sul1_5, tet(D)_1
NEL0136_790163_1_F_C_79	790163		0	female	CSF	1979	567	B2		mdf(A)_1
NEL0137_790163_2_F_B_79	790163		0	female	blood	1979	567	B2		mdf(A)_1
NEL0138_790194_1_M_C_79	790194		0	male	CSF	1979	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0139_790195_1_F_C_79	790195		2	female	CSF	1979	59	F	parE_I355T	mdf(A)_1
NEL0140_790206_1_F_C_79	790206	57		female	CSF	1979	95	B2		mdf(A)_1
NEL0141_790219_1_M_C_79	790219		0	male	CSF	1979	95	B2		blaTEM-1A_1, mdf(A)_1
NEL0142_790249_1_M_C_79	790249		1	male	CSF	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0143_790249_2_M_B_79	790249		1	male	blood	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0144_790305_1_M_C_79	790305		1	male	CSF	1979	95	B2		mdf(A)_1
NEL0146_790348_1_M_C_79	790348		0	male	CSF	1979	95	B2		mdf(A)_1
NEL0149_790536_1_M_C_79	790536		0	male	CSF	1979	62	F		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0150_790565_1_M_C_79	790565		0	male	CSF	1979	567	B2		mdf(A)_1
NEL0151_790580_1_M_C_79	790580		0	male	CSF	1979	567	B2		mdf(A)_1
NEL0152_790606_1_F_C_79	790606	22		female	CSF	1979	1434	A		mdf(A)_1
NEL0153_790625_1_F_C_79	790625		0	female	CSF	1979	567	B2		mdf(A)_1
NEL0154_790641_1_M_C_79	790641		0	male	CSF	1979	95	B2		mdf(A)_1
NEL0156_790647_1_U_C_79	790647		1	unknown	CSF	1979	567	B2		mdf(A)_1
NEL0157_790679_1_F_C_79	790679	0	0	female	CSF	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0158_790715_1_M_C_79	790715		0	male	CSF	1979	80	B2		mdf(A)_1
NEL0159_790771_1_M_C_79	790771		0	male	CSF	1979	390	B2		mdf(A)_1
NEL0162_790814_1_F_C_79	790814		0	female	CSF	1979	95	B2		mdf(A)_1
NEL0163_790848_1_M_C_79	790848	2		male	CSF	1979	73	B2		mdf(A)_1
NEL0164_790848_2_M_O_79	790848	2		male	other	1979	73	B2		mdf(A)_1
NEL0165_790895_1_F_C_79	790895		0	female	CSF	1979	73	B2		aph(6)-Id_1, mdf(A)_1
NEL0166_790967_1_M_C_79	790967		0	male	CSF	1979	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0167_791002_1_F_C_79	791002		0	female	CSF	1979	23	C		mdf(A)_1
NEL0168_791013_1_M_B_79	791013		1	male	blood	1979	12	B2		mdf(A)_1
NEL0169_791013_2_M_O_79	791013		1	male	other	1979	12	B2		mdf(A)_1
NEL0170_791014_1_M_C_79	791014		0	male	CSF	1979	95	B2		mdf(A)_1
NEL0171_791058_1_M_C_79	791058	59		male	CSF	1979	12	B2		mdf(A)_1
NEL0172_791063_1_U_C_79	791063		1	unknown	CSF	1979	567	B2		aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0173_791073_1_U_C_79	791073	77		unknown	CSF	1979	70	D		aph(3'')-Ib_5, aph(3')-IIa_2, aph(6)-Ic_1, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0175_800014_1_M_C_80	800014		1	male	CSF	1980	62	F		mdf(A)_1
NEL0177_800133_1_M_C_80	800133		0	male	CSF	1980	62	F		mdf(A)_1
NEL0178_800154_1_M_C_80	800154		0	male	CSF	1980	567	B2		mdf(A)_1
NEL0179_800290_1_F_C_80	800290	2		female	CSF	1980	1057	B2		mdf(A)_1
NEL0180_800325_1_M_C_80	800325		1	male	CSF	1980	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0182_800341_1_U_C_80	800341		0	unknown	CSF	1980	95	B2		mdf(A)_1
NEL0183_800495_1_F_C_80	800495		1	female	CSF	1980	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0184_800518_1_M_C_80	800518		3	male	CSF	1980	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0185_800540_1_M_C_80	800540		0	male	CSF	1980	773	A		mdf(A)_1
NEL0186_800569_1_M_C_80	800569		5	male	CSF	1980	23	C		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0187_800662_1_M_C_80	800662		0	male	CSF	1980	62	F		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0188_800753_1_M_B_80	800753		1	male	blood	1980	10	A		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5, sul2_2
NEL0189_800753_2_M_O_80	800753		1	male	other	1980	10	A		ant(3'')-Ia_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, sul2_2
NEL0190_800792_1_M_C_80	800792		1	male	CSF	1980	95	B2		mdf(A)_1
NEL0191_800792_2_M_B_80	800792		1	male	blood	1980	95	B2		mdf(A)_1
NEL0192_800807_1_M_C_80	800807		0	male	CSF	1980	390	B2		mdf(A)_1
NEL0193_800831_1_M_B_80	800831		1	male	blood	1980	14	B2		mdf(A)_1
NEL0194_800888_1_M_C_80	800888	12		male	CSF	1980	550	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0195_800904_1_M_C_80	800904		1	male	CSF	1980	62	F		aph(3'')-Ib_5, aph(6)-Id_1, catA2_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0196_800904_2_M_B_80	800904		1	male	blood	1980	62	F		aph(3'')-Ib_5, aph(6)-Id_1, catA2_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0197_800941_1_M_C_80	800941		0	male	CSF	1980	390	B2		mdf(A)_1
NEL0198_800968_1_M_C_80	800968		1	male	CSF	1980	73	B2		blaTEM-1C_1, mdf(A)_1
NEL0199_801100_1_M_C_80	801100		0	male	CSF	1980	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0200_801118_1_M_C_80	801118		0	male	CSF	1980	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0201_801125_1_M_C_80	801125		0	male	CSF	1980	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0202_801130_1_M_C_80	801130		0	male	CSF	1980	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0204_801132_2_F_B_80	801132	15		female	blood	1980	130	E	parC_S57T	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_5, sul2_2
NEL0205_801173_1_M_C_80	801173		0	male	CSF	1980	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0206_801210_1_F_C_80	801210		0	female	CSF	1980	12	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, catA1_1, mdf(A)_1, sul1_5, sul2_2
NEL0207_801210_2_F_B_80	801210		0	female	blood	1980	12	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, catA1_1, mdf(A)_1, sul1_5, sul2_2
NEL0208_801227_1_F_C_80	801227	6		female	CSF	1980	12	B2	parE_I355T	blaTEM-1A_1, mdf(A)_1, sul2_2
NEL0209_801240_1_F_C_80	801240		0	female	CSF	1980	57	E		mdf(A)_1
NEL0210_801260_1_F_C_80	801260	1		female	CSF	1980	14	B2		mdf(A)_1
NEL0211_801335_1_M_C_80	801335	70		male	CSF	1980	130	E	parC_S57T	aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1A_1, dfrA16_2, mdf(A)_1, sul1_5, tet(A)_6
NEL0213_801411_1_M_C_80	801411		0	male	CSF	1980	144	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0214_810028_1_M_C_81	810028		0	male	CSF	1981	2628	B1		mdf(A)_1
NEL0215_810033_1_F_C_81	810033		11	female	CSF	1981	1858	B2		mdf(A)_1
NEL0216_810052_1_M_C_81	810052		0	male	CSF	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0217_810060_1_M_B_81	810060		0	male	blood	1981	4580	B1		mdf(A)_1
NEL0218_810065_1_F_C_81	810065		1	female	CSF	1981	2346	B2		aph(3')-Ia_9, blaTEM-1A_1, mdf(A)_1
NEL0219_810100_1_M_C_81	810100		1	male	CSF	1981	62	F		mdf(A)_1
NEL0220_810100_2_M_B_81	810100		1	male	blood	1981	62	F		mdf(A)_1
NEL0221_810135_1_F_C_81	810135		0	female	CSF	1981	1252	B1		mdf(A)_1
NEL0222_810146_1_F_C_81	810146		2	female	CSF	1981	62	F		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0223_810250_1_M_C_81	810250	68		male	CSF	1981	641	B1		ant(3'')-Ia_1, aph(3')-Ia_7, aph(6)-Id_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0224_810338_1_M_C_81	810338		0	male	CSF	1981	95	B2		mdf(A)_1
NEL0225_810351_1_F_C_81	810351		19	female	CSF	1981	14	B2		mdf(A)_1
NEL0226_810390_1_F_C_81	810390		0	female	CSF	1981	95	B2		mdf(A)_1
NEL0227_810390_2_F_B_81	810390		0	female	blood	1981	95	B2		mdf(A)_1
NEL0228_810458_1_M_C_81	810458		0	male	CSF	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0229_810458_2_M_B_81	810458		0	male	blood	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0230_810614_1_F_B_81	810614		0	female	blood	1981	144	B2		mdf(A)_1
NEL0231_810658_1_M_C_81	810658		0	male	CSF	1981	567	B2		mdf(A)_1
NEL0232_810679_1_F_B_81	810679		0	female	blood	1981	62	F		mdf(A)_1
NEL0233_810724_1_M_C_81	810724		19	male	CSF	1981	1618	B2		mdf(A)_1
NEL0234_810724_2_M_B_81	810724		19	male	blood	1981	1618	B2		mdf(A)_1
NEL0235_810733_1_F_C_81	810733		0	female	CSF	1981	567	B2		mdf(A)_1
NEL0236_810776_1_M_C_81	810776		0	male	CSF	1981	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0237_810827_1_F_C_81	810827		0	female	CSF	1981	-	B2		mdf(A)_1
NEL0238_810894_1_M_C_81	810894		0	male	CSF	1981	567	B2		mdf(A)_1
NEL0239_810894_2_M_B_81	810894		0	male	blood	1981	567	B2		mdf(A)_1
NEL0240_810911_1_M_C_81	810911	50		male	CSF	1981	6257	C		mdf(A)_1
NEL0241_810947_1_M_C_81	810947		0	male	CSF	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0242_810955_1_M_C_81	810955		0	male	CSF	1981	10	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul1_5, sul2_2, tet(C)_3
NEL0243_810972_1_F_C_81	810972		0	female	CSF	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0244_811058_1_F_C_81	811058	12		female	CSF	1981	75	B1		ant(3'')-Ia_1, fosA7_1, mdf(A)_1, sul1_5
NEL0245_811067_1_M_C_81	811067		0	male	CSF	1981	390	B2		mdf(A)_1, tet(A)_6
NEL0246_811067_2_M_B_81	811067		0	male	blood	1981	390	B2		mdf(A)_1, tet(A)_6
NEL0247_811089_1_M_C_81	811089		2	male	CSF	1981	12	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(C)_3
NEL0248_811155_1_M_C_81	811155		4	male	CSF	1981	567	B2		mdf(A)_1
NEL0249_811155_2_M_B_81	811155		4	male	blood	1981	567	B2		mdf(A)_1
NEL0250_811156_1_F_C_81	811156		0	female	CSF	1981	-	B2		mdf(A)_1
NEL0251_811171_1_M_C_81	811171		0	male	CSF	1981	95	B2		mdf(A)_1
NEL0252_811171_2_M_B_81	811171		0	male	blood	1981	95	B2		mdf(A)_1
NEL0254_811195_1_M_C_81	811195		0	male	CSF	1981	95	B2		mdf(A)_1
NEL0255_811204_1_M_C_81	811204		0	male	CSF	1981	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0256_811204_2_M_B_81	811204		0	male	blood	1981	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0257_811206_1_M_C_81	811206	73		male	CSF	1981	-	E		mdf(A)_1
NEL0258_811227_1_F_C_81	811227		0	female	CSF	1981	421	B2		mdf(A)_1
NEL0259_811255_1_M_C_81	811255		0	male	CSF	1981	567	B2		mdf(A)_1
NEL0260_811255_2_M_B_81	811255		0	male	blood	1981	567	B2		mdf(A)_1
NEL0261_811321_1_M_C_81	811321		0	male	CSF	1981	95	B2		mdf(A)_1
NEL0262_811353_1_F_C_81	811353		0	female	CSF	1981	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0263_820024_1_F_C_82	820024	90		female	CSF	1982	95	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0264_820141_1_M_C_82	820141		1	male	CSF	1982	95	B2		mdf(A)_1
NEL0265_820144_1_M_C_82	820144	51		male	CSF	1982	23	C		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0266_820215_1_M_C_82	820215		0	male	CSF	1982	500	B1		aac(3)-Ia_1 , aph(3')-Ia_7, blaOXA-1_1, blaTEM-1D_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_5, tet(A)_6, tet(B)_2
NEL0267_820317_1_F_C_82	820317		0	female	CSF	1982	95	B2		mdf(A)_1
NEL0268_820346_1_M_C_82	820346		0	male	CSF	1982	567	B2		mdf(A)_1
NEL0269_820346_2_M_B_82	820346		0	male	blood	1982	567	B2		mdf(A)_1
NEL0270_820426_1_F_C_82	820426		0	female	CSF	1982	567	B2		mdf(A)_1
NEL0271_820516_1_U_C_82	820516		0	unknown	CSF	1982	70	D		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0272_820522_1_M_C_82	820522		0	male	CSF	1982	500	B1		aac(3)-Ia_1 , aadA1_4, aph(3'')-Ib_5, aph(3')-Ia_7, aph(6)-Id_1, blaOXA-1_1, blaTEM-1D_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_5, sul2_2, tet(A)_6, tet(B)_2
NEL0273_820531_1_F_C_82	820531		0	female	CSF	1982	95	B2		mdf(A)_1
NEL0274_820531_2_F_B_82	820531		0	female	blood	1982	95	B2		mdf(A)_1
NEL0275_820531_3_F_O_82	820531		0	female	other	1982	95	B2		mdf(A)_1
NEL0276_820713_1_F_C_82	820713	66		female	CSF	1982	550	B2		mdf(A)_1
NEL0277_820750_1_F_C_82	820750		0	female	CSF	1982	10	A		mdf(A)_1
NEL0278_820841_1_F_C_82	820841		0	female	CSF	1982	390	B2		mdf(A)_1
NEL0279_820904_1_M_C_82	820904	68		male	CSF	1982	88	C		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0280_820977_1_M_C_82	820977		0	male	CSF	1982	372	B2		mdf(A)_1
NEL0281_820985_1_M_C_82	820985		0	male	CSF	1982	567	B2		mdf(A)_1, sul2_14
NEL0282_820985_2_M_B_82	820985		0	male	blood	1982	567	B2		mdf(A)_1
NEL0283_821001_1_M_C_82	821001	78		male	CSF	1982	73	B2		mdf(A)_1
NEL0284_821077_1_M_C_82	821077		1	male	CSF	1982	538	B2	parE_D475E	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0285_821098_1_M_B_82	821098		0	male	blood	1982	62	F		mdf(A)_1, tet(A)_6
NEL0286_821099_1_M_B_82	821099		0	male	blood	1982	10	A		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(C)_3
NEL0287_821100_1_F_B_82	821100		0	female	blood	1982	95	B2		mdf(A)_1
NEL0288_821102_1_F_B_82	821102		0	female	blood	1982	59	F	parE_I355T	aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0289_821103_1_M_B_82	821103		1	male	blood	1982	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0290_821104_1_M_B_82	821104		0	male	blood	1982	201	B1		mdf(A)_1
NEL0291_821105_1_M_B_82	821105		0	male	blood	1982	79	B2		mdf(A)_1
NEL0292_821106_1_F_B_82	821106		0	female	blood	1982	144	B2		mdf(A)_1
NEL0293_821107_1_M_B_82	821107		0	male	blood	1982	500	B1		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, tet(B)_2
NEL0294_821108_1_M_C_82	821108		0	male	CSF	1982	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(C)_2
NEL0295_821146_1_F_B_82	821146		0	female	blood	1982	567	B2		mdf(A)_1
NEL0296_821192_1_M_C_82	821192		0	male	CSF	1982	62	F		mdf(A)_1
NEL0297_821266_1_F_C_82	821266	75		female	CSF	1982	57	E		ant(3'')-Ia_1, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(B)_2
NEL0298_821266_2_F_B_82	821266	75		female	blood	1982	57	E		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(B)_2
NEL0299_821271_1_F_C_82	821271		0	female	CSF	1982	416	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0300_821271_2_F_B_82	821271		0	female	blood	1982	416	B2		mdf(A)_1
NEL0301_821310_1_U_C_82	821310	70		unknown	CSF	1982	393	D	parC_S57T, gyrA_D87G	mdf(A)_1
NEL0302_830005_1_F_C_83	830005		0	female	CSF	1983	62	F		mdf(A)_1
NEL0303_830037_1_M_C_83	830037		1	male	CSF	1983	62	F		mdf(A)_1
NEL0304_830073_1_F_C_83	830073		0	female	CSF	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1, sul2_2
NEL0305_830073_2_F_B_83	830073		0	female	blood	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1, sul2_2
NEL0306_830074_1_M_B_83	830074		0	male	blood	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1, sul2_2
NEL0307_830094_1_F_C_83	830094		0	female	CSF	1983	86	B1	parE_I355T	aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(B)_2
NEL0308_830097_1_F_C_83	830097		0	female	CSF	1983	567	B2		mdf(A)_1
NEL0309_830191_1_F_B_83	830191		0	female	blood	1983	95	B2		blaTEM-1B_1, mdf(A)_1
NEL0310_830214_1_M_C_83	830214		0	male	CSF	1983	-	B2		mdf(A)_1
NEL0311_830214_2_M_B_83	830214		0	male	blood	1983	-	B2		mdf(A)_1
NEL0312_830294_1_F_C_83	830294		0	female	CSF	1983	567	B2		mdf(A)_1
NEL0313_830294_2_F_B_83	830294		0	female	blood	1983	567	B2		mdf(A)_1
NEL0314_830329_1_U_C_83	830329		0	unknown	CSF	1983	567	B2		mdf(A)_1
NEL0315_830383_1_M_C_83	830383		0	male	CSF	1983	567	B2		mdf(A)_1
NEL0316_830383_2_M_B_83	830383		0	male	blood	1983	567	B2		mdf(A)_1
NEL0317_830396_1_F_C_83	830396		0	female	CSF	1983	567	B2		mdf(A)_1
NEL0318_830396_2_F_B_83	830396		0	female	blood	1983	567	B2		mdf(A)_1
NEL0319_830425_1_F_C_83	830425		0	female	CSF	1983	567	B2		mdf(A)_1
NEL0320_830543_1_F_C_83	830543		3	female	CSF	1983	10	A		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0321_830554_1_M_C_83	830554	48		male	CSF	1983	12	B2		aac(3)-IIa_1, ant(3'')-Ia_1, aph(3')-Ia_7, aph(6)-Id_1, blaTEM-110_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_9, tet(B)_1
NEL0322_830583_1_M_C_83	830583		0	male	CSF	1983	421	B2		mdf(A)_1
NEL0323_830583_2_M_B_83	830583		0	male	blood	1983	421	B2		mdf(A)_1
NEL0324_830595_1_M_B_83	830595		0	male	blood	1983	10	A		mdf(A)_1
NEL0325_830680_1_M_C_83	830680		0	male	CSF	1983	567	B2		mdf(A)_1
NEL0326_830680_2_M_B_83	830680		0	male	blood	1983	567	B2		mdf(A)_1
NEL0327_830695_1_M_C_83	830695	16		male	CSF	1983	-	C		mdf(A)_1
NEL0328_830748_1_F_C_83	830748		6	female	CSF	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0329_830748_2_F_B_83	830748		6	female	blood	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0330_830785_1_M_C_83	830785		0	male	CSF	1983	117	G		mdf(A)_1
NEL0331_830785_2_M_B_83	830785		0	male	blood	1983	117	G		mdf(A)_1
NEL0332_830798_1_F_C_83	830798		0	female	CSF	1983	59	F	parE_I355T	mdf(A)_1
NEL0333_830818_1_M_C_83	830818		2	male	CSF	1983	73	B2		mdf(A)_1
NEL0334_830897_1_F_C_83	830897	75		female	CSF	1983	390	B2		mdf(A)_1
NEL0335_830942_1_M_C_83	830942	68		male	CSF	1983	409	A		mdf(A)_1
NEL0336_830942_2_M_B_83	830942	68		male	blood	1983	409	A		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0337_830955_1_M_C_83	830955		0	male	CSF	1983	95	B2		mdf(A)_1
NEL0338_830955_2_M_B_83	830955		0	male	blood	1983	95	B2		mdf(A)_1
NEL0339_830975_1_F_C_83	830975		0	female	CSF	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0340_830975_2_F_B_83	830975		0	female	blood	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0341_830992_1_F_C_83	830992		0	female	CSF	1983	101	B1		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6, tet(C)_3
NEL0342_830992_2_F_B_83	830992		0	female	blood	1983	101	B1		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0343_830994_1_F_C_83	830994		0	female	CSF	1983	95	B2		mdf(A)_1
NEL0344_831026_1_M_C_83	831026		0	male	CSF	1983	416	B2		mdf(A)_1
NEL0345_831048_1_M_C_83	831048	65		male	CSF	1983	-	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0346_831050_1_F_C_83	831050	83		female	CSF	1983	-	D		mdf(A)_1
NEL0347_831120_1_F_C_83	831120		0	female	CSF	1983	10	A		mdf(A)_1
NEL0348_831128_1_M_C_83	831128		0	male	CSF	1983	363	B2	parE_D475E	mdf(A)_1, tet(A)_6
NEL0349_831176_1_F_C_83	831176	54		female	CSF	1983	88	C		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0350_831188_1_M_C_83	831188		0	male	CSF	1983	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-IIa_2, aph(6)-Ic_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0351_831236_1_F_C_83	831236		0	female	CSF	1983	59	F	parE_I355T	mdf(A)_1
NEL0352_831236_2_F_B_83	831236		0	female	blood	1983	59	F	parE_I355T	mdf(A)_1
NEL0353_831366_1_F_C_83	831366		0	female	CSF	1983	73	B2	parC_S57T	mdf(A)_1
NEL0354_831366_2_F_B_83	831366		0	female	blood	1983	73	B2	parC_S57T	mdf(A)_1
NEL0355_831390_1_M_C_83	831390		0	male	CSF	1983	567	B2		mdf(A)_1
NEL0356_831390_2_M_B_83	831390		0	male	blood	1983	567	B2		mdf(A)_1
NEL0357_831391_1_M_C_83	831391		0	male	CSF	1983	390	B2		mdf(A)_1
NEL0358_831391_2_M_B_83	831391		0	male	blood	1983	390	B2		mdf(A)_1
NEL0359_831412_1_F_C_83	831412		0	female	CSF	1983	59	F	parE_I355T	mdf(A)_1
NEL0360_831412_2_F_B_83	831412		0	female	blood	1983	59	F	parE_I355T	mdf(A)_1
NEL0361_831452_1_F_B_83	831452		0	female	blood	1983	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0362_831459_1_M_C_83	831459		0	male	CSF	1983	127	B2		mdf(A)_1
NEL0363_831469_1_F_C_83	831469		0	female	CSF	1983	95	B2		mdf(A)_1
NEL0364_831497_1_M_C_83	831497		0	male	CSF	1983	2218	B2		mdf(A)_1
NEL0365_831497_2_M_B_83	831497		0	male	blood	1983	2218	B2		mdf(A)_1
NEL0366_840074_1_M_C_84	840074		0	male	CSF	1984	59	F	parE_I355T	mdf(A)_1
NEL0367_840074_2_M_B_84	840074		0	male	blood	1984	59	F	parE_I355T	mdf(A)_1
NEL0368_840173_1_M_C_84	840173		0	male	CSF	1984	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0369_840201_1_F_C_84	840201		0	female	CSF	1984	88	C		mdf(A)_1
NEL0370_840201_2_F_B_84	840201		0	female	blood	1984	88	C		mdf(A)_1
NEL0371_840204_1_F_C_84	840204		0	female	CSF	1984	567	B2		mdf(A)_1
NEL0372_840254_1_M_C_84	840254		0	male	CSF	1984	95	B2		mdf(A)_1
NEL0373_840254_2_M_B_84	840254		0	male	blood	1984	95	B2		mdf(A)_1
NEL0374_840258_1_U_C_84	840258	73		unknown	CSF	1984	14	B2		mdf(A)_1
NEL0375_840269_1_F_C_84	840269	49		female	CSF	1984	73	B2		mdf(A)_1
NEL0376_840269_2_F_B_84	840269	49		female	blood	1984	73	B2		mdf(A)_1
NEL0377_840321_1_U_C_84	840321	1		unknown	CSF	1984	10	A		aph(3'')-Ib_2, mdf(A)_1, tet(B)_1
NEL0378_840382_1_M_C_84	840382	77		male	CSF	1984	5389	B2		mdf(A)_1
NEL0379_840516_1_F_C_84	840516		1	female	CSF	1984	144	B2		mdf(A)_1
NEL0380_840516_2_F_B_84	840516		1	female	blood	1984	144	B2		mdf(A)_1
NEL0381_840553_1_F_C_84	840553		0	female	CSF	1984	62	F		mdf(A)_1
NEL0382_840554_1_F_C_84	840554		0	female	CSF	1984	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0383_840562_1_M_C_84	840562	24		male	CSF	1984	62	F		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1, tet(A)_6
NEL0384_840647_1_M_C_84	840647		0	male	CSF	1984	88	C		mdf(A)_1
NEL0385_840670_1_M_B_84	840670		0	male	blood	1984	101	B1		mdf(A)_1, tet(A)_6
NEL0386_840681_1_M_C_84	840681		0	male	CSF	1984	12	B2		aac(3)-Ia_1, ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, 8188, catA1_1, mdf(A)_1, sul1_5, tet(B)_1
NEL0387_840681_2_M_B_84	840681		0	male	blood	1984	12	B2		aac(3)-Ia_1, ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, 8188, catA1_1, mdf(A)_1, sul1_5, tet(B)_1
NEL0388_840811_1_M_C_84	840811	79		male	CSF	1984	969	B2	parE_D475E	mdf(A)_1
NEL0389_840854_1_F_B_84	840854		0	female	blood	1984	127	B2		ant(3'')-Ia_1, aph(3')-Ia_7, dfrA1_10, mdf(A)_1, sul1_5
NEL0390_840862_1_F_B_84	840862	4		female	blood	1984	-	A		mdf(A)_1, tet(A)_6
NEL0391_840863_1_M_B_84	840863		0	male	blood	1984	59	F	parE_I355T	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0392_840919_1_F_B_84	840919		0	female	blood	1984	10	A		blaTEM-1B_1, mdf(A)_1
NEL0393_840974_1_M_C_84	840974		0	male	CSF	1984	95	B2		mdf(A)_1
NEL0394_840974_2_M_B_84	840974		0	male	blood	1984	95	B2		mdf(A)_1
NEL0395_841041_1_F_C_84	841041		0	female	CSF	1984	12	B2		aac(3)-Ia_1, ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_1
NEL0396_841060_1_M_C_84	841060		0	male	CSF	1984	2218	B2		mdf(A)_1
NEL0397_841060_2_M_B_84	841060		0	male	blood	1984	2218	B2		mdf(A)_1
NEL0398_841132_1_M_C_84	841132		0	male	CSF	1984	95	B2		mdf(A)_1
NEL0399_841132_2_M_B_84	841132		0	male	blood	1984	95	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0400_841137_1_M_C_84	841137		0	male	CSF	1984	95	B2		mdf(A)_1
NEL0401_841137_2_M_B_84	841137		0	male	blood	1984	95	B2		mdf(A)_1
NEL0402_841175_1_F_C_84	841175	1		female	CSF	1984	95	B2		mdf(A)_1
NEL0403_841237_1_F_C_84	841237		0	female	CSF	1984	62	F		mdf(A)_1
NEL0404_841328_1_F_C_84	841328		8	female	CSF	1984	73	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(C)_2
NEL0405_841410_1_U_B_84	841410		0	unknown	blood	1984	363	B2	parE_D475E	mdf(A)_1
NEL0406_850007_1_F_C_85	850007	1		female	CSF	1985	95	B2		mdf(A)_1
NEL0407_850042_1_U_C_85	850042		0	unknown	CSF	1985	567	B2		mdf(A)_1
NEL0408_850054_1_F_C_85	850054		0	female	CSF	1985	567	B2		mdf(A)_1
NEL0409_850153_1_M_C_85	850153		0	male	CSF	1985	59	F		mdf(A)_1
NEL0410_850155_1_M_C_85	850155		0	male	CSF	1985	59	F	parE_I355T	ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0411_850155_2_M_B_85	850155		0	male	blood	1985	59	F	parE_I355T	ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0413_850294_1_M_C_85	850294		0	male	CSF	1985	95	B2		blaTEM-1A_1, mdf(A)_1
NEL0415_850424_2_M_B_85	850424		0	male	blood	1985	95	B2		mdf(A)_1
NEL0416_850464_1_F_C_85	850464		0	female	CSF	1985	2628	B1		mdf(A)_1
NEL0417_850542_1_F_C_85	850542		1	female	CSF	1985	390	B2		mdf(A)_1
NEL0418_850639_1_F_C_85	850639		0	female	CSF	1985	62	F		mdf(A)_1
NEL0419_850639_2_F_B_85	850639		0	female	blood	1985	62	F		mdf(A)_1
NEL0420_850697_1_F_C_85	850697		1	female	CSF	1985	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0421_850697_2_F_B_85	850697		1	female	blood	1985	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0422_850763_1_M_C_85	850763	71		male	CSF	1985	12	B2		mdf(A)_1
NEL0423_850781_1_M_C_85	850781		0	male	CSF	1985	62	F		mdf(A)_1
NEL0424_850781_2_M_B_85	850781		0	male	blood	1985	62	F		mdf(A)_1
NEL0425_850812_1_F_C_85	850812		0	female	CSF	1985	95	B2		mdf(A)_1
NEL0426_850814_1_F_C_85	850814		0	female	CSF	1985	52	A		mdf(A)_1
NEL0427_850836_1_M_C_85	850836		0	male	CSF	1985	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0428_850836_2_M_B_85	850836		0	male	blood	1985	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0429_850873_1_F_C_85	850873		0	female	CSF	1985	10	A		mdf(A)_1
NEL0430_850873_2_F_B_85	850873		0	female	blood	1985	10	A		mdf(A)_1
NEL0431_850918_1_F_B_85	850918	2		female	blood	1985	641	B1		mdf(A)_1
NEL0432_850969_1_M_C_85	850969		0	male	CSF	1985	95	B2		mdf(A)_1
NEL0433_851038_1_M_C_85	851038		0	male	CSF	1985	95	B2		mdf(A)_1
NEL0434_851056_1_F_C_85	851056	89		female	CSF	1985	187	B1		mdf(A)_1
NEL0435_851060_1_U_C_85	851060		0	unknown	CSF	1985	95	B2		blaTEM-1A_1, mdf(A)_1
NEL0436_851060_2_U_B_85	851060		0	unknown	blood	1985	95	B2		blaTEM-1A_1, mdf(A)_1
NEL0437_851147_1_F_C_85	851147		0	female	CSF	1985	73	B2		mdf(A)_1
NEL0438_851163_1_U_C_85	851163		1	unknown	CSF	1985	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0439_851242_1_U_C_85	851242	28		unknown	CSF	1985	126	B2		mdf(A)_1
NEL0440_851271_1_F_C_85	851271		0	female	CSF	1985	-	B2		mdf(A)_1
NEL0441_851285_1_M_B_85	851285	1		male	blood	1985	73	B2		mdf(A)_1
NEL0442_851322_1_F_C_85	851322	67		female	CSF	1985	95	B2		mdf(A)_1
NEL0443_851326_1_F_C_85	851326		0	female	CSF	1985	62	F		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL0444_851326_2_F_B_85	851326		0	female	blood	1985	62	F		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL0445_851434_1_F_B_85	851434		1	female	blood	1985	95	B2		mdf(A)_1
NEL0446_860006_1_M_B_86	860006		1	male	blood	1986	80	B2		mdf(A)_1
NEL0448_860072_2_M_B_86	860072		0	male	blood	1986	95	B2		mdf(A)_1
NEL0449_860079_1_M_C_86	860079		4	male	CSF	1986	127	B2		mdf(A)_1
NEL0450_860098_1_F_C_86	860098		0	female	CSF	1986	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0452_860222_1_M_C_86	860222		0	male	CSF	1986	95	B2		mdf(A)_1
NEL0453_860222_2_M_B_86	860222		0	male	blood	1986	95	B2		mdf(A)_1
NEL0454_860274_1_M_C_86	860274	49		male	CSF	1986	127	B2		mdf(A)_1
NEL0456_860360_1_M_C_86	860360	74		male	CSF	1986	14	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL0457_860434_1_M_C_86	860434		0	male	CSF	1986	543	E		aph(3'')-Ib_5, mdf(A)_1, sul2_2
NEL0458_860451_1_F_B_86	860451		0	female	blood	1986	73	B2		mdf(A)_1
NEL0459_860464_1_F_C_86	860464	1		female	CSF	1986	88	C		ant(3'')-Ia_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0460_860514_1_F_C_86	860514		0	female	CSF	1986	144	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0461_860514_2_F_B_86	860514		0	female	blood	1986	144	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0462_860536_1_F_C_86	860536		0	female	CSF	1986	390	B2		mdf(A)_1
NEL0463_860599_1_M_C_86	860599		0	male	CSF	1986	95	B2		mdf(A)_1
NEL0464_860706_1_M_C_86	860706		0	male	CSF	1986	567	B2		aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0465_860706_2_M_B_86	860706		0	male	blood	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0466_860753_1_F_C_86	860753		0	female	CSF	1986	567	B2		mdf(A)_1
NEL0467_860794_1_F_C_86	860794		0	female	CSF	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0468_860794_2_F_B_86	860794		0	female	blood	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0469_860794_3_F_O_86	860794		0	female	other	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0470_860876_1_F_C_86	860876		0	female	CSF	1986	-	B2		mdf(A)_1, sul2_2
NEL0471_860952_1_M_C_86	860952		0	male	CSF	1986	95	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0472_860952_2_M_B_86	860952		0	male	blood	1986	95	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0473_860968_1_M_C_86	860968		3	male	CSF	1986	14	B2		mdf(A)_1
NEL0474_860996_1_M_C_86	860996		0	male	CSF	1986	95	B2		mdf(A)_1
NEL0475_860996_2_M_B_86	860996		0	male	blood	1986	95	B2		mdf(A)_1
NEL0478_861220_1_F_C_86	861220	62		female	CSF	1986	405	D		mdf(A)_1
NEL0479_861239_1_M_C_86	861239		0	male	CSF	1986	144	B2		mdf(A)_1
NEL0480_861305_1_M_C_86	861305		0	male	CSF	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0481_861305_2_M_B_86	861305		0	male	blood	1986	567	B2		aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0482_861315_1_F_C_86	861315		0	female	CSF	1986	95	B2		mdf(A)_1
NEL0483_861379_1_M_C_86	861379	62		male	CSF	1986	73	B2		mdf(A)_1
NEL0484_861526_1_F_C_86	861526		0	female	CSF	1986	390	B2		mdf(A)_1
NEL0485_861526_2_F_B_86	861526		0	female	blood	1986	390	B2		mdf(A)_1
NEL0486_861526_3_F_O_86	861526		0	female	other	1986	390	B2		mdf(A)_1
NEL0487_861578_1_M_C_86	861578		0	male	CSF	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0488_861578_2_M_B_86	861578		0	male	blood	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0489_861578_3_M_O_86	861578		0	male	other	1986	416	B2		mdf(A)_1
NEL0490_861583_1_M_C_86	861583		0	male	CSF	1986	390	B2		mdf(A)_1
NEL0491_861583_2_M_B_86	861583		0	male	blood	1986	390	B2		mdf(A)_1
NEL0492_861773_1_F_B_86	861773		0	female	blood	1986	10	A		mdf(A)_1
NEL0493_870006_1_M_C_87	870006		0	male	CSF	1987	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0494_870006_2_M_B_87	870006		0	male	blood	1987	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0495_870007_1_F_C_87	870007		0	female	CSF	1987	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0496_870099_1_M_C_87	870099		1	male	CSF	1987	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0497_870111_1_M_C_87	870111		0	male	CSF	1987	416	B2		blaTEM-1B_1, mdf(A)_1
NEL0498_870111_2_M_B_87	870111		0	male	blood	1987	416	B2		blaTEM-1B_1, mdf(A)_1
NEL0499_870130_1_M_C_87	870130		0	male	CSF	1987	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0500_870130_2_M_B_87	870130		0	male	blood	1987	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0501_870419_1_F_C_87	870419	72		female	CSF	1987	73	B2		mdf(A)_1
NEL0502_870423_1_F_C_87	870423	54		female	CSF	1987	127	B2		mdf(A)_1
NEL0503_870528_1_M_C_87	870528		0	male	CSF	1987	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0504_870528_2_M_B_87	870528		0	male	blood	1987	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0505_870654_1_F_C_87	870654		0	female	CSF	1987	567	B2		mdf(A)_1
NEL0506_870656_1_M_C_87	870656		0	male	CSF	1987	390	B2		mdf(A)_1
NEL0507_870656_2_M_B_87	870656		0	male	blood	1987	390	B2		mdf(A)_1
NEL0508_870721_1_F_C_87	870721		0	female	CSF	1987	95	B2		mdf(A)_1
NEL0509_870721_2_F_B_87	870721		0	female	blood	1987	95	B2		mdf(A)_1
NEL0510_870742_1_U_C_87	870742		0	unknown	CSF	1987	567	B2		mdf(A)_1
NEL0511_870816_1_F_B_87	870816		0	female	blood	1987	73	B2		ant(3'')-Ia_1, blaOXA-1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL0512_870841_1_F_C_87	870841	4		female	CSF	1987	12	B2		dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL0513_870841_2_F_B_87	870841	4		female	blood	1987	12	B2		dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL0514_870863_1_F_C_87	870863	2		female	CSF	1987	88	C		mdf(A)_1, tet(C)_2
NEL0515_870863_2_F_B_87	870863	2		female	blood	1987	101	B1		mdf(A)_1, tet(C)_2
NEL0516_870917_1_M_C_87	870917		0	male	CSF	1987	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0517_870917_2_M_B_87	870917		0	male	blood	1987	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0518_870989_1_M_B_87	870989		0	male	blood	1987	144	B2		mdf(A)_1
NEL0519_871039_1_F_C_87	871039		0	female	CSF	1987	95	B2		mdf(A)_1
NEL0520_871039_2_F_B_87	871039		0	female	blood	1987	95	B2		mdf(A)_1
NEL0521_871360_1_M_C_87	871360		6	male	CSF	1987	537	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0524_871486_1_M_C_87	871486	72		male	CSF	1987	117	G		mdf(A)_1, sul2_2, tet(B)_2
NEL0525_871524_1_M_B_87	871524		0	male	blood	1987	538	B2	parE_D475E	mdf(A)_1
NEL0526_871534_1_M_C_87	871534		0	male	CSF	1987	95	B2		mdf(A)_1
NEL0527_871534_2_M_B_87	871534		0	male	blood	1987	95	B2		mdf(A)_1
NEL0528_871568_1_M_B_87	871568		0	male	blood	1987	297	B1		mdf(A)_1
NEL0529_871578_1_M_C_87	871578	56		male	CSF	1987	12	B2		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1, sul1_5
NEL0530_871578_2_M_B_87	871578	56		male	blood	1987	12	B2		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1, sul1_5
NEL0531_871692_1_F_C_87	871692		0	female	CSF	1987	95	B2		mdf(A)_1
NEL0532_871692_2_F_B_87	871692		0	female	blood	1987	95	B2		mdf(A)_1
NEL0533_871707_1_F_B_87	871707		0	female	blood	1987	73	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(B)_2
NEL0534_871857_1_M_C_87	871857		0	male	CSF	1987	390	B2		mdf(A)_1
NEL0535_880035_1_M_C_88	880035		0	male	CSF	1988	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0536_880149_1_M_B_88	880149		0	male	Blood	1988	625	B2	parE_D475E	ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0537_880184_1_M_C_88	880184		0	male	CSF	1988	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0538_880243_1_F_C_88	880243		0	female	CSF	1988	95	B2		mdf(A)_1
NEL0539_880278_1_F_C_88	880278		0	female	CSF	1988	95	B2		mdf(A)_1
NEL0540_880278_2_F_B_88	880278		0	female	Blood	1988	95	B2		mdf(A)_1
NEL0541_880284_1_U_C_88	880284	27		unknown	CSF	1988	2494	B2		mdf(A)_1, sul2_2
NEL0542_880287_1_F_C_88	880287	88		female	CSF	1988	73	B2		ant(3'')-Ia_1, blaTEM-1D_1, dfrA1_8, mdf(A)_1, mph(E)_1, msr(E)_1
NEL0543_880287_2_F_B_88	880287	88		female	Blood	1988	73	B2		ant(3'')-Ia_1, blaTEM-1D_1, dfrA1_8, mdf(A)_1, mph(E)_1, msr(E)_1
NEL0544_880477_1_F_C_88	880477	37		female	CSF	1988	405	D		ant(3'')-Ia_1, mdf(A)_1
NEL0545_880478_1_F_C_88	880478		0	female	CSF	1988	390	B2		mdf(A)_1
NEL0546_880478_2_F_B_88	880478		0	female	Blood	1988	390	B2		mdf(A)_1
NEL0547_880602_1_F_C_88	880602		0	female	CSF	1988	1828	B2		mdf(A)_1
NEL0548_880621_1_M_C_88	880621	1		male	CSF	1988	537	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0549_880634_1_F_C_88	880634		1	female	CSF	1988	567	B2		mdf(A)_1
NEL0550_880787_1_M_B_88	880787		0	male	Blood	1988	12	B2		mdf(A)_1
NEL0551_880798_1_F_C_88	880798		0	female	CSF	1988	567	B2		mdf(A)_1
NEL0552_880798_2_F_B_88	880798		0	female	Blood	1988	567	B2		mdf(A)_1
NEL0553_880821_1_M_C_88	880821		1	male	CSF	1988	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0554_880853_1_F_C_88	880853		2	female	CSF	1988	95	B2		mdf(A)_1
NEL0555_880869_1_M_C_88	880869		7	male	CSF	1988	696	B1		mdf(A)_1
NEL0556_880893_1_F_C_88	880893		8	female	CSF	1988	363	B2	parE_D475E	ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL0557_881084_1_M_C_88	881084		2	male	CSF	1988	429	B2		mdf(A)_1
NEL0558_881084_2_M_B_88	881084		2	male	Blood	1988	429	B2		mdf(A)_1
NEL0559_881236_1_F_C_88	881236		0	female	CSF	1988	95	B2		mdf(A)_1
NEL0560_881236_2_F_B_88	881236		0	female	Blood	1988	95	B2		mdf(A)_1
NEL0561_881299_1_M_C_88	881299		0	male	CSF	1988	416	B2		blaTEM-1A_1, mdf(A)_1
NEL0562_881507_1_M_C_88	881507	48		male	CSF	1988	537	B2		mdf(A)_1
NEL0563_881516_1_M_C_88	881516		0	male	CSF	1988	62	F		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL0564_881521_1_M_B_88	881521		0	male	Blood	1988	95	B2		mdf(A)_1, tet(A)_6
NEL0565_881532_1_M_B_88	881532		0	male	Blood	1988	62	F		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL0566_881536_1_U_C_88	881536	57		unknown	CSF	1988	88	C		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1A_1, dfrA1_10, mdf(A)_1, sul1_5, tet(B)_1
NEL0567_881598_1_F_C_88	881598		0	female	CSF	1988	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(C)_2
NEL0568_881598_2_F_B_88	881598		0	female	Blood	1988	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(C)_2
NEL0569_881598_3_F_O_88	881598		0	female	urine	1988	295	B1		mdf(A)_1
NEL0570_881638_1_M_C_88	881638		0	male	CSF	1988	421	B2		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5
NEL0571_881638_2_M_B_88	881638		0	male	Blood	1988	421	B2		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5
NEL0572_881661_1_U_C_88	881661		3	unknown	CSF	1988	12	B2		mdf(A)_1
NEL0574_881677_1_M_C_88	881677	75		male	CSF	1988	88	C		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL0575_881915_1_M_C_88	881915	21		male	CSF	1988	73	B2		mdf(A)_1
NEL0576_881927_1_F_C_88	881927	49		female	CSF	1988	62	F		blaTEM-1B_1, mdf(A)_1
NEL0577_881927_2_F_B_88	881927	49		female	Blood	1988	62	F		blaTEM-1B_1, mdf(A)_1
NEL0578_882068_1_M_C_88	882068		0	male	CSF	1988	62	F		ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1
NEL0579_882068_2_M_B_88	882068		0	male	Blood	1988	62	F		ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1
NEL0580_882178_1_M_B_88	882178	50		male	Blood	1988	10	A		mdf(A)_1
NEL0581_890142_1_M_C_89	890142	75		male	CSF	1989	2218	B2		mdf(A)_1
NEL0582_890142_2_M_B_89	890142	75		male	Blood	1989	2218	B2		mdf(A)_1
NEL0583_890165_1_F_C_89	890165		0	female	CSF	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0584_890165_2_F_B_89	890165		0	female	Blood	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0585_890424_1_F_B_89	890424		0	female	Blood	1989	2803	E		mdf(A)_1
NEL0586_890557_1_M_C_89	890557		1	male	CSF	1989	10	A		blaTEM-1A_1, mdf(A)_1
NEL0587_890562_1_F_B_89	890562		0	female	Blood	1989	2678	B2		mdf(A)_1
NEL0588_890706_1_M_C_89	890706		0	male	CSF	1989	-	B2		mdf(A)_1
NEL0589_890706_2_M_B_89	890706		0	male	Blood	1989	-	B2		mdf(A)_1
NEL0590_890706_3_M_O_89	890706		0	male	ne/throa	1989	-	B2		mdf(A)_1
NEL0591_890706_4_M_O_89	890706		0	male	ne/throa	1989	-	B2		mdf(A)_1
NEL0592_890736_1_M_C_89	890736		0	male	CSF	1989	567	B2		mdf(A)_1
NEL0593_890746_1_F_C_89	890746	80		female	CSF	1989	367	C		mdf(A)_1
NEL0594_890795_1_F_B_89	890795		0	female	Blood	1989	59	F	parE_I355T	mdf(A)_1
NEL0595_890854_1_M_C_89	890854		8	male	CSF	1989	963	D		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0596_890900_1_M_C_89	890900		0	male	CSF	1989	416	B2		mdf(A)_1, sul2_2
NEL0598_890934_1_M_B_89	890934		0	male	Blood	1989	416	B2		mdf(A)_1, sul2_2
NEL0599_890936_1_F_C_89	890936		1	female	CSF	1989	59	F	parE_I355T	mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0600_890936_2_F_B_89	890936		1	female	Blood	1989	59	F	parE_I355T	mdf(A)_1
NEL0601_890968_1_F_C_89	890968		0	female	CSF	1989	126	B2		mdf(A)_1
NEL0602_890968_2_F_B_89	890968		0	female	Blood	1989	126	B2		mdf(A)_1
NEL0603_891063_1_M_C_89	891063		0	male	CSF	1989	-	C		mdf(A)_1
NEL0605_891153_1_M_C_89	891153	34		male	CSF	1989	62	F		mdf(A)_1
NEL0606_891311_1_F_C_89	891311		0	female	CSF	1989	416	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL0607_891449_1_M_C_89	891449		0	male	CSF	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0608_891449_2_M_B_89	891449		0	male	Blood	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0609_891460_1_F_C_89	891460	11		female	CSF	1989	390	B2		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0610_891503_1_F_C_89	891503		0	female	CSF	1989	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0611_891525_1_M_C_89	891525		1	male	CSF	1989	62	F		blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL0612_891525_2_M_B_89	891525		1	male	Blood	1989	62	F		blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL0613_891555_1_M_C_89	891555		1	male	CSF	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0614_891555_2_M_B_89	891555		1	male	Blood	1989	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0615_891663_1_M_B_89	891663		0	male	Blood	1989	10	A		mdf(A)_1
NEL0616_891669_1_M_B_89	891669		0	male	Blood	1989	973	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0617_891711_1_F_B_89	891711		0	female	Blood	1989	-	A		mdf(A)_1
NEL0618_891736_1_M_C_89	891736	11		male	CSF	1989	390	B2		ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0619_892071_1_F_C_89	892071	8		female	CSF	1989	-	B2		aac(3)-Ia_1 , aac(6')-Ib_1, ant(3'')-Ia_1, blaOXA-10_1, blaTEM-1D_1, catB2_1, dfrA1_10, floR_2, mdf(A)_1, sul1_5, sul2_2
NEL0620_892072_1_F_C_89	892072	8		female	CSF	1989	141	B2		ant(3'')-Ia_1, dfrA14_5, mdf(A)_1, tet(B)_2
NEL0621_892129_1_M_C_89	892129		0	male	CSF	1989	567	B2		mdf(A)_1
NEL0622_892160_1_F_C_89	892160	77		female	CSF	1989	14	B2		aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0623_892308_1_M_C_89	892308		0	male	CSF	1989	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0624_892308_2_M_B_89	892308		0	male	Blood	1989	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0625_892450_1_M_C_89	892450	62		male	CSF	1989	14	B2		mdf(A)_1
NEL0626_892538_1_M_C_89	892538		1	male	CSF	1989	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1
NEL0627_892623_1_M_C_89	892623		0	male	CSF	1989	390	B2		mdf(A)_1
NEL0628_892623_2_M_B_89	892623		0	male	Blood	1989	390	B2		mdf(A)_1
NEL0629_892647_1_M_C_89	892647	72		male	CSF	1989	847	B1		mdf(A)_1
NEL0630_900007_1_M_C_90	900007		0	male	CSF	1990	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0631_900007_2_M_B_90	900007		0	male	Blood	1990	567	B2		mdf(A)_1
NEL0632_900356_1_M_B_90	900356		0	male	Blood	1990	59	F	parE_I355T	mdf(A)_1
NEL0634_900357_2_F_B_90	900357		0	female	Blood	1990	10	A		mdf(A)_1
NEL0635_900362_1_F_B_90	900362		0	female	Blood	1990	349	D		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0636_900372_1_M_B_90	900372		0	male	Blood	1990	95	B2		mdf(A)_1
NEL0637_900397_1_M_C_90	900397		2	male	CSF	1990	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_14
NEL0638_900562_1_F_C_90	900562	62		female	CSF	1990	-	B2		mdf(A)_1
NEL0639_900681_1_M_C_90	900681		2	male	CSF	1990	95	B2		mdf(A)_1
NEL0640_900681_2_M_B_90	900681		2	male	Blood	1990	95	B2		mdf(A)_1
NEL0641_900710_1_M_C_90	900710		1	male	CSF	1990	362	D		ant(3'')-Ia_1, blaTEM-1A_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0642_900710_2_M_B_90	900710		1	male	Blood	1990	362	D		ant(3'')-Ia_1, blaTEM-1A_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0643_900925_1_F_C_90	900925		0	female	CSF	1990	95	B2		mdf(A)_1
NEL0644_900925_2_F_B_90	900925		0	female	Blood	1990	95	B2		mdf(A)_1
NEL0645_901267_1_M_C_90	901267		1	male	CSF	1990	95	B2		mdf(A)_1
NEL0646_901280_1_M_C_90	901280		1	male	CSF	1990	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0647_901348_1_F_C_90	901348		1	female	CSF	1990	567	B2		mdf(A)_1
NEL0648_901443_1_M_C_90	901443		1	male	CSF	1990	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0649_901443_2_M_B_90	901443		1	male	Blood	1990	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0650_901634_1_F_C_90	901634		1	female	CSF	1990	62	F		mdf(A)_1
NEL0651_901634_2_F_B_90	901634		1	female	Blood	1990	62	F		mdf(A)_1
NEL0652_901682_1_M_B_90	901682		1	male	Blood	1990	17	B1		mdf(A)_1
NEL0653_901702_1_M_C_90	901702		0	male	CSF	1990	95	B2		ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5
NEL0654_901717_1_M_B_90	901717		1	male	Blood	1990	25	B1		mdf(A)_1
NEL0655_901760_1_F_C_90	901760		1	female	CSF	1990	1001	B1		mdf(A)_1
NEL0656_901782_1_M_B_90	901782		1	male	Blood	1990	12	B2	parE_I355T	ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5
NEL0657_901783_1_U_O_90	901783		0	unknown	puth/skin	1990	10	A		mdf(A)_1
NEL0659_901810_1_M_C_90	901810		1	male	CSF	1990	567	B2		mdf(A)_1
NEL0660_901810_2_M_B_90	901810		1	male	Blood	1990	567	B2		mdf(A)_1
NEL0661_901946_1_F_C_90	901946		3	female	CSF	1990	14	B2		mdf(A)_1
NEL0662_901987_1_U_C_90	901987		0	unknown	CSF	1990	10	A		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL0663_901987_2_U_B_90	901987		0	unknown	Blood	1990	10	A		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL0665_901995_2_M_B_90	901995		0	male	Blood	1990	4360	B1		mdf(A)_1
NEL0666_902034_1_F_C_90	902034		1	female	CSF	1990	12	B2		aadA2_1, aph(3')-Ia_1, blaTEM-1A_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL0667_902034_2_F_B_90	902034		1	female	Blood	1990	12	B2		aadA2_1, aph(3')-Ia_1, blaTEM-1A_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL0668_902043_1_F_C_90	902043		1	female	CSF	1990	127	B2		mdf(A)_1
NEL0669_902047_1_F_C_90	902047	6		female	CSF	1990	-	cladeIII	parC_S57T, parE_I355T	mdf(A)_1
NEL0670_902177_1_M_C_90	902177		0	male	CSF	1990	95	B2		mdf(A)_1
NEL0671_902260_1_M_C_90	902260		1	male	CSF	1990	393	D	parC_S57T	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0672_902270_1_F_C_90	902270		0	female	CSF	1990	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0673_902270_2_F_B_90	902270		0	female	Blood	1990	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0674_902412_1_M_B_90	902412		0	male	Blood	1990	23	C		mdf(A)_1, tet(A)_6
NEL0675_902473_1_M_B_90	902473		1	male	Blood	1990	390	B2		mdf(A)_1
NEL0676_902509_1_M_B_90	902509		0	male	Blood	1990	59	F	parE_I355T	mdf(A)_1
NEL0677_902710_1_F_O_90	902710	33		female	lochia	1990	141	B2		mdf(A)_1
NEL0678_910007_1_F_C_91	910007	84		female	CSF	1991	62	F		mdf(A)_1
NEL0679_910051_1_F_C_91	910051	70		female	CSF	1991	14	B2		mdf(A)_1
NEL0680_910053_1_F_C_91	910053		0	female	CSF	1991	10	A		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL0681_910053_2_F_B_91	910053		0	female	Blood	1991	10	A		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL0682_910053_3_F_O_91	910053		0	female	skin	1991	10	A		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL0683_910207_1_M_C_91	910207	51		male	CSF	1991	12	B2		ant(3'')-Ia_1, blaTEM-1A_1, mdf(A)_1, sul1_5
NEL0684_910207_2_M_B_91	910207	51		male	Blood	1991	12	B2		ant(3'')-Ia_1, blaTEM-1A_1, mdf(A)_1, sul1_5
NEL0685_910222_1_F_C_91	910222	71		female	CSF	1991	126	B2		mdf(A)_1
NEL0686_910367_1_F_C_91	910367	71		female	CSF	1991	126	B2		mdf(A)_1
NEL0687_910459_1_F_C_91	910459		0	female	CSF	1991	10	A		
NEL0688_910644_1_M_C_91	910644		0	male	CSF	1991	390	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0689_910644_2_M_B_91	910644		0	male	Blood	1991	390	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0690_910696_1_M_C_91	910696	46		male	CSF	1991	95	B2		mdf(A)_1
NEL0691_910738_1_M_B_91	910738		0	male	Blood	1991	95	B2		mdf(A)_1
NEL0692_910784_1_F_B_91	910784		0	female	Blood	1991	34	A		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0693_910965_1_F_C_91	910965		2	female	CSF	1991	88	C		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL0694_910974_1_M_B_91	910974		1	male	Blood	1991	95	B2		mdf(A)_1
NEL0695_911089_1_M_B_91	911089		0	male	Blood	1991	3714	B1		mdf(A)_1
NEL0698_911241_1_M_C_91	911241		1	male	CSF	1991	62	F		mdf(A)_1
NEL0699_911251_1_M_C_91	911251		0	male	CSF	1991	62	F		blaTEM-1C_1, mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0701_911422_1_M_C_91	911422		1	male	CSF	1991	62	F		blaTEM-1C_1, mdf(A)_1
NEL0702_911422_2_M_B_91	911422		1	male	Blood	1991	62	F		blaTEM-1C_1, mdf(A)_1
NEL0703_911462_1_M_C_91	911462	47		male	CSF	1991	12	B2		mdf(A)_1
NEL0704_911480_1_F_B_91	911480		0	female	Blood	1991	73	B2		mdf(A)_1
NEL0705_911630_1_M_C_91	911630	65		male	CSF	1991	538	B2	parE_D475E	mdf(A)_1
NEL0707_911679_1_F_B_91	911679		1	female	Blood	1991	448	B1		mdf(A)_1
NEL0708_911718_1_M_C_91	911718	64		male	CSF	1991	6896	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0709_911730_1_F_B_91	911730		0	female	Blood	1991	567	B2		mdf(A)_1
NEL0710_911781_1_M_B_91	911781		0	male	Blood	1991	73	B2		aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1
NEL0711_911986_1_F_C_91	911986		0	female	CSF	1991	95	B2		mdf(A)_1
NEL0712_911986_2_F_B_91	911986		0	female	Blood	1991	95	B2		mdf(A)_1
NEL0713_912164_1_M_B_91	912164		0	male	Blood	1991	95	B2		mdf(A)_1
NEL0714_920167_1_M_C_92	920167		0	male	CSF	1992	95	B2		mdf(A)_1
NEL0715_920167_2_M_B_92	920167		0	male	Blood	1992	95	B2		mdf(A)_1
NEL0716_920607_1_M_C_92	920607		1	male	CSF	1992	10	A		mdf(A)_1
NEL0717_920607_2_M_B_92	920607		1	male	Blood	1992	10	A		mdf(A)_1
NEL0718_920710_1_M_C_92	920710		0	male	CSF	1992	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0719_920821_1_M_C_92	920821		2	male	CSF	1992	10	A		mdf(A)_1
NEL0720_920944_1_U_C_92	920944		0	unknown	CSF	1992	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0721_920944_2_U_B_92	920944		0	unknown	Blood	1992	390	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0722_921098_1_M_C_92	921098		1	male	CSF	1992	398	A		mdf(A)_1
NEL0723_921162_1_M_C_92	921162		0	male	CSF	1992	62	F		mdf(A)_1
NEL0724_921162_2_M_B_92	921162		0	male	Blood	1992	62	F		mdf(A)_1
NEL0725_921162_3_M_O_92	921162		0	male	ylic hema	1992	62	F		mdf(A)_1
NEL0726_921329_1_M_B_92	921329		0	male	Blood	1992	127	B2		mdf(A)_1
NEL0727_921495_1_M_B_92	921495		0	male	Blood	1992	62	F		mdf(A)_1
NEL0728_921500_1_F_B_92	921500		0	female	Blood	1992	95	B2		mdf(A)_1
NEL0729_921516_1_M_C_92	921516			male	CSF	1992	416	B2		mdf(A)_1
NEL0730_921516_2_M_B_92	921516			male	Blood	1992	416	B2		mdf(A)_1
NEL0731_921625_1_M_C_92	921625		0	male	CSF	1992	379	F	parE_I355T	blaTEM-1A_1, mdf(A)_1
NEL0732_921625_2_M_B_92	921625		0	male	Blood	1992	379	F	parE_I355T	blaTEM-1A_1, mdf(A)_1
NEL0733_921630_1_M_B_92	921630		0	male	Blood	1992	62	F		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0734_921699_1_M_C_92	921699			male	CSF	1992	504	B2		mdf(A)_1
NEL0735_921699_2_M_B_92	921699			male	Blood	1992	504	B2		mdf(A)_1
NEL0736_921721_1_U_B_92	921721		0	unknown	Blood	1992	357	B2		mdf(A)_1
NEL0737_921800_1_F_C_92	921800		11	female	CSF	1992	117	G		mdf(A)_1, tet(C)_2
NEL0738_921961_1_M_C_92	921961		0	male	CSF	1992	95	B2		mdf(A)_1
NEL0739_921961_2_M_B_92	921961		0	male	Blood	1992	95	B2		mdf(A)_1
NEL0740_922003_1_F_C_92	922003		1	female	CSF	1992	95	B2		mdf(A)_1
NEL0741_922003_2_F_B_92	922003		1	female	Blood	1992	95	B2		mdf(A)_1
NEL0742_922058_1_U_C_92	922058		0	unknown	CSF	1992	416	B2		mdf(A)_1
NEL0743_922058_2_U_B_92	922058		0	unknown	Blood	1992	416	B2		mdf(A)_1
NEL0744_922090_1_M_C_92	922090		0	male	CSF	1992	95	B2		mdf(A)_1, sul2_2
NEL0745_922090_2_M_B_92	922090		0	male	Blood	1992	95	B2		mdf(A)_1, sul2_2
NEL0746_922203_1_M_C_92	922203		0	male	CSF	1992	144	B2		mdf(A)_1
NEL0747_930085_1_U_C_93	930085		2	unknown	CSF	1993	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0748_930255_1_U_C_93	930255	71		unknown	CSF	1993	372	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0749_930267_1_U_B_93	930267		1	unknown	Blood	1993	62	F		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_8, mdf(A)_1, sul2_2, tet(A)_6
NEL0750_930313_1_M_C_93	930313	67		male	CSF	1993	10	A		aadA5_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA16_2, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL0751_930418_1_M_C_93	930418		2	male	CSF	1993	362	D		mdf(A)_1
NEL0752_930554_1_F_C_93	930554		16	female	CSF	1993	390	B2		blaTEM-1B_1, dfrA8_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0753_930983_1_M_C_93	930983		19	male	CSF	1993	416	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0754_931023_1_F_C_93	931023		1	female	CSF	1993	95	B2		mdf(A)_1
NEL0755_931150_1_F_B_93	931150			female	Blood	1993	372	B2		blaTEM-1A_1, mdf(A)_1
NEL0756_931224_1_M_B_93	931224			male	Blood	1993	95	B2		mdf(A)_1
NEL0759_931422_1_M_C_93	931422			male	CSF	1993	567	B2		mdf(A)_1
NEL0760_931422_2_M_B_93	931422			male	Blood	1993	567	B2		mdf(A)_1
NEL0761_931486_1_M_C_93	931486		1	male	CSF	1993	-	B2		mdf(A)_1
NEL0762_931486_2_M_B_93	931486		1	male	Blood	1993	-	B2		mdf(A)_1
NEL0763_931513_1_F_C_93	931513		2	female	CSF	1993	95	B2		mdf(A)_1
NEL0764_931513_2_F_B_93	931513		2	female	Blood	1993	95	B2		mdf(A)_1
NEL0765_931548_1_F_C_93	931548		0	female	CSF	1993	372	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0766_931548_2_F_B_93	931548		0	female	Blood	1993	372	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0767_931549_1_M_C_93	931549		0	male	CSF	1993	390	B2		mdf(A)_1
NEL0768_931549_2_M_B_93	931549		0	male	Blood	1993	390	B2		mdf(A)_1
NEL0769_931679_1_U_B_93	931679	66		unknown	Blood	1993	155	B1		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(B)_1
NEL0770_931754_1_M_C_93	931754		0	male	CSF	1993	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL0771_931819_1_F_C_93	931819	70		female	CSF	1993	-	D		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0772_931955_1_U_C_93	931955			unknown	CSF	1993	80	B2		mdf(A)_1
NEL0773_931955_2_U_B_93	931955			unknown	Blood	1993	80	B2		mdf(A)_1
NEL0774_932042_1_M_C_93	932042			male	CSF	1993	95	B2		mdf(A)_1
NEL0775_932042_2_M_B_93	932042			male	Blood	1993	95	B2		mdf(A)_1
NEL0776_940051_1_U_C_94	940051			unknown	CSF	1994	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL0777_940051_2_U_B_94	940051			unknown	Blood	1994	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL0778_940123_1_F_B_94	940123			female	Blood	1994	372	B2		mdf(A)_1, tet(A)_6
NEL0779_940133_1_U_C_94	940133		1	unknown	CSF	1994	95	B2		mdf(A)_1
NEL0780_940188_1_F_C_94	940188			female	CSF	1994	95	B2		mdf(A)_1
NEL0781_940198_1_U_B_94	940198			unknown	Blood	1994	95	B2		aac(3)-IVa_1, ant(3'')-Ia_1, aph(4)-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0782_940238_1_M_C_94	940238			male	CSF	1994	453	B1		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0783_940238_2_M_B_94	940238			male	Blood	1994	453	B1		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0784_940258_1_U_C_94	940258		1	unknown	CSF	1994	421	B2		mdf(A)_1
NEL0785_940270_1_M_B_94	940270		0	male	Blood	1994	420	B2	parE_D475E, gyrA_S83L	mdf(A)_1, tet(A)_6
NEL0786_940322_1_M_B_94	940322			male	Blood	1994	10	A		mdf(A)_1
NEL0787_940478_1_M_B_94	940478		0	male	Blood	1994	1858	B2		mdf(A)_1
NEL0788_940508_1_M_C_94	940508			male	CSF	1994	62	F		blaTEM-1C_1, mdf(A)_1
NEL0789_940508_2_M_B_94	940508			male	Blood	1994	62	F		blaTEM-1C_1, mdf(A)_1
NEL0790_940619_1_M_C_94	940619			male	CSF	1994	567	B2		mdf(A)_1
NEL0791_940619_2_M_B_94	940619			male	Blood	1994	567	B2		mdf(A)_1
NEL0792_940621_1_F_C_94	940621			female	CSF	1994	95	B2		mdf(A)_1
NEL0793_940621_2_F_B_94	940621			female	Blood	1994	95	B2		mdf(A)_1
NEL0794_940625_1_M_B_94	940625		0	male	Blood	1994	973	D		aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(B)_1
NEL0795_940703_1_M_C_94	940703		0	male	CSF	1994	73	B2		ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5
NEL0796_940768_1_M_C_94	940768			male	CSF	1994	144	B2		mdf(A)_1
NEL0797_940881_1_F_C_94	940881		0	female	CSF	1994	62	F		aph(6)-Id_1, blaTEM-1B_1, dfrA14_5, mdf(A)_1, sul2_2, tet(B)_2
NEL0798_940922_1_M_B_94	940922		0	male	Blood	1994	127	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL0799_941004_1_F_C_94	941004			female	CSF	1994	-	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0800_941068_1_M_C_94	941068			male	CSF	1994	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0801_941068_2_M_B_94	941068			male	Blood	1994	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0802_941243_1_M_B_94	941243		0	male	Blood	1994	357	B2		mdf(A)_1
NEL0803_941260_1_M_C_94	941260	19		male	CSF	1994	-	B2		mdf(A)_1
NEL0804_941293_1_M_C_94	941293			male	CSF	1994	1531	B2		mdf(A)_1
NEL0805_941410_1_U_C_94	941410		5	unknown	CSF	1994	73	B2		mdf(A)_1
NEL0806_941515_1_F_C_94	941515		0	female	CSF	1994	12	B2		blaTEM-1A_1, mdf(A)_1, tet(A)_6
NEL0807_941515_2_F_B_94	941515		0	female	Blood	1994	12	B2		blaTEM-1A_1, mdf(A)_1, tet(A)_6
NEL0808_941589_1_F_B_94	941589		0	female	Blood	1994	567	B2		aadA24_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_8, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0809_941609_1_U_C_94	941609		0	unknown	CSF	1994	3101	C		mdf(A)_1
NEL0810_941609_2_U_B_94	941609		0	unknown	Blood	1994	3101	C		mdf(A)_1
NEL0811_941834_1_F_C_94	941834	68		female	CSF	1994	14	B2		mdf(A)_1
NEL0812_941834_2_F_B_94	941834	68		female	Blood	1994	14	B2		mdf(A)_1
NEL0813_941836_1_M_B_94	941836		0	male	Blood	1994	95	B2		mdf(A)_1
NEL0814_950094_1_M_B_95	950094		0	male	Blood	1995	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL0815_950108_1_U_C_95	950108		0	unknown	CSF	1995	978	B2		mdf(A)_1
NEL0816_950255_1_M_B_95	950255		0	male	Blood	1995	10	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL0817_950322_1_F_B_95	950322		0	female	Blood	1995	349	D		ant(3'')-Ia_1, blaOXA-1_1, mdf(A)_1, sul1_5
NEL0818_951154_1_M_B_95	951154		0	male	Blood	1995	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0819_951155_1_M_C_95	951155		1	male	CSF	1995	95	B2		mdf(A)_1
NEL0820_951209_1_M_B_95	951209		0	male	Blood	1995	73	B2		ant(3'')-Ia_1, blaSHV-102_1, mdf(A)_1, sul1_5
NEL0821_951510_1_M_C_95	951510		0	male	CSF	1995	131	B2	parE_I529L	mdf(A)_1
NEL0822_951510_2_M_B_95	951510		0	male	Blood	1995	131	B2	parE_I529L	mdf(A)_1
NEL0823_951533_1_F_B_95	951533		0	female	Blood	1995	567	B2		mdf(A)_1
NEL0824_951681_1_M_B_95	951681		0	male	Blood	1995	73	B2		mdf(A)_1
NEL0825_951740_1_M_C_95	951740	21		male	CSF	1995	968	B2		mdf(A)_1
NEL0826_960174_1_F_O_96	960174		0	female	eye	1996	10	A		mdf(A)_1
NEL0827_960237_1_U_C_96	960237	38		unknown	CSF	1996	73	B2		mdf(A)_1
NEL0828_960286_1_M_C_96	960286		0	male	CSF	1996	95	B2		mdf(A)_1
NEL0829_960286_2_M_B_96	960286		0	male	Blood	1996	95	B2		mdf(A)_1
NEL0830_960551_1_M_C_96	960551	1		male	CSF	1996	95	B2		mdf(A)_1
NEL0831_960618_1_M_C_96	960618		0	male	CSF	1996	95	B2		mdf(A)_1
NEL0832_960618_2_M_B_96	960618		0	male	Blood	1996	95	B2		mdf(A)_1
NEL0833_960642_1_F_B_96	960642		0	female	Blood	1996	95	B2	gyrA_S83L	mdf(A)_1
NEL0834_960679_1_F_C_96	960679		0	female	CSF	1996	567	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0835_960683_1_M_C_96	960683		2	male	CSF	1996	59	F	parE_I355T	mdf(A)_1
NEL0836_960727_1_M_B_96	960727		1	male	Blood	1996	88	C		ant(3'')-Ia_1, blaTEM-1C_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL0837_960747_1_M_C_96	960747		2	male	CSF	1996	59	F	parE_I355T	mdf(A)_1
NEL0839_960771_2_F_B_96	960771		1	female	Blood	1996	127	B2		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, catA1_1, mdf(A)_1, sul1_5, tet(E)_2
NEL0840_960901_1_M_C_96	960901	79		male	CSF	1996	372	B2		mdf(A)_1, tet(A)_6
NEL0841_960997_1_F_C_96	960997		2	female	CSF	1996	10	A		blaTEM-1A_1, mdf(A)_1
NEL0842_960997_2_F_B_96	960997		2	female	Blood	1996	10	A		blaTEM-1A_1, mdf(A)_1
NEL0843_961026_1_F_C_96	961026	57		female	CSF	1996	88	C		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0844_961140_1_U_C_96	961140		1	unknown	CSF	1996	405	D		mdf(A)_1
NEL0845_961448_1_F_C_96	961448		0	female	CSF	1996	390	B2		mdf(A)_1
NEL0846_961499_1_F_C_96	961499		0	female	CSF	1996	88	C		ant(3'')-Ia_1, blaTEM-1A_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL0847_961499_2_F_B_96	961499		0	female	Blood	1996	88	C		ant(3'')-Ia_1, blaTEM-1A_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL0848_961551_1_F_B_96	961551	75		female	Blood	1996	393	D	parC_S57T	mdf(A)_1
NEL0849_961564_1_M_C_96	961564		0	male	CSF	1996	538	B2	parE_D475E	mdf(A)_1
NEL0850_961573_1_M_C_96	961573		1	male	CSF	1996	10	A		mdf(A)_1
NEL0851_961597_1_F_C_96	961597	68		female	CSF	1996	73	B2		mdf(A)_1
NEL0852_961597_2_F_B_96	961597	68		female	Blood	1996	73	B2		mdf(A)_1
NEL0853_961604_1_U_B_96	961604		0	unknown	Blood	1996	75	B1		fosA7_1, mdf(A)_1
NEL0854_961771_1_F_C_96	961771	53		female	CSF	1996	73	B2		blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL0855_961799_1_F_C_96	961799		0	female	CSF	1996	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0856_961799_2_F_B_96	961799		0	female	Blood	1996	95	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL0857_961805_1_M_C_96	961805		0	male	CSF	1996	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0858_961805_2_M_B_96	961805		0	male	Blood	1996	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0859_961854_1_F_C_96	961854	83		female	CSF	1996	69	D		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(B)_2
NEL0860_961984_1_M_C_96	961984		0	male	CSF	1996	416	B2		mdf(A)_1
NEL0861_961984_2_M_B_96	961984		0	male	Blood	1996	416	B2		mdf(A)_1
NEL0862_962124_1_M_C_96	962124		1	male	CSF	1996	416	B2		mdf(A)_1
NEL0863_962137_1_M_B_96	962137		0	male	Blood	1996	95	B2		mdf(A)_1
NEL0864_970136_1_M_C_97	970136	64		male	CSF	1997	69	D	parC_S57T	blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL0865_970207_1_M_C_97	970207		0	male	CSF	1997	95	B2		mdf(A)_1
NEL0866_970207_2_M_B_97	970207		0	male	Blood	1997	95	B2		mdf(A)_1
NEL0867_970306_1_M_C_97	970306		1	male	CSF	1997	95	B2		blaTEM-1B_1, catA1_1, mdf(A)_1, tet(B)_2
NEL0868_970348_1_M_C_97	970348		0	male	CSF	1997	95	B2		mdf(A)_1
NEL0869_970349_1_F_C_97	970349		0	female	CSF	1997	421	B2		mdf(A)_1
NEL0870_970349_2_F_B_97	970349		0	female	Blood	1997	421	B2		mdf(A)_1
NEL0871_970508_1_M_B_97	970508		1	male	Blood	1997	390	B2		mdf(A)_1
NEL0872_970533_1_M_B_97	970533		0	male	Blood	1997	59	F	parE_I355T	ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL0873_970773_1_M_B_97	970773		0	male	Blood	1997	62	F		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_2
NEL0874_970879_1_M_C_97	970879		0	male	CSF	1997	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0875_970879_2_M_B_97	970879		0	male	Blood	1997	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2
NEL0876_970923_1_F_C_97	970923	75		female	CSF	1997	14	B2		ant(3'')-Ia_1, blaSHV-1_1, dfrA1_8, mdf(A)_1, sul1_5, tet(D)_1
NEL0877_970923_2_F_B_97	970923	75		female	Blood	1997	14	B2		ant(3'')-Ia_1, blaSHV-1_1, dfrA1_8, mdf(A)_1, sul1_5, tet(D)_1
NEL0878_971123_1_M_B_97	971123		0	male	Blood	1997	372	B2		mdf(A)_1, tet(A)_6
NEL0879_971147_1_F_B_97	971147		0	female	Blood	1997	73	B2		mdf(A)_1
NEL0880_971185_1_M_C_97	971185		0	male	CSF	1997	95	B2		mdf(A)_1
NEL0881_971185_2_M_B_97	971185		0	male	Blood	1997	95	B2		mdf(A)_1
NEL0882_971187_1_M_C_97	971187	63		male	CSF	1997	73	B2		mdf(A)_1
NEL0883_971268_1_F_C_97	971268		0	female	CSF	1997	390	B2		mdf(A)_1
NEL0884_971268_2_F_B_97	971268		0	female	Blood	1997	390	B2		mdf(A)_1
NEL0885_971315_1_F_C_97	971315		1	female	CSF	1997	95	B2		mdf(A)_1
NEL0886_971315_2_F_B_97	971315		1	female	Blood	1997	95	B2		mdf(A)_1
NEL0887_971351_1_M_C_97	971351		0	male	CSF	1997	62	F		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0888_971372_1_U_C_97	971372		2	unknown	CSF	1997	91	B2		mdf(A)_1
NEL0889_971384_1_M_C_97	971384		1	male	CSF	1997	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0890_971384_2_M_B_97	971384		1	male	Blood	1997	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1
NEL0891_971421_1_M_B_97	971421		0	male	Blood	1997	91	B2		mdf(A)_1
NEL0892_971422_1_F_C_97	971422		1	female	CSF	1997	390	B2		mdf(A)_1
NEL0893_971437_1_F_C_97	971437		0	female	CSF	1997	7092	B2		mdf(A)_1
NEL0894_971444_1_U_B_97	971444		0	unknown	Blood	1997	357	B2		mdf(A)_1
NEL0895_971478_1_F_C_97	971478		0	female	CSF	1997	95	B2		mdf(A)_1
NEL0896_971478_2_F_B_97	971478		0	female	Blood	1997	95	B2		mdf(A)_1
NEL0897_971494_1_F_B_97	971494		0	female	Blood	1997	640	B2		mdf(A)_1
NEL0898_971520_1_M_C_97	971520		2	male	CSF	1997	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0899_971527_1_F_C_97	971527		0	female	CSF	1997	567	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL0900_971537_1_M_C_97	971537		0	male	CSF	1997	216	A		mdf(A)_1
NEL0901_971579_1_M_C_97	971579	75		male	CSF	1997	141	B2		mdf(A)_1
NEL0902_971633_1_M_C_97	971633		0	male	CSF	1997	10	A		aph(3'')-Ib_5, aph(6)-Id_1, blaOXA-2_1, blaTEM-1B_1, catA1_1, dfrA29_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0903_971633_2_M_B_97	971633		0	male	Blood	1997	10	A		aph(3'')-Ib_5, aph(6)-Id_1, blaOXA-2_1, blaTEM-1B_1, catA1_1, dfrA29_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0904_971634_1_M_B_97	971634		0	male	Blood	1997	10	A		aph(3'')-Ib_5, aph(6)-Id_1, blaOXA-2_1, blaTEM-1B_1, catA1_1, dfrA29_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL0905_971731_1_M_C_97	971731	51		male	CSF	1997	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA8_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0906_971759_1_M_C_97	971759		1	male	CSF	1997	6807	A		mdf(A)_1
NEL0907_971876_1_F_C_97	971876		0	female	CSF	1997	95	B2		mdf(A)_1
NEL0908_971876_2_F_B_97	971876		0	female	Blood	1997	95	B2		mdf(A)_1
NEL0909_971882_1_F_C_97	971882		2	female	CSF	1997	135	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0910_971946_1_F_B_97	971946		0	female	Blood	1997	73	B2		mdf(A)_1
NEL0911_972005_1_M_C_97	972005			male	CSF	1997	80	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0912_972006_1_M_B_97	972006			male	Blood	1997	73	B2		mdf(A)_1
NEL0913_980075_1_F_C_98	980075	79		female	CSF	1998	88	C		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL0914_980363_1_F_B_98	980363		0	female	Blood	1998	7092	B2		mdf(A)_1
NEL0915_980848_1_F_C_98	980848		0	female	CSF	1998	95	B2		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL0916_980862_1_M_C_98	980862		0	male	CSF	1998	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0917_980862_2_M_B_98	980862		0	male	Blood	1998	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL0918_980965_1_M_C_98	980965		0	male	CSF	1998	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0919_981008_1_M_C_98	981008	77		male	CSF	1998	567	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2, tet(B)_2
NEL0920_981149_1_M_C_98	981149	72		male	CSF	1998	23	C		mdf(A)_1
NEL0921_981149_2_M_B_98	981149	72		male	Blood	1998	23	C		mdf(A)_1
NEL0922_981203_1_F_B_98	981203		0	female	Blood	1998	23	C		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL0923_981221_1_F_C_98	981221		0	female	CSF	1998	1807	C		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL0924_981304_1_M_C_98	981304		0	male	CSF	1998	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0925_981586_1_M_B_98	981586		0	male	Blood	1998	10	A		aph(6)-Id_1, blaTEM-1D_1, dfrA14_5, mdf(A)_1, sul2_2
NEL0926_981597_1_F_C_98	981597		0	female	CSF	1998	416	B2		mdf(A)_1
NEL0927_981597_2_F_B_98	981597		0	female	Blood	1998	416	B2		mdf(A)_1
NEL0928_981679_1_M_B_98	981679		0	male	Blood	1998	95	B2		mdf(A)_1
NEL0929_981709_1_F_C_98	981709		0	female	CSF	1998	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0930_981709_2_F_B_98	981709		0	female	Blood	1998	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL0931_981744_1_F_B_98	981744		0	female	Blood	1998	69	D		mdf(A)_1
NEL0932_981798_1_U_B_98	981798		0	unknown	Blood	1998	648	F		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6, tet(B)_2
NEL0933_981804_1_M_C_98	981804	70		male	CSF	1998	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL0934_981844_1_F_C_98	981844	61		female	CSF	1998	69	D		mdf(A)_1
NEL0935_981844_2_F_B_98	981844	61		female	Blood	1998	69	D		mdf(A)_1
NEL0936_981907_1_M_B_98	981907		0	male	Blood	1998	69	D		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, tet(A)_6
NEL0938_982051_1_U_B_98	982051		1	unknown	Blood	1998	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA8_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0939_982177_1_F_C_98	982177	76		female	CSF	1998	405	D	parC_S80I, gyrA_D87N, gyrA_S83L	aac(3)-IId_1, aadA5_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul2_2, tet(B)_2
NEL0940_982177_2_F_B_98	982177	76		female	Blood	1998	405	D	parC_S80I, gyrA_D87N, gyrA_S83L	aac(3)-IId_1, aadA5_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul2_2, tet(B)_2
NEL0941_982228_1_M_B_98	982228		0	male	Blood	1998	95	B2		mdf(A)_1
NEL0942_982367_1_M_C_98	982367		0	male	CSF	1998	62	F		mdf(A)_1
NEL0943_990112_1_F_C_99	990112	83		female	CSF	1999	58	B1		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(B)_2
NEL0944_990113_1_M_C_99	990113		0	male	CSF	1999	88	C		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(B)_1
NEL0945_990113_2_M_B_99	990113		0	male	Blood	1999	88	C		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(B)_1
NEL0946_990213_1_U_C_99	990213		2	unknown	CSF	1999	567	B2		mdf(A)_1
NEL0947_990213_2_U_B_99	990213		2	unknown	Blood	1999	567	B2		mdf(A)_1
NEL0948_990213_3_U_O_99	990213		2	unknown	throat	1999	567	B2		mdf(A)_1
NEL0949_990430_1_F_C_99	990430		0	female	CSF	1999	144	B2		mdf(A)_1
NEL0950_990484_1_F_B_99	990484		2	female	Blood	1999	14	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5
NEL0951_990490_1_F_C_99	990490	30		female	CSF	1999	38	D		mdf(A)_1
NEL0952_990609_1_F_B_99	990609		0	female	Blood	1999	-	B2		mdf(A)_1
NEL0953_990610_1_M_C_99	990610	65		male	CSF	1999	38	D		aph(3')-Ia_1, dfrA5_1, mdf(A)_1, tet(A)_6
NEL0954_990610_2_M_B_99	990610	65		male	Blood	1999	38	D		aph(3')-Ia_1, dfrA5_1, mdf(A)_1, tet(A)_6
NEL0955_990885_1_M_C_99	990885		0	male	CSF	1999	390	B2		mdf(A)_1
NEL0956_990885_2_M_B_99	990885		0	male	Blood	1999	390	B2		mdf(A)_1
NEL0957_990914_1_M_B_99	990914		0	male	Blood	1999	95	B2		mdf(A)_1
NEL0958_991000_1_M_B_99	991000		0	male	Blood	1999	-	A		mdf(A)_1
NEL0959_991001_1_M_C_99	991001		0	male	CSF	1999	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0960_991001_2_M_B_99	991001		0	male	Blood	1999	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL0961_991122_1_F_B_99	991122		0	female	Blood	1999	95	B2		mdf(A)_1
NEL0962_991134_1_F_B_99	991134		0	female	Blood	1999	567	B2		mdf(A)_1
NEL0963_991325_1_F_C_99	991325		5	female	CSF	1999	62	F		mdf(A)_1
NEL0964_991325_2_F_B_99	991325		5	female	Blood	1999	62	F		mdf(A)_1
NEL0965_991423_1_M_C_99	991423		0	male	CSF	1999	10	A		mdf(A)_1
NEL0966_991578_1_F_B_99	991578		0	female	Blood	1999	131	B2	parE_I529L	aph(3')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL0967_991613_1_M_C_99	991613		0	male	CSF	1999	131	B2	parE_I529L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA8_1, mdf(A)_1, sul2_3, tet(A)_6, tet(B)_2
NEL0968_991719_1_M_B_99	991719		0	male	Blood	1999	10	A		blaTEM-1A_1, mdf(A)_1
NEL0969_992097_1_M_C_99	992097		0	male	CSF	1999	95	B2		mdf(A)_1
NEL0970_992097_2_M_B_99	992097		0	male	Blood	1999	95	B2		mdf(A)_1
NEL0971_992143_1_F_C_99	992143	52		female	CSF	1999	57	E		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL0972_2000051_1_F_C_00	2000051		1	female	CSF	2000	538	B2	parE_D475E	blaTEM-1A_1, mdf(A)_1
NEL0973_2000066_1_F_C_00	2000066		0	female	CSF	2000	73	B2		ant(3'')-Ia_1, aph(3')-Ia_1, blaOXA-1_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_5
NEL0974_2000084_1_M_C_00	2000084		1	male	CSF	2000	372	B2		mdf(A)_1
NEL0975_2000204_1_F_C_00	2000204		0	female	CSF	2000	95	B2		mdf(A)_1
NEL0976_2000204_2_F_B_00	2000204		0	female	Blood	2000	95	B2		mdf(A)_1
NEL0979_2000417_1_M_B_00	2000417		0	male	Blood	2000	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2, tet(A)_6
NEL0980_2000461_1_M_B_00	2000461		0	male	Blood	2000	73	B2		mdf(A)_1
NEL0981_2000580_1_F_C_00	2000580		0	female	CSF	2000	95	B2		mdf(A)_1
NEL0983_2001041_1_M_B_00	2001041		0	male	Blood	2000	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL0984_2001061_1_M_C_00	2001061		1	male	CSF	2000	95	B2		mdf(A)_1
NEL0985_2001061_2_M_B_00	2001061		1	male	Blood	2000	95	B2		mdf(A)_1
NEL0986_2001146_1_M_B_00	2001146		0	male	Blood	2000	95	B2		mdf(A)_1
NEL0987_2001225_1_F_C_00	2001225		0	female	CSF	2000	95	B2		blaTEM-1B_1, mdf(A)_1
NEL0988_2001230_1_M_C_00	2001230		1	male	CSF	2000	59	F	parE_I355T	mdf(A)_1
NEL0989_2001231_1_F_C_00	2001231	35		female	CSF	2000	135	B2		mdf(A)_1
NEL0990_2001402_1_U_C_00	2001402	1		unknown	CSF	2000	74	B2		mdf(A)_1
NEL0991_2001568_1_M_C_00	2001568		1	male	CSF	2000	550	B2		mdf(A)_1
NEL0992_2001611_1_F_C_00	2001611	80		female	CSF	2000	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA17_1, mdf(A)_1, sul1_5, sul2_3
NEL0993_2001611_2_F_B_00	2001611	80		female	Blood	2000	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA17_1, mdf(A)_1, sul1_5, sul2_3
NEL0994_2001636_1_M_B_00	2001636		0	male	Blood	2000	569	B2	parE_D475E	mdf(A)_1
NEL0995_2001638_1_F_B_00	2001638		0	female	Blood	2000	59	F	parE_I355T	mdf(A)_1
NEL0996_2001654_1_U_C_00	2001654		0	unknown	CSF	2000	567	B2		mdf(A)_1
NEL0997_2001698_1_M_B_00	2001698			male	blood	2000	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1000_2010376_1_F_C_01	2010376			female	CSF	2001	95	B2		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1001_2010376_2_F_B_01	2010376			female	blood	2001	95	B2		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1002_2010393_1_U_B_01	2010393			unknown	blood	2001	73	B2		mdf(A)_1
NEL1003_2010420_1_M_B_01	2010420			male	blood	2001	95	B2		mdf(A)_1
NEL1004_2010471_1_M_B_01	2010471			male	blood	2001	95	B2		mdf(A)_1
NEL1005_2010477_1_F_C_01	2010477			female	CSF	2001	95	B2		ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5
NEL1006_2010536_1_M_C_01	2010536			male	CSF	2001	390	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1007_2010539_1_F_B_01	2010539			female	blood	2001	59	F	parE_I355T	mdf(A)_1
NEL1008_2010613_1_F_B_01	2010613	63		female	blood	2001	23	C		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1009_2010713_1_M_B_01	2010713			male	blood	2001	453	B1		mdf(A)_1, tet(B)_2
NEL1010_2011073_1_F_B_01	2011073			female	blood	2001	1308	B1		mdf(A)_1
NEL1011_2011089_1_M_C_01	2011089			male	CSF	2001	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1012_2011089_2_M_B_01	2011089			male	blood	2001	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1013_2011291_1_F_B_01	2011291			female	blood	2001	10	A		mdf(A)_1
NEL1014_2011303_1_F_B_01	2011303			female	blood	2001	362	D		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1015_2011348_1_F_B_01	2011348			female	blood	2001	52	A		mdf(A)_1
NEL1016_2011424_1_F_B_01	2011424			female	blood	2001	567	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL1017_2011484_1_M_B_01	2011484			male	blood	2001	127	B2		mdf(A)_1
NEL1018_2011500_1_M_C_01	2011500			male	CSF	2001	3727	cladeV	parC_S57T, parE_I355T	blaTEM-1B_1, mdf(A)_1
NEL1019_2011561_1_M_B_01	2011561			male	blood	2001	567	B2		mdf(A)_1
NEL1020_2011590_1_M_B_01	2011590			male	blood	2001	95	B2		mdf(A)_1
NEL1021_2011626_1_M_B_01	2011626	41		male	blood	2001	636	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_3, tet(B)_2
NEL1022_2011668_1_F_B_01	2011668			female	blood	2001	127	B2		mdf(A)_1
NEL1023_2011701_1_M_C_01	2011701	70		male	CSF	2001	405	D		mdf(A)_1, tet(B)_2
NEL1024_2011812_1_M_B_01	2011812			male	blood	2001	93	A	parE_I355T	mdf(A)_1
NEL1025_2011918_1_U_C_01	2011918			unknown	CSF	2001	390	B2		mdf(A)_1
NEL1026_2011952_1_F_C_01	2011952			female	CSF	2001	567	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1027_2012165_1_M_C_01	2012165			male	CSF	2001	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1028_2012186_1_M_B_01	2012186			male	blood	2001	12	B2		mdf(A)_1
NEL1029_2012288_1_F_B_01	2012288			female	blood	2001	95	B2		mdf(A)_1
NEL1030_2012295_1_M_B_01	2012295			male	blood	2001	453	B1		mdf(A)_1, tet(B)_2
NEL1031_2012296_1_M_B_01	2012296			male	blood	2001	59	F	parE_I355T	mdf(A)_1
NEL1032_2012320_1_M_B_01	2012320			male	blood	2001	95	B2		mdf(A)_1
NEL1033_2012333_1_M_B_01	2012333			male	blood	2001	95	B2		mdf(A)_1
NEL1034_2012368_1_F_C_01	2012368	53		female	CSF	2001	393	D	parC_S57T, parC_S80I, gyrA_D87N, gyrA_S83L	mdf(A)_1
NEL1035_2012368_2_F_B_01	2012368	53		female	blood	2001	393	D	parC_S57T, parC_S80I, gyrA_D87N, gyrA_S83L	mdf(A)_1
NEL1036_2020162_1_F_C_02	2020162			female	CSF	2002	62	F		mdf(A)_1
NEL1037_2020162_2_F_B_02	2020162			female	blood	2002	62	F		mdf(A)_1
NEL1038_2020173_1_M_B_02	2020173			male	blood	2002	80	B2		mdf(A)_1
NEL1039_2020176_1_F_B_02	2020176			female	blood	2002	95	B2		aph(3')-Ia_1, dfrA5_1, mdf(A)_1, tet(A)_6
NEL1040_2020290_1_M_C_02	2020290			male	CSF	2002	95	B2		mdf(A)_1
NEL1041_2020290_2_M_B_02	2020290			male	blood	2002	95	B2		mdf(A)_1
NEL1042_2020493_1_F_C_02	2020493	53		female	CSF	2002	550	B2		mdf(A)_1
NEL1043_2020624_1_M_C_02	2020624			male	CSF	2002	10	A		mdf(A)_1
NEL1044_2020651_1_F_C_02	2020651			female	CSF	2002	567	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1045_2020651_2_F_B_02	2020651			female	blood	2002	567	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1046_2020682_1_M_B_02	2020682			male	blood	2002	543	E		mdf(A)_1
NEL1047_2020889_1_U_B_02	2020889			unknown	blood	2002	4040	D		mdf(A)_1
NEL1048_2021099_1_M_C_02	2021099			male	CSF	2002	12	B2		mdf(A)_1
NEL1049_2021099_2_M_B_02	2021099			male	blood	2002	12	B2		mdf(A)_1
NEL1050_2021412_1_M_C_02	2021412			male	CSF	2002	73	B2		mdf(A)_1
NEL1051_2021412_2_M_B_02	2021412			male	blood	2002	73	B2		mdf(A)_1
NEL1052_2021557_1_M_C_02	2021557			male	CSF	2002	567	B2		aadA2_1, ant(3'')-Ia_1, aph(3')-Ia_1, catA1_1, cmlA1_1, dfrA1_10, mdf(A)_1, tet(B)_2
NEL1053_2021557_2_M_B_02	2021557			male	blood	2002	567	B2		aadA2_1, ant(3'')-Ia_1, aph(3')-Ia_1, catA1_1, cmlA1_1, dfrA1_10, mdf(A)_1, tet(B)_2
NEL1054_2021653_1_F_C_02	2021653			female	CSF	2002	401	A		mdf(A)_1
NEL1055_2021662_1_M_C_02	2021662			male	CSF	2002	59	F	parE_I355T	mdf(A)_1
NEL1056_2021677_1_M_C_02	2021677			male	CSF	2002	88	C		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(A)_6
NEL1057_2021762_1_M_B_02	2021762			male	blood	2002	131	B2	parE_I529L	mdf(A)_1
NEL1058_2021800_1_M_C_02	2021800			male	CSF	2002	567	B2		mdf(A)_1
NEL1059_2021800_2_M_B_02	2021800			male	blood	2002	567	B2		mdf(A)_1
NEL1060_2021868_1_F_C_02	2021868			female	CSF	2002	62	F		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2, tet(B)_2
NEL1061_2022022_1_M_C_02	2022022			male	CSF	2002	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1062_2022022_2_M_B_02	2022022			male	blood	2002	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1063_2022264_1_F_B_02	2022264			female	blood	2002	73	B2	parC_S57T	mdf(A)_1
NEL1064_2022286_1_F_C_02	2022286			female	CSF	2002	-	B1		mdf(A)_1
NEL1065_2022286_2_F_B_02	2022286			female	blood	2002	-	B1		mdf(A)_1
NEL1066_2022320_1_M_B_02	2022320			male	blood	2002	140	B2		mdf(A)_1
NEL1067_2022351_1_M_B_02	2022351			male	blood	2002	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(B)_2
NEL1068_2022403_1_F_C_02	2022403			female	CSF	2002	95	B2		mdf(A)_1
NEL1069_2022403_2_F_B_02	2022403			female	blood	2002	95	B2		mdf(A)_1
NEL1070_2030268_1_F_C_03	2030268			female	CSF	2003	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL1071_2030268_2_F_B_03	2030268			female	blood	2003	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL1072_2030269_1_M_B_03	2030269			male	blood	2003	1123	B1		mdf(A)_1
NEL1073_2030269_2_M_O_03	2030269			male	tissue	2003	1123	B1		mdf(A)_1
NEL1074_2030294_1_M_C_03	2030294	66		male	CSF	2003	14	B2		mdf(A)_1
NEL1075_2030420_1_M_B_03	2030420			male	blood	2003	73	B2		mdf(A)_1
NEL1076_2030544_1_F_C_03	2030544	2		female	CSF	2003	973	D		mdf(A)_1
NEL1077_2030567_1_F_B_03	2030567			female	blood	2003	95	B2		mdf(A)_1
NEL1078_2030631_1_M_C_03	2030631			male	CSF	2003	59	F	parE_I355T	mdf(A)_1, tet(A)_6
NEL1079_2030673_1_F_B_03	2030673			female	blood	2003	369	C		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL1080_2030973_1_M_C_03	2030973			male	CSF	2003	62	F		mdf(A)_1
NEL1081_2030999_1_F_B_03	2030999			female	blood	2003	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1082_2031278_1_M_C_03	2031278			male	CSF	2003	95	B2		mdf(A)_1
NEL1083_2031278_2_M_B_03	2031278			male	blood	2003	95	B2		mdf(A)_1
NEL1084_2031308_1_F_C_03	2031308			female	CSF	2003	95	B2		mdf(A)_1
NEL1085_2031672_1_M_C_03	2031672			male	CSF	2003	95	B2		mdf(A)_1
NEL1086_2031672_2_M_B_03	2031672			male	blood	2003	95	B2		mdf(A)_1
NEL1087_2031844_1_M_C_03	2031844			male	CSF	2003	357	B2		mdf(A)_1
NEL1088_2031844_2_M_B_03	2031844			male	blood	2003	357	B2		mdf(A)_1
NEL1089_2031850_1_M_C_03	2031850			male	CSF	2003	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1090_2031850_2_M_B_03	2031850			male	blood	2003	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1091_2031872_1_M_C_03	2031872			male	CSF	2003	428	B2		mdf(A)_1
NEL1092_2031887_1_F_C_03	2031887			female	CSF	2003	59	F	parE_I355T	mdf(A)_1
NEL1093_2031928_1_F_C_03	2031928			female	CSF	2003	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1094_2031928_2_F_B_03	2031928			female	blood	2003	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1095_2031933_1_M_B_03	2031933			male	blood	2003	416	B2		mdf(A)_1
NEL1096_2031997_1_F_C_03	2031997	35		female	CSF	2003	34	A	gyrA_S83L	ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul2_2, tet(A)_6
NEL1097_2032333_1_M_B_03	2032333			male	blood	2003	73	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL1098_2032491_1_F_C_03	2032491			female	CSF	2003	567	B2		ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1
NEL1099_2032491_2_F_B_03	2032491			female	blood	2003	567	B2		ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1100_2032677_1_M_B_03	2032677			male	blood	2003	141	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(B)_2
NEL1101_2040381_1_F_C_04	2040381			female	CSF	2004	416	B2		mdf(A)_1
NEL1102_2040381_2_F_B_04	2040381			female	blood	2004	416	B2		mdf(A)_1
NEL1103_2040701_1_F_C_04	2040701	78		female	CSF	2004	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL1104_2040701_2_F_B_04	2040701	78		female	blood	2004	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL1105_2040705_1_M_C_04	2040705	58		male	CSF	2004	538	B2	parE_D475E	mdf(A)_1
NEL1106_2040935_1_F_O_04	2040935	31		female	ear	2004	69	D		aadA5_1, catA1_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, tet(B)_2
NEL1107_2040951_1_M_C_04	2040951	62		male	CSF	2004	567	B2		aph(3'')-Ib_5, mdf(A)_1, sul2_2
NEL1108_2041158_1_F_C_04	2041158			female	CSF	2004	567	B2		mdf(A)_1
NEL1109_2041158_2_F_B_04	2041158			female	blood	2004	567	B2		mdf(A)_1
NEL1110_2041180_1_F_C_04	2041180			female	CSF	2004	131	B2	parC_E84V, parC_S80I, parE_I529L, gyrA_D87N, gyrA_S83L	aac(6')-Ib-cr_1, blaOXA-1_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1111_2041180_2_F_B_04	2041180			female	blood	2004	131	B2	parC_E84V, parC_S80I, parE_I529L, gyrA_D87N, gyrA_S83L	aac(6')-Ib-cr_1, blaOXA-1_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1112_2041271_1_F_C_04	2041271			female	CSF	2004	357	B2		mdf(A)_1
NEL1113_2041342_1_F_C_04	2041342			female	CSF	2004	357	B2		mdf(A)_1
NEL1114_2041399_1_M_B_04	2041399			male	blood	2004	357	B2		mdf(A)_1
NEL1115_2041551_1_M_C_04	2041551			male	CSF	2004	73	B2		ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1, sul1_5
NEL1116_2041575_1_M_B_04	2041575			male	blood	2004	141	B2		mdf(A)_1
NEL1117_2041659_1_F_B_04	2041659			female	blood	2004	681	B2	parE_D475E	blaTEM-1B_1, mdf(A)_1
NEL1118_2050086_1_F_C_05	2050086	83		female	CSF	2005	404	B2		blaTEM-30_1, mdf(A)_1, sul2_2
NEL1119_2050102_1_M_B_05	2050102			male	blood	2005	95	B2		dfrA5_1, mdf(A)_1, tet(A)_6
NEL1120_2050193_1_M_B_05	2050193			male	blood	2005	93	A		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA8_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1121_2050198_1_M_C_05	2050198			male	CSF	2005	73	B2		blaTEM-1A_1, mdf(A)_1
NEL1122_2050211_1_M_C_05	2050211	70		male	CSF	2005	101	B1		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1123_2050211_2_M_B_05	2050211	70		male	blood	2005	101	B1		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1124_2050353_1_F_C_05	2050353			female	CSF	2005	357	B2		mdf(A)_1
NEL1125_2050354_1_F_B_05	2050354			female	blood	2005	95	B2		mdf(A)_1
NEL1126_2050355_1_F_B_05	2050355			female	blood	2005	421	B2		mdf(A)_1
NEL1127_2050444_1_M_C_05	2050444			male	CSF	2005	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1128_2050444_2_M_B_05	2050444			male	blood	2005	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1129_2050469_1_M_O_05	2050469			male	urine	2005	95	B2		mdf(A)_1
NEL1130_2050486_1_F_C_05	2050486			female	CSF	2005	372	B2		mdf(A)_1
NEL1131_2050496_1_F_O_05	2050496			female	ear	2005	127	B2		blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1132_2050496_2_F_C_05	2050496			female	CSF	2005	59	F	parE_I355T	aph(6)-Id_1, blaTEM-1C_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1133_2050496_3_F_B_05	2050496			female	blood	2005	59	F	parE_I355T	aph(6)-Id_1, blaTEM-1C_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1134_2050637_1_M_C_05	2050637			male	CSF	2005	12	B2		mdf(A)_1
NEL1135_2050652_1_M_B_05	2050652			male	blood	2005	567	B2		mdf(A)_1
NEL1136_2050692_1_F_C_05	2050692	68		female	CSF	2005	448	B1	parC_S80I, parE_S458T, gyrA_D87N, gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1137_2050692_2_F_B_05	2050692	68		female	blood	2005	448	B1	parC_S80I, parE_S458T, gyrA_D87N, gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1138_2051042_1_M_B_05	2051042			male	blood	2005	-	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(B)_2
NEL1139_2051059_1_M_B_05	2051059			male	blood	2005	91	B2		mdf(A)_1
NEL1140_2051145_1_M_B_05	2051145			male	blood	2005	547	B2		mdf(A)_1
NEL1141_2051179_1_M_B_05	2051179			male	blood	2005	538	B2	parE_D475E	mdf(A)_1
NEL1142_2051207_1_F_B_05	2051207			female	blood	2005	144	B2		mdf(A)_1
NEL1143_2051217_1_M_B_05	2051217			male	blood	2005	131	B2	parE_I529L	mdf(A)_1
NEL1144_2051551_1_F_B_05	2051551			female	blood	2005	95	B2		mdf(A)_1
NEL1145_2051569_1_M_C_05	2051569	58		male	CSF	2005	117	G	gyrA_S83L	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1146_2051569_2_M_B_05	2051569	58		male	blood	2005	117	G	gyrA_S83L	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1147_2051675_1_F_C_05	2051675			female	CSF	2005	140	B2	gyrA_S83L	aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(B)_2
NEL1148_2051705_1_M_B_05	2051705			male	blood	2005	491	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL1149_2060157_1_M_B_06	2060157			male	blood	2006	127	B2		mdf(A)_1
NEL1150_2060463_1_M_B_06	2060463			male	blood	2006	73	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul2_3
NEL1151_2060805_1_U_C_06	2060805			unknown	CSF	2006	394	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1152_2060805_2_U_B_06	2060805			unknown	blood	2006	394	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1153_2060854_1_M_C_06	2060854	54		male	CSF	2006	73	B2		blaTEM-1B_1, mdf(A)_1
NEL1154_2060936_1_F_C_06	2060936			female	CSF	2006	73	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(B)_1
NEL1155_2061084_1_M_B_06	2061084			male	blood	2006	14	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL1156_2061136_1_M_B_06	2061136			male	blood	2006	73	B2		mdf(A)_1
NEL1157_2061143_1_U_B_06	2061143			unknown	blood	2006	973	D	gyrA_S83L	ant(3'')-Ia_1, blaOXA-1_1, floR_2, mdf(A)_1, sul1_5, sul2_2
NEL1158_2061220_1_U_B_06	2061220			unknown	blood	2006	73	B2		mdf(A)_1
NEL1159_2061249_1_M_B_06	2061249			male	blood	2006	57	E	parC_S80I, gyrA_D87N, gyrA_S83L	ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL1160_2061303_1_F_B_06	2061303			female	blood	2006	404	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1161_2061377_1_F_C_06	2061377	78		female	CSF	2006	62	F		mdf(A)_1
NEL1162_2061412_1_F_C_06	2061412			female	CSF	2006	140	B2	gyrA_D87N	mdf(A)_1
NEL1163_2061412_2_F_B_06	2061412			female	blood	2006	140	B2	gyrA_D87N	mdf(A)_1
NEL1164_2061530_1_M_C_06	2061530	73		male	CSF	2006	847	B1		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, fosA7_1, mdf(A)_1, sul2_3
NEL1165_2061530_2_M_B_06	2061530	73		male	blood	2006	847	B1		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, fosA7_1, mdf(A)_1, sul2_3
NEL1166_2070648_1_M_C_07	2070648			male	CSF	2007	95	B2		mdf(A)_1
NEL1167_2070709_1_F_B_07	2070709	33		female	blood	2007	10	A		mdf(A)_1, tet(B)_2
NEL1168_2070732_1_F_C_07	2070732			female	CSF	2007	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1169_2070732_2_F_B_07	2070732			female	blood	2007	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1170_2070937_1_M_B_07	2070937			male	blood	2007	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1171_2070968_1_M_B_07	2070968			male	blood	2007	10	A		mdf(A)_1
NEL1172_2070969_1_F_B_07	2070969	27		female	blood	2007	141	B2		mdf(A)_1
NEL1173_2070969_2_F_O_07	2070969	27		female	vagina	2007	141	B2		mdf(A)_1
NEL1174_2071034_1_F_B_07	2071034			female	blood	2007	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1175_2071049_1_M_C_07	2071049			male	CSF	2007	131	B2	parE_I529L	aph(3')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1176_2071073_1_M_C_07	2071073			male	CSF	2007	131	B2	parE_I529L	aph(3')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1177_2071073_2_M_B_07	2071073			male	blood	2007	131	B2	parE_I529L	aph(3')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1178_2071083_1_F_C_07	2071083	85		female	CSF	2007	93	A	gyrA_S83L	mdf(A)_1
NEL1179_2071122_1_M_C_07	2071122			male	CSF	2007	10	A		mdf(A)_1
NEL1180_2071127_1_F_B_07	2071127			female	blood	2007	162	B1		mdf(A)_1
NEL1181_2071135_1_M_C_07	2071135	22		male	CSF	2007	131	B2	parE_I529L	mdf(A)_1
NEL1182_2071232_1_M_B_07	2071232			male	blood	2007	95	B2		mdf(A)_1
NEL1183_2071234_1_M_B_07	2071234			male	blood	2007	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1184_2071286_1_M_C_07	2071286			male	CSF	2007	95	B2	gyrA_S83L	blaCMY-2_1, blaTEM-1B_1, mdf(A)_1
NEL1185_2071286_2_M_B_07	2071286			male	blood	2007	95	B2	gyrA_S83L	blaCMY-2_1, blaTEM-1B_1, mdf(A)_1
NEL1186_2071331_1_M_C_07	2071331			male	CSF	2007	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1187_2071376_1_F_C_07	2071376	69		female	CSF	2007	62	F		mdf(A)_1
NEL1188_2071376_2_F_B_07	2071376	69		female	blood	2007	62	F		mdf(A)_1
NEL1189_2071406_1_F_C_07	2071406			female	CSF	2007	6807	A		ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL1190_2071406_2_F_B_07	2071406			female	blood	2007	6807	A		ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL1191_2071411_1_M_C_07	2071411			male	CSF	2007	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1192_2071411_2_M_B_07	2071411			male	blood	2007	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1193_2071468_1_F_C_07	2071468	81		female	CSF	2007	695	A	gyrA_S83L	ant(3'')-Ia_1, blaCMY-2_1, blaTEM-1B_1, lnu(F)_1, mdf(A)_1, tet(A)_6
NEL1194_2071468_2_F_B_07	2071468	81		female	blood	2007	695	A	parE_I464F, gyrA_S83L	ant(3'')-Ia_1, blaCMY-2_1, lnu(F)_1, mdf(A)_1, tet(A)_6
NEL1195_2071476_1_M_B_07	2071476			male	blood	2007	73	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL1196_2071484_1_M_C_07	2071484			male	CSF	2007	154	B1		mdf(A)_1
NEL1197_2071506_1_M_C_07	2071506	87		male	CSF	2007	69	D	parC_E84G, gyrA_S83L	aadA5_1, blaTEM-1B_1, catA1_1, dfrA17_1, mdf(A)_1, sul1_5, sul2_3, tet(B)_2
NEL1198_2071792_1_M_B_07	2071792			male	blood	2007	95	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1200_2071937_1_U_B_07	2071937			unknown	blood	2007	73	B2		mdf(A)_1
NEL1201_2072057_1_M_C_07	2072057	54		male	CSF	2007	69	D		mdf(A)_1
NEL1202_2080012_1_M_B_08	2080012			male	blood	2008	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1203_2080067_1_F_C_08	2080067			female	CSF	2008	59	F	parE_I355T	mdf(A)_1
NEL1204_2080147_1_M_B_08	2080147			male	blood	2008	1057	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA8_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1205_2080364_1_F_B_08	2080364			female	blood	2008	12	B2		blaTEM-1C_1, mdf(A)_1
NEL1206_2080540_1_M_C_08	2080540	64		male	CSF	2008	6375	B2		mdf(A)_1
NEL1207_2080562_1_F_C_08	2080562			female	CSF	2008	390	B2		aadA2_1, ant(3'')-Ia_1, cmlA1_1, dfrA12_8, mdf(A)_1, sul3_2, tet(A)_6
NEL1208_2080744_1_M_B_08	2080744			male	blood	2008	131	B2	parE_I529L, parE_S458A, gyrA_S83L	blaCTX-M-14_1, blaTEM-1B_1, erm(B)_18, mdf(A)_1
NEL1209_2080781_1_F_C_08	2080781	87		female	CSF	2008	141	B2		mdf(A)_1
NEL1210_2080839_1_F_B_08	2080839			female	blood	2008	131	B2	parE_I529L	aph(3')-Ia_1, blaTEM-1A_1, mdf(A)_1, tet(B)_2
NEL1211_2080908_1_F_C_08	2080908			female	CSF	2008	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA8_1, mdf(A)_1, sul2_2
NEL1212_2080909_1_F_C_08	2080909			female	CSF	2008	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1213_2080909_2_F_B_08	2080909			female	blood	2008	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1214_2080919_1_M_B_08	2080919			male	blood	2008	95	B2		mdf(A)_1
NEL1215_2081068_1_F_C_08	2081068			female	CSF	2008	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1216_2081191_1_F_C_08	2081191	67		female	CSF	2008	1682	B1		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1217_2081191_2_F_B_08	2081191	67		female	blood	2008	1682	B1		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1218_2081577_1_M_C_08	2081577			male	CSF	2008	131	B2	parE_I529L	ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL1219_2081577_2_M_B_08	2081577			male	blood	2008	131	B2	parE_I529L	ant(3'')-Ia_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, tet(A)_6
NEL1221_2081643_1_M_C_08	2081643	52		male	CSF	2008	767	B1		aph(3')-Ia_1, blaTEM-1B_1, mdf(A)_1
NEL1222_2081680_1_M_B_08	2081680			male	blood	2008	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1223_2081722_1_F_B_08	2081722			female	blood	2008	550	B2		mdf(A)_1
NEL1224_2081795_1_M_B_08	2081795			male	blood	2008	73	B2		mdf(A)_1
NEL1225_2081984_1_M_B_08	2081984			male	blood	2008	95	B2		mdf(A)_1
NEL1226_2082145_1_F_B_08	2082145			female	blood	2008	-	B2		mdf(A)_1
NEL1227_2082286_1_F_C_08	2082286			female	CSF	2008	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1228_2082286_2_F_B_08	2082286			female	blood	2008	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1229_2082355_1_U_B_08	2082355			unknown	blood	2008	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA14_5, mdf(A)_1, mph(A)_2, sul2_2, tet(B)_2
NEL1230_2090314_1_F_C_09	2090314	66		female	CSF	2009	23	C	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1231_2090321_1_F_C_09	2090321			female	CSF	2009	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1232_2090370_1_F_B_09	2090370			female	blood	2009	80	B2		blaTEM-1A_1, mdf(A)_1
NEL1233_2091169_1_F_B_09	2091169			female	blood	2009	59	F	parE_I355T	mdf(A)_1
NEL1234_2091243_1_F_C_09	2091243	43		female	CSF	2009	131	B2	parE_I529L	mdf(A)_1
NEL1235_2091278_1_M_B_09	2091278			male	blood	2009	95	B2		mdf(A)_1
NEL1236_2091609_1_M_B_09	2091609			male	blood	2009	95	B2		mdf(A)_1
NEL1237_2091808_1_M_B_09	2091808			male	blood	2009	95	B2		mdf(A)_1
NEL1238_2092080_1_F_C_09	2092080			female	CSF	2009	390	B2	gyrA_D87G	mdf(A)_1
NEL1239_2092080_2_F_B_09	2092080			female	blood	2009	390	B2	gyrA_D87G	mdf(A)_1
NEL1240_2092090_1_F_C_09	2092090	49		female	CSF	2009	95	B2		mdf(A)_1
NEL1241_2092090_2_F_B_09	2092090	49		female	blood	2009	95	B2		mdf(A)_1
NEL1242_2092121_1_M_B_09	2092121			male	blood	2009	491	B2		mdf(A)_1
NEL1243_2092624_1_M_B_09	2092624			male	blood	2009	140	B2	gyrA_S83L	mdf(A)_1
NEL1244_2093061_1_M_C_09	2093061	56		male	CSF	2009	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3
NEL1245_2093122_1_M_B_09	2093122			male	blood	2009	127	B2		mdf(A)_1
NEL1246_2093371_1_M_C_09	2093371			male	CSF	2009	62	F		mdf(A)_1
NEL1247_2093404_1_U_B_09	2093404			unknown	blood	2009	1882	D		mdf(A)_1
NEL1248_2093913_1_F_C_09	2093913			female	CSF	2009	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1249_2093948_1_F_C_09	2093948			female	CSF	2009	10	A		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL1250_2093977_1_F_C_09	2093977	79		female	CSF	2009	73	B2		mdf(A)_1
NEL1251_2094037_1_M_B_09	2094037			male	blood	2009	73	B2		aph(3')-Ia_9, blaTEM-1B_1, mdf(A)_1
NEL1252_2094066_1_F_C_09	2094066			female	CSF	2009	10	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL1253_2094080_1_F_B_09	2094080			female	blood	2009	-	F	parE_I355T	mdf(A)_1
NEL1254_2094240_1_F_B_09	2094240			female	blood	2009	95	B2		mdf(A)_1
NEL1255_2100057_1_M_C_10	2100057			male	CSF	2010	95	B2	gyrA_S83L	blaTEM-1B_1, mdf(A)_1, qnrS1_1
NEL1256_2100300_1_M_B_10	2100300			male	blood	2010	354	F	parC_S80I, parE_I355T, gyrA_D87N, gyrA_S83L	aac(3)-IId_1, ant(3'')-Ia_1, blaOXA-1_1, catA1_1, floR_2, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL1257_2100336_1_F_O_10	2100336	27		female	vagina	2010	925	D	parC_S57T	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3
NEL1258_2100337_1_U_O_10	2100337			unknown	ear	2010	925	D	parC_S57T	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3
NEL1259_2100516_1_F_B_10	2100516			female	blood	2010	12	B2		mdf(A)_1
NEL1260_2100540_1_U_C_10	2100540			unknown	CSF	2010	567	B2		mdf(A)_1
NEL1261_2102367_1_F_B_10	2102367			female	blood	2010	95	B2		mdf(A)_1
NEL1262_2102542_1_M_B_10	2102542			male	blood	2010	95	B2		mdf(A)_1
NEL1263_2102615_1_F_C_10	2102615			female	CSF	2010	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1264_2102995_1_M_B_10	2102995			male	blood	2010	95	B2		mdf(A)_1
NEL1265_2103117_1_F_C_10	2103117			female	CSF	2010	847	B1		fosA7_1, mdf(A)_1
NEL1266_2103120_1_M_C_10	2103120	66		male	CSF	2010	2604	B2		mdf(A)_1
NEL1267_2103242_1_M_C_10	2103242			male	CSF	2010	95	B2		dfrA5_1, mdf(A)_1
NEL1268_2103255_1_M_B_10	2103255			male	blood	2010	1279	C	parC_S80I, parE_S458A, gyrA_D87N, gyrA_S83L	ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL1269_2103268_1_M_C_10	2103268			male	CSF	2010	420	B2	parE_D475E	mdf(A)_1
NEL1270_2103268_2_M_B_10	2103268			male	blood	2010	420	B2	parE_D475E	mdf(A)_1
NEL1271_2103397_1_F_B_10	2103397			female	blood	2010	418	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3
NEL1272_2103398_1_F_B_10	2103398			female	blood	2010	418	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3
NEL1273_2103399_1_M_B_10	2103399			male	blood	2010	73	B2		ant(3'')-Ia_1, dfrA1_10, mdf(A)_1, sul1_5
NEL1274_2103429_1_F_C_10	2103429			female	CSF	2010	95	B2		mdf(A)_1
NEL1275_2103429_2_F_B_10	2103429			female	blood	2010	95	B2		mdf(A)_1
NEL1276_2103487_1_M_C_10	2103487	68		male	CSF	2010	666	B1		mdf(A)_1
NEL1277_2103487_2_M_B_10	2103487	68		male	blood	2010	666	B1		mdf(A)_1
NEL1278_2103603_1_F_C_10	2103603			female	CSF	2010	69	D		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1279_2103616_1_F_C_10	2103616			female	CSF	2010	95	B2		mdf(A)_1
NEL1280_2103632_1_F_B_10	2103632			female	blood	2010	349	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1281_2103638_1_U_B_10	2103638			unknown	blood	2010	155	B1		mdf(A)_1
NEL1282_2103639_1_M_B_10	2103639			male	blood	2010	80	B2		mdf(A)_1
NEL1283_2104182_1_M_C_10	2104182			male	CSF	2010	10	A		mdf(A)_1
NEL1284_2104314_1_M_B_10	2104314			male	blood	2010	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3
NEL1285_2110017_1_M_B_11	2110017			male	blood	2011	95	B2		mdf(A)_1
NEL1286_2110110_1_M_C_11	2110110	59		male	CSF	2011	405	D	gyrA_S83L	mdf(A)_1, tet(B)_2
NEL1287_2110254_1_F_C_11	2110254	63		female	CSF	2011	90	C		mdf(A)_1
NEL1288_2110380_1_F_C_11	2110380			female	CSF	2011	567	B2		mdf(A)_1
NEL1289_2110380_2_F_B_11	2110380			female	blood	2011	567	B2		mdf(A)_1
NEL1290_2110513_1_M_C_11	2110513			male	CSF	2011	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1291_2110620_1_M_B_11	2110620			male	blood	2011	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1D_1, dfrA1_8, mdf(A)_1, sul2_2
NEL1292_2110751_1_M_B_11	2110751			male	blood	2011	59	F	parE_I355T	mdf(A)_1
NEL1293_2111032_1_M_C_11	2111032	42		male	CSF	2011	-	D		mdf(A)_1
NEL1294_2111081_1_M_B_11	2111081			male	blood	2011	131	B2	parE_I529L	mdf(A)_1
NEL1295_2111147_1_F_B_11	2111147			female	blood	2011	69	D		aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(B)_1
NEL1296_2111255_1_U_B_11	2111255			unknown	blood	2011	648	F		aadA2_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1297_2111312_1_M_C_11	2111312			male	CSF	2011	428	B2		blaTEM-1B_1, catA1_1, mdf(A)_1, tet(A)_6
NEL1298_2111312_2_M_B_11	2111312			male	blood	2011	428	B2		blaTEM-1B_1, catA1_1, mdf(A)_1, tet(A)_6
NEL1299_2111342_1_F_C_11	2111342			female	CSF	2011	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1300_2111434_1_M_B_11	2111434			male	blood	2011	744	A	parC_A56T, parC_S80I, gyrA_D87N, gyrA_S83L	aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, catA1_1, lnu(G)_1, mdf(A)_1, sul2_3, tet(B)_2
NEL1301_2111644_1_F_C_11	2111644			female	CSF	2011	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1302_2111644_2_F_B_11	2111644			female	blood	2011	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1303_2120427_1_M_C_12	2120427			male	CSF	2012	95	B2		mdf(A)_1
NEL1304_2120730_1_M_B_12	2120730			male	blood	2012	95	B2		mdf(A)_1
NEL1305_2120787_1_M_C_12	2120787	47		male	CSF	2012	73	B2		mdf(A)_1
NEL1306_2120849_1_M_B_12	2120849			male	blood	2012	73	B2		mdf(A)_1
NEL1307_2120860_1_F_B_12	2120860			female	blood	2012	12	B2		blaTEM-1A_1, mdf(A)_1
NEL1308_2120906_1_M_C_12	2120906			male	CSF	2012	95	B2		dfrA5_1, mdf(A)_1, tet(A)_6
NEL1309_2120906_2_M_B_12	2120906			male	blood	2012	95	B2		dfrA5_1, mdf(A)_1, tet(A)_6
NEL1310_2120925_1_U_B_12	2120925			unknown	blood	2012	95	B2		mdf(A)_1
NEL1311_2121153_1_M_B_12	2121153			male	blood	2012	130	E		blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul1_5
NEL1312_2121201_1_F_C_12	2121201			female	CSF	2012	95	B2		mdf(A)_1
NEL1313_2121343_1_M_B_12	2121343			male	blood	2012	95	B2		mdf(A)_1
NEL1314_2121414_1_M_B_12	2121414			male	blood	2012	2639	B2		aac(3)-IId_1, aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-14_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6
NEL1315_2121541_1_F_B_12	2121541			female	blood	2012	73	B2		blaTEM-1B_1, mdf(A)_1
NEL1316_2121621_1_F_C_12	2121621	65		female	CSF	2012	3032	D		aadA2_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, sul2_2
NEL1317_2121628_1_F_B_12	2121628			female	blood	2012	59	F		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL1318_2130122_1_M_B_13	2130122			male	blood	2013	131	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1319_2130253_1_M_B_13	2130253			male	blood	2013	7092	B2		mdf(A)_1
NEL1320_2130305_1_M_B_13	2130305			male	blood	2013	144	B2		mdf(A)_1
NEL1321_2130348_1_M_B_13	2130348			male	blood	2013	10	A		mdf(A)_1
NEL1322_2130797_1_M_B_13	2130797			male	blood	2013	95	B2		mdf(A)_1
NEL1323_2131026_1_M_B_13	2131026			male	blood	2013	59	F		mdf(A)_1
NEL1324_2131366_1_M_B_13	2131366			male	blood	2013	95	B2		mdf(A)_1
NEL1325_2131403_1_M_B_13	2131403			male	blood	2013	-	B2		mdf(A)_1
NEL1326_2131554_1_M_C_13	2131554			male	CSF	2013	390	B2		mdf(A)_1
NEL1327_2131554_2_M_B_13	2131554			male	blood	2013	390	B2		mdf(A)_1
NEL1328_2131632_1_F_C_13	2131632	56		female	CSF	2013	88	C		ARR-3_4, aac(6')-Ib-cr_1, aadA16_1, ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_10, dfrA27_1, mdf(A)_1, sul1_5, sul2_3
NEL1329_2131656_1_M_C_13	2131656			male	CSF	2013	569	B2		mdf(A)_1
NEL1330_2131656_2_M_B_13	2131656			male	blood	2013	569	B2		mdf(A)_1
NEL1331_2131703_1_M_B_13	2131703			male	blood	2013	95	B2		mdf(A)_1
NEL1332_2131815_1_M_C_13	2131815			male	CSF	2013	95	B2		aadA2_1, ant(3'')-Ia_1, blaTEM-1B_1, cmlA1_1, dfrA12_8, mdf(A)_1, sul3_2
NEL1333_2131815_2_M_B_13	2131815			male	blood	2013	95	B2		aadA2_1, ant(3'')-Ia_1, blaTEM-1B_1, cmlA1_1, dfrA12_8, mdf(A)_1, sul3_2
NEL1334_2131838_1_F_C_13	2131838	30		female	CSF	2013	58	B1		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-15_1, blaTEM-1B_1, dfrA14_5, dfrA1_10, mdf(A)_1, mph(B)_1, qnrB1_1, sul1_5, sul2_3, tet(A)_6
NEL1335_2131846_1_F_C_13	2131846			female	CSF	2013	390	B2		mdf(A)_1
NEL1336_2131854_1_F_B_13	2131854			female	blood	2013	69	D		mdf(A)_1
NEL1337_2131906_1_M_B_13	2131906			male	blood	2013	421	B2		mdf(A)_1
NEL1338_2131921_1_M_B_13	2131921			male	blood	2013	-	B2		mdf(A)_1
NEL1339_2132405_1_F_B_13	2132405			female	blood	2013	69	D		blaCTX-M-1_1, mdf(A)_1
NEL1340_2132406_1_F_B_13	2132406			female	blood	2013	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1341_2132454_1_M_B_13	2132454			male	blood	2013	69	D		blaTEM-1B_1, mdf(A)_1
NEL1342_2132455_1_M_C_13	2132455			male	CSF	2013	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1343_2132496_1_F_B_13	2132496			female	blood	2013	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1344_2132505_1_F_B_13	2132505			female	blood	2013	428	B2		aac(3)-IId_1, ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1345_2132505_2_F_C_13	2132505			female	CSF	2013	428	B2		aac(3)-IId_1, ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1346_2132511_1_F_B_13	2132511			female	blood	2013	428	B2		aac(3)-IId_1, ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1347_2132537_1_M_B_13	2132537			male	blood	2013	62	F		mdf(A)_1
NEL1348_2140141_1_M_C_14	2140141			male	CSF	2014	95	B2	gyrA_S83L	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul1_5
NEL1349_2140141_2_M_B_14	2140141			male	blood	2014	95	B2	gyrA_S83L	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul1_5
NEL1350_2140179_1_F_C_14	2140179			female	CSF	2014	416	B2		mdf(A)_1
NEL1351_2140179_2_F_B_14	2140179			female	blood	2014	416	B2		mdf(A)_1
NEL1352_2140873_1_F_B_14	2140873			female	blood	2014	12	B2		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1353_2140874_1_M_B_14	2140874			male	blood	2014	12	B2		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1354_2140875_1_M_B_14	2140875			male	blood	2014	73	B2		mdf(A)_1
NEL1355_2141122_1_M_B_14	2141122			male	blood	2014	69	D		mdf(A)_1
NEL1356_2141151_1_F_B_14	2141151			female	blood	2014	390	B2		mdf(A)_1
NEL1357_2141178_1_M_B_14	2141178			male	blood	2014	88	C		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1358_2141216_1_M_C_14	2141216			male	CSF	2014	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1359_2141216_2_M_B_14	2141216			male	blood	2014	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1360_2141288_1_F_C_14	2141288			female	CSF	2014	95	B2		mdf(A)_1
NEL1361_2141308_1_M_B_14	2141308			male	blood	2014	131	B2	parC_E84V, parC_S80I, parE_I529L, gyrA_D87N, gyrA_S83L	blaCTX-M-15_1, mdf(A)_1
NEL1362_2141325_1_M_B_14	2141325			male	blood	2014	428	B2		mdf(A)_1
NEL1363_2141361_1_M_B_14	2141361			male	blood	2014	131	B2	parE_I529L	blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1364_2141434_1_F_B_14	2141434			female	blood	2014	1333	B2		mdf(A)_1
NEL1365_2141477_1_M_B_14	2141477			male	blood	2014	5956	A		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1366_2141481_1_F_B_14	2141481			female	blood	2014	10	A		mdf(A)_1
NEL1367_2141482_1_M_B_14	2141482			male	blood	2014	1431	B1		aadA2_1, aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1C_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1368_2141483_1_F_B_14	2141483			female	blood	2014	95	B2		mdf(A)_1
NEL1369_2141515_1_M_C_14	2141515			male	CSF	2014	95	B2		mdf(A)_1
NEL1370_2141516_1_M_C_14	2141516			male	CSF	2014	569	B2	parE_D475E	mdf(A)_1
NEL1371_2141516_2_M_B_14	2141516			male	blood	2014	569	B2	parE_D475E	mdf(A)_1
NEL1372_2141529_1_M_B_14	2141529			male	blood	2014	95	B2		dfrA5_1, mdf(A)_1
NEL1373_2141584_1_M_B_14	2141584			male	blood	2014	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1A_1, mdf(A)_1, mph(A)_2, sul2_2, tet(B)_2
NEL1374_2141639_1_M_C_14	2141639			male	CSF	2014	453	B1		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1375_2141658_1_M_B_14	2141658			male	blood	2014	95	B2		blaTEM-1C_1, mdf(A)_1, tet(A)_6
NEL1376_2141924_1_M_B_14	2141924			male	blood	2014	5484	B2		mdf(A)_1
NEL1377_2141953_1_U_B_14	2141953			unknown	blood	2014	95	B2		mdf(A)_1
NEL1378_2141978_1_F_C_14	2141978			female	CSF	2014	95	B2		mdf(A)_1
NEL1379_2141978_2_F_B_14	2141978			female	blood	2014	95	B2		mdf(A)_1
NEL1380_2142037_1_F_B_14	2142037			female	blood	2014	69	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1381_2142044_1_F_B_14	2142044			female	blood	2014	69	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1382_2142058_1_F_B_14	2142058			female	blood	2014	69	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1384_2142155_1_M_B_14	2142155			male	blood	2014	69	D	gyrA_S83L	aac(3)-IId_1, aadA5_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, tet(B)_2
NEL1385_2150132_1_F_C_15	2150132			female	CSF	2015	95	B2	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1386_2150164_1_F_B_15	2150164			female	blood	2015	69	D		mdf(A)_1
NEL1387_2150180_1_F_B_15	2150180			female	blood	2015	141	B2		mdf(A)_1
NEL1388_2150307_1_F_C_15	2150307	80		female	CSF	2015	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1389_2150373_1_F_B_15	2150373			female	blood	2015	569	B2	parE_D475E	mdf(A)_1
NEL1390_2150481_1_F_B_15	2150481			female	blood	2015	95	B2		mdf(A)_1
NEL1391_2150652_1_M_B_15	2150652			male	blood	2015	95	B2		mdf(A)_1
NEL1392_2150686_1_M_C_15	2150686	67		male	CSF	2015	-	D		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1393_2150718_1_F_B_15	2150718			female	blood	2015	-	B2		mdf(A)_1
NEL1394_2150719_1_F_C_15	2150719	64		female	CSF	2015	95	B2		mdf(A)_1
NEL1395_2150719_2_F_B_15	2150719	64		female	blood	2015	95	B2		mdf(A)_1
NEL1396_2150765_1_M_B_15	2150765			male	blood	2015	127	B2		ant(3'')-Ia_1, blaTEM-1A_1, catA1_1, dfrA1_8, mdf(A)_1, sul1_5, sul2_2, tet(B)_2
NEL1397_2150835_1_M_B_15	2150835			male	blood	2015	3478	A	parE_I355T, gyrA_S83L	mdf(A)_1
NEL1398_2150872_1_M_B_15	2150872			male	blood	2015	69	D	gyrA_S83L	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, tet(B)_2
NEL1399_2150872_2_M_C_15	2150872			male	CSF	2015	69	D	gyrA_S83L	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, tet(B)_2

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1400_2150891_1_F_B_15	2150891			female	blood	2015	567	B2		mdf(A)_1
NEL1401_2150909_1_M_C_15	2150909			male	CSF	2015	95	B2		mdf(A)_1
NEL1402_2150927_1_M_B_15	2150927			male	blood	2015	69	D		mdf(A)_1
NEL1403_2150949_1_M_B_15	2150949			male	blood	2015	95	B2		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul1_5, sul2_2
NEL1404_2151086_1_M_B_15	2151086			male	blood	2015	676	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(B)_2
NEL1405_2151151_1_M_B_15	2151151			male	blood	2015	69	D		mdf(A)_1
NEL1406_2151205_1_M_C_15	2151205			male	CSF	2015	428	B2		mdf(A)_1
NEL1407_2151205_2_M_B_15	2151205			male	blood	2015	428	B2		mdf(A)_1
NEL1408_2151226_1_F_B_15	2151226			female	blood	2015	1193	B2	parC_S80I, parE_L416F, gyrA_D87N, gyrA_S83L	blaTEM-1B_1, mdf(A)_1
NEL1409_2151250_1_M_B_15	2151250			male	blood	2015	141	B2		mdf(A)_1
NEL1410_2151369_1_M_B_15	2151369			male	blood	2015	127	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, ere(A)_2, mdf(A)_1, sul2_3
NEL1411_2151449_1_M_B_15	2151449			male	blood	2015	73	B2		mdf(A)_1
NEL1412_2151472_1_M_C_15	2151472			male	CSF	2015	95	B2	gyrA_S83L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1413_2151472_2_M_B_15	2151472			male	blood	2015	95	B2	gyrA_S83L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1414_2151473_1_M_B_15	2151473			male	blood	2015	95	B2	gyrA_S83L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1415_2151487_1_M_B_15	2151487			male	blood	2015	69	D		mdf(A)_1, tet(B)_2
NEL1416_2151571_1_F_B_15	2151571			female	blood	2015	95	B2	gyrA_S83L	mdf(A)_1
NEL1417_780401_2_F_C_78	780401		0	female	CSF	1978	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1418_780401_3_F_C_78	780401		0	female	CSF	1978	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1419_780665_3_M_C_78	780665	1		male	CSF	1978	2944	D	parE_I355T	ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-Ia_7, aph(6)-Id_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1420_780727_2_M_C_78	780727	1		male	CSF	1978	127	B2		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL1421_780817_2_M_C_78	780817		1	male	CSF	1978	390	B2		mdf(A)_1
NEL1422_780817_3_M_C_78	780817		1	male	CSF	1978	390	B2		mdf(A)_1
NEL1423_790194_2_M_C_79	790194		0	male	CSF	1979	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1A_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1424_790580_2_M_C_79	790580		1	male	CSF	1979	567	B2		mdf(A)_1
NEL1425_790679_2_M_C_79	790679		1	male	CSF	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1426_790848_3_M_O_79	790848	2		male	other	1979	73	B2		mdf(A)_1
NEL1427_791002_2_M_C_79	791002		0	male	CSF	1979	23	C		mdf(A)_1
NEL1428_791058_2_M_C_79	791058	59		male	CSF	1979	12	B2		mdf(A)_1
NEL1429_791063_2_F_C_79	791063		0	female	CSF	1979	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1430_800010_2_F_C_80	800010		3	female	CSF	1980	3168	A		mdf(A)_1
NEL1431_831048_2_M_C_83	831048	65		male	CSF	1983	-	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL1432_831048_3_M_C_83	831048	65		male	CSF	1983	-	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3, tet(A)_6
NEL1433_831188_2_M_C_83	831188		0	male	CSF	1983	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(3')-IIa_2, aph(6)-Ic_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL1434_840201_3_F_B_84	840201		0	female	Blood	1984	88	C		mdf(A)_1
NEL1435_840201_4_F_C_84	840201		1	female	CSF	1984	88	C		mdf(A)_1
NEL1436_840201_5_F_B_84	840201		1	female	Blood	1984	88	C		mdf(A)_1
NEL1437_840553_2_F_C_84	840553		0	female	CSF	1984	62	F		mdf(A)_1
NEL1438_841175_2_F_C_84	841175	1		female	CSF	1984	95	B2		mdf(A)_1
NEL1439_850294_2_M_C_85	850294		1	male	CSF	1985	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1440_850294_3_M_B_85	850294		1	male	Blood	1985	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1441_851056_2_F_C_85	851056	89		female	CSF	1985	187	B1		mdf(A)_1
NEL1442_860222_3_M_B_86	860222		1	male	Blood	1986	95	B2		mdf(A)_1
NEL1443_860222_4_M_B_86	860222		1	male	Blood	1986	95	B2		mdf(A)_1
NEL1444_860794_4_F_O_86	860794		0	female	other	1986	567	B2		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_2
NEL1445_860952_3_M_C_86	860952		2	male	CSF	1986	95	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1446_860952_4_M_B_86	860952		2	male	Blood	1986	95	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1447_861379_2_M_B_86	861379	62		male	Blood	1986	73	B2		mdf(A)_1
NEL1448_881532_2_M_B_88	881532		0	male	Blood	1988	62	F		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_2, tet(B)_2
NEL1449_910051_2_F_C_91	910051	70		female	CSF	1991	14	B2		mdf(A)_1
NEL1450_911422_3_M_B_91	911422		1	male	Blood	1991	62	F		blaTEM-1C_1, mdf(A)_1
NEL1451_921800_2_F_C_92	921800		11	female	CSF	1992	-	A		mdf(A)_1
NEL1452_930085_2_U_C_93	930085		1	unknown	CSF	1993	95	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1453_931224_2_M_B_93	931224		0	male	Blood	1993	95	B2		mdf(A)_1
NEL1454_940508_3_M_O_94	940508		0	male	unknown	1994	62	F		blaTEM-1C_1, mdf(A)_1
NEL1455_2001402_2_U_C_00	2001402		11	unknown	CSF	2000	74	B2		mdf(A)_1
NEL1456_2010477_2_F_B_01	2010477		0	female	Blood	2001	95	B2		ant(3'')-Ia_1, blaTEM-1B_1, catA1_1, mdf(A)_1, sul1_5
NEL1457_2021677_2_M_C_02	2021677		0	male	CSF	2002	5476	D	parC_S57T, parC_S80I, parE_L416F, gyrA_D87N, gyrA_S83L	aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5
NEL1458_2120906_3_M_B_12	2120906		0	male	Blood	2012	95	B2		dfrA5_1, mdf(A)_1, tet(A)_6
NEL1459_2140141_3_M_C_14	2140141		0	male	CSF	2014	95	B2	gyrA_S83L	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul1_5
NEL1460_2150132_2_F_C_15	2150132		0	female	CSF	2015	95	B2	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1461_2160136_1_M_B_16	2160136		0		Blood	2016	95	B2		mdf(A)_1
NEL1463_2160175_2_F_B_16	2160175		0		Blood	2016	967	F		mdf(A)_1
NEL1464_2160231_1_M_C_16	2160231		7		CSF	2016	8186	B2	parE_D475E	mdf(A)_1
NEL1465_2160291_1_M_B_16	2160291		1		Blood	2016	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1466_2160361_1_M_C_16	2160361		1		CSF	2016	1193	B2	gyrA_D87N	aph(3'')-Ib_5, aph(6)-Id_1, dfrA17_1, mdf(A)_1, sul2_3, tet(B)_2
NEL1467_2160361_2_M_B_16	2160361		1		Blood	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aph(3'')-Ib_5, aph(6)-Id_1, dfrA17_1, mdf(A)_1, sul2_3, tet(B)_2
NEL1468_2160550_1_F_B_16	2160550		2		Blood	2016	-	B1		mdf(A)_1
NEL1469_2160639_1_M_B_16	2160639		0		Blood	2016	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1470_2160881_1_F_B_16	2160881		0		Blood	2016	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1471_2160895_1_M_B_16	2160895		0		Blood	2016	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1472_2160989_1_M_B_16	2160989		0		Blood	2016	141	B2		mdf(A)_1
NEL1473_2161053_1_M_C_16	2161053		743		CSF	2016	357	B2		mdf(A)_1
NEL1474_2161062_1_F_B_16	2161062		0		Blood	2016	95	B2		blaTEM-1B_1, dfrA5_1, mdf(A)_1
NEL1475_2161077_1_F_C_16	2161077		944		CSF	2016	-	B2	gyrA_S83L, parE_I529L	aadA2_1, ant(3'')-Ia_1, cmlA1_1, dfrA12_8, mdf(A)_1, sul3_2
NEL1476_2161204_1_F_B_16	2161204		2		Blood	2016	69	D		mdf(A)_1
NEL1477_2161213_1_M_C_16	2161213		804		CSF	2016	69	D	gyrA_S83L, parC_E84G	blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1478_2161216_1_F_B_16	2161216		0		Blood	2016	404	B2		blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1479_2161238_1_F_B_16	2161238		0		Blood	2016	141	B2		mdf(A)_1
NEL1480_2161242_1_M_C_16	2161242		0		CSF	2016	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1481_2161242_2_M_B_16	2161242		0		Blood	2016	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1482_2161315_1_M_C_16	2161315		665		CSF	2016	23	C		ant(3'')-Ia_1, lnu(F)_1, mdf(A)_1
NEL1483_2161315_2_M_B_16	2161315		665		Blood	2016	23	C		ant(3'')-Ia_1, lnu(F)_1, mdf(A)_1
NEL1484_2161324_1_M_B_16	2161324		1		Blood	2016	95	B2		mdf(A)_1
NEL1485_2161328_1_F_B_16	2161328		0		Blood	2016	357	B2		mdf(A)_1
NEL1486_2161333_1_M_B_16	2161333		0		Blood	2016	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1487_2161363_1_M_C_16	2161363		1041		CSF	2016	59	F	parE_I355T	mdf(A)_1
NEL1488_2161381_1_M_C_16	2161381		0		CSF	2016	131	B2	parE_I529L	mdf(A)_1
NEL1489_2161426_1_F_C_16	2161426		0		CSF	2016	23	C	gyrA_S83L	mdf(A)_1
NEL1490_2161434_1_F_B_16	2161434		0		Blood	2016	23	C	gyrA_S83L	mdf(A)_1
NEL1491_2161489_1_M_B_16	2161489		0		Blood	2016	141	B2		mdf(A)_1
NEL1494_2161522_1_M_C_16	2161522		752		CSF	2016	-	C	gyrA_S83L	mdf(A)_1
NEL1495_2161522_2_M_B_16	2161522		752		Blood	2016	-	C	gyrA_S83L	mdf(A)_1
NEL1497_2161567_1_M_C_16	2161567		454		CSF	2016	58	B1		aadA2_1, ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, cmlA1_1, dfrA5_1, dfrA8_1, mdf(A)_1, sul2_3, sul3_2
NEL1498_2161568_1_M_C_16	2161568		785		CSF	2016	88	C		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, catA1_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1499_2161582_1_U_B_16	2161582		9		Blood	2016	73	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3, tet(B)_2

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1500_2161624_1_M_B_16	2161624		0		Blood	2016	131	B2	parE_I529L	catA1_1, mdf(A)_1
NEL1501_2161648_1_M_C_16	2161648		2		CSF	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_3, tet(B)_2
NEL1502_2161649_1_M_B_16	2161649		0		Blood	2016	-	B1		mdf(A)_1
NEL1503_2161650_1_M_C_16	2161650		0		CSF	2016	567	B2		mdf(A)_1
NEL1504_2161664_1_M_B_16	2161664		0		Blood	2016	95	B2		mdf(A)_1
NEL1505_2161719_1_F_B_16	2161719		4		Blood	2016	2165	B1		mdf(A)_1
NEL1506_2161721_1_F_C_16	2161721		0		CSF	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	blaDHA-1_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, qnrB4_1, sul1_5
NEL1507_2161721_2_F_B_16	2161721		0		Blood	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	blaDHA-1_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, qnrB4_1, sul1_5
NEL1508_2161735_1_M_B_16	2161735		0		Blood	2016	6331	B1		mdf(A)_1
NEL1509_2161805_1_M_B_16	2161805		0		Blood	2016	131	B2	gyrA_S83L, parE_I529L	blaTEM-1B_1, mdf(A)_1
NEL1510_2161810_1_M_B_16	2161810		1		Blood	2016	104	B2		mdf(A)_1
NEL1511_2161811_1_M_B_16	2161811		0		Blood	2016	95	B2		mdf(A)_1
NEL1512_2161812_1_F_B_16	2161812		0		Blood	2016	10	A		mdf(A)_1
NEL1513_2162050_1_M_B_16	2162050		0		Blood	2016	95	B2		mdf(A)_1
NEL1514_2162114_1_M_C_16	2162114		0		CSF	2016	141	B2		mdf(A)_1
NEL1515_2162127_1_M_B_16	2162127		0		Blood	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, blaCTX-M-55_1, blaTEM-1B_1, mdf(A)_1
NEL1516_2162193_1_M_B_16	2162193		0		Blood	2016	3877	A		blaTEM-1A_1, mdf(A)_1
NEL1517_2170193_1_F_B_17	2170193		0		Blood	2017	393	D	gyrA_D87N, gyrA_S83L, parC_S57T, parC_S80I, parE_L416F	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1518_2170199_1_M_B_17	2170199		1		Blood	2017	357	B2		mdf(A)_1
NEL1519_2170238_1_F_B_17	2170238		1		Blood	2017	73	B2		ant(3'')-Ia_1, mdf(A)_1, sul1_5
NEL1520_2170247_1_F_B_17	2170247		0		Blood	2017	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, mdf(A)_1
NEL1521_2170306_1_M_B_17	2170306		2		Blood	2017	73	B2		blaTEM-1D_1, mdf(A)_1, tet(B)_2
NEL1522_2170314_1_M_B_17	2170314		0		Blood	2017	-	D		mdf(A)_1
NEL1523_2170508_1_F_B_17	2170508		0		Blood	2017	428	B2		blaTEM-1C_1, mdf(A)_1
NEL1524_2170939_1_F_B_17	2170939		1		Blood	2017	-	B1		mdf(A)_1
NEL1525_2170992_1_F_B_17	2170992		1		Blood	2017	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA8_1, mdf(A)_1, sul2_2
NEL1526_2171165_1_M_B_17	2171165		0		Blood	2017	95	B2		aph(3'')-Ib_5, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1527_2171166_1_U_B_17	2171166		2		Blood	2017	127	B2		mdf(A)_1
NEL1528_2171263_1_F_B_17	2171263		0		Blood	2017	4481	B1		mdf(A)_1
NEL1529_2171282_1_M_B_17	2171282		0		Blood	2017	4481	B1		mdf(A)_1
NEL1530_2171387_1_F_C_17	2171387		0		CSF	2017	80	B2		mdf(A)_1
NEL1531_2171387_2_F_B_17	2171387		0		Blood	2017	80	B2		mdf(A)_1
NEL1532_2171388_1_M_B_17	2171388		0		Blood	2017	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1533_2171389_1_F_B_17	2171389		0		Blood	2017	144	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_2
NEL1534_2171406_1_M_B_17	2171406		1		Blood	2017	73	B2		blaTEM-1B_1, mdf(A)_1
NEL1535_2171410_1_M_B_17	2171410		0		Blood	2017	56	B1		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1536_2171478_1_M_B_17	2171478		0		Blood	2017	69	D		mdf(A)_1
NEL1538_2171515_1_M_C_17	2171515		913		CSF	2017	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1539_2171515_2_M_B_17	2171515		913		Blood	2017	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1540_2171620_1_M_B_17	2171620		5		Blood	2017	127	B2		aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA14_5, dfrA8_1, mdf(A)_1, sul2_2
NEL1541_2171625_1_M_B_17	2171625		9		Blood	2017	69	D		blaTEM-1A_1, mdf(A)_1
NEL1542_2171770_1_F_B_17	2171770		0		Blood	2017	101	B1		blaTEM-1B_1, mdf(A)_1, qnrS1_1
NEL1543_2171781_1_F_C_17	2171781		936		CSF	2017	10	A		aph(3')-Ia_3, aph(6)-Id_1, blaCTX-M-32_2, dfrA14_5, mdf(A)_1, sul2_2, tet(B)_1
NEL1544_2171797_1_M_C_17	2171797		0		CSF	2017	-	A		ant(3'')-Ia_1, mdf(A)_1, tet(B)_2
NEL1545_2171801_1_M_B_17	2171801		6		Blood	2017	73	B2		mdf(A)_1
NEL1546_2171827_1_M_B_17	2171827		0		Blood	2017	2200	D		mdf(A)_1
NEL1547_2171835_1_F_B_17	2171835		0		Blood	2017	543	E		mdf(A)_1
NEL1548_2171842_1_M_B_17	2171842		0		Blood	2017	543	E		mdf(A)_1
NEL1549_2171896_1_M_B_17	2171896		0		Blood	2017	12	B2		mdf(A)_1
NEL1550_2171901_1_M_B_17	2171901		0		Blood	2017	73	B2	gyrA_D87G	mdf(A)_1
NEL1551_2171948_1_M_B_17	2171948		1		Blood	2017	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1552_2171955_1_F_C_17	2171955		0		CSF	2017	10	A		mdf(A)_1
NEL1553_2171955_2_F_B_17	2171955		0		Blood	2017	10	A		mdf(A)_1
NEL1554_2172037_1_M_B_17	2172037		0		Blood	2017	543	E		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1, tet(A)_4
NEL1561_2172170_1_M_C_17	2172170		0		CSF	2017	80	B2		mdf(A)_1
NEL1565_2172447_1_M_B_17	2172447		0		Blood	2017	6630	F		mdf(A)_1
NEL1566_2172470_1_M_B_17	2172470		0		Blood	2017	7576	E		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1567_2172593_1_M_B_17	2172593		0		Blood	2017	59	F	parE_I355T	blaTEM-1D_1, mdf(A)_1
NEL1568_2172618_1_M_B_17	2172618		2		Blood	2017	141	B2		mdf(A)_1
NEL1569_2180140_1_M_B_18	2180140		0		Blood	2018	415	F	parE_I355T	aph(3'')-Ib_5, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1570_2180228_1_M_B_18	2180228		1		Blood	2018	95	B2		aph(3'')-Ib_5, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1571_2180245_1_M_B_18	2180245		0		Blood	2018	362	D		mdf(A)_1
NEL1572_2180339_1_M_B_18	2180339		0		Blood	2018	95	B2		mdf(A)_1
NEL1573_2180359_1_M_B_18	2180359		0		Blood	2018	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5
NEL1574_2180464_1_F_C_18	2180464		0		CSF	2018	117	G	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1578_2180820_1_F_C_18	2180820		585		CSF	2018	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1579_2180862_1_F_C_18	2180862		947		CSF	2018	62	F		aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1
NEL1580_2180884_1_F_B_18	2180884		0		Blood	2018	635	A		mdf(A)_1
NEL1581_2181121_1_F_B_18	2181121		0		Blood	2018	95	B2		mdf(A)_1
NEL1582_2181133_1_M_B_18	2181133		0		Blood	2018	95	B2	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, dfrA17_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1583_2181182_1_F_B_18	2181182		0		Blood	2018	69	D		mdf(A)_1
NEL1584_2181189_1_M_B_18	2181189		0		Blood	2018	95	B2		mdf(A)_1
NEL1585_2181323_1_M_B_18	2181323		1		Blood	2018	429	Unknown		mdf(A)_1
NEL1586_2181364_1_M_B_18	2181364		6		Blood	2018	95	B2		mdf(A)_1
NEL1587_2181365_1_F_B_18	2181365		0		Blood	2018	390	B2		mdf(A)_1
NEL1588_2181393_1_F_B_18	2181393		0		Blood	2018	1851	B2		mdf(A)_1
NEL1589_2181472_1_F_B_18	2181472		1		Blood	2018	390	B2		mdf(A)_1
NEL1590_2181491_1_M_C_18	2181491		806		CSF	2018	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-15_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, qnrS1_1, sul1_5, sul2_3, tet(A)_6
NEL1591_2181491_2_M_B_18	2181491		806		Blood	2018	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-15_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, qnrS1_1, sul1_5, sul2_3, tet(A)_6
NEL1592_2181552_1_M_B_18	2181552		5		Blood	2018	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, tet(B)_2
NEL1593_2181614_1_M_B_18	2181614		1		Blood	2018	390	B2		mdf(A)_1
NEL1594_2181615_1_F_B_18	2181615		0		Blood	2018	2144	B1		mdf(A)_1
NEL1595_2181678_1_M_B_18	2181678		0		Blood	2018	73	B2		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1596_2181679_1_F_C_18	2181679		0		CSF	2018	410	C		aac(3)-IVa_1, aph(3'')-Ib_5, aph(4)-Ia_1, aph(6)-Id_1, blaCTX-M-1_1, mdf(A)_1, mph(A)_2
NEL1597_2181679_2_F_B_18	2181679		0		Blood	2018	410	C		aph(3'')-Ib_5, aph(4)-Ia_1, aph(6)-Id_1, blaCTX-M-1_1, mdf(A)_1
NEL1599_2181694_1_F_B_18	2181694		0		Blood	2018	73	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, tet(B)_2

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1600_2181788_1_M_C_18	2181788		1		CSF	2018	141	B2		mdf(A)_1
NEL1602_2181918_1_F_B_18	2181918		2		Blood	2018	1851	B2		mdf(A)_1
NEL1604_2182028_1_M_C_18	2182028		1		CSF	2018	390	B2		mdf(A)_1
NEL1605_2182028_2_M_B_18	2182028		1		Blood	2018	390	B2		mdf(A)_1
NEL1606_2182033_1_F_B_18	2182033		0		Blood	2018	929	B2	parE_D475E	mdf(A)_1
NEL1607_2182075_1_M_B_18	2182075		2		Blood	2018	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3, tet(B)_2
NEL1608_2182081_1_M_B_18	2182081		0		Blood	2018	1861	B2		mdf(A)_1
NEL1609_2182209_1_M_C_18	2182209		2		CSF	2018	390	B2	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, floR_2, mdf(A)_1, sul2_2
NEL1610_2182229_1_M_B_18	2182229		0		Blood	2018	567	B2		mdf(A)_1
NEL1611_2182250_1_M_B_18	2182250		1		Blood	2018	95	B2		mdf(A)_1
NEL1612_2182259_1_M_B_18	2182259		3		Blood	2018	73	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, blaTEM-1B_1, dfrA1_10, mdf(A)_1, sul1_5, sul2_3
NEL1614_2182271_2_M_B_18	2182271		1		Blood	2018	59	F	parE_I355T	mdf(A)_1
NEL1615_2182307_1_M_B_18	2182307		0		Blood	2018	362	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1616_2182347_1_M_B_18	2182347		0		Blood	2018	3779	E		mdf(A)_1
NEL1617_2182370_1_M_B_18	2182370		0		Blood	2018	95	B2		mdf(A)_1
NEL1618_2182417_1_M_B_18	2182417		0		Blood	2018	3779	E		mdf(A)_1
NEL1619_2182516_1_M_B_18	2182516		1		Blood	2018	73	B2		blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1620_2182644_1_M_B_18	2182644		0		Blood	2018	69	D		aadA5_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul1_5, tet(B)_2
NEL1621_2182678_1_M_B_18	2182678		0		Blood	2018	59	F	parE_I355T	mdf(A)_1
NEL1622_2182681_1_F_B_18	2182681		0		Blood	2018	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA8_1, mdf(A)_1, sul2_2, tet(B)_2
NEL1623_2182682_1_M_B_18	2182682		8		Blood	2018	69	D		mdf(A)_1
NEL1624_2182708_1_M_B_18	2182708		0		Blood	2018	131	B2	parE_I529L	aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_3
NEL1625_2182709_1_M_B_18	2182709		2		Blood	2018	-	B2		mdf(A)_1
NEL1626_2182742_2_M_B_18	2182742		0		Blood	2018	453	B1	gyrA_D87N, gyrA_S83L, parC_S80I	blaTEM-1B_1, floR_2, mdf(A)_1, tet(A)_6
NEL1628_2182743_1_M_B_18	2182743		0		Blood	2018	62	F		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL1629_2182756_1_M_B_18	2182756		1		Blood	2018	73	B2		mdf(A)_1
NEL1630_2182809_1_F_C_18	2182809		1		CSF	2018	141	B2	gyrA_S83L	mdf(A)_1
NEL1631_2182846_1_F_B_18	2182846		0		Blood	2018	428	B2		mdf(A)_1
NEL1632_2182909_1_M_C_18	2182909		1		CSF	2018	62	F		aph(3'')-Ib_2, aph(6)-Id_1, mdf(A)_1
NEL1633_2182915_1_U_B_18	2182915		0		Blood	2018	12	B2		mdf(A)_1
NEL1634_2182931_1_M_C_18	2182931		0		CSF	2018	10	A	gyrA_S83L	blaCMY-138_1, dfrA14_5, mdf(A)_1, tet(A)_6
NEL1635_2182982_1_M_B_18	2182982		0		Blood	2018	131	B2	parE_I529L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1636_2183101_1_M_B_18	2183101		0		Blood	2018	95	B2		mdf(A)_1
NEL1637_2190009_1_U_B_19	2190009		0		Blood	2019	14	B2		mdf(A)_1
NEL1638_2190238_1_M_B_19	2190238		0		Blood	2019	10	A		mdf(A)_1
NEL1639_2190383_1_M_B_19	2190383		4		Blood	2019	95	B2		mdf(A)_1
NEL1640_2190384_1_M_B_19	2190384		12		Blood	2019	73	B2		blaTEM-1B_1, catA1_1, mdf(A)_1
NEL1641_2190401_1_F_B_19	2190401		2		Blood	2019	69	D		aadA2_1, aph(6)-Id_1, blaTEM-1C_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5
NEL1642_2190402_1_F_B_19	2190402		2		Blood	2019	95	B2		aadA2_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1643_2190466_1_M_B_19	2190466		0		Blood	2019	131	B2		mdf(A)_1, tet(B)_1
NEL1644_2190522_1_F_C_19	2190522		0		CSF	2019	131	B2	parE_I529L	mdf(A)_1
NEL1645_2190523_1_F_B_19	2190523		0		Blood	2019	59	F	parE_I355T	mdf(A)_1, qnrB19_1
NEL1646_2190544_1_M_B_19	2190544		2		Blood	2019	-	B2	gyrA_D87N, gyrA_S83L, parC_E84V, parC_S80I, parE_I529L	mdf(A)_1
NEL1647_2190588_1_M_B_19	2190588		1		Blood	2019	69	D		mdf(A)_1, tet(B)_2
NEL1648_2190661_1_M_B_19	2190661		4		Blood	2019	59	F	parE_I355T	mdf(A)_1
NEL1649_2190763_1_M_B_19	2190763		1		Blood	2019	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1650_2190809_1_F_B_19	2190809		1		Blood	2019	2914	D		mdf(A)_1
NEL1651_2190836_1_M_B_19	2190836		0		Blood	2019	428	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1652_2190850_1_F_C_19	2190850		0		CSF	2019	504	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1653_2190850_2_F_B_19	2190850		0		Blood	2019	504	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1
NEL1654_2190874_1_M_B_19	2190874		0		Blood	2019	80	B2		mdf(A)_1
NEL1655_2190881_1_F_C_19	2190881		1		CSF	2019	131	B2	parE_I529L	mdf(A)_1
NEL1656_2190881_2_F_B_19	2190881		1		Blood	2019	131	B2	parE_I529L	mdf(A)_1
NEL1658_2190894_1_F_B_19	2190894		0		Blood	2019	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1659_2191002_1_F_B_19	2191002		0		Blood	2019	117	G		mdf(A)_1
NEL1660_2191003_1_M_B_19	2191003		0		Blood	2019	12	B2	gyrA_S83L	mdf(A)_1
NEL1661_2191041_1_M_C_19	2191041		1		CSF	2019	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, floR_2, mdf(A)_1, sul2_2
NEL1662_2191041_2_M_B_19	2191041		1		Blood	2019	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, floR_2, mdf(A)_1, sul2_2
NEL1663_2191061_1_M_C_19	2191061		0		CSF	2019	69	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1664_2191061_2_M_B_19	2191061		0		Blood	2019	69	D		blaTEM-1B_1, mdf(A)_1, tet(A)_6
NEL1665_2191062_1_M_B_19	2191062		0		Blood	2019	95	B2	gyrA_S83L	blaTEM-1C_1, mdf(A)_1
NEL1666_2191090_1_F_B_19	2191090		0		Blood	2019	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1667_2191149_1_M_B_19	2191149		0		Blood	2019	95	B2	gyrA_S83L	ant(3'')-Ia_1, catA1_1, mdf(A)_1, sul1_5
NEL1669_2191204_1_F_B_19	2191204		1		Blood	2019	95	B2		aph(6)-Id_1, dfrA14_5, mdf(A)_1, sul2_2
NEL1670_2191218_1_M_B_19	2191218		0		Blood	2019	14	B2	gyrA_S83L	blaTEM-1C_1, mdf(A)_1
NEL1671_2191244_1_M_B_19	2191244		0		Blood	2019	69	D		aph(3'')-Ib_5, aph(6)-Id_1, mdf(A)_1, sul2_3
NEL1672_2191245_1_M_B_19	2191245		0		Blood	2019	10	A		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, dfrA7_5, mdf(A)_1, sul1_5, sul2_3, tet(A)_6
NEL1673_2191387_1_M_B_19	2191387		0		Blood	2019	73	B2		blaTEM-1D_1, mdf(A)_1
NEL1675_2191604_1_U_C_19	2191604		0		CSF	2019	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1676_2191604_2_U_B_19	2191604		0		Blood	2019	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1677_2191622_1_M_C_19	2191622		0		CSF	2019	131	B2	gyrA_S83L, parE_I529L	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6
NEL1678_2191671_1_M_C_19	2191671		462		CSF	2019	73	B2		mdf(A)_1
NEL1679_2191690_1_M_B_19	2191690		2		Blood	2019	95	B2	gyrA_S83L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1680_2191691_1_M_B_19	2191691		0		Blood	2019	538	B2	parE_D475E	mdf(A)_1
NEL1681_2191713_1_M_C_19	2191713		0		CSF	2019	95	B2		mdf(A)_1
NEL1682_2191713_2_M_B_19	2191713		0		Blood	2019	95	B2		mdf(A)_1
NEL1683_2191733_1_M_C_19	2191733		0		CSF	2019	131	B2	gyrA_S83L	mdf(A)_1
NEL1684_2191733_2_M_B_19	2191733		0		Blood	2019	131	B2	gyrA_S83L	mdf(A)_1
NEL1685_2191734_1_F_B_19	2191734		0		Blood	2019	131	B2	parE_I529L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1686_2191828_1_M_B_19	2191828		1		Blood	2019	131	B2	parE_I529L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1687_2191851_1_M_B_19	2191851		0		Blood	2019	69	D	parC_S80I	mdf(A)_1
NEL1688_2191934_1_F_B_19	2191934		0		Blood	2019	69	D		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1690_2191936_1_F_B_19	2191936		5		Blood	2019	95	B2		ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA14_5, dfrA5_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6
NEL1691_2191944_1_M_B_19	2191944		1		Blood	2019	69	D		mdf(A)_1
NEL1692_2191962_1_M_B_19	2191962		3		Blood	2019	69	D		mdf(A)_1
NEL1693_2192057_1_M_B_19	2192057		0		Blood	2019	95	B2		mdf(A)_1
NEL1694_2192058_1_M_B_19	2192058		2		Blood	2019	73	B2		mdf(A)_1
NEL1696_2192080_1_F_B_19	2192080		1		Blood	2019	404	B2		aadA2_1, dfrA12_8, mdf(A)_1, sul1_5
NEL1697_2192081_1_M_B_19	2192081		0		Blood	2019	10	A		aph(3'')-Ib_5, aph(6)-Id_1, dfrA1_8, mdf(A)_1, sul2_2
NEL1698_2192107_1_M_B_19	2192107		0		Blood	2019	69	D		mdf(A)_1
NEL1699_2192124_1_F_B_19	2192124		0		Blood	2019	80	B2		mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1700_2192173_1_F_B_19	2192173		unknown		Blood	2019	80	B2		mdf(A)_1
NEL1701_2192187_1_F_B_19	2192187		7		Blood	2019	3877	A		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, sul2_2, tet(A)_6
NEL1702_2192207_1_F_B_19	2192207		2		Blood	2019	449	D	parC_S57T	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA7_5, mdf(A)_1, qnrB19_1, sul1_5, sul2_3
NEL1703_2192216_1_M_B_19	2192216		0		Blood	2019	-	B2		blaTEM-1C_1, mdf(A)_1
NEL1704_2192217_1_M_B_19	2192217		0		Blood	2019	4481	B1		mdf(A)_1
NEL1705_2192228_1_F_B_19	2192228		0		Blood	2019	12	B2		mdf(A)_1
NEL1706_2192284_1_F_B_19	2192284		6		Blood	2019	-	B2	gyrA_S83L	mdf(A)_1
NEL1707_2192308_1_M_C_19	2192308		0		CSF	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, blaTEM-1B_1, mdf(A)_1
NEL1708_2192308_2_M_B_19	2192308		0		Blood	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, blaTEM-1B_1, mdf(A)_1
NEL1709_2192389_1_M_B_19	2192389		0		Blood	2019	131	B2	parE_I529L	mdf(A)_1
NEL1710_2192402_1_M_B_19	2192402		0		Blood	2019	-	E		mdf(A)_1
NEL1711_2192403_1_M_B_19	2192403		1		Blood	2019	127	B2		mdf(A)_1
NEL1712_2192442_1_M_B_19	2192442		0		Blood	2019	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, catA1_1, floR_2, mdf(A)_1, sul2_3, tet(A)_6
NEL1714_2192459_1_M_C_19	2192459		770		CSF	2019	127	B2		aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1D_1, mdf(A)_1, tet(B)_2
NEL1715_2192470_1_F_B_19	2192470		0		Blood	2019	101	B1		mdf(A)_1
NEL1716_2192485_1_U_B_19	2192485		2		Blood	2019	-	B2		blaTEM-1C_1, mdf(A)_1
NEL1717_2192486_1_M_C_19	2192486		1		CSF	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, blaTEM-1B_1, mdf(A)_1
NEL1718_2192503_1_M_B_19	2192503		1		Blood	2019	83	B2		mdf(A)_1
NEL1719_2192516_1_M_C_19	2192516		241		CSF	2019	69	D		mdf(A)_1, tet(B)_2
NEL1720_2192614_1_M_B_19	2192614		0		Blood	2019	95	B2		blaTEM-1B_1, mdf(A)_1
NEL1721_2192649_1_F_B_19	2192649		0		Blood	2019	69	D	gyrA_S83L	blaCTX-M-15_1, dfrA14_5, mdf(A)_1, mph(A)_2, tet(B)_2
NEL1722_2192650_1_F_B_19	2192650		1		Blood	2019	141	B2		mdf(A)_1
NEL1723_2192686_1_M_B_19	2192686		0		Blood	2019	62	F		mdf(A)_1
NEL1724_2192748_1_M_B_19	2192748		0		Blood	2019	80	B2		blaTEM-1C_1, mdf(A)_1
NEL1725_2192804_1_F_C_19	2192804		0		CSF	2019	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1726_2192804_2_F_B_19	2192804		0		Blood	2019	95	B2		aph(3'')-Ib_5, dfrA14_5, mdf(A)_1, sul2_2, tet(A)_6
NEL1727_2192822_1_M_B_19	2192822		0		Blood	2019	1982	B2	parE_I529L	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2
NEL1729_2192836_2_F_B_19	2192836		0		Blood	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1
NEL1731_2193089_1_M_B_19	2193089		0		Blood	2019	69	D		mdf(A)_1
NEL1733_2193170_1_F_B_19	2193170		0		Blood	2019	88	C		ant(3'')-Ia_1, aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, mdf(A)_1, mph(B)_1, sul1_5, sul2_3
NEL1734_2193185_1_F_B_19	2193185		0		Blood	2019	69	D	gyrA_S83A	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5
NEL1735_2193210_1_M_B_19	2193210		0		Blood	2019	10	A		dfrA1_8, mdf(A)_1, tet(B)_2
NEL1736_2193211_1_F_B_19	2193211		0		Blood	2019	69	D	gyrA_S83A	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5
NEL1737_2193213_1_M_C_19	2193213		0		CSF	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1
NEL1738_2193213_2_M_B_19	2193213		0		Blood	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1
NEL1739_2193244_1_F_B_19	2193244		6		Blood	2019	405	D		dfrA14_5, mdf(A)_1, sul2_2
NEL1740_2193271_1_M_C_19	2193271		0		CSF	2019	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F,	mdf(A)_1
NEL1741_2193272_1_F_B_19	2193272		0		Blood	2019	10	A		mdf(A)_1
NEL1742_2193277_1_M_B_19	2193277		3		Blood	2019	12	B2		aph(3')-Ia_1, blaTEM-1C_1, mdf(A)_1, tet(B)_2
NEL1745_2200037_1_F_B_20	2200037		0		Blood	2020	141	B2		mdf(A)_1, tet(B)_2
NEL1746_2200078_1_F_B_20	2200078		0		Blood	2020	95	B2		dfrA5_1, mdf(A)_1, tet(A)_6
NEL1747_2200095_1_F_C_20	2200095		0		CSF	2020	95	B2		dfrA5_1, mdf(A)_1
NEL1748_2200096_1_M_C_20	2200096		1		CSF	2020	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1
NEL1749_2200358_1_F_B_20	2200358		0		Blood	2020	144	B2		blaTEM-1B_1, mdf(A)_1
NEL1750_2200421_1_M_B_20	2200421		0		Blood	2020	131	B2	gyrA_S83L, parE_I529L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1751_2200596_1_F_B_20	2200596		1		Blood	2020	62	F		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1752_2200693_1_M_B_20	2200693		0		Blood	2020	69	D		mdf(A)_1
NEL1753_2200730_1_F_B_20	2200730		0		Blood	2020	38	D	gyrA_S83L, parC_S57T	aph(3'')-Ib_2, aph(6)-Id_1, dfrA7_5, mdf(A)_1, sul2_2
NEL1754_2200782_1_M_B_20	2200782		0		Blood	2020	83	B2		mdf(A)_1
NEL1755_2200801_1_F_C_20	2200801		0		CSF	2020	95	B2		aph(3'')-Ib_5, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1756_2200801_2_F_B_20	2200801		0		Blood	2020	95	B2		dfrA5_1, mdf(A)_1, sul2_3
NEL1757_2200816_1_F_B_20	2200816		0		Blood	2020	95	B2		blaTEM-1B_1, mdf(A)_1, tet(C)_3
NEL1758_2200898_1_M_B_20	2200898		0		Blood	2020	95	B2		mdf(A)_1
NEL1759_2200932_1_F_B_20	2200932		0		Blood	2020	127	B2	gyrA_S83L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1760_2200989_1_M_B_20	2200989		1		Blood	2020	131	B2	parE_I529L	mdf(A)_1, tet(B)_2
NEL1761_2200990_1_M_B_20	2200990		2		Blood	2020	69	D		aac(3)-IVa_1, aph(4)-Ia_1, blaTEM-1C_1, mdf(A)_1, tet(31)_1, tet(A)_6
NEL1762_2201046_1_F_B_20	2201046		2		Blood	2020	131	B2	gyrA_D87N, gyrA_S83L, parC_E84V, parC_S80I, parE_I529L	aac(3)-IId_1, aac(6')-Ib-cr_1, aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-15_1, blaOXA-1_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6
NEL1763_2201047_1_M_B_20	2201047		0		Blood	2020	5484	B2		mdf(A)_1
NEL1764_2201056_1_M_B_20	2201056		0		Blood	2020	12	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1765_2201061_1_F_B_20	2201061		0		Blood	2020	58	B1		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3, tet(A)_6
NEL1766_2201075_1_M_B_20	2201075		0		Blood	2020	144	B2		mdf(A)_1
NEL1767_2201156_1_F_B_20	2201156		0		Blood	2020	131	B2	parE_I529L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1768_2201233_1_M_B_20	2201233		0		Blood	2020	40	B1		mdf(A)_1
NEL1769_2201247_1_M_B_20	2201247		0		Blood	2020	537	B2		mdf(A)_1
NEL1770_2201248_1_F_B_20	2201248		0		Blood	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1771_2201249_1_M_B_20	2201249		5		Blood	2020	648	F		mdf(A)_1
NEL1772_2201264_1_F_B_20	2201264		0		Blood	2020	127	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, tet(B)_2
NEL1773_2201265_1_F_B_20	2201265		5		Blood	2020	357	B2		mdf(A)_1
NEL1774_2201273_1_F_B_20	2201273		0		Blood	2020	421	B2		
NEL1775_2201279_1_M_B_20	2201279		0		Blood	2020	127	B2	gyrA_S83L	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6, tet(B)_2
NEL1776_2201284_1_F_B_20	2201284		2		Blood	2020	550	B2		blaTEM-1B_1, mdf(A)_1
NEL1777_2201323_1_M_B_20	2201323		0		Blood	2020	95	B2		mdf(A)_1, tet(A)_6
NEL1778_2201324_1_F_B_20	2201324		0		Blood	2020	69	D	parC_S80I	aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, dfrA5_1, mdf(A)_1, sul2_14, tet(B)_2
NEL1779_2201351_1_M_B_20	2201351		1		Blood	2020	40	B1		mdf(A)_1
NEL1780_2201357_1_M_B_20	2201357		0		Blood	2020	62	F		mdf(A)_1
NEL1781_2201396_1_M_B_20	2201396		0		Blood	2020	73	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, mph(A)_2, sul2_3, tet(A)_6
NEL1782_2201405_1_M_B_20	2201405		1		Blood	2020	95	B2	gyrA_D87N, gyrA_S83L, parC_S80I	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_8, mdf(A)_1, sul2_3, tet(A)_6
NEL1783_2201422_1_M_B_20	2201422		1		Blood	2020	421	B2		mdf(A)_1
NEL1784_2201433_1_M_B_20	2201433		0		Blood	2020	6767	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA14_5, mdf(A)_1, sul2_2, tet(A)_6
NEL1785_2201434_1_F_B_20	2201434		4		Blood	2020	69	D		ant(3'')-Ia_1, blaTEM-1B_1, mdf(A)_1, sul1_5, tet(A)_6
NEL1786_2201442_1_M_C_20	2201442		0		CSF	2020	95	B2		mdf(A)_1
NEL1787_2201442_2_M_B_20	2201442		0		Blood	2020	95	B2		mdf(A)_1
NEL1788_2201467_1_M_C_20	2201467		738		CSF	2020	69	D		ant(3'')-Ia_1, blaOXA-1_1, blaTEM-1B_1, floR_2, mdf(A)_1, sul1_5, sul2_2, tet(A)_6
NEL1789_2201480_1_F_B_20	2201480		0		Blood	2020	69	D		blaCTX-M-15_1, mdf(A)_1, qnrS1_1
NEL1790_2201491_1_F_B_20	2201491		0		Blood	2020	95	B2		mdf(A)_1, qnrS1_1
NEL1791_2201510_1_F_C_20	2201510		0		CSF	2020	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1792_2201515_1_F_C_20	2201515		0		CSF	2020	3346	G		mdf(A)_1
NEL1793_2201556_1_F_B_20	2201556		0		Blood	2020	14	B2		blaTEM-1D_1, mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1800_2201722_1_M_C_20	2201722		0		CSF	2020	416	B2		mdf(A)_1
NEL1801_2201746_1_M_B_20	2201746		0		Blood	2020	416	B2		mdf(A)_1
NEL1802_2201748_1_F_B_20	2201748		0		Blood	2020	141	B2		aadA5_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, tet(B)_2
NEL1803_2201758_1_M_C_20	2201758		0		CSF	2020	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	aac(3)-IId_1, aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6
NEL1804_2201769_1_U_B_20	2201769		1		Blood	2020	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1805_2201791_1_M_C_20	2201791		1049		CSF	2020	95	B2		mdf(A)_1
NEL1806_2201804_1_M_B_20	2201804		0		Blood	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, qnrB19_1, sul2_3
NEL1807_2201817_1_F_B_20	2201817		0		Blood	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, sul2_3
NEL1808_2201864_1_M_B_20	2201864		0		Blood	2020	69	D	parC_S80I	aph(3'')-Ib_2, aph(3')-Ia_3, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, tet(B)_1
NEL1809_2201909_1_M_B_20	2201909		0		Blood	2020	69	D	gyrA_S83L	blaTEM-1B_1, mdf(A)_1
NEL1810_2201936_1_M_B_20	2201936		0		Blood	2020	73	B2		mdf(A)_1
NEL1811_2201948_1_M_B_20	2201948		8		Blood	2020	69	D		mdf(A)_1
NEL1812_2201962_1_F_B_20	2201962		0		Blood	2020	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1
NEL1813_2201978_1_M_B_20	2201978		0		Blood	2020	69	D		aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_3
NEL1814_2201991_1_M_B_20	2201991		0		Blood	2020	127	B2		mdf(A)_1
NEL1816_2202032_1_F_B_20	2202032		12		Blood	2020	38	D		blaTEM-1B_1, mdf(A)_1
NEL1817_2202088_1_M_C_20	2202088		644		CSF	2020	357	B2		mdf(A)_1
NEL1818_2202089_1_M_C_20	2202089		0		CSF	2020	95	B2		mdf(A)_1
NEL1819_2202089_2_M_B_20	2202089		0		Blood	2020	95	B2		mdf(A)_1
NEL1820_2202149_1_F_B_20	2202149		0		Blood	2020	131	B2	parE_I529L	aac(3)-IId_1, aadA2_1, blaTEM-1B_1, dfrA12_8, mdf(A)_1, mph(A)_2, sul1_5, tet(A)_6
NEL1821_2202150_1_F_B_20	2202150		0		Blood	2020	95	B2		mdf(A)_1
NEL1822_2202160_1_M_C_20	2202160		695		CSF	2020	73	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL1823_2202160_2_M_B_20	2202160		695		Blood	2020	73	B2		ant(3'')-Ia_1, blaOXA-1_1, catA1_1, mdf(A)_1, sul1_5, tet(B)_2
NEL1824_2202165_1_F_B_20	2202165		11		Blood	2020	8026	B2		mdf(A)_1
NEL1825_2202196_1_F_C_20	2202196		0		CSF	2020	95	B2	gyrA_S83L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1826_2202196_2_F_B_20	2202196		0		Blood	2020	95	B2	gyrA_S83L	aac(3)-IId_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, sul2_2, tet(A)_6
NEL1827_2202256_1_M_B_20	2202256		10		Blood	2020	73	B2		mdf(A)_1
NEL1828_2202270_1_F_B_20	2202270		0		Blood	2020	349	D		mdf(A)_1
NEL1829_2202271_1_M_B_20	2202271		0		Blood	2020	95	B2		blaTEM-1C_1, mdf(A)_1
NEL1830_2202310_1_M_C_20	2202310		0		CSF	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1C_1, mdf(A)_1, sul2_2
NEL1831_2202318_1_M_B_20	2202318		0		Blood	2020	5771	D		mdf(A)_1
NEL1832_2202324_1_M_B_20	2202324		0		Blood	2020	141	B2		mdf(A)_1, tet(A)_6
NEL1833_2202333_1_F_C_20	2202333		0		CSF	2020	95	B2		ant(3'')-Ia_1, dfrA1_8, mdf(A)_1
NEL1834_2202334_1_F_B_20	2202334		0		Blood	2020	95	B2		mdf(A)_1
NEL1835_2202341_1_F_B_20	2202341		2		Blood	2020	101	B1		mdf(A)_1
NEL1836_2202374_1_M_B_20	2202374		0		Blood	2020	416	B2		mdf(A)_1
NEL1838_2202416_1_U_B_20	2202416		0		Blood	2020	95	B2		mdf(A)_1
NEL1839_2202417_1_F_B_20	2202417		0		Blood	2020	141	B2		mdf(A)_1
NEL1840_2202426_1_F_B_20	2202426		1		Blood	2020	12	B2		mdf(A)_1
NEL1841_2202434_1_M_C_20	2202434		2		CSF	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1842_2202434_2_M_B_20	2202434		2		Blood	2020	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1843_2202435_1_F_B_20	2202435		4		Blood	2020	95	B2		mdf(A)_1
NEL1844_2202436_1_M_B_20	2202436		0		Blood	2020	95	B2		blaTEM-1A_1, mdf(A)_1
NEL1845_2202456_1_M_B_20	2202456		0		Blood	2020	95	B2		mdf(A)_1
NEL1846_2202469_1_M_B_20	2202469		0		Blood	2020	963	D		mdf(A)_1
NEL1847_2210021_1_M_C_21	2210021		0		CSF	2021	416	B2		mdf(A)_1
NEL1848_2210021_2_M_B_21	2210021		0		Blood	2021	416	B2		mdf(A)_1
NEL1850_2210023_1_M_C_21	2210023		484		CSF	2021	131	B2	gyrA_D87N, gyrA_S83L, parC_E84V, parC_S80I, parE_I529L	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaCTX-M-27_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2, tet(A)_6
NEL1851_2210044_1_F_B_21	2210044		3		Blood	2021	95	B2		aph(3'')-Ib_5, aph(3')-Ia_1, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, qnrB19_1, sul2_3
NEL1852_2210048_1_F_O_21	2210048		410		Vagina	2021	73	B2		mdf(A)_1
NEL1853_2210060_1_F_B_21	2210060		0		Blood	2021	69	D	parC_S80I	blaTEM-1B_1, mdf(A)_1
NEL1854_2210061_1_M_B_21	2210061		1		Blood	2021	131	B2	gyrA_S83L, parE_I529L, parE_S458A	aadA5_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, sul1_5, sul2_2
NEL1855_2210070_1_F_B_21	2210070		0		Blood	2021	95	B2		mdf(A)_1
NEL1856_2210071_1_M_B_21	2210071		0		Blood	2021	95	B2	gyrA_S83L, parC_G78C	dfrA5_1, mdf(A)_1, tet(A)_6
NEL1857_2210078_1_F_B_21	2210078		0		Blood	2021	421	B2		mdf(A)_1
NEL1859_2210094_1_M_B_21	2210094		0		Blood	2021	95	B2		mdf(A)_1
NEL1860_2210100_1_M_B_21	2210100		0		Blood	2021	69	D		mdf(A)_1
NEL1861_2210119_1_M_C_21	2210119		740		CSF	2021	73	B2		mdf(A)_1
NEL1862_2210123_1_F_B_21	2210123		0		Blood	2021	69	D	parC_S80I	ant(3'')-Ia_1, aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA1_10, floR_2, mdf(A)_1, sul2_2, tet(A)_6
NEL1863_2210126_1_F_B_21	2210126		0		Blood	2021	93	A	gyrA_D87N, gyrA_S83L, parC_S80I	aac(3)-IIa_1, blaTEM-1A_1, mdf(A)_1, mef(C)_1, mph(G)_1, tet(B)_2
NEL1864_2210131_1_M_B_21	2210131		0		Blood	2021	95	B2		mdf(A)_1
NEL1865_2210132_1_M_C_21	2210132		740		CSF	2021	73	B2		mdf(A)_1
NEL1868_2210158_1_F_B_21	2210158		0		Blood	2021	69	D		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, sul2_2, tet(A)_6
NEL1869_2210159_1_M_B_21	2210159		0		Blood	2021	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	mdf(A)_1, mph(A)_2
NEL1872_2210216_1_M_B_21	2210216		0		Blood	2021	12	B2		blaTEM-1C_1, mdf(A)_1
NEL1889_2210548_1_M_B_21	2210548		0		Blood	2021	59	F	parE_I355T	mdf(A)_1
NEL1890_2210647_1_M_B_21	2210647		1		Blood	2021	-	A		ant(3'')-Ia_1, aph(6)-Id_1, blaOXA-1_1, blaTEM-1C_1, catA1_1, dfrA14_5, mdf(A)_1, sul2_2, tet(B)_2
NEL1891_2210713_1_F_B_21	2210713		0		Blood	2021	69	D		mdf(A)_1
NEL1892_2210724_1_M_B_21	2210724		0		Blood	2021	421	B2		mdf(A)_1
NEL1893_2210737_1_F_B_21	2210737		1		Blood	2021	95	B2		mdf(A)_1
NEL1894_2210769_1_M_B_21	2210769		0		Blood	2021	421	B2		mdf(A)_1
NEL1895_2210809_1_F_C_21	2210809		0		CSF	2021	1851	B2		mdf(A)_1
NEL1896_2210809_2_F_B_21	2210809		0		Blood	2021	1851	B2		mdf(A)_1
NEL1897_2210867_1_M_B_21	2210867		0		Blood	2021	1851	B2		mdf(A)_1
NEL1898_2210929_1_M_B_21	2210929		0		Blood	2021	38	D		ant(3'')-Ia_1, blaCTX-M-14b_1, dfrA1_8, mdf(A)_1
NEL1899_2210966_1_M_B_21	2210966		0		Blood	2021	410	C		aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1

Final ID	NRLBM number	Age (years)	Age (months)	Gender	Source	Year	ST	Phylogroup	par and gyr mutations (AMRFinder)	Abricate Resfinder
NEL1901_2210985_1_M_B_21	2210985		0		Blood	2021	95	B2		aph(3'')-Ib_5, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul2_3
NEL1902_2210986_1_F_B_21	2210986		0		Blood	2021	5281	E		mdf(A)_1
NEL1903_2210987_1_M_B_21	2210987		0		Blood	2021	62	F		mdf(A)_1
NEL1904_2210988_1_F_B_21	2210988		4		Blood	2021	95	B2		mdf(A)_1
NEL1905_2210996_1_F_C_21	2210996		1		CSF	2021	1851	B2		mdf(A)_1
NEL1906_2210996_2_F_B_21	2210996		1		Blood	2021	1851	B2		mdf(A)_1
NEL1908_2211011_1_M_B_21	2211011		0		Blood	2021	95	B2		mdf(A)_1
NEL1910_2211076_1_F_B_21	2211076		0		Blood	2021	-	D	parC_S57T	mdf(A)_1
NEL1911_2211086_1_M_B_21	2211086		0		Blood	2021	127	B2		mdf(A)_1
NEL1912_2211098_1_F_B_21	2211098		0		Blood	2021	538	B2	parE_D475E	mdf(A)_1
NEL1913_2211113_1_M_B_21	2211113		0		Blood	2021	69	D		mdf(A)_1
NEL1914_2211174_1_M_C_21	2211174		0		CSF	2021	69	D		mdf(A)_1
NEL1915_2211184_1_F_B_21	2211184		0		Blood	2021	-	F		mdf(A)_1
NEL1916_2211185_1_F_B_21	2211185		6		Blood	2021	569	B2	parE_D475E	mdf(A)_1
NEL1917_2211191_1_M_B_21	2211191		0		Blood	2021	569	B2	parE_D475E	mdf(A)_1
NEL1918_2211192_1_M_B_21	2211192		0		Blood	2021	569	B2	parE_D475E	mdf(A)_1
NEL1919_2211193_1_M_B_21	2211193		1		Blood	2021	95	B2		mdf(A)_1
NEL1921_2211253_1_M_B_21	2211253		0		Blood	2021	1618	B2		mdf(A)_1
NEL1922_2211263_1_M_B_21	2211263		0		Blood	2021	349	D		aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1B_1, mdf(A)_1, tet(B)_2
NEL1933_2161721_3_F_C_16	2161721	-	0		CSF	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	blaDHA-1_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, qnrB4_1, sul1_5
NEL1934_2161721_4_F_B_16	2161721	-	0		Blood	2016	1193	B2	gyrA_D87N, gyrA_S83L, parC_S80I, parE_L416F	blaDHA-1_1, blaTEM-1B_1, dfrA17_1, mdf(A)_1, mph(A)_2, qnrB4_1, sul1_5
NEL1935_2171282_2_M_B_17	2171282	-	0		Blood	2017	4481	B1		mdf(A)_1
NEL1936_2171620_2_M_B_17	2171620	-	5		Blood	2017	127	B2		aph(6)-Id_1, blaTEM-1B_1, catA1_1, dfrA14_5, dfrA8_1, mdf(A)_1, sul2_2
NEL1937_2171625_2_M_B_17	2171625	-	9		Blood	2017	174	G		aph(3'')-Ib_2, aph(6)-Id_1, blaTEM-1B_1, dfrA5_1, mdf(A)_1, sul1_5, tet(A)_6

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